Control of Verticillium dahliae causing sunflower wilt using Brassica cover crops

Celia Seassau, Diane Desserre, Jérémy Desplanques, Emmanuelle Mestries, Gregory Dechamp-Guillaume, Lionel Alletto

19TH INTERNATIONAL SUNFLOWER CONFERENCE

TRAKYA ÜNİVERSİTESİ 1982

ISC 2016

29 MAY – 3 JUNE, 2016

EDIRNE, TURKEY

ISC 2016

PROCEEEDINGS
OF
19TH INTERNATIONAL SUNFLOWER CONFERENCE

29 MAY – 3 JUNE, 2016

EDİRNE, TURKEY

19TH INTERNATIONAL SUNFLOWER CONFERENCE

29 MAY – 3 JUNE, 2016,

EDIRNE, TURKEY

In

Trakya University Balkan Congress Center,

Edirne, Turkey

Organized by

Trakya University

and

International Sunflower Association

WELCOME from the CHAIR

You are welcome to our conference that will be jointly organized by Trakya University and International Sunflower Association. The aim of our conference is to present scientific subjects of a broad interest to the sunflower community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global sunflower production and trade. Our goal is to bring three communities, namely science, research, and private investment together in a friendly environment of Edirne, Turkey in order to share their interests and ideas and to benefit from the interaction with each other.

Our Conference held with record participation with over 600 people working on sunflower as researchers, scientists from seed companies, from oil industry and machinery coming from all part of the World. We have 300 papers which is a record number and almost doubles the previous meetings.

Due to many inquiries about combining our activities with oil industries in ISC 2016, International Sunflower Oil Quality Symposium are organized as one day as a side event during the conference. Sunflower farmers and growers will join also to our conference, so it will be also interesting as an initial attempt to bring together triangle dimensions as scientist, growers and industry in our conference.

Conference activities;

Plenary sessions with oral and poster presentations are on 30th, 31st of May and 1st of June 2016. Besides, the field day and the Sightseeing tours are on June 2nd – 3rd June 2016.

Agriculture is an important sector feeding all humankind, but it needs new developments and technologies to supply enough food for increasing world population year by year. Turkey is one of the most important contries on sunflower production and trade and an example to the leading agricultural economies in the world. Therefore, we hope that this conference will help to solve the problems encountered in the Sunflower community with establishing good network collaborations, joint projects and better relationships among countries with sharing our knowledge and experience together. We wish success to this meeting and hope a great scientific achievement together with your contributions.

Edirne is not only a very nice, lovely and historical city at the edge of Europe, but located just at the heart of Balkan region and history endowed with monuments reminding imperial past. We are much pleased to host you all in Edirne and in Turkey.

We would like to thank you to join this conference and we would like to give also special thanks our sponsors and collaborators for giving us big supports to organize this event.

We wish you nice stay in Edirne for truly rewarding days.

Assoc Prof Dr Yalcin KAYA

Head of Organizing Committee
President of International Sunflower Association
May 2016

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19TH INTERNATIONAL SUNFLOWER CONFERENCE
29 MAY – 3 JUNE, 2016
EDIRNE, TURKEY

CONFERENCE PROGRAM

GENERAL SESSION

SUNDAY, MAY 29th, 2016	
14 00 - 20 30	**Registration at Hotels and Balkan Congress Center**
MONDAY, MAY 30th, 2016	
08 30 - 09 30	Registration at Balkan Congress Center
09 30 - 10 30	Opening Ceremony Balkan Synphony Orchestra Slide Show: Sunflower from Soil to Table:Our Yellow Bride in the fields Giving Appreciation Certficates to our Sponsors
10 30 – 11 00	Coffee break
11 00 - 12 30	**OPENING SESSION:** Session Chair: ***PROF DR MARIA DUCA*** – Rector of University of Moldova Academy of Science
11 00 – 11 40	***Invited Speaker*** **Prof Dr. Dragan Skoric "HISTORY OF SUNFLOWER BREEDING IN THE WORLD"**
11 40 – 12 20	***Invited Speaker*** **Dr. Lili Qİ "MOLECULAR MAPPING OF THE DISEASE RESISTANCE GENES AND ITS IMPACT ON SUNFLOWER BREEDING"**
12 20 – 12 30	DISCUSSION
12 30 – 13 30	**LUNCH ((Courtesy of Nidera Semillas)**

	GENETIC AND BREEDING	BIOTIC AND ABIOTIC STRESS TOLERANCE	CROP PRODUCTION AND MANAGEMENT	MOLECULAR GENETICS
	(Main Meeting Room)	**(2nd Floor Senate Meeting Room)**	**(2nd Floor Left Meeting Room)**	**(2nd Floor Right Meeting Room)**
30.05.2016 MONDAY		**30.05.2016 MONDAY**	**30.05.2016 MONDAY**	**30.05.2016 MONDAY**
13^{30} -15^{30}	***1st Session Chair: CARLOS FEOLI***	***1st Session Chair: DR MARIA JOITA- PACUREANU***	***1stSession Chair:DR VALENTINA ENCHEVA***	***1stSession Chair: DR RENATE HORN***
13^{30} -13^{50}	***Invited Speaker*** **DR BRANISLAV DOZET** Contemporary Challenges in Sunflower Breeding	The genetics and evolution of solar tracking – B. BLACKMAN, S. HARMER	Use of polymer hydrogel in soil moisture conservation for sunflower cultivation in rainfed situations of Northern Karnataka, India: A case study – U. SHANWAD, B. CHITTAPUR, SHANKERGOUD I, B. DESAI, GOVİNDAPPA MR., V. KULKARNI	The cultivated sunflower pan genome provides insights on the wild sources of introgressions and their role in breeding – S. HUBNER, E. ZIGLER, J.R. MANDEL, D. SWANEVELDER, P. VINCOURT, N. LANGLADE, J. M. BURKE, L. H. RIESEBERG
13^{50} -14^{10}		Impact of exogenously applied glycine betaine on physiological attributes of sunflower under drought stress- NOSHIN I., NADIA Z., N. BATOOL, Q. BANO	Determination of the yield and yield components performance of some sunflowers (*Helianthus annuus* L.) under rainfed conditions – I. DEMIR	Principal Component Analysis for Carbon Isotope Discrimination-Related Traits in Recombinant Inbred Lines of Sunflower – A. L. ADIREDJO, T. LAMAZE, P. GRIEU
14^{10} -14^{30}	Genetic analysis of seed yield related traits under optimum and limited irrigation in sunflower – M. GHAFFARI	Rapid invitro screening of sunflower genotypes for moisture stress tolerance using PEG 6000 - SHANKERGOUD I., SHESHAIAH K. C.	Appropriate nitrogen (N) and phosphorus (P) fertilizer regime for sunflower (Helianthus annuus L.) in the humid tropics – E. AKPOJOTOR, V. OLOWE	Molecular Studies of Sunflower Responses to Abiotic Stresses – I. TINDAS, R. I. AYTEKIN, S. ÇALIŞKAN
14^{30} -14^{50}	Breeding for sunflower hybrids adapted to climate change: the SUNRISE collaborative and multi-disciplinary Project - LUBRANO-LAVADERA A.S., M. COQUE, MUNOS S., DEBAEKE P., MANGIN B., GOUZY J., KEPHALİACOS C., PIQUEMAL J., PINOCHET X.,	Exploring drought tolerance related traits in *Helianthus argophyllus*, *Helianthus annuus* and their hybrids – M. MUBASHAR HUSSAIN, M. KAUSAR, M. KHAN, P. MONNEVEUX	Interactive Effects of Different Intra-Row spacing and Nitrogen Levels on Yield and Yield Compenents of confectionery sunflower (*Helianthus annuus* L.) genotype (Alaca) Under Ankara conditions – S. DAY, O. KOLSARICI	Comparative assessment of androgenic response in sunflower (*Helianthus annus*) – N. AKGUL, E. ÇABUK ŞAHIN, Y. AYDIN, A. ALTINKUT UNCUOGLU, G. EVCI, A GÜREL

	LANGLADE N.			
14^{50}-15^{00}	Discussion	Discussion	Discussion	Discussion
15^{00}-15^{30}	Coffee break	Coffee break	Coffee break	Coffee break
15^{30}-17^{00}	***2nd Session: Chair: DR VLADIMIR MIKLIC***	***2nd Session: Chair: DR FELICITY VEAR***	***2nd Session Chair: PROF DR GIAN PAOLO VANNOZZI***	***2nd Session Chair: DR PHILIPPE DEBAEKE***
15^{30}-15^{50}	Assessment of sunflower germplasm selected for cold tolerance under autumn planting conditions in Morocco - HOUMANAT K., MAZOUZ H., EL FECHTALI M., NABLOUSSI A.	***Invited Speaker*** **PROF DR STEVAN MAŠIREVIĆ** Sunflower diseases research progress and management	Global change adaptation: what future for sunflower crops and products? A foresight study for oilseed chains at 2030 horizon – E. PILORGE, A. M. TREMBLAY, F. MUEL	Molecular and genetic aspects of sunflower defensive response to downy mildew - T. ȘESTACOVA, A.PORT, M. DUCA
15^{50}-16^{10}	Perspective and challenges to develop high yielding, disease resistant and oil quality sunflower hybrids in India - R.K.SHEORAN		Bioactivity and Phytochemical Evaluation of Sunflower (*Helianthus annuus* L.) Leaf Extract – Y. BIBI, A. QAYYUM, S. NISA	Recent Molecular Studies on Downy Mildew Disease – A. K. TURKMEN, S. CALISKAN
16^{10}-16^{30}	Stability performance of new introduced sunflower hybrids for seed yield and its components under Sudan conditions – A. A. M. ABDALLA	Control of Verticillium dahliae causing sunflower wilt using Brassica green manures - DESSERRE D., MESTRIES E., DECHAMP-GUILLAUME G., SEASSAU C.	Effects of Different Organomineral and Inorganic Compound Fertilizers on Seed Yield and Some Yield Components of Sunflower (*H. annuus* L.) – S. SUZER, E. CULHACI	Molecular Studies involved in sunflower responses in drought stres - I. ALTINDAS, E. AKSOY, S. CALISKAN
16^{30}16 ^{45}	Discussion	Discussion	Discussion	Discussion
16^{45}-18 ^{00}	***Poster Session***	***Poster Session***	***Poster Session***	***Poster Session***
19 ^{30} -	**Dinner Party (Courtesy of Syngenta)**	**Dinner Party (Courtesy of Syngenta)**	**Dinner Party (Courtesy of Syngenta)**	**Dinner Party (Courtesy of Syngenta)**

	31.05.2016 TUESDAY	31.05.2016 TUESDAY	31.05.2016 TUESDAY	31.05.2016 TUESDAY
09^{30} -10^{10}	***3RD Session Chair: DR OLIVIER COTTET***	***3RD Session Chair: PROF DR* STEVAN MASIREVIC**	***3RD Session Chair: DR AMELIA BERTERO DE ROMANO***	***3RD Session Chair: DR DRAGANA MILADINOVIC***
09^{30} -09^{50}	Collection of wild *Helianthus anomalus* and *deserticola* sunflower from the desert southwest USA – G. SEILER, L. MAREK	Isolation and identification of pathogen of Sunflower *Fusarium* Wilt - JING G. YUAN YUAN Z., GUİ Z., JİAN Z., KAİ W., JUN Z.	***Invited Speaker*** **DR PHILIPPE DEBAEKE** Sunflower and climate change in Europe: crop vulnerability, adaptation, and mitigation potential	Proteomic response of sunflower to drought stres – M. GHAFFARI, M. TOORCHI, M. VALIZADEH
09^{50} -10^{10}	The b1 locus that controls apical shoot branching in *H. annuus* exhibits a molecular diversity linked to the breeding history of hybrids - DURIEZ P., BONIFACE, M. C., POUILLY N., VAUTRIN S., MAYJ., RODDE N., BERGES H., CARRERE S., GOUZY J., P. VINCOURT, J. PIQUEMAL, S. MUNOS	Distribution of *Plasmopara halstedii* pathotypes in Hungary – R. BÁN, A. KOVÁCS, G. BAGLYAS, M. PERCZEL, G. TUROCZI, K. KOROSI		Identification of HaDELLA, HaGID1 as well as HaSLEEPY and HaSNEEZY genes involved in gibberellin signaling in sunflower - R. EWALD, N. GEHM, L. POPIOLKOWSKI, A. ANTELMANN, R. HORN
10^{10} -10^{30}	Phenotypic and genotypic characterization of 400 new sunflower pre-bred lines – G. BAUTE, W. ANYANGA, E. ALBRECHT, L. H. RIESEBERG	Exploitation of the knowledge on oomycete effectors to drive the discovery of durable disease resistance to downy mildew in sunflower – Y. PECRIX, L. BUENDIA, Q. GASCUEL, C. PENOUILH-SUZETTE, L. GODIARD	Chemical Broomrape (*Orobanche cumana*) control in Clearfield® sunflower with different Imazamox containing herbicide formulations – M. PFENNING, M. VALTIN, S. SASCHA, J. BESSAI	Characterization of sunflower inbred lines with high oleic acid content by DNA markers – B. B. BILGEN
10^{30} -10^{50}	Developing well adapted hybrids in Europe by using a G*E approach - GAUTIER F., HELOISE H., MILAGROS G., SAUVAIRE D.	Response to sunflower (*Helianthus annuus* L.) plant at early growth stage to cadmium toxicity – Y. CIKILI, H. SAMET, N. C. ATIKMEN	Pulsar® Plus and Eurolightning® Plus - herbicides for enhanced weed control in Clearfield® Plus sunflower – J. BESSAİ, SCHLÄFER S., PFENNING M., MORAN D., CARTIN J.	Evaluation of WRKY and MYB transcription factors in some downy mildew infected sunflower lines; microarray data analysis – E. FILIZ, I. I. ÖZYİĞİT, R. VATANSEVER

10^{50} -11^{00}	Discussion	Discussion	Discussion	Discussion
11^{00} -11^{20}	Coffee break	Coffee break	Coffee break	Coffee break
11^{20}-12^{30}	***4th Session: Chair: DR SINISA JOCIC***	***4th Session Chair: DR MICHAEL FOLEY***	***4th Session Chair: DR SUJATHA MULPURI***	***4th Session Chair: PROF DR RISHI BEHL***
11^{20}-11^{40}	Correlation studies between SSR marker based genetic distance and heterosis in sunflower (*Helianthus annuus* L.) – V. KULKARNI, SHANKERGOUD I., SUPRIYA S.M, SURESHA P.G.	PCR combined with GFP tagged *Verticillium dahliae* confirmed the seeds transmission of Sunflower *Verticillium* Wilt - YUAN YUAN Z., GUI Z., JIAN Z., JUN Z.	Relationships between Germination and Vigor Tests with Field Emergence of Sunflower in Iran – H. SADEGHI, S. SHEIDAEI	***Invited Speaker*** **DR STEPHANE MUNOS** De novo sequencing of the *Helianthus annuus* and *Orobanche cumana* genomes
11^{40}-12^{00}	Optimization of Agrobacterium-mediated gene transfer systems in Turkish sunflower (*Helianthus annuus* L.) varieties – I. I. ÖZYİĞİT, S. KARADENIZ, H. TOMBULOGLU, E. FILIZ	Stability of the level of partial resistance to white rot in sunflower – M. ANABELLA DINON, F. CASTAÑO, S. SAN MARTINO, J. LÚQUEZ, F. QUIROZ	Pest Monitoring and Handling System Based on 4G Mobile System – C. ATLIG	
12^{00}-12^{20}	Inclusion of dominance effect in genomic selection model to improve predictive ability for sunflower hybrid performance – F. BONNAFOUS, N. LANGLADE, B. MANGIN	Genetic divergence among sunflower inbred lines and their convergent improvement for yield, quality and disease resistance- R. RANI - R. K. SHEORAN – S. CHANDER – R. K. BEHL	New seed treatment solutions for *Plasmospora* Resistance Management in Sunflower – F. BRANDL	Comparison of cytoplasmic male sterility based on PET1 and PET2 cytoplasm in sunflower (*Helianthus annuus* L.) - HORN R., REDDEMANN A., DRUMEVA M
12^{20}-12^{30}	Discussion	Discussion	Discussion	Discussion
13^{30}-13^{30}	**Lunch (Courtesy of Edirne Farmer Union)**	**Lunch (Courtesy of Edirne Farmer Union)**	**Lunch (Courtesy of Edirne Farmer Union)**	**Lunch(Courtesy of Edirne Farmer Union)**
13^{30}-15^{30}	***5th Session Chair: DR THIERRY ANDRE***	***5th Session Chair: DR ROBERT NEMETH***	***5th Session Chair: PROF DR BENJAMIN BLACKMAN***	***5th Session Chair: PROF DR DEJANA PANKOVIC***
13^{30}-13^{50}	***Invited Speaker*** **DR MARIA JOITA-PACUREANU** Broomrape (*Orobanche cumana* Wallr.) - Update on racial	Cadmium-potassium interrelationships in sunflower (*Helianthus annuus* L.) – H. SAMET, Y. CIKILI, N. C. ATIKMEN	Performance of sunflower hybrids in black cotton soils of Northern Karnataka, India – U. SHANWAD, SHANKERGOUD I, S. N. SUDHAKARBABU, V. KULKARNI, GOVINDAPPA MR, VIJAYKUMAR G.	Approaches for improvement of resistance to powdery mildew in sunflower (*Helianthus annuus* L.) – S. MULPURI, K. PALCHAMY, C. R. SANKARANENI, V. KODEBOYİNA

13^{50} -14^{10}	composition and distribution, host resistance and management	Effects of Micro Nutrients (Fe, Zn, B and Mn) on Yield and Yield Components of Two Sunflower (*Helianthus annuus* L.) Cultivars in Urmia Condition – A. RAHIMI, J. JALILIAN	Modeling sunflower fungal complex to help design integrated pest management strategies - AUBERTOT J. N., MESTRIES E., M. A. VEDY-ZECCHINI, P. DEBAEKE	Genetic engineering studies on sunflower- M. E. ÇALIŞKAN, S. DAS DANGOL
14^{10} -14^{30}	Testing annual wild sunflower species for resistance to *Orobanche cumana* Wallr – S. TERZIĆ, B. DEDIĆ, J. ATLAGIĆ, S. JOCIĆ, D. MILADINOVIĆ, M. JOCKOVIĆ	Quantification of drought tolerance levels of sunflower inbred lines by means of *chlorophyll*-a fluorescence - A. S. BALKAN, NALCAIYI, S. CULHA ERDAL - O. GUNDUZ, V. PEKCAN, O. ARSLAN, N. CICEK, Y. KAYA, Y. EKMEKCI	Escape to tiny bug (*Nysius simulans* Stål) attack across planting date adjustment in sunflower hybrid seed crops from southern BuenosAires province, Argentine – J. RENZI, O. REINOSO, M. BRUNA, M. AVALOS, M. CANTAMUTTO	***Invited Speaker*** **DR NICOLAS LANGLADE** Genome-wide association of oil yield plasticity to drought, nitrogen and chilling stresses in sunflower
14^{30} -14^{50}	Determination of superior hybrid combinations in sunflower and testing of their resistance to broomrape (*Orobanche cumana* Wallr.) In infested areas – O. GÜNDÜZ, A. T. GOKSOY	The effect of climate factors and climate change on the yield of sunflower (*Helianthus annuus* L.) in Marmara region – H. GURKAN, H. BULUT, N. BAYRAKTAR, M. DEMIRCAN, O. ESKİOĞLU, N. KOÇAK	Current Situation, Problems and Solutions of Sunflower in the Central Anatolian Region – C. YAVUZ, S. CALISKAN	
14^{50} -15^{00}	Discussion	Discussion	Discussion	Discussion
15^{00} -15^{30}	Coffee break	Coffee break	Coffee break	Coffee break
15^{30} -17^{00}	***6th Session Chair: DR CHAO CHIEN JAN***	***6th Session: Chair: DR GERALD SEILER***	***6th Session Chair: PROF DR MICHELLE GILLEY***	***6th Session Chair: DR STEPHANE MUNOS***
15^{30} -15^{50}	***Invited Speaker*** **DR GORAN MALIDZA** Integrated weed management in sunflower: Challenges and opportunities	Effects of Naphthalene Acetic Acid and N6-Benzyladenine on Androgenesis in *Helianthus annuus* L. Anthers - S. DAYAN, H. ARDA	Microbial Dressing of Sunflower Seeds with *Trichoderma harzianum* KUEN 1585 – Y. S. YONSEL, M. SEVİM	QTL mapping for broomrape (*Orobanche cumana* Wallr.) resistance in sunflower – I. CELİK, D. ZARARSIZ, A. FRARY, S. DOGANLAR
15^{50} -16^{10}		Do cell wall proteins affect the setting of grains and their potential weight in sunflower? – D. CALDERINI, S. VASQUEZ, F. CASTILLO, P.	Green and brown bridges aid survival of multiple *Diaporthe / Phomopsis* species with a range of virulences on sunflower, soybeans,	Determination the genetic characterization of different lines of sunflower (*Helianthus annuus* l.)by using genetic resources

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KEYNOTE PAPERS

HISTORY OF SUNFLOWER BREEDING IN THE WORLD

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ABSTRACT

The sunflower originates from North America. Native Americans were the first breeders of sunflower. Sunflower arrived in Europe in 1510 as an ornamental plant in a botanical garden in Madrid, Spain. Later, sunflowers spread across Western Europe as a decorative plant. There is written evidence the sunflower arrived in Russia in 1599 and in Ukraine in 1613. In the 1830s in the Russia sunflowers began to be grown as and oilseed crop. Sunflower breeding on scientific basis began in imperial Russia in the early 20th century Kharkiv Station in 1910, Kruglik Station in Krasnodar in 1912, Saratov Station in 1913, and a little bit later in Rostov on Don, Armavir and others. In the first of 20th century at the above institutions a large number of varieties were developed that were highly productive and increased oil content, resistance to the sunflower head moth, and the existing races of broomrape. In the 1960s productive varieties with an oil content of above 50% were developed (Pustavoit and Zhdanov). Among these varieties, the most well known ones were Peredovik, VNIIMK 8931, Majak and others. They contributed to the spread of sunflower across the world. Using interspecific hybridization between the cultivated sunflower and *H. tuberosus*, Galina Pustavoit developed a large number of varieties with a broader resistance to diseases. The first inbreeding efforts were begun by Plachek at the Saratov Station, while the manifestation of heterosis for the major traits based on diallel crosses was first implemented by Morozov. In the 1950s in several centers there was intensive investigation of inbreeding and heterosis in sunflower. This research was carried out by Putt in Canada, Habura and Schuster in Germany, Vranceanu and Stoenescu in Romania, and Gundaev, Zhdanov, Wolf and others in the former USSR. Leclercq obtained the first stable source of CMS by crossing the wild species *H. petiolaris* Nutt. with the cultivated sunflower. Kinman, Enns et al., Leclercq, Vranceanu and Stoenescu and some others discovered the Rf genes, which enabled the development of commercial sunflower hybrids. At that time in public institutions and numerous private companies intensive programs were established on the development of sunflower hybrids, which quickly led to the introduction of sunflower hybrids into large-scale production and an increase in areas under this crop. In the paper proper we will discuss in detail the main centers of sunflower breeding in the world and their achievements. Induced and spontaneous mutations helped develop mutants with different levels of fatty acids (high-oleic) and different tocopherols at VNIIMK, Krasnodar and Cordoba, Spain, which enabled the development of hybrids with novel oils. It should be noted here that the wild sunflower species through the use of interspecific hybrids played a significant role in the increase of genetic variability of the cultivated sunflower, especially in the discovery of sources of resistance to different pathogens. Also, the wild species were used to identify resistance to the herbicides imidazolinones and sulfonylurea. Lately, the use of molecular methods, especially marker genes, is well under way in sunflower breeding worldwide.

Key Words : History, sunflower, breeding, varieties, hybrids, resistance

CONTEMPORARY CHALLENGES IN SUNFLOWER BREEDING

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ABSTRACT

The discovery of novel agronomically- and commercially-significant traits has changed the segmentation of the sunflower crop over the last years and has triggered higher complexity in sunflower breeding. Further, the development of the palm oil sector has changed the vegetable oil market. To remain competitive in this market, sunflower breeding needs to accelerate efforts to improve yield potential as well as adaptation to abiotic-/biotic-stress. Molecular genetics and associated technologies play significant roles in the discovery and understanding of novel traits. Together with high-throughput sequencing, molecular technologies will drive breeding to high-efficiency in the current era. Marker Assisted Recurrent Selection (MARS) and Genome Wide Selection (GWS) will allow breeders to better predict the breeding value, as manifest by genetic gain, of crosses and breeding programs. The application of new tools (*e.g*. digital phenotyping, hyperspectral-imaging, drones, and tractor-sensory-platforms) to facilitate complex data acquisition will significantly improve the precision of measurements and increase breeding efficiency. Interspecific-hybridization and induced mutations are, and will continue to be, major development areas for the identification of new traits and crop improvement. Doubled-haploidization (DH) is a methodology currently unavailable to the sunflower breeding process. This is likely to remain the situation for the foreseeable future as the development of DH for sunflower faces substantial technical and biological challenges. In January 2015, the "bronze" version of the first genomic reference sequence for sunflower (based on accession HA412-HO) was published and, by June, made publicly available. This milestone represents a clear advantage for sunflower breeding and is the foundation for increasing our knowledge of the sunflower genome. As our understanding of the sunflower genome improves, we will, over the next few years, transition to the post-genomic era in sunflower.

MARKET SEGMENTATION AND BREEDING GOALS

Oil crops take up around 10% of the total cultivable area worldwide, and other than the main cultivation aim, which is the production of oil, they also play an important part in food production, as well as in the sector of energy for the production of biodiesel. On the list of the most important oil crops, sunflowers hold a high position at number four. Allowing for the exception of breeding modifications geared towards the yield of seed and oil as the primary aim of breeding programs in the private sector (the primary goal of non-profit organisations can vary), the increase in market segmentation has had a great impact on breeding goals in the last few years. This refers, in particular, to the increase in demand for sunflower varieties with higher oil yields, as well as the introduction of herbicide-tolerant hybrids. In comparison to the situation 15 to 20 years ago, when there were 12 basic segments, there is greater complexity with 24 basic segments present in the contemporary market. Complexity is further increased with the sub-segmentation of: herbicide-tolerance (HT) between sulphonylurea (SU) and imidazolinone (IMI) resistances; disease races present

in specific regions (*e.g.* downy mildew- or broomrape-races); or by allowing for the segmentation distinguishing between oil production and confectionary as an end-use. This type of segmentation tends to be more global and is not applicable to every market. The segmentation of certain markets is far simpler than that of others: *e.g.* Argentina and the USA have market segmentation patterns that are much simpler than in Europe. This does not, however, diminish the importance of primary breeding goals which are seed- and oil-yield, as well as resistance to economically important diseases. Another segmentation type exists in India, where seasonal cultivation (spring and winter) informs the primary segmentation, and where an HT segment does not functionally exist. On the other hand, in the Russian and Ukrainian markets (which make up over 50% of the total sunflower cultivation areas), nearly all market segments are observed.

Addressing this type of segmentation requires the participation of all functions in the hybrid breeding process ranging from strategic planners, marketing teams, sales managers, research and development teams, production and even external consulting companies which help the team make sound strategic decisions. The programs must be balanced towards certain market segments and financial capabilities. It should never be forgotten that the selective breeding process takes a long time and that any wrong decision made today can bare implications which are made visible only after a few years have passed. Although segmentation is relatively stable, it is still dynamic in its own right, and strategic choices are to be adjusted according to the development of certain segments. Sunflower is, above all, an industrial crop whose segment development depends heavily on industrial demand. The increase in demand for high oil-yield sunflower can drastically affect the priorities and strategies of segment development in relation to high oil-yield varieties, especially after the implementation of labeling regulations in the EU. This is particularly important when developing segments for export-oriented markets such as Ukraine and Argentina.

A highly segmented market, such as that of sunflower, can rapidly change the demands placed on breeders and their programs. Further, this environment changes the attitude of breeders themselves, by shifting the process from distinct programs based on the efforts of an individual to team-based programs. Effective delivery requires/will require:

- increases in breeding efficiency;
- the introduction of new methods and techniques;
- expedition of the selection process using methods such as Marker Assistant Selection (MAS) and MARS;
- efficient intra-/extraorganizational material transfer (including the drafting and validation of material transfer agreements where necessary);
- strict breeder rights enforcement wherever possible;
- the creation of joint programs in collaboration with public and non-profit institutions;

BEYOND THE GENE

One of the greatest challenges breeders face is improving the analysis and understanding of gene expression/regulation and the subsequent impact on individual phenotypes, the phenome, and the metabolome given the context of environment. The development of "-omics" data and capabilities will be crutial in the coming decade and help improve the understanding of the complex processes that take place within the whole organism. Genomics, phenomics, and metabolomics bare a particular importance in sunflower research going forward.

Phenomics is a relatively new discipline whose name derives from the word "phenome". Coined in middle of the 20th century (Davis, 1949), phenome refers to the set of all potentially observable phenotypes of a cell, tissue, organ, organism, or species. Phenomics explores a wide array of processes influencing the phenome and individual phenotypes. Phenomics can also be defined as a highly detailed dissection of breed traits and their connection to the gene or genes (Furbank and Tester, 2011). The identification of QTL within the genome of differing plant species is a difficult and demanding task and the factors which can influence QTL detection can vary greatly: the genetic QTL basis causal to the trait in question, enviorment influence, population size, experimental error, and *etc*. The precision of phenotypic rating and the robustness of QTL mapping are intimately linked: a reasonable and actionable QTL map cannot be generated without accurate phenotype rating data. During the last 20 years or so, progress in precision phenotyping was more limited than that of molecular techniques, thus reducing the utility of QTL, especially in relation to traits such as seed yield. The research into *Phoma macdonaldii*, done by Maleki *et al*. (2014), showed a sizable genetic variability within the F_3 generations. 14 main QTLs were found, localized within seven related groups, with a phenotype variation ranging from 4 to 24 percent. The most important QTL identified in this study related to partial resistance to the three fungus isolates applied in the study. This QTL is a strong candidate for introduction of *Phoma macdonaldii* resistance using MAS. Sunflowers were also subjected to QTL research for other traits such as: water content and chlorophyll (Abdi *et al*., 2012), seed yield traits (Kiani *et al*., 2009), photosynthesis and CO_2 concentracion (Herve *et al*., 2001), flowering period (Cadic *et al*., 2013), and overall phenotype (Wills *et al*., 2010), *etc*.

A number of newer integrated solutions exist to tackle complex messurements and generate robust phenotypic ratings. Phenomics is based on the use of most of these technologies/methodologies: this paper will point out the significance and application aspects of some of the most critical:

Digital phenotyping is one of the cheapest, and most commonly used methods. With the aim of adequately processing digital imagery data and interpreting the results. There are many variants in application in relation to where and how these technologies are being used, dependent upon the crop culture being investigated. The device and methods developed by Van der Heiden *et al*. (2012), is a sterling example of digital phenotyping done on tall cultures in a greenhouse environment. The experiment was done on the recombined inbred lines of peppers, in the aim of determining heretability of QTLs. By applying this technique, three QTLs were determined for leaf size, and one for the leaf angle. Using statistical analysis, it was confirmed that this method has a high degree of correlation (0.93) with empirical, classical measurement of plant height, and total leaf surface.

Hyperspectral imaging or imaging spectroscopy combines the power of digital imaging and spectroscopy. Hyperspectral remote sensors collect image data simultaneously and it makes it possible to derive a continuous spectrum for each image cell. This technology is highly applicable to an array of scientific areas. It is extensively applied in plant disease analysis, as well as drought resilience research. The method proved itself succesful in detecting symptoms of different diseases found in sugar beet, because pathogens cause differing spectral signals dependant on interaction with plant tissues (Mahlein *et al*., 2012).

Drone application: In the past, drones were available mainly for millitary purposes, but in recent times they have become less expensive and more popular instruments for research, as well as in farmers' daily lives. There are a variety of designs, such as mini fixed-wing aircraft, and miniature multi-propeller helicopters. What all of them have in common is

their miniaturized stature and integrated GPS and camera systems. They are controlled remotely and operated by a single user. Agricultural producers are able to use them to optimize irrigation, detect diseases, and damage in the fields. The use of drones is widespread in a variety of developing projects including precision plant phenotyping.

Studying the germination process using cameras and computer monitoring. Germination is one of the most important processes in production. At the same time it presents a great challenge in precision phenotyping and undestanding the vigor phenomenon. Wagner *et al*. (2011) developed a method which represents a combination of the Jakobsen table (germination system known also as the "Copenhagen table" which provide constant temperature and moisture level for all seed samples) and four calibrated cameras which are able to analyze a sample of up to 400 seeds per camera, or individual seeds. Special software was developed for the process in the aim of controlling the germination parameters, as well as taking photographs. Another piece of software, *ImageJ*, analyzes the photographs and turns them into data which is later analyzed in detail. Although primarily developed for the use in sunflower germination, the system has been found to be useful in other plant species as well.

Multi-sensor tractor platforms. Various groups of authors have, by now, developed multifunctional tractor platforms. Equipped with a wide array of sensors, such as 3-D cameras, laser and hyperspectral measurement sensors, *etc*., the platforms provide an opportunity for the installation of many other types of sensors, depending on the type of research conducted: for instance, moisture content in plants, lodging, biomass yield and similar (Busemeyer *et al.*, 2013). One of the limiting factors is their use on tall cultures at later developmentalstages.

METABOLOMICS

Metabolomics is an area of research which combines strategies to identify and quantify cellular metabolites using sophisticated analytical technologies with the application of statistical methods for information extraction and data interpretation (Roessner and Bacic, 2010). Due to the complexity of analytics involved in their determination, a series of extractions, detections, quantification and metabolite identifications must be performed. As metabolite profiles are directly related to phenotype, metabolomics can be widely applied to determine gene functions and of the interaction of function with environmental factors such as biotic and abiotic stress. Today, metabolomics is increasingly used to analyze the tolerance of plants to: increased salinity; low temperatures; micronutrient deficiency; their toxicity, and *etc*. Cultivated sunflower, along with wild relatives, is well known as a rich source of plant metabolites of varying classes such as terpenoids. Broomrape (*Orobanche cumana* Wallr.) is a parasitic flowering plant known to cause extensive damage to sunflower and is probably the biggest obstacle in sunflower cultivation on globally. Very little is currently known about the specific processes involved in the changes in metabolism resulting from the plant’s reaction to this parasite. Using the Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry (UPLC-MS) method, A.-L. Hepp *et al*. (2013) distinguished key differences in the metabolism of specific metabolites present after the plant's exposure to the parasite. The biosynthesis of coumarines, lignans, and alkaloids also varied significantly depending on the plant’s defense response. This research, and others alike, will significantly help the understanding of plant metabolism when interacting with broomrape and point to a path which can lead to a better means of controlling the parasite.

Uniseriate linear grandular trichomes (LGT) are a part of the stem, leaves, and flower cluster of the *Heliathus* family. Their biological role and metabolic activity is still under investigaton. The results demostrated by Spring *et al*. (2015) pointed to the fact that LGTs

accumulate a variety of sesquiterpens (ST) and that ST synthesis requires sun exposure. Flavonoids are syntesized in parallel with ST, and are presumed to have a protective role against UV radiation. The authors also determined the existence of four known nevandesin types of flavonoids, none of which were found previously in *H. annuus*.

Metabolic markers can potentially be used in plant breeding (Stenfath *et al*., 2010).

GENOME SELECTION

In the broad sense, a genome is all the genetic material contained within a single organism. Genome-based selection as a methodology is of a more recent date and has great perspectives in the future. Although MARS (Marker Assistant Recurrent Selection) is still a developing method, genome selection (GS) already has the upper hand. While the marker assistant recurrent selection is based solely on markers that bare significant effects, GS encompasses all known markers in the entire genome. A large number of research papers have shown that the application of MARS is more successful than the regular phenotypic selection methods, and that GS is even more successful than MARS, which is especially true for traits that have a multi-genic origin and are heavily influenced by the enviorment. Information relating to the phenotype and genotype of a reference population enables the prediction of model parameters. Models, in turn, predict phenotypes in the plant population based on genotype results and produce genome breeding value (GEBV). This forms the base for selection of target phenotypes (A.M. Pérez-de-Castro *et al*. 2012).

DEVELOPMENT OF DH TECHNOLOGY

The creation of inbred lines using double-haploids has many benefits not only for shortering the plant cycle but haploids can also be utilized to provide researchers with genetic information not possible with normal diploid individuals. Since haploids possess only a single dose of their respective genomes this signifcantly facilitates the search and selection of favorable genes and the development of superior breeding genotypes. Sadly the technology is not available to sunflower breeders yet. By using this technology, it is possible to create homozygote lines in a single generation, thus the requirement for several generations of selfing. A number of published DH protocols exist in other plant cultures and this technology is today used routinely in barley, oilseed rape, wheat, and, more recently, the application of induced DH is being successfully used in corn. After years of research into anther culture, microspore cultures or radiation induced haploids, it can be concluded that none of the methods listed have shown promising results in sunflower, but there is hope that further research into radiation induced haploidisation will bare results in the near future (Dr. Jan C.C., personal communication). One of the possible solutions is to create a DH protocol similar to corn, by creating a line which possesses a haploidisation inducer, a marker and the abillity to eliminate the maternal chromosome (Dr Hulke B., personal communication). Although both projects are currently in motion, it is likely that a few more years will pass before a protocol is developed to satisfy the needs of selection programs.

MUTATIONAL BREEDING

In the broadest sense, mutations are heredetary changes in genomes. The subdivision of mutations is complex - mutations, and resulting changes in gene function, can arise from a single base change (point mutation) or from structural changes to the genome (insertion/deletion, duplication, translocation, inversion, and *etc*.). They can be spontaneous or induced, lethal, sublethal or vital. For breeding purposes, without a doubt the most

important mutations are the vital ones. Induced mutations bare high significance in plant breeding. This branch is called mutational breeding. To induce mutations, both physical mutagens, such as gamma rays, or UV rays, and chemical mutagens, such as ethyl methane sulphonate (EMS) and methyl methane sulphonate (MMS), can be used. A majority of agriculturally-important induced mutations have been created using EMS. The success of mutational breeding is not guaranteed. Desirable mutations appear at very low frequencies and always with undesirable background mutations. Although a lot of work has gone into sunflower mutations, only a few are commercially applicable. The application of purposeful mutations is still in its developing stages. Chemical mutagen methodologies have been applied most frequently, likely due to the high rate of mutagensis, and reduced rates of chromosomal aberration/restructuring. Causal mutations have been identified for important morphological traits such as plant height, leaf surface, shortening of the growth season, sterility, disease resistance/tolerance, oil content, and changes in oil quality. The frequency of induced mutation depends, not only on the agents, but also on the base genotype used in the mutation program (Gvozdenovic *et al*., 2009).

It is highly likely that the first succesful commercialization of mutants in sunflowers was a spontaneous mutation resulting in the chrysanthemum sunflower, but still, the availability of spontaneous, commercially relevant mutations is limited. Because of this, induced mutations are widely applied in modern breeding. Many sunflower mutations have been published to date, but very few have found commercial application. One of the most well-known, and economically most significant induced mutations affects acetohydroxyacid synthase (Al khatib *et al*., 1998). Sunflower tolerance to imidazolinones and sulfonylurea herbicide chemistries has significantly increased competitivness of sunflower in production and enabled manufacturers easier control of weed growth and, with the use of Clearfield technology, the control of broomrape. The ClearField-Plus mutation (Sala *et al*., 2008) patented by Nidera and BASF as well as the +M7 SU trait (Gebard and Huby, 2004) patented by DuPont are widely used in breeding today. Fatty acid composition mutations have also found their commercial use. The best known and most widely used is the Pervenats mutation (Soldatov, 1976) obtained by treating the seed of VNIIMK 8931 with 0.5% dimethyl sulfate. It led to the increase in oleic acid to 80-90%. The Pervenets variety is used to obtain gene lines and hybrids of high oleic content.

One of the notable mutations impacts tocopherol content. The LG17 line manifests a gamma tocopherol content of 95% (Popov and Demurin, 1987), while the T2100 line (Velasco and Fernandez-Martinez, 2003) obtained by an EMS mutation of the Peredovik seed showed a gamma tocopherol content upwards of 85%. Sunflowers are the first plant system in which tocopherol controlling genes, known as Tph1 and Tph2, have been identified (Popov *et al*., 1988). There are many methods to rapidly identify mutations, but TILLING (Targeting Induced Local Lesions in Genomes) can efficiently establish the existence of mutations in plant populations (McCallum *et al*., 2000). This technique certainly has its own array of benefits: widespread application, efficacy when applied to small populations, and the ability to target any gene (Henry and Comai, 2014).

ELUCIDATING THE IMPACT OF COMBINING TABLE-STAKES AND NOVEL TRAITS USING CROP MODELING

One of the greatest challenges found in breeding today is the selection of genotypes which bare higher yields in ever more volatile and complex climate conditions. The creation of a breeding ideotype is one of the main goals required to reach maximum expression in

traits which influence, most of all, seed yield. The main paradigm is found in the efficient usage of the basic GxExM model which is little different than standard GxE because management options allow genetics to fully express their potential. An ideotype is defined as a combination of morphological and/or physiological traits optimizing crop performances to a particular biophysical environment and crop management. The adaptabillity of a genotype depends largely on introducing new traits into hybrids which provide better productivity and stability. In broader sense, the ideotype is a plant with ideal characteristics for a certain climate zone accounting for climate changes (Donald, 1968; Semenov and Stratonovitch, 2013).

Applied crop modeling should allow the integration of different techniques (Struick *et al*. 2007). As described by Yin and al. (2004, 2003), crop physiology should support the discovery of complex traits by taking advantage of data and knowledge integration under a Genotype by Environment approach. The final aim being transversal integration, where the link between the phenotype and the genotype would be fulfilled.

POST-GENOMIC ERA

The main goal of the Sunflower Genomic Resources Consortium (formed in 2012, a collaboration between the Genome Canada project, the University of Georgia, the French National Institute for Agricultural Research, and private partners) was development of the first "high quality genomic sequence of sunflower genotype HA412-HO". Even with minor problems, the project has delivered, and the first bronze version of HA412-HO has been published. So what can we expect in the future? There are lots of challenges, possibilities, and benefits to be found in the sunflower post-genomic era. Using high-throughput sequencing technologies, we will enhance our ability to detect mutation, small regulatory RNA species (miRNA, siRNA, ncRNA), and we will progress towards whole transcriptome analysis. This will allow characterization and elucidation of mechanisms for gene regulation in sunflower (Aoki *et al*., 2013) with high impacts for our scientific, agricultural, commercial aspirations.

Another post-genomic era application is gene-editing technology. The access to one or more (complete) genomic sequences and the support for cDNA and other transcribed sequences allows not only for the determination of copy numbers and splice-variants within the species itself, but also related homologs and orthologs found in other species, which can be used to infer the functions of native allele(s), as well as changes in function resulting from modifications made through editing (Ledford H. 2015). These are just a few examples. As time progresses, we are witnessing that genomic information is no longer a bottleneck or a limiting factor in sunflower.

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MOLECULAR MAPPING OF THE DISEASE RESISTANCE GENE AND ITS IMPACT ON SUNFLOWER BREEDING

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ABSTRACT

Rust, downy mildew (DM), and Sclerotinia diseases are the major yield limiting factors in global sunflower production. The use of resistant hybrids, where available, is the most efficient measure of controlling these diseases. Development of DNA markers linked to the resistance genes will facilitate molecular breeding of disease-resistant hybrids in sunflower.

We have molecularly mapped seven rust *R*-gene loci, R_2, R_4, R_5, R_{11}, R_{12}, R_{13a}, and R_{13b} to linkage groups (LG) of the sunflower genome, developed both simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers linked to these *R*-genes, and used them in marker-assisted gene pyramiding in sunflower.

Two new DM *R*-genes, Pl_{17} and Pl_{18}, were mapped to LGs 4 and 2 of the sunflower genome, respectively, different from all known DM *R*-genes previously mapped to LGs 1, 8, and 13. Pl_{18} was recently transferred from *H. argophyllus* into cultivated sunflower. We also identified diagnostic SNP markers linked to the DM *R*-genes, Pl_{Arg} and Pl_8.

Quantitative trait loci (QTL) for *Sclerotinia* basal stalk rot resistance were identified in a sunflower recombinant inbred line population derived from the cross HA 441/RHA 439 using genotyping-by-sequencing approach. A total of six QTL were identified, one each on linkage groups (LGs) 4, 9, 10, 11, 16 and 17, each explaining between 6 and 29% of the observed phenotypic variance in the RIL population. The QTL on LGs 10 and 17 were detected in multiple environments with very high LOD values (5.49-12.01), while the remaining QTL were detected in single environment. A combined analysis with integrated phenotypic data across environments also detected the QTL on LGs 10 and 17, each explaining 32 and 20%, respectively of the phenotypic variation for the trait.

Key words: Sunflower, disease, resistance gene, genetic mapping, quantitative trait loci

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the fifth most important oilseed crop in the world and provides about 13 % of the world's edible oil. In the United States, the majority of sunflower produced is the oil-type, whereas 10-20% of production is confection, a high value seed product used primarily in human diets as a snack. Rust, downy mildew (DM), and Sclerotinia diseases are the major yield limiting factors in sunflower production of North

America and the world. The development of disease resistant sunflowers is a major goal of sunflower breeders because this effort may increase yield stability.

Rust and downy mildew, caused by the fungus, *Puccinia helianthi* Schwein. and the oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni, respectively, are serious sunflower diseases in the world. Genetic studies of resistance to rust and downy mildew in sunflower have indicated that the sources of resistance to both diseases are usually controlled by single dominant genes. The rust and DM resistance genes (*R* genes) have frequently been deployed singly in sunflower production, and as a consequence, the commercial life of resistant hybrids is quickly challenged as new *P. helianthi* and *P. halstedii* races with increased virulence evolve (Gulya and Markell 2009; Gulya et al. 2011; Moreno et al. 2012; Viranyi et al. 2015). Effective breeding strategies are needed to avoid rapid breaking down of resistance mediated by race-specific genes in sunflower. Gene pyramiding, which aims to combine different *R*-genes from multiple parents into a single genotype is considered an effective strategy to increase the durability of disease resistance (Singh et al. 2001; Mago et al. 2011). However, phenotype-based gene pyramiding cannot track the accumulation of resistance genes, especially when different *R*-gene cannot be distinguished using pathogen bioassays. Thus, mapping rust and DM *R* genes and developing of robust DNA markers that are closely linked to the specific genes are required to facilitate this breeding approach and add precision to selection.

Sclerotinia sclerotiorum (Lib.) de Bary, a necrotrophic fungus, causes three distinctly different diseases on sunflower, basal stalk rot (BSR) or wilt, mid-stalk rot (MSR), and head rot (HR). Unlike other hosts, BSR or wilt symptom starts from root infection resulting from myceliogenic germination of sclerotia. MSR commonly begins as a leaf infection, while HR infection begins on capitula; both symptoms are incited by airborne ascospores released from carpogenic germination of sclerotia. The MSR is not as commonly observed in the United States as the BSR and HR, and the latter two are serious problem in sunflower-growing areas of the humid temperate, as well as tropical and sub- tropical regions of the world. As the mode of infection for the two important sunflower diseases caused by *S. sclerotiorum* varies, the underlying genetics of resistance for the two diseases also appears to be different, effectively doubling the effort needed to combat the pathogens (Talukder et al., 2014a). The BSR resistance is genetically complex and conditioned by multiple genes, each having a small effect (Talukder et al., 2014b). Little is known about the quantitative trait loci (QTL) for resistance to Sclerotinia BSR in sunflower. Here, we reported development of a genetic map of sunflower using SNP markers generated using genotyping-by-sequencing (GBS) approach and identify QTL associated with BSR resistance in a high resolution genetic location of the cultivated sunflower genome.

MATERIALS AND METHODS

Mapping populations

Populations for mapping of the rust and DM *R*-genes are listed in Table 1. A population of 106 F_7 RILs for QTL mapping of Sclerotinia BSR was developed by single seed descent from a cross between sunflower inbred lines, HA 441 (PI 639164) and RHA 439 (PI 639162). Greenhouse and field screening trials at multiple locations in North Dakota, South Dakota, and Minnesota from 2008 to 2014 revealed that HA 441 and RHA 439 were moderately to highly tolerant to BSR.

Population for rust resistance gene pyramiding in confection sunflower

The confection line HA-R6 carrying a rust *R*-gene R_{13a} was crossed to a BC_3F_2-derived line "12-105" with the pedigree CONFSCLB1*4/HA-R2 (R_5) and a BC_4F_2-derived line '12-55' with the pedigree CONFSCLB1*5/MC29 (R_2) in a greenhouse in 2012, respectively. CONFSCLB1 is a narrow-based maintainer line composite of the confection sunflower that is susceptible to rust. The two F_2 populations were developed to pyramid R_{13a} with R_2 and R_5, respectively, and select homozygous double-resistant plants carrying the *R*-genes from both parents.

Table 1. Susceptible and resistant parents used for development of the mapping populations.

Susceptible parent	Resistant parent	Combination	No. of individuals	F_2 *R*-gene
HA 89	MC29 (USDA)	HA 89/MC29 F_2	120	R_2
HA 89	HA-R3	HA 89/HA-R3 F_2	94	R_4
HA 89	HA-R2	HA 89/HA-R2 F_2	118	R_5
HA 89	*H. annuus* PI 613748	HA 89/PI 613748 BC_1F_2	192	R_{11}
HA 89	RHA 464	HA 89/RHA 464 F_2	140	R_{12}
HA 89	HA-R6	HA 89/HA-R6 F_2	140	R_{13a}
HA 89	RHA 397	HA 89/RHA 397 F_2	140	R_{13b}
HA 89	RHA 464	HA 89/RHA 464 F_2	140	Pl_{Arg}
HA 434	RHA 340	HA 89/RHA 340 F_2	130	Pl_8
HA 234	HA 458	HA 89/HA 458 F_2	186	Pl_{17}
HA 89	*H. argophyllus* PI 494573	HA 89/PI 494573 BC_1F_2	142	Pl_{18}

Plant disease inoculation and score

For rust phenotyping, a North America (NA) rust race 336 was used to inoculate all F_2 and F_3 populations (20 plants for each F_3 family) as described by Qi et al. (2011). Rust evaluations were made at 12–14 d post inoculation to allow full development of symptoms evaluated using both pustule size or infection type (IT) (Yang et al. 1986) and percentage of leaf area covered with pustules (severity) on all inoculated leaves (Gulya et al. 1990). IT 0, 1, and 2 combined with pustule coverage of 0 to 0.5% were classified as resistant, while IT 3 and 4 with pustule coverage more than 0.5% were considered susceptible.

For DM phenotyping, the whole seedling immersion method described by Gulya et al. (1991) was applied for all F_3 population tests (30 seeds for each F_3 family) using the NA DM race 734, which is a new, virulent race identified in the US in 2010 (Gulya et al. 2011). The F_3 families were classified as homozygous resistant if none of the seedlings had sporulation, segregating if some seedlings (about one-quarter in a F_3 family) had sporulation on the cotyledons and true leaves, and homozygous susceptible if all seedlings had sporulation on cotyledons and true leaves.

All 106 RILs from the cross between HA 441 and RHA 439 along with the parents were evaluated for BSR resistance at five environments (locations and/or years) in North Dakota and Minnesota. Field trials were conducted at Carrington, ND in 2012 and 2014, at

Crookston, MN in 2012 and 2013, and at Grandin, ND in 2014. All field screening trials were conducted using a randomized complete block design (RCBD). The 2012 and 2013 field trials were conducted with two replications, while the 2014 trials had four replications. Fields were artificially inoculated at the V-6 growth stage following the method proposed by Gulya et al. (2008) depositing 90 gm of *S. sclerotiorum* mycelia grown on proso millet in furrows of 5 to 7 cm depth on one side next to the row. Disease incidence (DI) was expressed as the percent of plants showing wilting and/or a basal stem rot lesion. An analysis of variance (ANOVA) of DI of the RIL population was performed across all five environments using PROC MIXED in SAS 9.3 (SAS Institute, 2011).

Genetic mapping of disease resistance genes and QTL

For genetic mapping of the rust and DM *R*-genes, simple sequence repeat (SSR) markers that were previously mapped were used first to screen the parents. Bulked segregant analysis (BSA, Michelmore et al. 1991) was performed with those SSRs showing polymorphism between resistant and susceptible parents. SSR markers showing associations with the resistance bulk in BSA were genotyped on F_2 individuals to confirm the marker-trait associations. JoinMap 4.1 was used for linkage analyses and map construction with a regression mapping algorithm and Kosambi's mapping function (Van Ooijen 2006). The Chi-Square (χ^2) test was used to assess goodness-of-fit to the expected segregation ratio for each marker. Newly developed single nucleotide polymorphism (SNP) markers were used to further saturate the region where the *R*-gene resides.

Genotyping-by-sequencing (GBS) using the NGS technology was used for simultaneous discovery and genotyping of SNP markers for the HA 441/RHA 439 RIL population. Genomic DNA from each of the 106 RILs and two parental lines were sent to the Biotechnology Resource Center (BRC) Genomic Diversity Facility at Cornell University for GBS as described by Elshire et al. (2011) and at http://www.biotech.cornell.edu/brc/genomic-diversity-facility/services. A total of 1,236 SNP markers were used for linkage analysis using JoinMap 4.1 (Van Ooijen 2006). The SNPs were named with a prefix of S1 to S17 based on the draft sunflower genome assembly that corresponds to the 17 sunflower linkage groups (LGs), followed by a number representing the physical position of the SNP on the genome. However, there are some SNPs with a prefix of S18, which were discovered in scaffolds yet to be assigned a physical position in the genome. Four-hundred bp nucleotide sequences flanking the SNP position were retrieved from the draft sunflower reference genome, HA412.v1.0.bronze.20140814.fasta.gz. Quantitative trait loci analysis was conducted each environment separately, and also with integrated data across environments. The composite interval mapping (CIM) (Zeng, 1994) as implemented in WinQTL cartographer version 2.5 (Wang et al., 2005) was used to detect QTL. The output of the QTL analysis was verified with PLABQTL version 1.2 (Utz and Melchinger, 2006).

RESULTS AND DISCUSSIONS

Molecular mapping of the rust *R*-genes

Since 2011, we have molecularly mapped seven rust *R*-gene loci, R_2, R_4, R_5, R_{11}, R_{12}, R_{13a}, and R_{13b} to four LGs of the sunflower genome; R_5 in LG2, R_{12} to LG11, R_4, R_{11}, R_{13a} and R_{13b} in LG13, and R_2 in LG14 (Table 2). The results were summarized as follows.

R_5: On the initial SSR map, the R_5 gene from HA-R2 was located on LG2, flanked by two SSR markers, ORS1197 and ORS653 at 3.3 and 1.8 cM of genetic distance, respectively

(Qi et al. 2012a). After screening the 67 LG2 SNP markers, two SNPs, SFW03654 and NSA_000267, were found flanking R_5 at a genetic distance of 0.6 cM and 1.2 cM, respectively. This flanking narrowed the genetic interval containing R_5 from 5.1 to 1.8 cM in length (Qi et al. 2015b).

R_{12}: Virulence phenotypes of seedlings for the F_2 population and $F_{2:3}$ families suggested that a single dominant gene confers rust resistance in RHA 464, and this gene was designated as R_{12}. Bulked segregant analysis identified 10 LG11 SSR markers polymorphic between resistant- and susceptible-bulks. In subsequent genetic mapping, two markers, CRT275 and ZVG53 delimited R_{12} in an interval of 10.6 cM (Gong et al. 2013a). Later, the same F_2 population was used to assign SNP markers to the genetic map. When rust phenotypic data were integrated with SNP marker data, seven linked SNP markers were identified, five on one side (NSA_000064, NSA_003320, NSA_003426, NSA_004155, NSA_008884), and two on the other side (NSA_001570, and NSA_001392), defining an interval less than 2.3 cM surrounding the previously mapped R_{12} gene in LG11 (Talukder et al. 2014c).

R_4, R_{11}, R_{13a}, and R_{13b}: The four rust *R*-gene loci were all mapped to the lower end of LG13. R_{11} from RfANN-1742 is mapped distal to SSR marker ORS316, a common marker among maps related to *R*-gene cluster in the lower end of LG13. This gene is closely linked to a restorer gene *Rf5* at a genetic distance of 1.6 cM, and shared a common marker, ORS728, which was mapped 1.3 cM proximal to *Rf5* and 0.3 cM distal to R_{11} (*Rf5*/ORS728/R_{11}). Two additional SSRs were linked to *Rf5* and R_{11}: ORS995 was 4.5 cM distal to *Rf5* and ORS45 was 1.0 cM proximal to R_{11} (Qi et al. 2012b).

Three rust *R*-genes, R_4 from HA-R3, R_{13a} from HA-R6, and R_{13b} from RHA 397, were all mapped proximal to ORS316 (Qi et al. 2011; Gong et al. 2013b). The allelic analysis indicated that that R_4 and R_{13a} are two distinct rust resistance genes, but very closely linked, whereas, R_{13a} and R_{13b} are the same rust *R*-gene (Gong et al. 2013b; Qi et al. 2015b). The SSR and SNP markers linked to R_4, R_{13a}, and R_{13b} are listed on Table 2.

***R_2*:** Based on phenotypic assessments and SSR marker analyses of the 117 F_2 individuals derived from a cross of HA 89 with MC29 (carrying R_2), R_2 was mapped to LG14 of the sunflower, and not to the previously reported location on LG9. The closest SSR marker HT567 was located at 4.3 cM distal to R_2. Furthermore, 36 selected SNP markers from LG14 were used to saturate the R_2 region. Two SNP markers, NSA_002316 and SFW01272, flanked R_2 at a genetic distance of 2.8 and 1.8 cM, respectively. Of the three closely linked markers, SFW00211 amplified an allele specific for the presence of R_2 in a marker validation set of 46 breeding lines, and SFW01272 was also shown to be diagnostic for R_2 (Qi et al. 2015a).

Molecular mapping of the DM *R*-genes

***Pl_{17}*:** DM resistance in HA 458 has been shown to be effective against all virulent races of *P. halstedii* currently identified in the United States. To determine the chromosomal location of this resistance, 186 $F_{2:3}$ families derived from a cross of HA 458 with HA 234 were phenotyped for their resistance to race 734 of *P. halstedii*. The segregation ratio of the population supported that the resistance was controlled by a single dominant gene, named as Pl_{17}. Bulked segregant analysis using 849 SSR markers located Pl_{17} to LG4, which is the first DM gene discovered in this linkage group. An F_2 population of 186 individuals was screened with polymorphic SSR and SNP primers from LG4. Two flanking markers, SNP SFW04052 and SSR ORS963, delineated Pl_{17} in an interval of 3.0 cM (Qi et al. 2015c). A search for the

physical location of flanking markers in sunflower genome sequences revealed that the *Pl17* region has a recombination frequency of 0.59 Mb/cM, which is a 4-fold higher recombination rate relative to the genomic average. This region can be considered amenable to molecular manipulation for further map-based cloning of *Pl17*.

***Pl18*:** A new dominant DM resistance gene (*Pl18*) transferred from wild *Helianthus argophyllus* (PI 494573) into cultivated sunflower was mapped to LG2 of the sunflower genome using bulked segregant analysis with 869 SSR markers. Since no other *Pl* gene has been mapped to LG2, this gene was novel and designated as *Pl18*. SSR markers CRT214 and ORS203 flanked *Pl18* at a genetic distance of 1.1 and 0.4 cM, respectively. Forty-six SNP markers that cover the *Pl18* region were surveyed for saturation mapping of the region. Six co-segregating SNP markers were 1.2 cM distal to *Pl18*, and another four co-segregating SNP markers were 0.9 cM proximal to *Pl18* (Qi et al. 2016a). The new BC_2F_4-derived germplasm, HA-DM1, carrying *Pl18* has been released to the public. This new line is highly resistant to all *P. halstedii* races identified in the US providing breeders with an effective new source of resistance against downy mildew in sunflower.

Table 2. List of DNA markers closely linked to the rust and downy mildew resistance genes.

Gene donor	Gene	LG	Linked marker	Map position (cM)	Reference
HA-R2	*R5*	2	NSA_001605 SFW03654 ***R5*** NSA_000267	14.4 14.9 15.5 16.7	Qi et al. 2012a, 2015b
RHA464	*R12*	11	NSA03320, NSA_003426, NSA_004155 ***R12*** NSA_001392, NSA_001570	44.6 45.4 46.8	Gong et al. 2013a Talukder et al. 2014C
HA-R3	*R4*	13	ORS316, ZVG61, SFW05240, SFW05630, SFW06095, SFW08283 ***R4*** SFW01497, SFW05453, SFW08875	3.5 4.1 4.8	Qi et al. 2011, 2015a
HA-R9	*R11*	13	ORS728, ***R11*** ORS45	6.1 7.1 9.1	Qi et al. 2012b
HA-R6	*R13a*	13	ORS316, ZVG61, SFW05832, SFW08188 *R13a* SFW05743	3.4 3.8 4	Gong et al. 2013b Qi et al. 2015b
RHA397	*R13b*	13	ORS316, ZVG61, SFW05832, SFW08188 ***R13b*** SFW00757	5.9 6.8 6.8	Gong et al. 2013b Qi et al. 2015b
MC29 (USDA)	*R2*	14	HT567	42.4	Qi et al. 2015a

			SFW00211	43.8	
			R₂	46.7	
			SFW01272	48.5	
RHA 464	*Pl_Arg*	1	NSA_002131, NSA_002798, NSA_008037, NSA_007595	29.7	Qi et al. unpublished data
			Pl_Arg	29.7	
			NSA_001835	30.0	
			NSA_006530	30.5	
H.argophyllus PI494573	*Pl18*	2	SFW03013	2.3	Qi et al. 2016a
			CRT214	2.4	
			Pl18	3.5	
			ORS203	3.9	
			SFW03060	4.4	
HA458	*Pl17*	4	SFW04052	14.3	Qi et al. 2015c
			Pl17	16.4	
			ORS963	17.2	
			SFW08268	18.2	
RHA 340	*Pl8*	13	NSA_000423, SFW01497, SFW08875	1.2	Qi et al. 2016b
			Pl8	1.7	
			SFW06597	2.6	
			NSA_002220, NSA_002251	3.0	

Development of diagnostic SNP markers linked to DM *R*-genes, *Pl_Arg* and *Pl8*

Pl_Arg and *Pl8* both originating from the wild *H. argophyllus* were previously mapped to LGs1 and 13, respectively (Dußle et al. 2004; Bachlava et al. 2011). An F_2 population of 140 individuals from the cross of HA 89 and RHA 464 harboring *Pl_Arg* and *R12* was previously used as a mapping population to map SNP markers in the sunflower genome of the National Sunflower Association (NSA) SNP Consortium project (Talukder et al. 2014c). DM phenotypic data from $F_{2:3}$ families of this population were integrated with SNP marker data. Seventy-eight co-segregating SNP markers were 0.01 cM distal to *Pl_Arg*, and SNP marker NSA_001835 was 0.31 cM proximal to *Pl_Arg*. Genotyping of 80 SNP markers flanking *Pl_Arg* in the 548 collected sunflower lines discovered diagnostic SNP markers for selection of *Pl_Arg* in most of the sunflower backgrounds (Table 2).

A total of 30 seeds from each of 120 F_3 families derived from the cross of HA 434 and RHA 340 (*Pl8*) were inoculated with NA DM race 734 and tested for their resistance in the greenhouse. Forty-one SNP markers were selected from the lower end of LG13 of two SNP maps, which covered the *Pl8* region (Bowers et al. 2012; Talukder et al. 2014c). Nine SNP markers that showed polymorphism between the parents were genotyped in the F_2 population. Three co-segregating SNPs were 0.4 cM distal to *Pl8* and SNP SFW06579 was 1.3 cM proximal to *Pl8* (Table 2, Qi et al. 2016b). Three SNPs, NSA_00423, NSA_02220, and NSA_02251 were used to genotype the 548 sunflower lines. More than 85% of sunflower lines did not share the resistant alleles of both NSA_00423 and NSA_02220 with RHA 340. These two SNPs can be potentially used as diagnostic markers for the selection of *Pl8* in sunflower breeding programs.

QTL mapping of Sclerotinia basal stock rot (BSR) resistance

Quantitative trait loci (QTL) for BSR resistance were identified in a sunflower recombinant inbred line (RIL) population derived from the cross HA 441/RHA 439 (Talukder et al. 2016). The genotyping-by-sequencing (GBS) approach was adapted to discover SNP markers and simultaneously genotyping the RIL population. A genetic linkage map was developed comprising of 1,053 SNP markers on 17 LGs spanning 1,401.36 cM. The RILs were tested in five environments (locations and/or years) for resistance to BSR. Due to the presence of significant genotype × environment interactions, QTL analyses were first performed for each of the five environments separately, followed by a combined QTL analysis using mean disease incidence across environments. A total of six QTL were identified in all five environments, one each on LGs 4, 9, 10, 11, 16 and 17 (Table 3).The QTL on LG10, *Qbsr-10.1* was detected at every environment between 57.9 and 66.5 cM genomic positions with LOD values ranging from 5.5-12.0. This QTL alone explaining 17-29% of the phenotypic variations across five environments. The QTL on LG17, *Qbsr-17.1*was detected in three of the five environments with LOD values ranging from 3.1-7.0. The remaining four QTL, *Qbsr-4.1*, *Qbsr-9.1*, *Qbsr-11.1* and *Qbsr-16.1* were detected in only one environment on LGs 4, 9, 11 and 16, respectively. Each of these QTL explains between 6 and 11% of the phenotypic variation in their respective environment. In the combined analysis with mean BSR disease incidence across environments, the *Qbsr-10.1* and *Qbsr-17.1* were detected with high LOD values each accounting for 32% and 20% of the phenotypic variation, respectively. Alleles conferring increased resistance were contributed by both parents (Table 3).

Table 3. Significant quantitative trait loci (QTL) for *Sclerotinia* basal stalk rot resistance identified in the HA 441/RHA 439 RIL sunflower population in five individual environments

QTL name	Linkage group	Peak QTL position (cM)	Flanking markers (cM position)		$R^{2\dagger}$	1-LOD‡ interval
			Left	Right		
Qbsr-4.1	4	32.0	S4_147688288 (29.1)	S4_135190076 (33.1)	6.4	6.5
Qbsr-9.1	9	45.0	S9_153762438 (45.0)	S9_158145790 (46.6)	9.3	1.5
Qbsr-10.1	10	66.5	S10_288646223 (66.5)	S10_281294015 (67.5)	31.6	1.6
Qbsr-11.1	11	83.2	S14_148877201 (83.2)	S14_148877253 (83.4)	7.7	3.5
Qbsr-16.1	16	87.3	S16_157591485 (87.3)	S16_137964301 (87.9)	10.5	10.4
Qbsr-17.1	17	23.9	SFW02170 (23.7)	S17_228661362 (24.0)	20.2	4.2

†Percentage of phenotypic variance explained by the QTL in the population.
‡LOD, logarithm of odds.
SNP markers nearest to the QTL pick position are underlined

Marker-assisted gene pyramiding in confection sunflower

The DNA markers linked to the genes R_2 and R_{13a} were used to screen 524 F_2 individuals from a cross of a confection R_2 line and HA-R6 carrying R_{13a}. Eleven homozygous double-resistant F_2 plants with the gene combination of R_2 and R_{13a} were obtained. Similarly, a total of 368 F_2 plants from the cross between confection R_5 with HA-R6 were screened by DNA markers linked to the R_5 and R_{13a}. Twelve F_2 plants were identified to be homozygous for a combination of R_5 and R_{13a}. These double-resistant lines will be extremely useful in confection sunflower, where few rust *R*-genes are available, risking evolution of new virulence phenotypes and further disease epidemics. The germplasms HA-R12 carrying R_2 and R_{13a} and HA-R13 harboring R_5 and R_{13a} have been released to the public (Ma et al. 2016).

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SUNFLOWER GENETIC RESOURCES

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ABSTRACT

Sunflower (*Helianthus*) collections around the world play a critical role supplying germplasm for crop security and improvement. Sunflower is native to North America and this presentation focuses on the collection of more than 5000 cultivated and wild *Helianthus* accessions maintained by the United States National Plant Germplasm System (USDA-NPGS) genebank in Ames, IA with some discussion of other national collections. The NPGS cultivated collection, curated in Ames since 1948, contains landraces, cultivars, breeding lines, populations, synthetic varieties, and pre-breeding lines. Exciting new material includes a 288 member association mapping population as well as more than 300 pre-breeding lines containing DNA introgressions from 11 wild species. The USDA wild sunflower collection, started in Bushland TX in 1976, was transferred to Ames in 1985. Holdings include accessions representing all 66 extant wild annual and perennial sunflower taxa except one subspecies endemic to Baja California. The accessions were primarily sourced from wild populations in the US and Canada with some representation from naturalized populations around the world. With increased awareness of the value of crop wild relatives as sources offer traits and genetic diversity for cultivar improvement and to enhance crop security, we have emphasized collection of wild species to maximize representation of geographic and genetic diversity. A third of the wild germplasm holdings has been added in the past ten years including explorations in 2015 to increase representation of species from the western, southwestern and southern US. The NPGS collection is freely available for research and educational purposes although some restrictions are imposed by import regulations in receiving countries.

Key words: Sunflower, Genebank, Wild, Cultivated, Explorations

INTRODUCTION

The National Plant Germplasm System (NPGS) in the United States maintains and manages genetic resources for agricultural crops in the United States. The sunflower collection is maintained at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA. The NPGS is an association of twenty genebanks (Fig 1) operating under the umbrella of United States Department of Agriculture Agricultural Research Service (USDA-ARS) united by common use of the Germplasm Resources Information Database (GRIN, GRIN-Global) and leadership from USDA National Program Staff in Beltsville, MD.

Most locations in the NPGS are partnered with a land grant university; for example, the station in Ames is a partnership with Iowa State University. There are four multi-crop plant introduction stations in the NPGS located in Pullman WA, Ames IA, Griffin GA and Geneva NY; a back-up storage and preservation research unit in Ft Collins CO (the National Laboratory for Genetic Resources Preservation); and 15 other repositories such as Sturgeon

Bay WI where the potato collection is curated, the National Small Grains Collection at Aberdeen ID, and College Station TX where the pecan collection is maintained. As illustrated in Figure 1, the stations cover a wide range of latitudes as well as temperature zones.

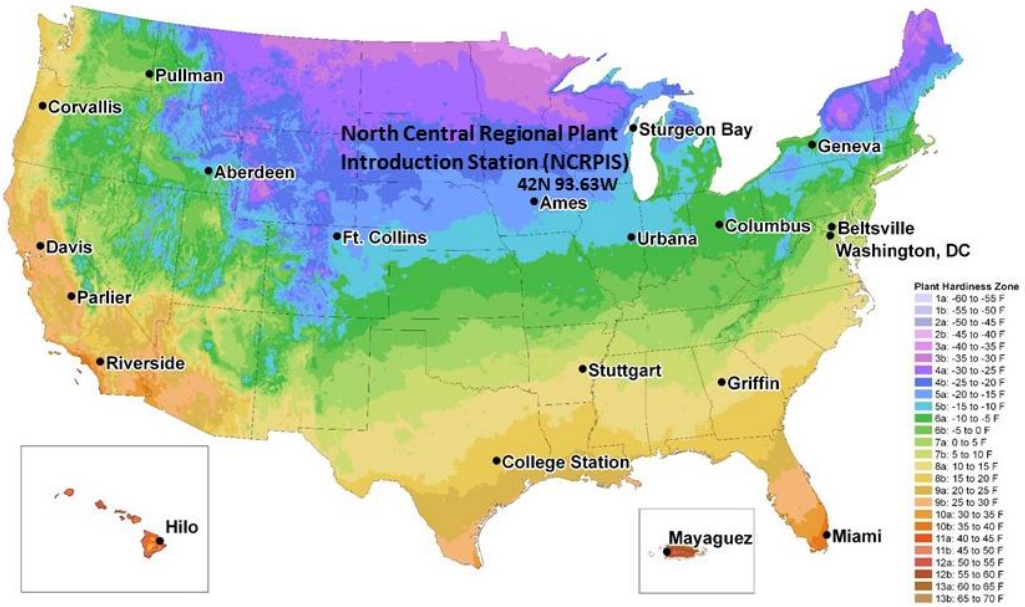


Figure 1: The 20 locations of the US National Plant Germplasm System positioned on the USDA's Plant Hardiness Zone map.

The NCRPIS is located in southwestern Ames on a little over 41 hectares of Iowa State University Experiment Station land at 42° north latitude, a comparable distance north of the equator as Edirne, Turkey although with greater average annual precipitation (910mm in Ames, 578mm in Edirne) and at a higher elevation (287 meters in Ames, 63 meters in Edirne). The NCRPIS is a joint project between the Agriculture Experiment Stations of the 12 North Central States, Iowa State University, and the USDA-ARS. There are five curatorial projects in Ames (amaranth, millets, quinoa, miscellaneous other crops; horticultural and medicinal crops; maize; oilseed crops; vegetable crops including carrots) and seven support and project teams (administration, entomology, farm maintenance and operations, information technology, pathology, management and distribution and storage of seeds, viability testing and the Germplasm Enhancement of Maize project).

The Oilseeds Project includes the sunflower collections as well as oilseed brassicaceae (the *Brassicas* such as canolas and rapeseed and miscellaneous new crop genera in the brassicaceae family such as *Camelina*, *Thlaspi*, *Crambe* and 20 others), flax, *Cuphea*, *Euphorbia* and a group of miscellaneous asters (36 genera). All of the support teams are critical to curatorial success and three in particular are described here. Our staff entomologist rears honeybees which are the primary pollinator for caged sunflowers as well as *Osmia* and alfalfa leaf cutter bees, bumble bees, and several species of flies. We have a plant pathologist on staff who inspects our fields and greenhouse plantings, conducts seed health testing, manages seed treatmenst, and issues the additional declaration statements often needed for international seed shipments. The viability team conducts germination testing on new accessions and regeneration lots before seed lots are stored as well as testing of distribution

lots at species specific intervals to help curators decide when seed lots need to be replaced with fresh seed.

THE SUNFLOWER COLLECTION AT THE NCRPIS, BASIC INFORMATION

It is the mission of the NCRPIS to expand the genetic diversity of priority plant genetic resource (PGR) collections, to improve associated information and information management tools that facilitate PGR conservation and utilization in research and crop improvement, and to provide these resources for research and educational objectives. The information presented will illustrated how that mission is fulfilled for sunflowers.

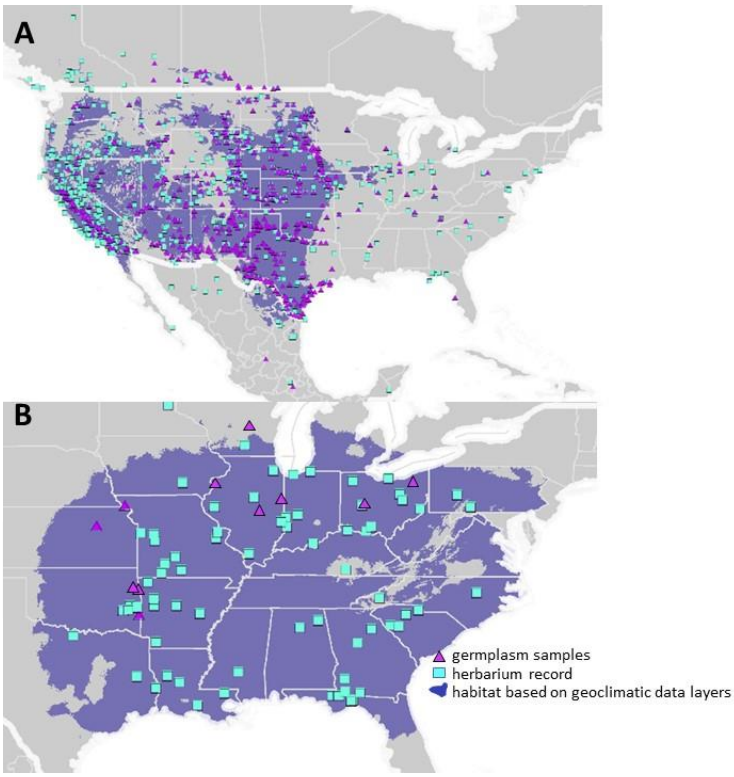
The USDA sunflower collection consists of a little over 5000 accessions, about 2500 cultivated accessions and 2500 accessions of wild species. As of May 2016, both groups were about 90% available for distribution.

The cultivated sunflower collection has been in Ames since the station opened in 1948 and consists of traditional open pollinated populations, North American native land races, inbred lines, composite and synthetic varieties, pre-breeding lines and an association mapping population (UGA-SAM1). The cultivated collection has been 90% or more available for distribution for many years.

The wild collection was transferred to Ames in 1985 from Bushland TX where it was initiated in the 1970s and where Dr. Gerald Seiler served the first curator. The wild collection contains samples from populations of all extant *Helianthus* taxa except *H. niveus* ssp *niveus* which is endemic to Baja California, Mexico as well as samples from naturalized populations in the US and around the world. The collection is curated as a living one; that is, seeds from active accessions can be germinated and plants will result. There had been less emphasis on the wild collection prior to 2004 when I became curator. Increasing species and accession availability, which was about 27% for all taxa except wild and cultivated *H. annuus* in 2004, became a priority. Increasing collection availability involves both regenerating seeds to distribution quantities and inactivating inviable accessions as well as collecting new samples to fill species and geographic gaps. About 700 wild accessions have been inactivated during the past 12 years when seed failed to germinate (primarily from the oldest original collections) during regeneration attempts.

EXPANDING GENETIC DIVERSITY IN THE NCRPIS WILD SUNFLOWER COLLECTION: UNDERPININGS

Identification of collection gaps involves comparing collected locations of existing accessions with known ranges and herbaria records which support potential active collection sites for species of interest. Rogers et al. (1982) published maps with range estimates for most of the wild *Helianthus* taxa, providing a starting point. The mapping group at the International Center for Tropical Agriculture (CIAT) in Cali, Columbia led by Chyrstian Sousa extended the concept of gap filling based on herbaria and germplasm sample records to include geoclimatic data layers (precipitation, temperature and soil characteristics, Kantar et al. 2015) and the resultant maps are intended to predict where populations could be expected to occur. For sunflower the soil data were critical to identifying species differentiation. As shown in Figure 2A for the direct progenitor of the crop plant, wild *Helianthus annuus*, samples and records overlap well and correspond to the areas of expected range based on the geoclimate data layers, although there are scattered geographic gaps across the US and gaps in Mexico and Canada.



In the case of *H. hirsutus* (Figure 2B), although herbaria voucher records agree with the expected range based on geoclimatic data layers, there are no germplasm samples from the southern and eastern portion of the species range. This analysis helped identify a species in need of further collection to achieve a full range of sampling; *H. hirsutus* will be a target of a future collection effort.

Based on this kind of information, I planned two explorations for 2015, both of which are described because they represent very different environments and dramatically illustrate the diversity present within the genus *Helianthus*. Each exploration offered a unique perspective as to the value of filling gaps in a collection during these times of climate change. Dr. Seiler was my co-collector for both of the 2015 explorations.

Planning and preparing a plant exploration is a complex activity. First an infrastructure is established based on existing herbaria voucher records for the species of interest. Herbaria records tend to be ten to twenty or more years old. A second critical step is to contact botanists at universities, often starting with the herbaria directors, public lands (federal, state, city and county, such at the national forests and Bureau of Land Management scientists), and non-government organizations such as The Nature Conservancy and appropriate departments in tribal nations to accumulate information about extant populations and current year data. A key requirement for seed coming to the NPGS is that landowners are identified and any necessary permits obtained. We must be permitted to collect germplasm and the seeds must be distributable, generally without restrictions. Once locations of populations to sample are identified, determining a collection route usually involves the use of multiple maps. Topographical maps can be critical in the case of older voucher records which were made pre-GPS and which often relied on landmarks such as a cemetery or a church named on the topo maps but not in google or on Gazetteers. Roads can be re-routed and names can change, making finding the location of old records sometimes very difficult. And finally, the decision when to collect is made based on current year weather conditions along with information from local botanists. At each collection location passport data are recorded for entry into the NPGS database: latitude and longitude, description of the site including associated vegetation and how it was accessed, population size, and number of plants sampled, and images of the population and surrounding habitat are taken. In addition a voucher specimen is prepared for each population if possible. Sunflower specimens are currently stored at the USDA-ARS Sunflower and Plant Biology Research Unit in Fargo, ND.

2015 EXPLORATIONS: INCREASING DIVERSITY AND FILLING GAPS

Figure 3. 2015 sunflower collection explorations funded by the Plant Exchange Office (PEO), National Germplasm Resources Laboratory, USDA-ARS, Beltsville, MD.

The general areas of the 2015 sunflower explorations are indicted on Figure 3. A mid-June 2015 sunflower exploration to southeastern Utah and northern Arizona targeted *H. anomalus*, a species under-represented in the NPGS collection and of interest because of its success growing in a very dry habitat. Populations of *H. deserticola*, *H. petiolaris* ssp *petiolaris* and *H. annuus* were sampled as well. The US desert southwest has been experiencing more frequent and longer lasting droughts. Filling collection gaps before climate change affects long term *in vivo* survival of these crop wild relatives is a real consideration. For several years prior to 2015 this region had experienced severe drought. In 2015, however, southeastern Utah and northern Arizona received more than their average annual precipitation (178 mm) by mid-May and there was explosive plant growth. It was a very fortunate year to be exploring, although, as is typical, we did not find all expected populations.

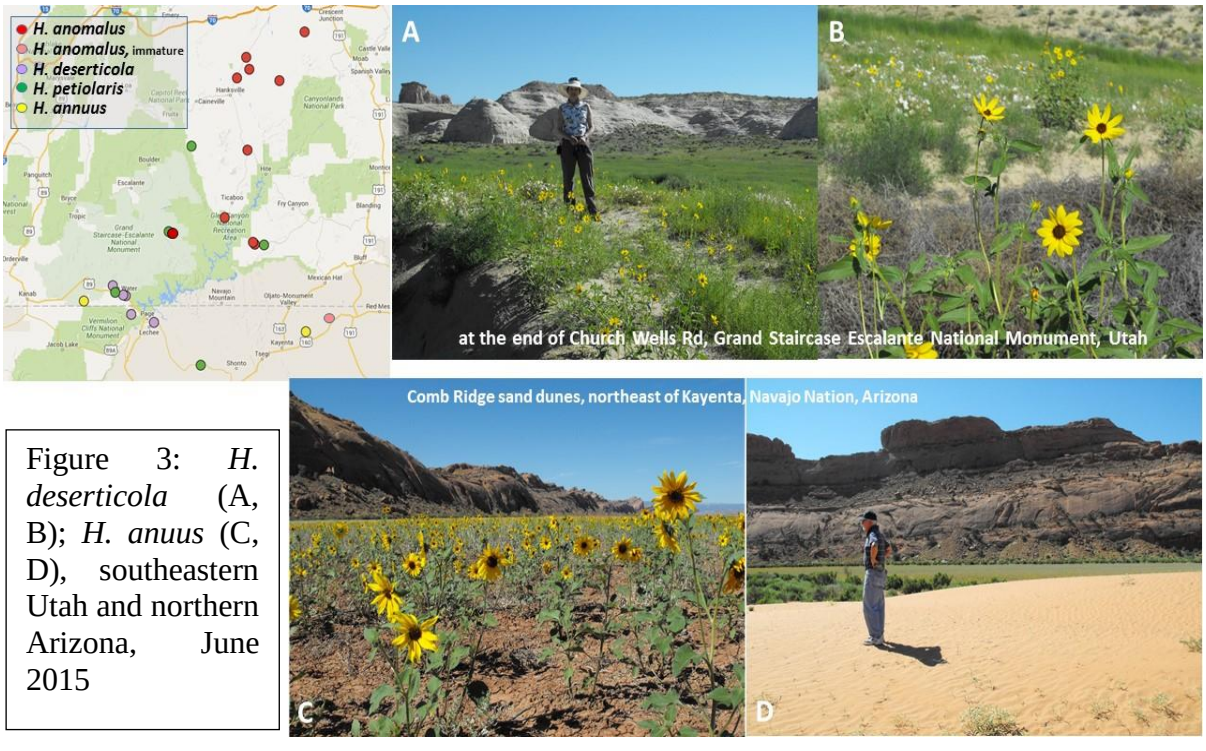


Figure 3: *H. deserticola* (A, B); *H. anuus* (C, D), southeastern Utah and northern Arizona, June 2015

Based on herbaria voucher records, we expected to find *H. anomalus* between Kanab, Utah and Page, Arizona. We did not locate any populations of the target species in this region but we did find *H. deserticola* (Fig 3, purple circles on map and images A and B) which added to genetic diversity in the NPGS wild sunflower collection for that species. We were also expecting to find *H. anomalus* in the Comb Ridge sand dunes northeast of Kayenta,

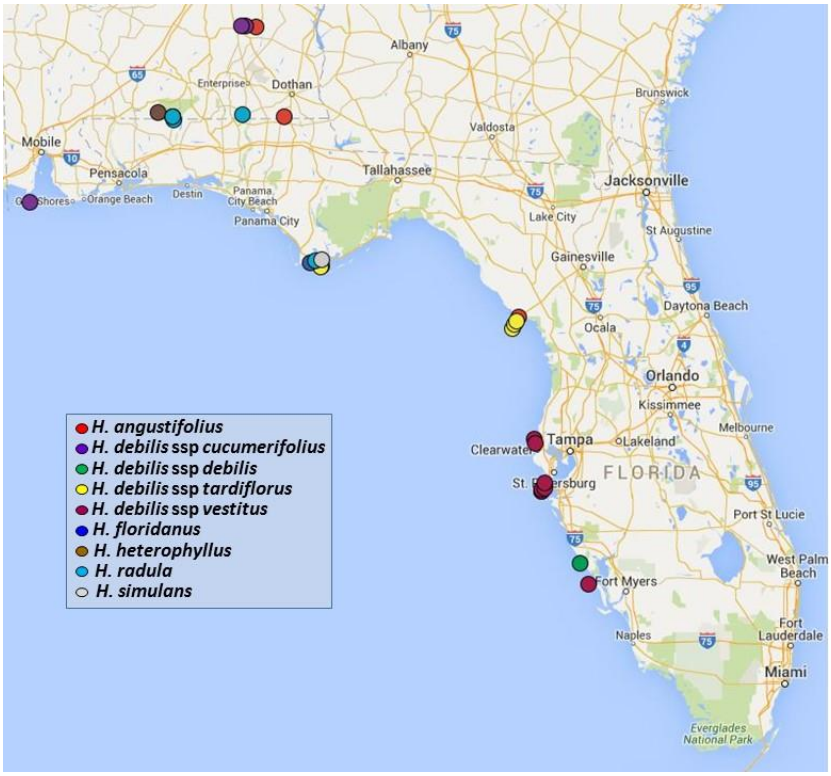
Arizona in the Navajo Nation. We observed an expansive population of *H. annuus* in the dried playa between the sandy dirt car track and the dunes but no *H. anomalus* plants in the dunes (images C and D, Fig 3.)

The first substantial populations of the exploration's target species, *H. anomalus*, were observed on the Nokai Dome plateau east of Lake Powell, Utah (Fig 4, image A; two populations) and eight additional populations were discovered and sampled as noted on the map (Figure 4, red circles on map). One unique feature of this exploration was that a freelance journalist, Nelson Harvey, accompanied us for two days. He was interested in crop wild relatives and their relationship to agriculture, a topic that seems to have had some presence in the popular press in the U. S. He had a professional quality camera and took many images which he agreed to share (Fig 4, images C, D and E). His article appeared in the Fall 2015 issue of Modern Farmer (N. Harvey, 2015).

Figure 4. *H. anomalus* in southeastern Utah, June 2015

The second exploration in 2015 took place in an area of the United States (Figure 5) where the climate concern is not drought but excess water due to rising sea levels and an increase in severe storms. This region of the United States continues to become increasingly urbanized, putting intense pressure on wild populations and creating some urgency to collect samples before populations disappear. In addition, revegetation work along the western coast and southern panhandle of Florida and southern Alabama often takes place without regard to use of appropriate endemic taxa. *H. debilis* ssp *debilis* is a popular species for dune stabilization and revegetation across the region despite it being native to eastern coastal Florida. As it is spread out of its traditional range, it has hybridized with the western coastal Florida subspecies *vestitus*. *H. debilis* ssp *debilis* and hybrids have been displacing *H. debilis* ssp *vestitus* in its traditional habitat (Bradley et al. 2004) which also lent some urgency to the second exploration. Valuable disease resistance traits have been transferred from several of the *H. debilis* subspecies to cultivated lines, notably resistance to infestation by the parasitic

plant *Orobanche cumana*, which was crossed into a cultivated background from the subspecies *tardiflorus* (Velasco et al. 2012). This discovery is especially interesting because *O. cumana* does not occur in the US. *O. cumana* is a primary concern in sunflower fields across many regions in Europe, Asia and now Africa and genetic resistance is the most reliable form of protection for the crop.



The target taxa for the fall 2015 exploration were three subspecies in the *H. debilis* complex (*vestitus*, *cucumerifolius* and *tardiflorus*). Other species were collected if interesting populations were observed in unusual habitats or in regions for which there was not an existing accession in the NPGS sunflower collection.

Figure 5. Overview map of fall 2015 collection exploration to western Florida and southern Alabama

All collected populations of *H. debilis* ssp *vestitus* were on islands off the coast of western peninsular Florida. Four islands were accessible by causeway and/or draw-bridge; three required the rental of boats and captains. Of particular note was one of the three populations sampled on Egmont Key (Figure 6 image B). The population on the western side of the island, not previously vouchered and first noted by the state park biologist the week before we arrived, was in an area where the rising sea level (salt water) has killed palm trees. The sunflower population was expansive and healthy. The only mainland based population of *H. debilis* ssp *vestitus* we observed was in Oscar Scherer State Park but it was smaller than the size permitted for collecting (Figure 6, pale pink circle on map map) so we could not make a collection.

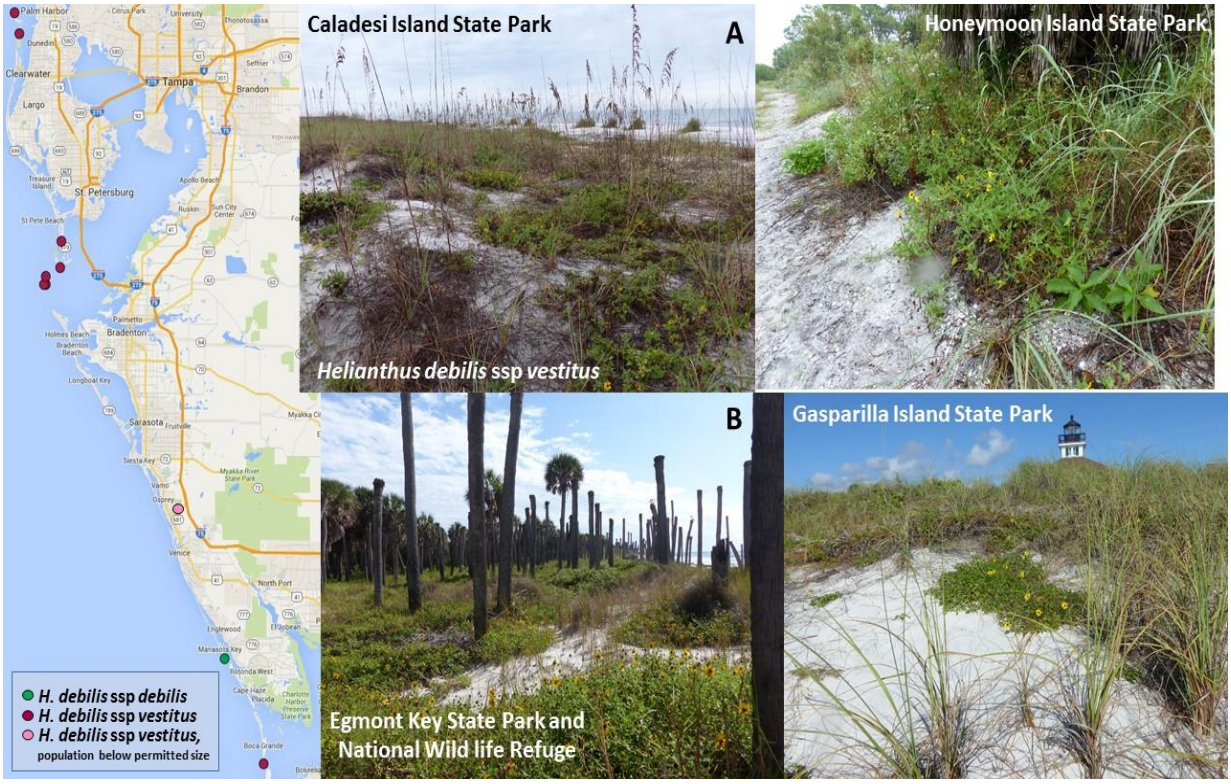


Figure 6. Collection area for *Helianthus debilis* ssp *vestitus*.

Figure 7. Collection area for *H. debilis* ssp *cucumerifolius* and *H. debilis* ssp *tardiflorus* and five additional sunflower species.

All the populations of *H. debilis* collected along southern Alabama and peninsular Florida were affected by the storm surge caused by Hurricane Patricia, initially the most intense tropical disturbance ever recorded, the week before we arrived. The hurricane ran aground into the mountains of western Mexico and did very little of the damage it was expected to cause, although it did cause a tidal surge across the Gulf of Mexico. Dauphin Island, Alabama was under water for about a week. Based on a herbarium voucher record, we expected a population of *H. debilis* ssp *cucumerifolius* in the general area of the street sign in Figure 7, image A. There were not plants visible at that location, although we did find a small population in a drier region some distance down the road (Figure 7, image B). St Vincent's Island, about 325 miles to the southeast of Dauphin Island and along the southern Florida panhandle was also largely underwater for about a week due to the Hurricane Patricia tidal surge. Extensive *H. debilis* ssp *tardiflorus* populations observed by the National Wildlife Refuge biologist just three weeks before the exploration were mostly destroyed; although we were able to collect limited numbers of seeds (Figure 7 image C).

Diversity in the NPGS wild sunflower collection was increased significantly in 2015. Dr. Seiler and I sampled 48 different wild populations of 13 taxa over 15 days of collecting in two separate PEO funded explorations in vastly different parts of the United States. We also received a significant donation of 140 accessions of five wild annual species, from a collecting exploration targeted for habitat type and species funded by Dr. Rieseberg's lab at the University of British Columbia. Dr. Dylan Burge made the collections during a three month exploration in the western and southwestern US.

PROVIDING RESOURCES FOR RESEARCH AND EDUCATIONAL PURPOSES

Identifying gaps, planning an exploration and collecting seed comprise the first steps in providing genetic resources for research and educational purposes. The next steps involve ensuring that seeds are available for use as expediently as possible. A goal is to collect enough seed to allow for at least some immediate distribution and back up storage in Ft Collins. We also send seed for long term back up to the Global Seed Vault in Svalbard, Norway when inventory supports a transfer. It is not always possible to collect significant quantities of original seed, in which case the accession is tagged for regeneration. Some accessions with significant quantities of original seed are scheduled for regeneration to ensure availability of all taxa for international distributions: we cannot make disease declarations for wild collected seed. We also schedule regenerations when distribution lots fall below some minimum (usually 500 to 1000 seeds) or when viability testing indicates failing quality.

We use standard protocols for regeneration at the NCRPIS. In addition to growing sunflowers in Ames, we have an alternate grow out location in the central valley of California southeast of Fresno at the San Joaquin Valley Agricultural Sciences Center, USDA-ARS. Parlier has a much longer growing season than Ames allowing late flowering southern species to complete their life cycles and it is hot and dry during the summer more closely mimicking the conditions expected by species native to the desert southwest. All wild sunflower regenerations take place in screened steel cages with added pollinators (usually honeybees). Cultivated sunflowers are grown either in rows with head bagging and hand sib pollination or in cages (multi-headed restorer lines, accessions with few seed, open pollinated populations, others) with added pollinators.

NCRPIS CULTIVATED SUNFLOWER COLLECTION

Not surprisingly the sunflower collection at the NCRPIS has an international origin. Although the crop wild relatives are native to North America, cultivated sunflower breeding had its beginnings in Russia. The USDA started a sunflower collection some decades before there was a significant USDA supported breeding effort in sunflowers. The first accessions in the NPGS cultivated collection were donated from Argentina, Uruguay, Lebanon and Turkey (Table 1). Over time, fifty-nine countries including US breeders have made contributions. By the late 1970's the USDA breeding programs were generating populations and inbred lines for public release and inclusion in the genebank, and since 1990 the primary source of new cultivated lines has been the USDA breeding program although there have been significant donations from Spain and very recently from Canada.

Table 1: International Origin of the NPGS cultivated sunflower collection: donor countries and years donated.

Source	# of accession	date(s)	Source	# of accession	date(s)
Afghanistan	2	1954, 1978	Lithuania	1	1974
Argentina	46	1948 - 1998	Mexico	9	1981 - 1992
Australia	5	1976-1983	Monaco	1	1995
Austria	2	1954	Mongolia	2	1992
Brazil	2	1965, 1967	Morocco	1	1989
Bulgaria	14	1959 - 1992	Netherlands	4	1989, 1997
Canada	452	1962 - 1993, 2016	Pakistan	4	1959 - 1978
Chile	7	1960s, 1995	Paraguay	1	1995
China	22	1950 - 1995	Peru	1	1984
Colombia	3	1969, 1996	Poland	37	1962 - 2000
Cuba	1	1996	Portugal	2	1967, 1989
Czechoslovakia	3	1989	Rhodesia	3	1979
Egypt	4	1958, 1979	Romania	45	1969 - 1995
Ethiopia	6	1951	Russian Federation	71	1981 - 1992
Former Serbia and Montenegro	69	1978 - 1989	Serbia	4	1949 - 1979
France	25	1963 -1997	South Africa	11	1964 - 1971
Georgia	1	1974	Soviet Union, Former	152	1988 - 1992
Germany	46	1952 - 1997	Spain	118	1958 - 2005
Hungary	83	1956 - 1989	Sweden	1	1995
India	3	1989	Syria	2	1948
Indonesia	1	1954	Tanzania, Tanga	1	1960
Iran	61	1955 - 1995	Turkey	122	1948 - 1965
Iraq	10	1958	Ukraine	16	1959 - 1985
Israel	4	1954 - 2000	United Kingdom	3	1954, 1997
Italy	3	1978 -1989	United States	592	1964 - 2014
Jordan	12	1957, 1980	Uruguay	7	1948 - 1995
Kazakhstan	1	1974	Zambia	28	1981 - 1985
Kenya	17	1971, 1972	Zimbabwe	36	1985 - 1987
South Korea	2	2012	Unknown	49	1959 - 1992
Lebanon	1	1948			

The breeding effort in Fargo has traditionally focused on disease resistance, early maturity, and oil quality. Disease resistance and oil quality continue to be a focus although there are lines pending release carrying insect resistance, lines with low saturated fat and high oleic acid, and material with altered tocopherol composition (reduced alpha and increased gamma and delta tocopherols).

Two groups of core accessions have been identified within the cultivated collection. In the first, described by Brothers and Miller (1999), 113 accessions were determined to represent the diversity in the collection at that time based on passport data, morphological traits, oil quality traits and isozymes. In 2011, Mandel et al. described a set of 12 to 288 nested accessions using 34 SSR markers (two per linkage group) and the 288 lines were estimated to capture 90% of the diversity present in cultivated sunflower. The NCRPIS is also distributing one sunflower association mapping population, UGA-SAM1, developed at the University of Georgia and described in Mandel et al. 2013. The two sets of 288 lines are not identical but have a 67% overlap. 10% of the 288 lines in both the 2011 nested core set and the UGA-SAM1 population were provided by the INRA sunflower genebank as representing diversity in the French collection.

The NPGS cultivated collection contains a significant number of lines with introgressions from wild species. 138 accessions have a wild sunflower mentioned in their descriptive data field in GRIN; a more detailed assessment of the pedigree of all the inbreds and addition of that information to the GRIN-Global database is planned. Fifty-five of the introgressed lines were recently genotyped as described by Greg Baute (2015). Dr. Baute also developed and genotyped over 400 pre-breeding lines with introgressions from 11 wild species (Baute, 2015) of which 350 were recently received by the NCRPIS (May 2016).

INTERNATIONAL COLLECTIONS

Most of the countries which helped start the NPGS cultivated sunflower collection have continued to breed and develop their sunflower programs several of which are detailed in Table 2.

Table 2. Seven international sunflower collections with genebanks.

Country	Genebank Location	database	Treaty* status	accessions
Argentina	Cordoba	under development	signature only	~1350 op populations, inbred lines, naturalized populations, wild species
Canada	Saskatoon	GRIN Canada	contracting party	~600 cultivated and wild accessions 162 H. tuberosus accessions not databased
France	Toulouse	under development	contracting party	~6100 inbred lines, RILs, interspecific lines, op populations, EMS mutant population, wild species
Russia	St Petersburg	VIR PGR		~2800 cultivated and wild accessions
Serbia	Novi Sad	wild sunflowers	contracting party	3000 inbred lines and wild species
Turkey	Izmir	under development	contracting party	~400 oilseed and confectionary landraces
United States	Ames	GRIN Global US	signature only	~5000 op populations, inbred lines, interspecific lines, association mapping population, wild species

*The International Treaty on Plant Genetic Resources for Food and Agriculture

Sunflower breeding as an oil crop began in Russia more than 100 years ago and a search of the database at the Vavilov Institute of Plant Genetic Resources (VIR) in May 2016 indicated close to 3000 cultivated and wild species accessions in the genebank there. The collection is curated by Dr. Vera Garvilova (v.gavrilova@vir.nw.ru) who participated in a sunflower exploration in the United States in the 1980s. Use of sunflowers as more than a garden curiosity spread quickly throughout Europe and to South America and back to North

America. Sunflower breeding in Argentina began in the 1930s and the Instituto Nacional de Tecnologia Agropecuaria (INTA) genebank, located in Cordoba, contains a collection of approximately 1350 open pollinated populations, inbred lines, naturalized populations and wild species curated by Dr. Daniel Alvarez (alvarez.daniel@inta.gob.ar). Argentina was an original contributor to the establishment of the USDA sunflower collection (Table1). A collection of 400 Turkish oilseed and confectionary land races is maintained in Izmir by Dr. Ahmet Tan (a_s_tan@Hotmail.com). Turkey was also an original contributor to the NPGS sunflower collection (Table 1). The Institute for Field and Vegetable Crops, Novi Sad, Serbia has a long history in sunflower breeding and a strong interest in wild species. Scientists from Novi Sad participated in seven collection explorations in the United States during the 1980s through 1991. Their collection of wild species is available through a public database and the collection is curated by Dr. Sreten Terzic (sretenterzic@gmail.com). The French Sunflower Genetic Resources Center has recently been consolidated at the Institut National de la Recherche Agronomique (INRA) in Toulouse. Dr. Stephane Munos (crb.tournesol@toulouse.inra.fr) is the scientific curator with assistance from Marie-Claude Boniface and Nicolas Pouilly. The INRA collection has about 6110 lines including an EMS mutant population as well as recombinant inbred lines, open pollinated populations, inbred lines, interspecific lines and wild species. The group at INRA is developing a database and is expecting to be able to distribute seeds under material transfer agreements. The Canadian sunflower collection of about 600 lines is databased in GRIN Canada through Agriculture and Agri-Food Canada. The group maintains a substantial *H. tuberosus* collection which will be accessible online once their database migrates to GRIN Global Canada. Dr. Kessler (Dallas.Kessler@AGRI.GC.CA) distributes limited numbers of *H. tuberosus* accessions as tubers (all Canadian genebank materials are distributed under the SMTA) domestically and internationally.

SEED DISTRIBUTION FOR CROP SECURITY AND IMPROVEMENT

Sunflower is an Annex 1 crop under the International Treaty on Genetic Resources for Food and Agriculture which has effected exchange of germplasm: germplasm can be exchanged freely between contracting parties but under the terms of the standard material transfer agreement (SMTA). Countries with significant sunflower industries have varied in their response to the treaty: 129 countries are contracting parties to the treaty, nine countries have signed but not ratified the treaty, and about 50 countries have not yet participated. Of the countries discussed in the previous paragraphs, Canada, France, Serbia, and Turkey are contracting parties, the US and Argentina have signed but not ratified the treaty and Russia has neither signed nor ratified the treaty (Table 2). The US NPGS does accept germplasm under the SMTA and will distribute that germplasm under the SMTA. Currently there are two sunflower accessions with SMTAs attached, both land race lines received from South Korea in 2012.

The NPGS distributes germplasm without charge and generally without restriction except that intellectual property rights cannot be applied to germplasm as it is received; some breeding and selection must be undertaken first. In addition, the US Fish and Wildlife Service puts restrictions on species that are on its Threatened and Endangered Species list which includes three sunflower species: *H. paradoxus* (threatened), *H. schwenitzii* (endangered) and *H. verticillatus* (endangered). The NCRPIS is currently permitted to distribute *H. paradoxus* accessions under the conditions that any plants raised and their derivatives cannot be commercialized and all research must occur in a laboratory or greenhouse. The US Treasury Department imposes sanctions on trade with some countries; these do not allow for seed distribution although there can be exceptions for humanitarian purposes.

The primary restrictions on seed distribution, however, are imposed by the importing countries. Frequently a phytosanitary certificate is required to accompany a seed shipment and some countries require an import permit. It is common for countries with sunflower industries to require that any cultivated sunflower seed shipped be either declared free from downy mildew or be treated for that pathogen. Our staff pathologist inspects our regeneration fields, removes any diseased plants, and he can make many declarations based on those observations. Sometimes our pathologist is required to perform seed testing to meet import requirements. Resource limitations, both seed inventory amounts and pathology group time and expense, generally limit the number of accessions that can be tested to not more than 30 on an order. Testing results are entered into the GRIN Global database and hold for that accession as long as the distribution lot remains the same. There are times we cannot meet requirements by either testing or field declaration and then the seeds cannot be sent unless the requester can receive a waiver from the relevant agency in his/her country.

Ordering germplasm from the NPGS takes place through the Germplasm Resources Information Network (GRIN) Database, which migrated on November 30, 2015 to GRIN Global. Unless the requester has ordered since November 30, 2015, the first step in the ordering process is for the requestor to set up a GRIN Global online profile which can be done by selecting My Profile at the GRIN Global home page (http://npgsweb.ars-grin.gov/gringlobal/search.aspx) and following prompts. An order can then be set up under the "Accession" sub-menu on the home page, using a shopping cart style of ordering, unless the requester is interested in accessions which are not available when it is necessary to contact the curator.

The distribution process from the NCRPIS is straight forward (Figure 8) but the length of time to complete the process varies depending on the number of accessions requested and whether the request is domestic or international and if international, if there are import restrictions, and sometimes other factors. Plan for at least two months for receipt of international orders.

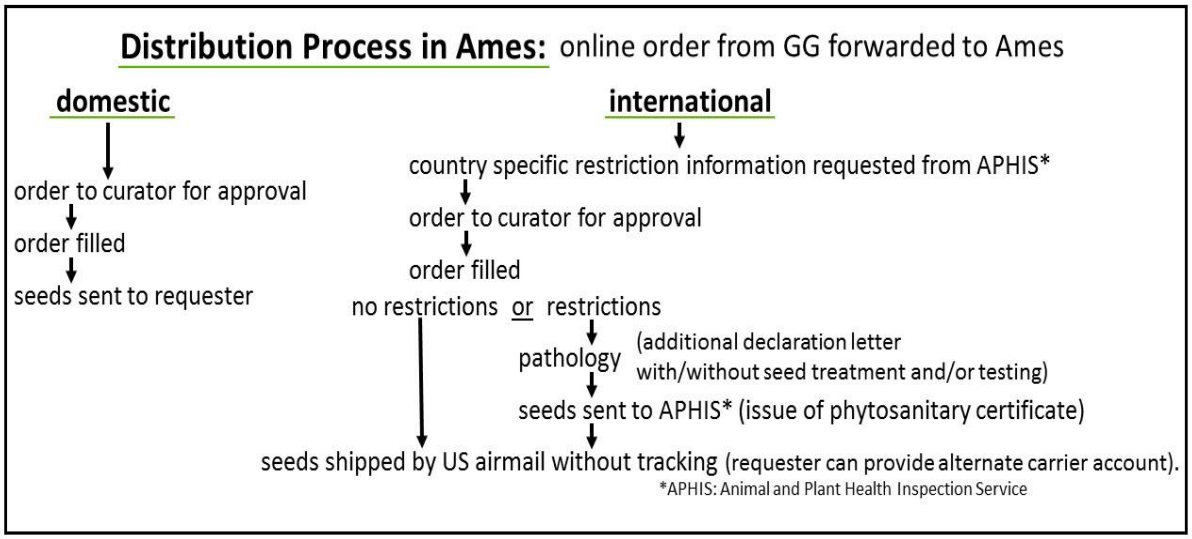


Figure 8: Schematic representation of seed distribution from the NCRPIS, Ames.

During the five year period from 2011 to 2015, 24,600 sunflower seed packets were distributed from the NCRPIS in 755 orders, 20% of which were sent internationally to 40 countries. The seed packets were split approximately 2/3 (16,000) cultivated accessions and 1/3 (8,600) wild sunflower accessions. About 50% of the wild packets were wild *H. annuus*

and 50% all other wild taxa. Demand for wild sunflower accessions has increased over the past 10 years as more accessions and all taxa have become available.

CONCLUSIONS

The USDA sunflower collection has had an international origin, beginning in 1948 with donations from agricultural programs in Turkey, Argentina, Syria, Uruguay and Lebanon. In subsequent years, 54 additional countries have made important contributions. The USDA sunflower breeding program, underway in the 1970s, began contributing inbred lines to the collection in the 1980s. Now the USDA sunflower collection is able to make significant resources available to the international community both cultivated germplasm as well as the substantial collection of all wild sunflower taxa (except *Helianthus niveus ssp niveus*) which we continue to expand to ensure maximum geographic and genetic representation. Sunflower crop wild relatives provide a valuable genetic resource which, along with a significant collection of cultivated accessions, the NPGS maintains and distributes free of charge for scientific and educational purposes.

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PRESENT STATUS AND FUTURE PROSPECTS OF GLOBAL CONFECTIONERY SUNFLOWER PRODUCTION

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ABSTRACT

Although sunflower is mainly grown for the production of vegetable oils in the world, there are many countries that prefer confectionery sunflower hybrids and varieties (landraces). Confectionery sunflower breeding is characterized by the fact that different markets have different demands when it comes to the seed size, hull color and other traits, which makes this process more difficult and costly. Confectionery low-oil protein type is used in the snack food industry in the form of roasted sunflower seeds or dehulledas a part of snacks and baker's wares, as wellas for bird and pet feed. It currently represents less than 10% of total global sunflower production. Seed of high protein sunflower usually varies in color, from black, black with white stripes, to white and colorful. It is significantly bigger than the seed of oil type sunflower, with thicker hull loosely connected to the kernel. The hull is easily separated from the kernel and allows the whole seed to be dehulled. When creating confectionery hybrids it is very important to combine genes responsible for high yield potential and good technical and technological traits of the seed. In order to successfully obtain high yields and adaptability for confectionery sunflower the main direction in breeding is defining an ideal ideotype of plantfor specific agro-ecological conditions, sef-fertility rate, larger seed, increased 1000 seed weight, protein content and quality. While lowering the seed oil content and hull ratio and at the same time introducing resistance genes in order to achieve stability of sunflower resistance to certain pathogens. Major breeding goals are tolerance to biotic and abiotic stress conditions, resistance to diseases (*Plasmopara halstedii, Phomopsis helianthi, Sclerotinia sclerotiorum*), broomrape and herbicides. In the resent period introgression of genes from wild *Helianthus* species for herbicides resistance of confectionery hybrids (Imidazolinone (IMI) and Sulfonyl urea (SU)) has become a crucial breeding objective. By growing confectionery IMI hybridsto the same time control both broomrape and the main broad leave weeds. They have recently effectively increased the market share in many countries which prefer confectionery hybrids. Developing confectionery hybrids with modified oil quality(higher oleic acid and tocopherols) to increase in the nutritional value in seeds is also an important breeding objective. By introducing molecular markers, genetic maps, genomics, bioinformatics data and other developing techniques, many relevant sunflower traits, such as oil and protein quality, cms, fertility restoration genes, and resistance to diseases and abiotic stress can be verified. Armed with this knowledge and the possibility of further development of these techniques, in the future we should be able to pinpoint the desirable genes and more efficiently trace their transfer to the confectionery sunflower.

Key words: Confectionery sunflower, Breeding, Seed and protein yield, Resistance to disease and herbicides, MAS

INTRODUCTION

The genus *Helianthus* is comprised of a large number of species. One of the species is the cultivated sunflower *Helianthus annuus* L., which is a globally significantoilseed crop and an important source of non-oil confectionery seed. Native Americans gradually changed the

genetic composition of the plant by repeatedly selecting the largest seeds (Yarnell, 1978). Sunflower oil and confectionery types arrived from the USA to Europe, first landing in Spain. They was transferred from Spain to France, England, Germany and other European countries and then spread along trade routes to Egypt, India, China and Russia (Brintall and Conner-Ogorzaly, 1986). In the mid-19th century, sunflower seeds oil and confectionery types were transferred to Bolivia, Paraguayand Argentina where sunflower was used for human consumption as a roasted and salted confection (Feoli and Ingaramo, 2015).

The agronomic development of sunflower for oil ("oilseed" types) and edible achenes ("confectionery" types) occurred in Eastern Europe and Russia, where by the late 1800s a number of landraces had been developed (Cronn, 1997). Russia is considered as a secondary domestication center for sunflower, as seeds of local sunflower varieties were reintroduced into the USA, and then spread to Canada and Argentina in the late 18th century by Russian Ukrainian and German immigrants. In commercial sunflower breeding there are two main types: oil and non-oil (confectionery) sunflower type (Duihua and Hoeft, 2009; Gontcharov and Beresneva, 2011; Hladni et al., 2011, 2012). Around 10% of the world's annual production of sunflower seed is used for non-oil purposes, mainly for confectionery and snack food, as well as for bird and pet food (National Sunflower Association, 2011). Market demands and production area of confectionery sunflower show a steady increase due to its nutritional value and use in human nutrition. Confectionery sunflower for human consumption has found its place in production in Turkey, China, USA, Canada, Spain, Russia, Ukraine, Israel, Argentina, Pakistan, Iran, and many other countries (Lofgren, 1997; Kaya, 2004; Zhang, 2004; Feoli, 2004; Dong, 2007; Kholghi, 2011). This type of sunflower has a small presence in the EU, which makes the EU (particularly Spain) a large importer of confectionery sunflower seeds (Nabloussi et al., 2011). In contrast to EU, confectionery sunflower represents more than 60% of sunflower production in China (Zhang, 2004).

Confectionery sunflower breeding is characterized by the fact that different markets have different demands regarding the seed size, hull color and other traits, which makes this process more difficult and costly. Although the favored seed color of confectionery hybrid in Turkey is white with gray stripes, in Balkan countries such as Serbia, Bulgaria, Moldova and Romania, as well as Russia black seeds are preferred (Ergen and Saglam 2005; Yaia et al., 2005; Sincik and Goksoy, 2014). Gray seeds with stripes are popular in the United States, Spain and China (Kaya, 2008).

Most customers prefer tasty, high-quality, and longer seeds for confectionery types. It is not easy to develop bigger kernels in breeding programs. For instance, consumers from China, Turkey, and some other countries require seeds that are at least 2 cm long, whereas in Eastern Europe such as the Balkans, Ukraine, and Russia consumers prefer big seeds with big kernels and reduced hull content. Confectionery sunflower is distinguished by a large hull ration, usually up to 40 or 50% (Jovanović, 2001), high mass of 1000 seeds which is usually higher than 100g (Hladni et al., 2011), should ideally contain less than 30% oil and has hull content up to 50% (Kaya et al., 2008). The hull is easily separated from the kernel and allows the whole seed to be dehulled (Gonzalez-Perez and Vereijken, 2007; Fernandez-Martinezet al., 2009; Hladni et al., 2012a; Kleingartner, 2015). Breeding advances allow confectionery sunflower to have similar yields to regular sunflowers (Feoli and Ingaramo, 2015). Based on its size confectionery sunflower is generally classified into three categories. The largest size seeds, called "in-hull seeds", go on hall market. They are salted, roasted and packaged for human consumption. Medium-size seeds, called "hulling seeds" are hulled and are usually the kernel market. Kernels are used, either roasted or not, as a snack food or in a number or confectionery or bakery products. Finally, smaller seeds, called "bird seeds", are mainly

intended for feeding wild birds and pets (Holfland and Kadrmas, 1989; Lofgren, 1997; de Figueiredo et al., 2011, 2014*)*.

Dehulled sunflower kernels are typically less expensive than some of the other nuts, used for confectionery, cakes, and other purposes in the food industry in North America (Miller and Fick and, 1997; Fernandez-Martinez et al., 2009; Škorić, 2012; Kaya et al., 2012). The bread and bakery industries are growing markets for sunflower kernel, which is also used for the fortification of foods by sunflower meal, especially meat and milk products, infant formulae, bakery and pasta products (Žilić et al., 2010). Growing confectionery sunflower for consumption is becoming more and more attractive in the whole world, currently production and research of confectionery sunflower is very low in comparison to the oil sunflower.The main goal in writing this paper was to present an overview of what has been done so far in confectionary sunflower breeding and production, along with the directions in which confectionery breeding is expected to develop in the future.

GENETICS AND BREEDING

An important quality of modern agricultural production is breeding for high yielding cultivars and hybrids tolerant to diseases, pests and unfavorable climate conditions. These cultivars and hybrids are developed primarily by employing different breeding techniques and methods which are based on the choice of favorable genotypes depending on the selection goals (Hladni, 2010).

Genetic resources

Land races/local populations have huge genetic variation and are well adapted to local soil types and climatic conditions as well as other environmental factors. They are the source of many desirable genes, especially those addressing higher adaptability to environmental conditions and resistance to certain diseases (Kaya, 2015). However, little is known about the levels and distribution of genetic variation within confectionery sunflower gene pool (Omar Gieco et al., 2013).There are several important confectionery sunflower collections in Turkey (Kaya et al., 2001), China (Jan et al., 1998), Spain (Velasco, 2014), Russia (Borodin, 2003; Mamonov, 2004), US (Marek, 2004) etc. Velasco et al. (2014) assessed variation in seed quality traits (seed weight, kernel percentage, oil content, fatty acid composition, squalene, tocopherol and phytosterol contents, and tocopherol and phytosterol composition) in a germplasm collection of 137 Spanish local landraces of confectionery sunflower, and found large variability for all traits evaluated. Other important genetic resources are: cultivars in production, breeding lines, synthetic varietiesand others. Cultivars in production are easier to use in breeding programs, good source of genes that confer high yield and quality disease resistance. The benefits of synthetic populations that came to be by recurrent selection maintained by open pollination are the development of inbred lines showing high values of combining ability as a result of hybrid combinations (Fernandez et al., 2009; Škorić, 2012; Kaya et al., 2012). That is why it is important in a breeding program for confectionery sunflower to create new synthetic population for creation of new confectionery lines and hybrids.

The directions of confectionery sunflower breeding

Sunflower breeding is directed towards the increase of: genetic potential for yield, yield stability, health safety and nutritive quality with the increase of production economy (Hladni, 2010). In creation of new sunflower hybrids, significant attention should be paid to increase of adaptability, stability, and attractiveness to pollinators and tolerant to dominant

diseases, broomrape, insects, and stress conditions (high temperatures and drought conditions) (Hladni, 2010; Jocić et al., 2015).

Confectionery sunflower breeding is mostly similar to oil sunflower breeding, especially in increase of seed yield and resistance to main disease, but there are also certain specificities Jocić et al. (2015). Specific breeding goals for confectionery sunflower are: the increase of protein content and quality (>25%), 1000 seed weight (>100 g), hectoliter mass 90kg/hl, oil stability with decrease of its content in the seed (<40%), large achene and kernel size, uniformity in kernel size, increase of kernel ratio and decrease of hull ratio (<35%), uniformity in seed size, shape and color, ease of dehulling, seed quality maintenance in long term storage as well as tolerance to dominant diseases in the cultivation region (Hladni et al., 2009,2015; Škorić, 2012; Kaya, 2015). Seed size, shape, and color are especially important for confectionery-type sunflowers depending on the market. Consumer demand varies widely, especially for seed color.

In recent years, introgression of genes for resistance to herbicides (Imidazolinones (IMI) and Sulfonyl Urea (SU)) from wild *Helianthus* species has become a topical breeding objective for both oil and confectionery sunflower (Škorić, 2012). Drought stress is one of the most key aspects for crop yield losses in recent years and it seems that will be active threats for restricting crop productivity in the years to come, due to recent climatic changes and global warming (Peckan et al., 2016). In recent years, there have been many changes in research techniques, in particular, the possibility of determining the genotype of a plant and not just its phenotype Vear (2016). Marker technology is currently being used in breeding. Great steps have been made in obtaining essential knowledge of inheritance and linkage of target traits for breeding confectionery sunflower adapted to Australian production environments. The identified markers can be used in practical application of molecular markers (MAS), and further enhance the breeding process (Sun, 2009).

Confectionery sunflower breeding has become more prominent over the last decade (Sincik and Goksoy, 2014). In the world currently there are not a lot of Institutes and companies that have a confectionery breeding program. Market demand for confectionery sunflower seeds made Institute of Field and Vegetable Crops, Novi Sad initiate a special breeding program with the aim to develop modern confectionery open-pollinated hybrids.

Breeding goals

Two important criteria for introducing confectionery hybrids into production are high seed and protein yield (Hladni et al., 2009). Breeding for a desirable plant architecture and yield components requires a study of the gene effects, the number of genes controlling the expression of a particular trait, the mode of inheritance of quantitative traits. As well as the examination of general and specific combining abilities and interdependence of morpho-physiological traits with yield is of utmost importance in order for their breeding programs to be successful (Hladni et al., 2006; Škorić et al., 2012). For the creation of new sunflower hybrids with high genetic potential for seed and protein yield, it's important to find traits that have the biggest influence on the seed and protein yield formation. Presence or absence of correlations can contribute to the right choice of examined traits so as to enhance the efficiency of some selection criteria. Plant breeders commonly prefer yield components that indirectly increase yield (Kaya et al., 2007).

The most important criteria for introducing new confectionery hybrids into production are: protein and seed yield, plant height, head diameter, seed protein content, seed oil content,

number of seeds per head, 1000 seed weight, seed size, color of seed, hull kernel ratio (Hladni et al., 2009,2016; Pekcan et al., 2015).

Protein and seed yield

Higher protein yield is an ultimate objective of confectionery sunflower researchers. When creating new confectionery hybrids it is important to find traits that are easily determined and at the same time show their interdependence and very strong direct effects with protein yield, based on which that those traits can become selection criteria (Hladni et al., 2011a, 2015). Traits such as seed yield, seed protein content, kernel ratio, 1000 seed weight have a very strong positive direct effect with protein yield and, that breeding for these traits simultaneously breeding for protein yield (Hladni et al., 2011; Sincik and Goskoy, 2014; Hladni, 2015). Seed oil content had a very strong negative direct effect on protein yield (Hladni, 2015). The greatest positive indirect effects on protein yield were exhibited by the 1000 seed weight, plant height, and head diameter though their impacts on seed yield (Sincik and Goksoy, 2014).

Selection for higher seed yield, and other traits should start during inbred line creation by defining the effects of heterosis and by analyzing and evaluating the correlations among them to develop a productive hybrid with the desired traits (Škorić et al., 2007; Škorić, 2012; Hladni, 2011b). Any increase in seed yield depends on increasing one of three main components: number of plants per hectare, seeds per plant, and 1000 seed weight traits (Kaya, 2015). One of the efficient ways of increasing seed yield is lowering hull ratio and increasing the kernel ratio. That is why inbreeding programs special attention is paid to the hull and kernel correlation (Jovanović, 2001). Kholghi et al. (2011) found that the head diameter and 1000 seed weight had positive direct effects on seed yields of confectionery sunflowers. According to Hladni (2015), path coefficientanalysis showed strong direct effect of kernel ratio on seed yield is shows that the kernel ratio important selection criterion for confectionery sunflower. A negative weak correlation between seed oil content and seed yield was determined by Kaya et al. (2008) and strong negative correlation between seed yield and seed oil content to the research performed by Hladni et al. (2008). A positive and important interdependence was determined among morphophisiological traits like plant height and head diameter with seed yield (Goksoy and Turan, 2007; Hladni et al., 2008,2010; Kaya et al., 2009).

Seed protein content is one of the indicators of sunflower seed quality. According to (Jovanović and Stanojević, 1996; Hladni et al., 2009a; Hladni, 2010), protein content varies and, depending on the genotype, agroecological conditions and the interaction of the genotype and environment conditions, it ranges from 16-28% with confectionery sunflower. With kernel increase, the amount of protein in the seed also increases so breeding for increased seed protein amount should be followed by the selection of genotypes with larger kernels (Hladni et al., 2009b). Proteins of sunflower seeds have high digestibility and high biological value and hence the increase in their use as a component of functional foods and a nutritionally balanced diet, especially in this day and age when consumers wish to protect themselves from genetically modified soy protein products (Dimić et al., 2006). When choosing the initial materials for selection, theyshould have enough variability before the application of adequate plant-breeding methods. Recurrent selection is one of the most appropriate methods to increase sunflower seed protein content (Fick and Miller, 1997; Fernandez-Martinez et al., 2009; Škorić, 2012; Kaya et al., 2012).

Plant architecture

Plant height plays a major role in the creation of new SC hybrids with different plant model and high genetic potential for seed yield, but it is strictly linked to total leaf area and petiole length, which are very important for seed yield per plant. At the same time it is a very important trait because it has influence on the stability of the plant i.e. the tolerant to lodging and some diseases (Hladni, 2010; Hladni et al., 2014; Kaya, 2015). Confectionery sunflower is normally a tall plant. The height of the plants is very dependenton climatic and soil conditions and while drought or poor soil drastically reduce it, irrigating and less water stress affect the plant height very positively (Kaya et al., 2012).

Head diameter is a very important trait in the sunflower seed yield structure greatly influenced by the environmental conditions similar to the plant height. Head size, expressed as head diameter (cm), is one of the sunflower yield components that directly influence hybrid model changes (Hladni, 2010). Sunflower breeders should consider optimal head size and head shape with optimum plant density to increase sunflower yield (Miller and Fick, 1997). Plant height showed significant and positive correlation with head diameter in confectionery sunflower consorted Sincik and Goksoy (2014).

Total number of seeds per sunflower head represents one of the most important components of sunflower seed yield. It is conditioned by the number of formed tubular flowers, the degree of self-compatibility, attractiveness towards the pollinators and the environmental conditions during flowering and pollination of sunflower (Hladni, 2010). To increase total number of seeds per head breeders should focus on developing bigger kernels which need to assimilate more during seed filling and should consider limiting factors for seed growth development during the flowering period (Pereira et al., 2000; Škorić, 2012).

1000 seed weight

Breeding for increase in 1000 seed weight, results in increased seed yield. Therefore it is considered an important criterion in the development of confectionery sunflower hybrids (Miller and Fick, 1997; Goksoy and Turan, 2007; Hladni et al., 2008; Yasin and Singh, 2010; Kholghiet al., 2011; Hladni et al., 2016). The analysis of simple correlation coefficient shows a very strong negative correlation between 1000 seed weight and kernel ratio, and very strong positive correlation with hull ratio (Kaya et al., 2008; Liet al., 2010; Hladni et al., 2015). Path coefficient analysis for 1000 seed weight at the phenotypic level showed that the length of seed and thickness of seed had a very strong direct positive effect on 1000 seed weight, which is in accordance with the simple correlation coefficient. Length and thickness of seed were the most important traits for 1000 seed weight, and can be used for the improvement of seed yield and evaluation of sunflower breeding materials (Hladni, 2016).

Seed size, shape, and color

Increased seed length is one of the main goals in confectionery sunflower breeding and it can be achieved by selection. Sun (2009) found that the polygenic system controls seed length in sunflower, but QTL analysis showed that only one or two major genes play an important role. In order to produce larger seeds, plants should first of all have good genetic potential for this trait. By studying the seed parameter inheritance in confectionery sunflower Dozet and Jovanović (1997) have found that the seed length and width were intermediary in F_1 generation in all hybrid combinations and in a three hybrid combinations expressed dominance and partial dominance by better parent for seed thickness. Shape is usually described from a side view of the kernel. Kernels may be round, oval, ovate, oblong with

edges that are relatively straight when viewed from one edge, and rounded the variousdegrees the others (Janick, 2013). Confectionery types have seeds of variable colors black, white, or striped grey/black and white.

Drought tolerance

Water stress is a major limiting factor for sunflower production in the many regions in the world especially when the frequency and amount of rainfall are often quite variable during sunflower growing season. Therefore, drought tolerance became one of the most important goals in the sunflower breeding programs in the world (Pecan et al., 2015). Generally, drought stress reduces leaf area, stem extension and physiological activities as well as photosynthesis rate of plants resulting in decreasing seed yield (Anjum et al., 2011). Breeding for tolerant to drought and high temperatures is an important objective in many sunflower programs. Drought stress decrease grain filling period, grain length and yield potential (Anonymous, 2013). When setting up a breeding program for sunflower resistance to drought, it is important to decide in advance whether to aim for adaptation to a specific environment, adaptation to a variable environment, or combined selection for drought tolerant traits and high yield potential (Fick and Miller, 1997; Škorić, 2009; Pecan et al., 2016). Škorić (2009) in sunflower breeding for drought tolerant in oil sunflower, best practical results have been achieved using the phenomenon of stay-green. Confectionery sunflower showed that seed yield decreased significantly due to water stress (Anjum et al., 2011). In order to evaluate morpho-physiological traits of confectionery sunflower under different irrigation regimes, tested fifty six confectionery sunflower landraces in Iran, the effect of genotype × irrigation regime was significant for seed yield, kernel/ seed ratio and kernel weight (Gholinezhad, 2013). Growing the drought tolerant genotypes will contribute to more stable sunflower production. Furthermore, the screening of the response of sunflower cultivars or breeding lines to drought stress can play a crucial role in breeding programs (Onemli and Gucer, 2010).

Resistance to diseases and broomrape.

Diseases are the main limiting factor in the production of sunflower (*Helianthus annuus* L.) and they cause poor realization of genetic yield potential of sunflower hybrids (Jocić et al., 2010). Different diseases are dominant in different regions, depending on the prevailing environmental conditions. More than 30 different pathogens that attack sunflowers and cause economic loss in production have been identified so far (Škorić et al., 2012). Due to confectionery sunflower production in different scattered areas, the damage from birds, such as crow, sparrow and starling, is another factor reducing yield (Kaya,2015). Breeding for resistance or tolerance to diseases is one of the most important goals in sunflower breeding (Kaya et al., 2015). Although some sunflower diseases occur only locally or in specific environments, some of them result in great yield losses in sunflower production. The most serious ones for oil and confectionery sunflower are Downy mildew (*Plasmopara halstedii*), Phomopsis (*Diaporthe helianthi*), Sclerotinia stalk and head rot (*Sclerotinia sclerotiorum*), Charcoal rot (*Macrophomina phaseolina*) Verticillium wilt (*Verticillium dahliae*), Rust(*Puccinia helianthi*), Phoma black stem (*Phoma macdonaldii*), Alternaria (*Alternaria spp*.) and Rhizopus head rot (*Rhizopus spp*.). Chemical application is effective in the control of some diseases, but developing resistance genes is considered the most effective and sustainable control in sunflower. Sunflower breeders have achieved significant results in finding genes for resistance or high tolerance to certain diseases in wild species and in incorporating them into the cultivated sunflower genotypes. Besides drought conditions during seed filling, different diseases, and the main problemlimiting sunflower yield is the occurrence of broomrape (*Orobanche Cumana* Wallr.) infestations. Broomrape spreading

rapidly to new areas in recent years leading to considerable yield losses up to 100% and reducing sunflower seed quality (Kaya et al., 2012; Pineda-Martos et al., 2013). Since broomrape is a highly variable parasite, the break down of resistance is a frequent phenomenon, and multiple sources of resistance are needed (Seiler, 2012). In Spain, broomrape was detected first in the Toledo Province (central plateau) in 1958, infecting confectionery sunflower (Gonzalez Torres et al., 1982). Herbicide-tolerant hybrids are in turn divided into two different classes: tolerant to Imidazolinones (IMI) and tolerant to Tribenuron methyl or Sulfonil urea (SU). The utilization of IMI sunflower along with herbicide treatment offers an effective control of broomrape what ever the path type might be (Alonso et al., 1998), since this combination prevents the multiplication and dissemination of the pathogen. One of our breeding projects aimed to incorporate desirable traits such as disease resistance and herbicide resistance into confection sunflower lines for public release (Yoe, 2007). The sunflower breeding program at Institute of Field and Vegetable Crops, Novi Sad has been directed towards creating lines and hybrids which are resistant to new broomrape races. Continued work on creating new sunflower hybrids resistant to broomrape demands the screening of breeding materials for resistance in both field conditions and in controlled conditions of a greenhouse (Hladni et al., 2012a). One of the main advantages of Clearfield hybridsis the simultaneous control of broomrape and a broad spectrum of weeds (Pfeninget al., 2008). The combination of both strategies of broomrape control, genetic resistance to broomrape and herbicide tolerance, will contribute to a more durable control of broomrape while simultaneously controlling a wide spectrum of weeds (Fernandez-Martinezetal, 2015). In particular, resistance to fungal diseases and broomrape will continue to be a key aspect of sunflower breeding

PRESENT SITUATION IN CONFECTIONERY SUNFLOWER BREEDING

Landraces/open-pollinated confectionery varieties are mainly used for confectionery sunflower production in the many countries such as China (Zhang, 2004), Turkey (Tan, 2010), Iran (Kholghi, 2011), Spain (Nabloussi, 2011). The main reason is that there is not enough certified seed production with desired quality. The landraces or local varieties are not suitable for combine harvesting because of their non-uniformity of plant development in the field (Tan, 2009; Tan, 2010). In Turkey, confectionery sunflower farmers do not get higher yields even under irrigated conditions, as farmers plant different local populations. In Hungary, the stripe-patterned confectionery sunflower is still produced at small farms. The traditional manual technologies do not involve use of chemicals. Spraying with field machines if needed can be carried out only in first few months of the growing period because of the height of plants, while the small field size excludes aerial spraying (Szabo et al., 2008, 2010). In Spain, confectionery sunflower production was maintained at a small scale, mainly based on local landraces (Nabloussi, 2011). Traditionally open-pollinated confectionery varieties of sunflower are cultivated almostevery where in China. These open-pollinated varieties have quite low average yield (Zhang, 2004). Open-pollinated varieties covered about 500 000 hectares in Russia (Goncharov and Beresneva, 2011). This type of seeds has special Russian name “mezheumok” and means intermediate. Their seeds are close to the oil-type one by structure but larger in size and 1000 seed weight, has bigger husk content and less oil content (Borodin, 2003; Mamonov, 2004). In Serbia large open-pollinated confectionery varieties were grown, in the last few years they have been replaced by NS confectionery hybrids which keep spreading. Several factors have contributed to this occurrence, including crop uniformity, suitability for mechanized harvesting, and optimalplant densityfor achieving the desired size, seed quality and color suitable for the Serbian market demands.

FUTURE PROSPECTS IN CONFESTIONERY SUNFLOWER BREEDING

Market demands and production area of confectionery sunflower show a steady increase due to its nutritional value and use in human nutrition. It is expected that a high productive confectionery hybrids will replace varieties, which will influence the increase of surfaces under confectionery sunflower. Newly developed confectionery hybrids should have higher yield potential, higher self-fertility rate, resistance to diseases, broomrape and herbicide, and larger seeds with high oleic acid and vitamin E (tocopherol) content to increase their nutritional value and prolong seed shelf life (Jocić et al., 2015; Kaya, 2015). Modern varieties of sunflower show great variability in height, but for achieving higher yields lower plants are preferred. Lower plants are also more convenient for mechanical harvesting. (Hladni et al., 2008). The main direction of sunflower breeding is creation of hybrids with high genetic potential for seed yield >5 t/ha and seed protein content >25%. In order to achieve high and stable confectionery hybrid yield it is important to create a model of a sunflower plant which would enable an increase of number of plants per hectare in the conditions of high agrotechnics and mechanized harvesting. It is necessary to pay more attention to the architecture of plant organs, like petiole angle, petiole length, plant height and number of leaves per plant, which directly influence the change of the photosynthetic apparatus. The optimal plant size of confectionery sunflower hybrids for mechanized harvesting is <175cm, while the optimal head diameter is 20-25cm. Direct yield components, like number of plants per unit area (ha):42-46000, seed oil content <35%, seed protein content >25%, number of seeds per plant >1500 seeds, 1000 seed weight up to 110g, and low husk percentage <25% also play an important role in obtaining high seed and protein yield. When breeding for confectionery hybrids it is important to create hybrids with different vegetation seasons: early (80–90 days), medium early (90–100 days), medium late (100–115 days).

Molecular markers have several advantages compared to classical morphological markers and enable increased efficiency of conventional breeding (Vasić, 2001). It can be expected that the marker assisted selection (MAS) and molecular markers will increasingly beused in confectionery sunflower breeding for introduction of many desirable and agronomically important traits, quality traits, disease resistance or stress tolerance.

Within the breeding program for confectionery sunflower, special attention needs to be directed towards creating hybrids for different types of consumption and production depending on the demands of the European, Russian, Ukrainian, US, Turkish and Chinese market. Testing confectionery sunflower under different production systems (classical or organic) can be useful in identifying hybrids with broad adaptability (Hladni et al, 2015a;2015b).

CONCLUSION

Confectionery sunflower breeding is characterized by the fact that different markets have different demands when it comes to the seed size, hull color and other traits, which makes this process more difficult and costly. When creating confectionery hybrids it is very important to combine genes responsible for high yield potential and good technical and technological traits of the seed. In order to successfully obtain high yields and adaptability for confectionery sunflower the main direction in breeding is defining an ideal ideotype of plant for specific agro-ecological conditions, sef-fertility rate, larger seed, increased 1000 seed weight, protein content and quality. In order to achieve high and stable confectionery hybrid yield it is important to create a model of a sunflower plant which would enable an increase of number of plants per hectare in the conditions of high agrotechnics and mechanized harvesting. Confectionery hybrids have siggnificantly higher seed yield than the open

pollinated varieties. The advantage of confectionery sunflower in comaprison to varieties are crop uniformity, suitability for mechanized harvesting, optimal plant density for achieving the desired size, seed quality and color suitable. It is expected that confectionery hybrids will continue to spread more in production and eventually replace the varieties. One of the most important goals in breeding is creation of resistance or tolerance of hybrids to diseases, broomrape, and droughtand to incorporate herbicide tolerant traits in the adapted confectionery hybrids.

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SUNFLOWER DISEASES RESEARCH PROGRESS AND MANAGEMENT

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ABSTRACT

Sunflower diseases have been and remained a major limiting factor in successful sunflower production in the world. From the history point of view, in last several years new disease agents did not occur, however, new races or pathotypes emerged in some diseases. The exception is *Phomopsis/Diaporthe complex* in which appeared several new species of the genus *Phomopsis*. Broomrape should be added to this, but as it will be discussed in another text, we shall restrict ourselves only to diseases. Many disease agents are present throughout sunflower producing regions and some of them, despite high turnover and migration of seeds in the world remained present in some of them. Their spread is evident just due to seed turnover. Considering all sunflower pathogens, it should be noted that 13 of them are significant to a greater extent for sunflower production, in terms of yield and oil quality reduction, although in the world history of this significant oil crop, much higher number of them have been described. Significant progress has been made in better comparison of certain parasite races in the world and some regions due to new research techniques at molecular level. Good international cooperation in the use of a series of isogenic lines in determination of the intensity of the appearance of certain disease inducing races, above all of downy mildew, rust, *Verticillum* wilting and others has contributed to this. As a new parasite races emerge, pesticides that have remained one of the more significant tools in control of disease agents, in addition to the other control measure evolved resistance. This requires joint effort of researches in plant protection, breeding, and others in the struggle for reduction of losses caused by disease agents. It is, therefore, important to organize periodic surveys that would uncover their exact distribution and harmfulness in certain regions to sunflower crops.

Key words: Sunflower diseases,pathogen identification, host resistance.

INTRODUCTION

Sunflower diseases have been and remained a major limiting factor in successful sunflower production in the world. From the historical point of view, in several last years, new disease agents did not emerge, expect several exceptions. However, "virulent pathotypes", usually called pathogenic races occurred in some diseases. Many disease agents have been present in the whole sunflower production region in the world, and despite great marketing of seeds in the world, they have persisted in some regions. Their spread is evident, mainly due to seed marketing on a global scale (Gulya et al, 1997).

Diseases are far the most important factor in yield reduction, although they emerge in different intensity per years and growing regions. (Chattopadhyay et al, 2015; Artokhin and Ignatova2013;Vear 2016)For example, in 2015 in the U.S, the most important diseases were sunflower rust, *Phomopsis* and *Rhizopus* head rot (Kandel and Gulya, 2016). Contrary to the period 2001 - 2011 with incidences (occurrence) of *Sclerotinia* head rot, followed by sunflower rust and then *Phomopsis* were the most important in relation to incidence and severity in U.S.

***Plasmopara halstedii* (Farlow) Berlese and Toni – Downy Mildew**.The oldest and sometimes the most devastating disease is downy mildew of sunflower (*Plasmoparahalstedii*) for which in 80s of the last century only two races were known(Viranyi, 2008). Since then the exact ″invasion″ of races has occurred, and nowadays in the world over 38 races are recorded. In regard to Europe, the greatest number of new races, 13 was established in the last decade and previously 14. In other countries, such as Italy and Germany, there are 9 new races. In last decade in Hungary, Italy and Romania 7 new races were established. In the U.S. 21 new races have been registered and in Canada 18. As a great sunflower producer Argentina in last decade established the presence of 5 new races (Viranyi et al, 2015).

In the Czech Republic, Trojanova et al. (2013) reported that downy mildew races 700, 704, 710, 714, 730 and 770 were found in the last decade and there are no new reports about races composition earlier and after. In Hungary, new race 704 was found in 2011 and race 714 was found in 2013 (Ban et al,2014).According to Viranyi (2015), race 700 and 730 are predominant.

Pacureanu (2010) reported that 310 and 710 as new races in Romania. Older ones 100, 300, 330 and 730 have been previously described. Among all races 100 and 300 are predominant. Interesting data is that in Turkey changes of the population has not been established after 2007, and since then, 9 downy mildew races have been recorded. In Italy race 704 (Tossi and Beccare, 2007) has been found in last decade and no more new races.

In Serbia races 100, 700, 730 have been found earlier, and in last decade race 730 has become dominant. Based on latest researches, in Serbia, some new races with a race composition similar as in neighboring countries are to be expected. (Masirević, unpublished). This disease has not been established in Australia.

Seed treatment by fungicides and use of resistant genotypes is widely accepted measure against this oomycete. The problem, in this case, is wide genetic variability of pathogens. It seems that winning combination are resistant hybridsand fungicide seed treatment. This measure has the best perspective, and in contemporary conditions, its duration should be the longest until the discovery of some new indicators. After all, this measure is the most used measure in Europe, Russia, Ukraine and the efficacy of this combination has been proven and downy mildew is not such a big problem.

Genetic resistance is most effective and important crop management manner to control the disease. The situation with confectionary sunflower is very bad because no commercial hybrids are yet available with for downy mildew resistance. Recent studies made in the U.S. are encouraging due to finding of some lines for further selection work that is expected to be finished by the beginning of 2017.

Pathogen virulence observed in all of nine differential lines in the U.S. and also on some additional differential candidates with additional resistance genes indicate that that inclusion of supplementarydifferentials is needed. The identification of new lines with resistance to *P. halstedii* is an important action for breeding resistance.

***Phoma macdonaldi* Boerema– Black Spot.** *Phoma macdonaldi* is present in almost all regions of cultivation but the intensity of infection and impact on yield remained on the level from previous years. Kandel and Gulya (2016) reported that *Phoma* black spot has been the most widely observed disease on sunflower across the all U.S. sunflower production area of the central Great Plains. And this was the case until the first occurrence of the disease in 1980. The reason for not so great damages is prescribed to the source of infection which is in America mainly on the stem surface and it does not penetrate deeper into the vascular tissues. The highest damages occurred in cases when *Phoma* is transmitted by seed or when black spots are on the surface level (Maširević 2014).

This fungi is very interesting because it has not been established in the perfect stadium in new regions expect in previously determined localities in Argentina, Former Yugoslavia and the U.S. New in systematics is that now this fungus is named *Plenodomuslindquisti* (Gruyteret.all, 2012). Narrow crop rotation and reduced tillage are highlighted as important factors in spread of the disease in the southwest parts of France (CETIOM, 2012). Many authors reported that the occurrence of black stem is in positive correlation with the increase in nitrogen fertilization (Dedić and Maširević, unpublished).

Although it is an important disease, in France, as well as in the remaining parts of Europe and in the U.S, specific control measures have not been conducted. The epidemic of this disease is possible to reduce and diminish by the disease escape. The significant and efficient way of the control of the disease is increase of resistance toward *Phomamacdonaldi*. Hybrid resistance is efficient, environmentally friendly and fairly inexpensive for sunflower growers. Some agronomic factors and cultivation methods could contribute to the easiest way of control, i.e. reduction of the disease. In the case of heavier infestation, it can be intervened by fungicides in the case of some important selection material, and some larger treatments of commercial crops have lately become rare. Maybe control of *Phomopsis complex* at the same time controls *Phoma,* also reducing the damage caused by this U.S.agent no.1.

***Phomopsis* Brown Stem Canker (*Diaporthe/Phomopsis spp. complex*).**One of the few fungi that are in expansion and a real renaissance in the emergence of new species is *Phomopsis complex* causer ofbrown gray, i.e. sunflower stem canker.

Phomopsis stem canker has become one of the most yield-limiting diseases worldwide on oilseed sunflower (Mathew et al. 2015b) It was described as *Phomopsis helianthi* in 1980, when *D. helianthi*Munt(syn. *Phomopsis helianthi*) was identified as the U.S.agent no.1, first in Yugoslavia, later in Europe and the rest of the world (Marić et al, 1987). This disease became a limiting factor for sunflower production because it not only causes oilseed yield losses but also caused sunflower oil deterioration (Masirević and Gulya, 1992). Several other new species of *Diaporthe*have been identified as stem pathogens of sunflower. Morphologically and symptom-wise they are almost indistinguishable and thus molecular methods are necessary to speciate the U.S.fungus no.1. A total of ten new *Diaporthe*species have thus been added as sunflower pathogens, including *D. Gulya*Shivas, Thompson and Young, *D. Kongii*Shivas, Thompson andYoung, *D. Kochmanii*Shivas, Thompson and Young, *D. Masirevicii*Shivas, Thompson and Tan, *D. Sackstonii*Shivas, Thompson and Tan (Thompson et al, 2015). Of these, *D. gulyae* is the most aggressive, comparable with *D. helianthi*, and the former occurs in Australia, Canada and in the U.S. (Mathew et al, 2015a; Mathew et al, 2015b). No studies have looked into the *Phomopsis*complex on sunflower in other countries.

*Diaporthe/Phomopsishelianthi*has also been recorded in the U.S. and Argentina, but remains exotic to Australia where other newly described *Diaporthe* species are responsible for damaging stem cankers. However in 2010 in Australia, after a period of sustained wet weather, patches of lodged sunflowers were reported from across the growing regions of New South Wales (NSW) and Queensland (Qld).Three new species, *D. gulyae, D. kongii* and *D. kochmanii*, were isolated from infected sunflowers in New South Wales and Queensland.

Thompson et al.(2011) found that some weed hosts are involved in the epidemiology of three species of*D.gulyae,D.kochmanii*and *D. kongii*. During experiments in 2014 and 2015, same author and collaborators found alternative hosts of *Diaporthe* species that can cause sunflower stem canker in Australia. Eight novel species were identified based on GCPSR criteria.The following species have been found: *D. charlesworthii, D.goulteri, D. macintoshii,*

D.middletonii, D. masirevicii, D. miriciaes, D.sackstonii, D. serafiniae(Thompson et all. 2015).

According to latest findings, *Phomopsis/Diaporthegulyae* became the main agent of *Diaporthe* stem canker in the U.S. and Canada. Indeed, this situation leads to a possible conclusion on ways of such mass occurrence of new *Diaporthe* species in Australia, and at the same time some of these species have become more present in America. CatheU.S.l agents of *Phomopsis* in Argentina that occurred in a large scale in this 2016 have not been sufficiently studied (Huget 2016). Researches accomplished in Australia and some other countries clearly showed that weeds and their remaining after vegetation are actually brown bridge for *Diaporthe*species that can be harbor for propagation of pathogenic, saprobic or endophytic.

This is all comparable with green bridge with alternative green plants, above all weeds that enable *Diaporthe* species to survive easier cropping phases.

Sowing of hybrids with high levels of resistance to *Phomopsis* stem canker (tolerant genotypes)is a good way of reducing stem canker damage (Škorić, 2012).The disease has proved prevalent in Europe and seed companies have incorporated resistance in some hybrids. Some seed companies rate their hybrids for *Phomopsis* resistance. In regions where the disease has been endemic for 20 years, such as Yugoslavia now Serbia (Masirević and Forgić, 2000), in the cases of heavy infections, an erosion of tolerance was observed, but despite this, farmers in areas with a history of the disease should consider the tolerant. In Australia trials studying hybrids of varying maturities comparing quicker and later maturing hybrids showed that plants with the earlier maturing hybrids developed more damaging lesions and lodged more readily than plants with later maturities.

Lower nitrogen fertilization will in turn minimize the possibility of *Phomopsis* infection (Debaekeet et al, 2003). While *D. helianthi* and other sunflower pathogens were thought to be host specific, recent evidence shows they can infect and survive on weeds and other hosts, both on live and dead plants (Thompson, 2015). Rotation will thus be of limited use, but eliminating weeds and wild sunflowers from the field and margins will potentially cut down on inoculum. Burying plant residue, at least 5 cm deep, will fasten plant decomposition and expose *Diaporthe*to biodegradation.Resistance is widely available in oilseed germplasm.

Winter cereals, sorghum and maize are consideredthe least likely hosts, although Thompson et al. (2015) report four species of *Diaporthe*, including the damaging *D. gulyae*, from asymptomatic maize suggesting that endophytic infection may also play a role in survival of *Diaporthe spp*. in cropping situations (Thompson et al, 2011; Thompson et al, 2015).Complete burial of infected plant residue, at least 5 cm deep, is most thoroughly accomplished by plowing and not disking. This ″strategic tillage″ need only be done after an infected crop. In Europe, it has been found that stalks need to be buried deeper than 15 cm to prevent perithecial maturation. In Australia, early stubble burial trials suggest shallow burial of 5-10 cm leads to rapid stubble breakdown perhaps because of the sustained warmer temperatures in that.

Delayed sowing significantly reduces leaf and stem infection and disease severity. The strategy of delayed sowing is needed to synchronize the most susceptible periods (bud formation) with low-rainfall periods.

Weed control -Thus, eliminating weeds and volunteer sunflowers, from field margins as well as sunflower fields will reduce inoculums (Thompson et al, 2015). However, due to lack of detailed information on the weed host range of *Phomopsis* species, a precaution in weed control is necessary for the purpose of limiting the disease build-up on possible weed hosts. Damaging *D. gulyae* has been isolated from multiple weed hosts in Australia including live Noogoora Burr (*Xanthium pungens*), Bathurst Burr (*X. spinosum*), Saffron Thistle (*Carthamus lanatus*) and Common or Sow Thistle (*Sonchus oleraceus*).

Therefore, creating conditions less favorable to the expression of the disease and damage by the pathogen through reduced stem density, and N-fertilization, increased row width, efficient weed control, reduction in water supply and non-limiting K-nutrition is the necessity. Excessive N supply induces opposite effects on the proportion of infected stems. So a combination of strategic trash burial and rotation will be the most effective strategy in limiting the soil inoculum.

Systemic strobilurin compounds are likely to be most effective for control combinations of preventive and curative fungicide produce better control. The best disease control is achieved with fungicides on the base of benomyl applied before symptoms occurrence. However some recommended benzidamidazoles have not been registered in the U.S, and should be applied twice, the first time at the beginning of budding, when the crop is no more than 55-60 cm high on average stage and the second at flowering stage (Deaeke and Estragnat, 2003). Later times treatments, or after the occurrence of symptoms are inefficient. Forecasting systems proved to be very important, for effective and economic fungicide control depends on it. These systems include spore traps and weather monitoring to advice grower when it is necessary to apply fungicides. In regions where *Phomopsis* is normally present, a fungicide treatment at the critical plant height for conventional sprayers (50-70 cm) or following the advice of the official Plant Protection Service is generally recommended even when using tolerant genotypes.

Sclerotinia spp. **–Stalk Rot, Head Rot, Wilt and White.** *Sclerotinia sclerotiorum* (Libert) de Bary (1884); Syn.: *Whetzelinia scleotiorum* (Lib.) Korf & Dumont (1972), *Sclerotinia minör* Jagger; Syn. S. *intremedia* Ramsey (1924) (Kohn, 1979).

S. sclerotiorum is global in distribution on various hosts' climates and is found in everysunflower producing country.Most frequently it can be found in temperate climates.

In the U.S. the pathogen is observed most frequently in northern growing areas. *S. minor* is more frequent in hot, dry climates, and is observed on sunflowers in Argentina, Australia, the Indian subcontinent and EU countries bordering the Mediterranean and Black seas. In the U.S. *S. minor* is reported on sunflower only in California and Texas, and is one-six as prevalent as *S. Sclerotiorum* (Gulya et al, 2012).

Sclerotinia-incited stem and head rots are the leading cause of yield losses to sunflower worldwide. Their impact is not only upon the existing crop, but the contamination of soil with long-lived *Sclerotia*, and their wide host range.

Diseases caused by either of the *Sclerotinia* species are very difficult to control, and the best management is aimed at prevention.

Since infected plants can spread *Sclerotina* via root-to-root contact, lower plant densities will minimize wilt incidence (Nelson et al, 1989). To minimize head infection, anything which decreases foliage, increases air movement, including lower nitrogen fertilization, lower plant densities. Many different biocontrol/cultural practices have been shown to be partially effective at decreasing soil populations of *Sclerotia* and thus minimizing both root and head infections.

Deep plowing, with a moldboard plow to completely invert the soil profile to > 15 cm, will bury *Sclerotia* into an aerobic environment where microbes should degrade the *Sclerotia* (Mueller et al, 2002). However, there is conflicting data on this practice, and most experimental trials suggest that shallow burial leads to faster degradation of *Sclerotia* (Cosić et al, 2012; Subbaroa et al, 1996). Planting dense cover crops of cereal grains will produce them microenvironment conducive to the apothecial formation, and if this crop is tilled under just prior to a susceptible crop, the net effect is to sap the *Sclerotia* of energy/biomass sufficient to produce more apothecia (Mueller et al, 2002).

Crop rotation is of minimal use, since there are so many susceptible crops, but if several years of a non-host monocot (grasses or cereals) are planted, this also would hasten lowering

the sclerotial soil population (Mueller et al, 2002). Once eitherstem rot or head rot are observed, there are no curative measures to save those plants. The best option at that point isto removephysically affected plants and dispose of them away from the field, thus minimizing soil contamination with more *Sclerotia*.

Many commercial biocontrol products are available, based on fungi such as *Coniothyrium, Gliocladium*, and *Trichoderma*, and bacteria such as *Bacillus*, and these applied as soil drenches immediately following a *Sclerotinia* infestation will hasten degradation of this fungi and shorten the interval between planting another susceptible crop (Radujkov et al, 2015).

Biofumigation by planting *Brassica* cover crops, and tilling them under will release isothiocyanates, which are toxic to a range of fungi (Griffiths et al, 2011).

In certain sunflower hybrids, some progress has been made in developing partial (incomplete) resistance or tolerance to *S. sclerotiorum*. There are no hybrids available with complete resistance to either of the two *Sclerotinia* spp.

***Puccinia helianthi* Schwein.–Rust.**Sunflower rust is found worldwide on oilseed, and wild sunflowers (*Helianthus*). During 2013 and 2014 in China sunflower rust was established in many production regions. Using the set of nine international differentials 15 races has been identified. Race 304 was the most prevalent. More virulent races group 700 were less present (Guo et al, 2016).

Since wild sunflower is also a host for rust, removal of any wild sunflower in the vicinity will minimize the production of inoculum. In the future, the problem of rust would probably be greater due to the growth of confectionary sunflower, more susceptible to rust agents and a higher number of races. In Europe situation is under control, for there are not many races established in the U.S. and Canada, and therefore, quarantine measures should be especially followed (Acevedo et al, 2011).

As with many other diseases, genetic resistance is available within oilseed germplasm and this has been obtained from many different annual and perennial *Helianthus* species (Gulya and Brothers 2000). Resistance is specific in order that resistance iseffective. Breeders must know which races are present in the area of interest and use the appropriate single, dominant genes (Gulya and Markell, 2009).Growing a rust resistant hybrid is an important management tool. However, new races of the pathogen can develop and overcome genetic resistance. Wild sunflowers can be ″rust reservoirs″ and provide spores to infect neighboring fields of cultivated sunflower. The presence of early spore stages on wild sunflower, allows more infection cycles to take place. This creates a greater yield loss potential. In addition, sexual recombination occurs when the pathogen completes its life cycle, which enables new races to overcome available resistance. Therefore, removal of wild sunflower populations around fields is strongly recommended. Also, the remaining of infected sunflower by rust must be deeply ploughed and in such a manner destroy inoculum for the following crop, and especially if it is confectionary sunflower.

Fungicides are the main tool for managing rust. Many newer systemic fungicides are effective as both protectants and eradicants against rust (Friskop et al, 2014; Friskop et al, 2015). The timing of fungicide applications is of more impact than the type of fungicide used (Mueller et al, 2004). It is known that products Tebuconazole, Pyraclostrobin and Azoxystrobin, will reduce rust. a fungicide application is most likely economical when average disease severity reaches 1 % on the upper four, fully expanded leaves prior to during bloom. Later applications have not been proven to impact yield positively. High clearance tractors could be used in the control of many diseases that can be combined with rust control (Markell, 2016).

Alternaria spp.– **Leaf Spot and Blight.** Alternaria leaf blight affects sunflower in many regions of the world. When looking at the whole sunflower growing region it is one of themajor defoliating pathogens in warm, humid climates. There are no reports on the occurrence of new species of Alternaria genus. It means that damages and the intensity of the phenomena are at a level of preceding years and previously published papers.

Symptoms of many *Alternaria* species are similar; making field identification almost impossible.Conidial morphology combined with the genetic analysis is the only sure means to delineate species.

Alternaria helianthi is the primary U.S.agent no.1 and most widespread, also there are eight other species reported on sunflower. Most of them are specific for sunflower (Allen et al, 1983a, 1983b, 1983c; Wu and Wu, 2003).

Removal of wild and volunteer sunflowers, removal or incorporation in the soil of previous sunflower residues, will all reduce disease.

Most growers rely on multiple applications of fungicides containing chlorothalonil, iprodione, porcymidone, or vinclozolin. In the world, protection is carried out according to need in selection material and in cases of extremely convenient weather conditions for disease occurrence. Disease resistance has been found.

Taking into account tendency of soil tillage, i.e. no tillage or minimum tillage, it leads to the fact that many parasites remain on the surface as dry - desiccated or in infected plant residues. They are truly brown bridges for many sunflower parasites. This primarily refers to Argentina, the U.S, part of France, Australia and many other countries. Increased problems in sunflower arise just in such situations. Deep tillage and incorporation of plant residues into deeper layers, and not leaving them on the soil surface eliminates infection pressure of many parasites. In this process, it is most important for *Diaporthe complex*, *Scletotinia*,*Phoma*, *Plasmopara* and some others.

Crop rotation as a system of a combat with diseases does not have such significance if no tillage technology, i.e. crop cultivation is performed.

Weed control - The same situation is with weeds that should be controlled for they are reservoirs for many disease agents including viruses, and therefore, they are to be eliminated from sunflower crops. Wild sunflower plants should be added to this, for wild plants are in fact weedy plants for all cultivated crops, and should be eliminated and destroyed as they often serve to parasites for crossing over and as one kind of a green bridge.

Use of tractors and sprayers with high clearance for other agricultural cultures enables their application for treatment of sunflower crops against diseases. This is a way to obtain particularly high yields, especially in confectionary sunflower type that has a higher price and can serve as a place for propagation of certain parasites because it is generally less resistant to many parasites.

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BROOMRAPE (*OROBANCHE CUMANA* WALLR.) IN SUNFLOWER – UPDATE ON RACIAL COMPOSITION AND DISTRIBUTION, HOST RESISTANCE AND MANAGEMENT

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ABSTRACT

The parasitic plant *Orobanche cumana* Wallr. is the most important biotic constraint to the production of sunflower crop, in all countries where sunflower is grown, except North and South America. The parasitism of *O. cumana* on sunflower dates back to the first half of 19th century in Russia, expanding to Moldova and Romania by the beginning of 20th century and later in others like Turkey, Spain, Serbia, Bulgaria and Ukraine. Currently, *O.cumana* is present in all countries in Southern Europe and areas around Black See, as well as in many countries in Asia. The first mention to different races within *O. cumana* dates back to the beginning of 20th century, in Russia, breeders developing sunflower varieties genetically resistant to races A and B. Later (1970-1980) in Romania it have been identified other three races: C,D,E as well as a set of sunflower genotypes, each of them carrying one single major gene of resistance. Races A to E were effectively controlled through genetic resistance for some decades, until race F have been identified. Along the last decades, a new break of the genetic control of *O. cumana* has occurred in Turkey, Romania, Bulgaria, Russia, Spain, as a consequence of the crop intensification and short crop rotation, together with the use of genetic material from foreign breeding programs. There have been identified new highly virulent races, as G, H or more. Because in the last years, in China sunflower was growing very much as an oil crop, broomrape parasite has developed new races. Important efforts of breeders have been devoted to the search of effective resistance against the increasingly virulent parasite populations and, as a result, resistant genotypes have been released. In each country, sunflower material identified as resistant to *O. cumana* has been used to differentiate local races of increasingly virulence and termed from A to E, or F, or G. Because no comparative studies have been conducted to test the correspondence of races among the countries, there is not knowledge of the pathogenic traits of the parasite. In spite that races A to G have been identified in many countries, few works asses the similarity of those populations from different geographic origin and characterized as belonging to the same race. Due to the diversity of *O. cumana* races identified worldwide, the use of the coded triplets as a simple and global method to internationally determine the races of the parasite seems to be imperative at this moment. Among the genotypes identified as differentials for races A to E, other inbred lines were identified by scientists as having a clearly resistance against the broomrape populations in different countries. Selection for sunflower resistance to broomrape started in the early 1910s through individual selection method, using open pollinated varieties. Extensive research was conducted by Vranceanu in Romania (1976 – 1980), being identified the dominant genes *Or1* to *Or5*. he appearance of new races considerably reduced the available sources of resistance of cultivated sunflower. A high level of resistance was found in wild *Helianthus* spp., mainly in perennial ones. The genetic resistance to *O. cumana* is more complex than previously thought, in addition to the known major dominant genes, other minor genes being identified, in different sunflower genotypes. At the start of program, breeder must determine which races are present in the region for which the hybrids are developing. The alternative breeding strategies are required to increase the durability of genetic resistance to *O. cumana*. These strategies will require QTL analysis and developing of molecular markers

linked to major and minor resistance genes, to ensure that they are simultaneously introgressed during backcross and a detailed characterization of the physiological mechanisms underlying genetic resistance. Broomrape can also managed by development of IMI resistant hybrids or by using biological control. Efforts should be made to biochemical parameters (mechanical barriers, germination inhibitors, phytoalexins, etc.).

Key words: *sunflower, broomrape, races, resistance, management.*

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr. – *Orobanche cernua* Loelf.) is an angiosperm that parasitizes sunflower roots and causes economic damage to sunflower production on a worldwide scale. The parasitism of broomrape on sunflower dates back to the first half of the 19th century in Russia. It was first observed in Voronezh region in the 1890s (Satiperov, 1913). Thereafter, the geographical spread of this parasite in the world followed the same pattern with some decades delay, as the expansion of the crop of sunflower in time and over different countries.

Teryokhin (1976, 1992) refers to *Orobanche cumana* Wallr. in Odessa region, as well as to *Orobanche cernua* Loefl. in Krasnodar. There have been disputed aspects regarding the distinction between *O. cumana* and *O. cernua*. Some authors consider these two forms, as variants of *O. cernua* or synonymous, some others are separated them in two distinct species. Using RAPD markers, Joel et al. (1996) have showed that *O. cumana* is an autonom taxonomic unit, closely to *O. cernua*. In present there is a consensus in considering *Orobanche cumana* as sunflower broomrape.

From Russia, *O.cumana* significantly spread all over the crop in the former USSR and expanded to Romania, Bulgaria, Turkey, Serbia (Iliescu, 1984; Acimovic, 1988 a; Bulbul, 1991). Particularly interesting is the occurrence of infections by broomrape in Spain. This specie was identified on confectionery sunflower, in Toledo province, in 1958 (Diaz-Celayeta, 1974), spreading in Cuenca and Malaga (Gonzales-Torres et al., 1980). Later, broomrape has spread on oil sunflower, specially in Andaluzia (Gonzales – Carrascoza, 1992; Melero – Vara et al., 1996). Outside of Europe, *O. cumana* was identified in China, in Jilin province (Tingrui et al., 1996).

Currently, *O. cumana* is present in all the countries of Southern Europe and areas around the Black Sea where sunflowers are grown (Antonova, 2014; Batchvarova, 2014; Duca, 2014; Hargitay, 2014; Jestin *et al*., 2014; Kaya, 2014; Miladinovic *et al*., 2014; Molinero-Ruiz & Dominguez, 2014; Pacureanu, 2014; Pototskyi, 2014), as well as in North Africa (Amri *et al*., 2012), Israel (Eizenberg *et al*., 2004) and China (Baichun *et al*., 1996; Ma & Jan, 2014; Shi *et al*., 2015). The objective of this paper was to make an overview of what has been achieved in knowledge referring to sunflower broomrape race structure and distribution, host genetic of resistance and management of the parasite control.

Broomrape (*Orobanche cumana*) contact with sunflower plants

Broomrape (*Orobanche cumana*) seed is very small and 1000 seeds weight is most often 0.001 grams. The seeds can survive in soil for up to 20 years. *Orobanche cumana* infects the roots of sunflower early in the growing season, obtaining water and inorganic compounds from the host plant through xylem to xylem contact (Heide – Jorgensen, 2008). Broomrape

seeds germination takes place in wet ground at 20-25^{0}C temperatures . It is affected by pH value of the soil as well as by the excretion of the host roots (germination stimulators).

Pancenko (1975) gave a detailed description of germination of broomrape seeds, the mechanism of penetration of the haustorium of susceptible genotypes of sunflower, as well as the process of dying of the haustorium in the roots of the resistant genotypes of sunflower. Haustorium penetrates the skin and parenchyma of the sunflower roots, all the way to the central cambium cylinder, only in the susceptible genotypes. In the resistant genotypes there is a layer of lignin between parenchyma and cambium which does not allow the penetration of haustoria of broomrape (Pancenko and Antonova, 1975; Antonova, 1978).

The utilization of host photoassimilates by the parasite results in depletion of resources which are necessary for the growth of sunflower and for the optimal development of the seeds. Broomrape stems have a long underground development stage, emerging aboveground around flowering of sunflower (Melero – Vara and Alonso, 1988). By the time of broomrape emergence, most of metabolic imbalance is already produced by the parasite to sunflower (Molinero – Ruiz et al., 2015). From the first emergence of broomrape stems on, the impact of broomrape in the yield of the crop is increasingly through absent or small sized heads, low number and small seeds, even death of the plants. Yield reduction due to the infestation with broomrape depends on level of soil infestation, aggressiveness of the parasite, sunflower genotype, earliness of broomrape emergence and soil depth, among others (Jestin, 2014; Molinero – Ruiz et al., 2015).

Race structure of *Orobanche cumana*

At the beginning of 20th century, broomrape spread across Russia significantly and endangered the mass production of sunflower. Efforts of soviet breeders at the Saratov experimental station resulted in the release of genetically resistant varieties of sunflower The first cultivar resistant to race A, Saratovski 169, was created by Placek (1918). In the years that followed, other cultivars resistant to race A were also produced (Kruglik A- 41; Zelenka and Fuksinka). As the mass production of sunflower spread quickly, it was followed by a new race, called B (Zhdanov, 1926). The race B was spread in Rostov and Krasnodar regions (Antonova, 2014). During 1925-1960, Pustovoit created in Russia, highly productive cultivars, resistant to race B.

After the cultivars resistant to race B, for years, nobody mentioned the occurrence of new races, even the composition of races had changed. The existing cultivars were assumed to have possessed several genes for resistance to broomrape (races C and D). Petrov (1970) announced the existence of a new race in Bulgaria (race C). At the same time, its existence was announced in Romania. Vranceanu et al. (1980) added significantly to the examination of broomrape races and helped to detect dominant genes by establishing the existence of five races which were controlled by dominant genes: A(Or1), B(Or2), C(Or3), D(Or4) and E(Or5). Pacureanu – Joita et al. (1998) announced the existence of race F (Or6).

Along the last decades a new break of the genetic control of *O. cumana* has occurred in the countries situated in Black See region, as a consequence of the crop intensification and short crop rotations, together with the use of genetic material from foreign breeding programs and therefore susceptible to the local ecotypes of the parasite. Current facts indicate that there are at least 7-8 (A to G and H) broomrape races in Turkey, Romania, Russia, Ukraine, Bulgaria and Moldova (Kaya, 2004, 2009 and 2014; Pacureanu-Joita et al., 2009 and 2012; Pacureanu-Joita, 2014; Goncharov, 2009; Antonova et al., 2009; Antonova, 2014; Pototsky, 2014; Batchvarova, 2014; Gasca et al., 2013; Duca, 2014).

Genetic variability and dynamics of changes occurring in the race composition of broomrape, in Spain, which so far has been dominated by races E and F, have been studied by Alonso et al. (1996), Dominguez et al. (1999), Sukno et al. (1999), Rodriguez-Ojeda et al. (2001), Akhtouch et al. (2002), Perez-Vich et al. (2002; 2004), Molinero-Ruiz and Melero-Vara (2005), Velasco et al. (2007), Fernandez-Martinez et al. (2009 and 2012), Molinero – Ruiz and Dominguez, 2014.

The first report of *O. cumana* in Serbia dates back to the 1950's; the populations detected were probably race B, because they were controlled by Russian oil varieties and hybrids with resistance to this race (in Miladinovic *et al.*, 2014). Economically important incidences of broomrape on sunflower were again observed in the country in the 1990's and identified as a race E (Mihaljčević, 1996 ;Skoric and Jocic, 2005). During the last 20 years, and although the parasite has spread to new sunflower production areas in the country, new races have not occurred so far (Miladinovic *et al.*, 2014).

In China, *Orobanche cumana* has been present for a long time but, the identification of race A has been done in 1990s (Baichun et al. 1996). A widespread increase in new virulent parasite races has occurred since then, so that the wide distribution of races A to F in the country, as well as the identification of race G in Inner Mongolia have been recently reported (Ma & Jan, 2014; Shi *et al.*, 2015).
Concerning other sunflower growing countries, in Hungary, populations of *O. cumana* were characterised as low to moderately virulent (races A–D) (Zoltán, 2001). Currently, race E is the most frequent one in the country (Hargitay, 2014) although race F has been also identified (Molinero-Ruiz *et al.*, 2014).).

The presence of *O. cumana* parasitizing sunflower in France was not reported until 2007 (Jestin, 2012). It is currently located mainly in the South and also in the West of the country (Jestin *et al.*, 2014), although information about race/s is not available. Because no comparative studies have been conducted to test the correspondence of races among countries, an unbundled knowledge of pathogenic traits of the parasite is currently occurring. In spite that races A to G were or have recently been identified in many countries, very few works assess the similarity of those populations from different geographic origin and characterized as belonging to the same race (Molinero-Ruiz *et al.*, 2014).

When determining races of crop pathogens, pathotype information frequently surpasses a certain level of complexity. When this happens, mathematical codes are, by far, advantageous over the use of consecutive numbers or letters given in chronological order of pathotypes discovery. To ease communication and comparisons of results about characterization of races, a universal adoption of the coded triplets system is frequent for many plant pathogens (Liebenberg & Pretorius, 2011; Gurung *et al.*, 2013; Dreiseitl, 2014) including *Plasmopara halstedii* Farl. Berl. & de Toni, which causes sunflower downy mildew. Molinero – Ruiz et al. (2015) proposed the characterization and nomenclature of *Orobanche cumana* populations, using the coded triplets system, which is based on the use of eight sunflower differentials, grouped into three sets (table 1). In table 2, it is presented the characterization of *Orobanche cumana* populations, using the coded triplets system and its correspondence with the method based on the use of capital letters, given in chronological order of races identification (Molinero – Ruiz et al., 2015). The use of the group of differentials presented in table 1, worldwide, will facilitate comparisons of results about characterization of broomrape races in different countries. The most virulent races of the parasite and their location will be known by scientists working on the achievement of sunflower material with resistance to *Orobanche cumana* in any of the countries where the parasite occurs.

Genetic studies of the parasite are extremely important in present, in order to bring knowledge on the parasite at the level of knowledge on the genetics of resistance in sunflower. Studies of the inheritance of resistance to *Orobanche cumana* have confirmed the gene for gene interaction in the parasite – host system for races E-F, but the inheritance of genes from other sources of resistance to the most virulent races of this parasite is still unknown.

Sources of resistance to *Orobanche cumana*

Genes for resistance to broomrape races A, B, C, D and E are present in varietal populations of sunflower developed in breeding programs from Saratov (former USSR), Krasnodar (former USSR), Odessa (Ukraine), Fundulea (Romania) and several other places. Some of these genes have been identified in certain wild species of the genus *Helianthus* and have been incorporated into cultivated sunflower genotypes by interspecific hybridization. A species of wild sunflower (*Helianthus tuberosus*) was first used as a source of *Orobanche* resistance (Vrânceanu et al., 1980; Škorić et al., 2010) as the donor of *Or* genes.

As new broomrape races were appearing, sources of resistance in cultivated sunflower became increasingly scarce. In Turkey, Gulya et al. (1994) found only 22 resistant entries in a field evaluation of 903 accessions, whereas in Spain, Domínguez et al. (1996) found 8 resistant and 33 segregating entries in the evaluation of 429 accessions of different origins for resistance to race E. Sources of resistance to the latest races have been scarce in germplasm of cultivated sunflower, although valuable resistant germplasm has been identified in breeding programs conducted in Spain (Fernández-Martínez et al., 2004; Rodríguez-Ojeda et al., 2001), Romania (Pacureanu-Joita et al., 2004, 2009b), Turkey (Kaya et al., 2009), and Russia (Gontcharov et al., 2004; Gontcharov, 2009). In most cases, the germplasm exhibited vertical or qualitative genetic resistance. In addition, genetic sources of horizontal or quantitative genetic resistance have been developed (Pérez-Vich et al., 2005). In contrast, a high level of resistance to the newest races has been found in wild *Helianthus* species. Fernández-Martínez et al. (2000) tested for race F resistance 54 wild sunflower accessions (representing 27 perennial and four annual species) and 55 cultivated sunflower accessions. Most of the perennial species proved fully resistant to this race. The only exceptions were some populations of four of the wild perennials, which had a certain percentage of susceptible plants. Among the wild annual species, *H. anomalus* and *H. agrestis* were completely resistant, while *H. debilis* ssp. *cucumerifolius* and *H. exilis* segregated with regard to *Orobanche* resistance. Jan and Fernandez-Martinez (2002) employed interspecific hybridization to incorporate genes for resistance to race F from several wild species into cultivated sunflower, and developed four populations (BR1-BR4) resistant to this race from the wild sunflower species *H. maximilianii* Schrad, *H. grosseserratus* Mart., and *H. divaricatus* L (Jan et al., 2002). Where necessary, they used embryo culture and chromosomal doubling by colchicine in order to bypass the barriers and enable the transfer of desirable genes. Christov et al. (1992, 1998, 2009) have achieved outstanding results in identifying genes for broomrape resistance in the wild species of the genus *Helianthus* and incorporating them into cultivated sunflower genotypes. Especially important are the findings reported in Christov et al. (2009), which concern the detection of *Or* genes in 11 perennial wild sunflower species and their incorporation into elite cultivated sunflower lines by means of interspecific hybridization. Hladni et al. (2009) developed five new restorer lines, to race E, from interspecific populations originating from *H. deserticola*. The resistance to a race classified as G has been transferred from *H. debilis* into cultivated sunflower by Velasco et al. (2012).

Sources of *Orobanche* resistance can also be found by the use of induced mutations. Venkov and Shindrova (1998) reported that they obtained a mutant with partial resistance to *O. cumana* using a 0.4% solution of the mutagen nitrosumethylurea.

Genetic of sunflower resistance to *Orobanche cumana*

In parallel with the appearance of new broomrape races and sources of broomrape resistance, the genetics of resistance to this parasitic plant has been studied. As sources of resistance to races A and B were identified, it was also determined that resistance to broomrape was controlled by dominant genes. Burlov and Kostyuk (1976) and Pogorletsky and Geshele (1976) studied the genetic basis of *Orobanche* resistance and discovered that it was controlled by a single dominant gene, which they named *Or*. Vrânceanu et al. (1980) conducted extensive genetic research as part of his study of broomrape in Romania from 1976 to 1980. They established that there were five pathogenic races of this parasite and labeled them A, B, C, D, and E. They also identified a set of differential lines that had cumulative resistance to the five successive races, conferred by the dominant genes Or_1, Or_2, Or_3, Or_4, and Or_5, respectively. When race F subsequently appeared in Romania and resistance to it was discovered in the line LC-1093 (Or_6) by Pacureanu-Joita et al. (1998), this cycle of genetic research was completed.

In some particular cases, the resistance seems to be controlled by a complex of genes. So, Pustovoit (1966) and Paleev (1983) refers to an intermediary inheritance of F1 hybrids. Krohin (1980) concluded that the inbred line 6540 – 1M has a resistance for the race B of the parasite, controlled by two complementary genes. The same results obtained Hatnianskii (1982) and Ciriaev (1987) for some lines selected from Peredovik variety.

The appearance of new broomrape races in Spain triggered a new cycle of large-scale genetic analyses. Dominquez et al. (1996) noted that there is a low frequency of genes for resistance to race E in cultivated sunflower and that this resistance is controlled by two dominant genes. Alonso (1998) noted that, the known dominant genes notwithstanding, resistance to *Orobanche* may be more complex than previously thought and that genes other than single dominant ones may also be involved. In some cases involving cultivated sunflower germplasm, resistance to race F is controlled by recessive genes. Thus, *Orobanche* resistance found in the lines P-96 and KI-534 is controlled by recessive alleles at two loci (Rodrigez-Ojeda et al., 2001; Akhtouch et al., 2002). The same recessive genes control resistance to race E in the line KI-534 (Rodrigez-Ojeda et al., 2001). Akhtouch et al. (2002) crossed lines resistant to race F with those that are susceptible to it and found segregation ratios of 1:15 [Resistant (R): Susceptible (S)] and 1:3 (R : S) in the F_2, and BC_1 generations, which in most cases indicates double dominant epistasis. Cases of segregation ratios of 3:13 (R : S) and 1:1 (R : S) were also recorded in the F_2s and BC_1s, which is indicative of dominant-recessive epistasis. Velasco et al. (2007) crossed a line resistant to race F (J1) with three susceptible lines and studied the inheritance of face F resistance, obtaining segregation ratios of 3:1, 13:3, and 15:1 (R + Moderately R : S) in the F_2, generations. These results indicated incomplete dominance of the Or_6 alleles and the presence of a second gene, Or_7, whose expression was influenced by the environment. Pacureanu-Joita et al. (2008) tested the latest, virulent race of broomrape from Romania through a cross between the resistant line AO-548 and the susceptible line AD-66 and segregation ratios of 15:1 (R:S) and 3:1 (R:S) were observed in the F_2 and BC_1 generations, respectively, indicating that the resistance in AO-548 is controlled by two independent dominant genes. In Spain, a single dominant gene controlling resistance to the most virulent race G in lines derived from interspecific crosses with *Helianthus debilis* subsp. *tardiflorus* has been reported by Velasco et al. (2012).

Most of the molecular research for characterizing broomrape resistance has been focused on mapping the *Or5* gene conferring resistance to races A to E. This gene has been mapped to a terminal, probably telomeric region of linkage group (LG) 3 of the sunflower genetic map (Lu et al., 2000; Pérez-Vich et al., 2004; Tang et al., 2003). The closest marker was identified at around 6 centimorgan downstream of *Or5* (Lu et al., 2000; Tang et al., 2003), but no flanking markers were found in the upper part of the LG. Márquez-Lema et al. (2008) identified a telomere-associated target region amplification polymorphism (TRAP) marker linked to *Or5*, probably flanking the gene in the upper telomeric side. In addition to the major role of *Or5* in race E resistance, Pérez-Vich et al. (2004) also identified a quantitative component of the race E resistance determined by four quantitative trait loci (QTL) with minor effect associated with the number of broomrape shoots per plant. Imerovski et al. (2013) demonstrated that simple sequence repeat (SSR) markers of LG 3 were also strongly associated with resistance genes *Or2*, *Or4*, and *Or6*. For race F resistance, QTL analysis in a population derived from line P-96, for which phenotypic analysis suggested the presence of two recessive loci (Akhtouch et al., 2002), revealed the presence of six QTL with small to moderate effects on reducing the number of broomrape shoots per plant, three of them being non-race specific (Pérez-Vich et al., 2004). More recently, Louarn et al. (2014) identified four QTL for broomrape (Spanish race F) resistance mechanisms in a population derived from LR1line selected from (*H. debilis* x *H. annuuss*). These results suggest that sunflower resistance to broomrape is controlled by a combination of qualitative, race-specific resistance effecting the presence or absence of broomrape and quantitative, non-race-specific resistance affecting the number of broomrape stalks per plant.

Mechanisms of sunflower resistance to broomrape

It is very important to know all the mechanisms involved in broomrape resistance (physiological, biochemical, mechanical, etc.). Getting information on the physiological basis of different sources of resistance will have physiological based breeding and resistance genes pyramiding, underlying different resistance mechanisms (Perez-Vich et al., 2013). The resistance mechanisms have been studied for a long time. Thus, Morozov (1947) cites the results of Richter (1924) that indicated that broomrape susceptible sunflowers had root systems with a low pH, and those of Suhorukov (1930) concerning the link between peroxidase values and sunflower susceptibility to broomrape, according to which increased soil acidity increased peroxidase activity and the susceptibility of sunflower plants to *Orobanche*. According to Morozov (1947), Barcinskiy (1932, 1935) reported that sunflower root cells contain substances that stimulate the germination and development of broomrape seeds and seedlings. Long after that, Wegmann (1998), Alonso (1998), Matusova et al. (2004), and Honiges et al. (2009) also pointed out the importance of broomrape germination stimulants. Joel et al. (2011) identified the natural broomrape germination stimulant from sunflower roots exudates as a dehydrocostus lactone. Low exudation of germination stimulants by sunflower roots has been described as a preattachment resistance mechanism (Labrousse et al., 2001). Another preattachment resistance mechanism is the exudation by sunflower roots of seed germination inhibitors and/or inhibitors of radicle exoenzymes (Höniges et al., 2008). Phytoalexins, in particular 7-hydroxilated simple coumarins, have been suggested to play a defensive role by preventing broomrape germination and subsequent connection with sunflower roots (Serghini et al., 2001).

Mechanical barriers like lignifications of the cell wall by peroxidase-catalyzed reactions have been proposed as postattachemt resistence mechanisms (Höniges et al., 2008). Panchenko and Antonova (1975) concluded that the protective response of different sunflower cultivars came down to the accumulation of lignin and its precompounds in injured host cells, resulting in the

haustoria losing the ability to supply themselves with water and nutrients from the host cells. Also, a physical barrier by reinforcement of the host cell walls through suberization and protein cross-linking that prevents parasite intrusion has been described in sunflower genotypes resistant to race F (Echevarría-Zomeño et al. 2006). This mechanism was also observed for race E, but in this case cell wall was reinforced by means of callose depositions (Letousey et al., 2007).

Some of the previously mentioned studies have revealed the simultaneous occurrence of several resistance mechanisms in genotypes exhibiting complete resistance (Echevarría-Zomeño et al., 2006; Labrousse et al., 2001; Letousey et al., 2007). Labrousse et al. (2000, 2001, 2004) discuss different criteria for assessing *Orobanche* resistance and the different mechanisms by which such resistance operates. The authors were able to distinguish between three types of broomrape resistance in their work: a) resistance acting at an early stage in broomrape development (*H. debilis* ssp. *debilis*), when broomrape seedlings were present on the sunflower root, but an impassable encapsulation layer blocked the intruding parasite, which then died; b) resistance found in the resistant line LR1, which involves two types of action: decreased stimulation of broomrape germination (a three-fold reduction compared to susceptible line 2603); and rapid necrosis that appeared as early as stage 2 of parasite development; c) resistance observed at a later stage of broomrape development in the line 92B6 (necrosis developing prior to broomrape flowering). Louarn et al. (2012) found that arbuscular mycorrhizal fungi could produce inhibitors of *O. cumana* germination, and that this inhibitory effect seemed restricted to broomrape seeds.

Sunflower breeding for resistance to *Orobanche cumana*

Methods used for evaluating broomrape resistance

Sunflower breeders must develop a breeding strategy, decide on a breeding method, secure the necessary germplasm and differential lines for broomrape race identification, and choose the appropriate inoculation method. In the years in which races A through E were discovered, sunflower breeders tested their breeding materials in naturally infested fields, usually on plots that had been severely infested by broomrape the year before. This method is still employed by some breeders. However, this approach does not always produce reliable results due to the influence of environmental factors and an inadequate amount of broomrape seeds in the soil. In an effort to avoid this, breeders resorted to collecting broomrape seeds and to carrying out artificial infestation in the field experiments, either by incorporating the seed into the soil using basic tillage (Vrânceanu et al., 1980) or by inoculating individual plants in small pots to be transplanted into the field after 2-3 weeks in the growth chamber (Velasco et al., 2007). However, this method is prone to producing experimental errors too, caused primarily by the effects of environmental factors. Much more accurate results can be obtained by putting broomrape seeds into containers filled with a pre-prepared soil medium which are then placed in a controlled environment (growth chamber or greenhouse). Panchenko (1975) developed a screening method for assessing resistance to broomrape in greenhouse conditions during autumn and winter. This method was further honed by Grezes-Besset (1994), who made testing using plastic test tubes part of the procedure. The advantage of this technique is that it provides a higher level of reliability and makes it possible to test a large number of genotypes in a short period of time.

Different methods have been developed for evaluating sunflower physiological mechanisms of resistance, which include the evaluation of the underground broomrape development in Petri dishes assays covered with glass fiber paper (Echevarría-Zomeño et al., 2006) or in two-

layer filter paper rolls (Antonova et al., 2011; Rodríguez-Ojeda et al., 2010), or the use of hydroponic co-culture (Labrousse et al., 2004).

Methods of breeding for resistance to Orobanche cumana

Breeding programs focused on the development of broomrape-resistant hybrids of sunflower were first based on single dominant *Or* genes. To ensure their success, the best way to go is to pick out an elite line and cross it with a source of *Or* genes, which should then be incorporated into the breeding material using certain techniques (recurrent cross-breeding together with screening for resistance in all BC generations). At the start of the program, the breeder must determine which race or races are present in the region for which the hybrids are being developed. A set of differential lines for races A, B, C, D, and E has been provided by Vrânceanu et al. (1980), while Pacureanu-Joita et al. (1998) have identified such a line for race F.

There are no public differential lines for the new, virulent races of broomrape that have appeared in the last few years.

The breeding strategies have been developed in order to increase the durability of genetic resistance to broomrape. Continuous search for new sources of resistance is important. The most significant results are achieved by interspecific hybridization in which wild species of genus *Helianthus* are used as donor of the gene of resistance. Transferring resistance genes from annual wild species is accomplished rather easily with a conventional crossing scheme, but, from perennial species is generally more difficult, due to problems associated with early hybrid embryo abortion and sterility in F_1 and BC_1F_1 generations. Such problem can be overcome with using of embryo rescue and chromosome doubling of the F_1. Also, alternative breeding strategies involving vertical resistance should incorporate gene pyramiding, alternation of several forms of a hybrid with different *Or* genes, or mixtures of these different forms grown together. Finally, to get the best use of these major genes, they need to be backed-up by quantitative, non-race specific resistance. These strategies will require QTL analysis and development of molecular markers linked to major and minor resistance genes to ensure that they are simultaneously introgressed during backcross, and a detailed characterization of the physiological mechanisms underlying genetic resistance.

Alternative methods for the control of *Orobanche cumana*

The rapid changes in broomrape race composition have forced sunflower breeders and geneticists to not only search for genes for resistance to the new races of *Orobanche* but to also look for alternative solutions to the problem of broomrape control. In the past 15 years, the development of sunflower hybrids resistant to the imidazolinone herbicides has made it possible to successfully control broomrape regardless of its race composition. This option is generally used in combination with the available *Orobanche cumana* resistant genes.

Wild *Helianthus annuus* L. resistant to imidazolinones (imazethapyr, pursuit) was first identified in Kansas (USA) in 1996 in a soybean field treated for seven consecutive years with a herbicide from this group (Al-Khatib et al., 1999). The use of imidazolinone resistance in sunflower breeding through the introduction of IMI-resistance genes into cultivated sunflower genotypes provides a broad spectrum of weed control (covering over 40 broadleaf species and over 20 grass weed species) and is especially effective in controlling *Orobanche* in sunflower, as discovered by Alonso et al. (1998). The USDA-ARS (NDSU) research group quickly transferred this genetic resistance into cultivated sunflowers and released the public populations IMISUN-1 and IMISUN-2. Similar programs were developed in parallel by

Alonso et al. (1998) in Spain, by Malidža et al. (2000) and Jocić et al. (2001) in Serbia, and by several private companies in Argentina. Bruniard and Miller (2001) reported that IMI-resistance is controlled by two genes (semi-dominant type of gene action). *Imr*$_1$ is the gene responsible for imidazolinone resistance, while *Imr*$_2$ has the modifier effect when the major gene is present. Malidža et al. (2000) and Jocić et al. (2001) showed that resistance to imidazolinones is controlled by a single, partially dominant gene. These differences in the mode of inheritance could perhaps be attributed to the presence of mutations on several different loci in the original population of wild *Helianthus annuus* L.

Sala et al. (2008) obtained another gene for resistance to imidazolinones through ethyl methane-sulfonate mutagenesis of seeds and selection with the imazapyr herbicide. They labeled the gene CLHA-PLUS. Based on genetic analysis (F_1, F_2 and BC_1F_1), the authors determined that the IMI-resistance gene CLHA-PLUS is controlled by a partially dominant nuclear gene. Using the SSR marker for the AHASL1 gene, they concluded that the mutation present in CLHA-PLUS is different from *Imr*$_1$, but that both these genes are allelic variants of the locus $AHASL_1$.

Other chemical options have effect against *Orobanche cumana*, such as inducers of seeds germination leading to suicidal germination of the parasite in the absence of sunflower (Lachia *et al*., 2014), or inhibitors of the germination process (Okazawa & Benesh, 2011). Extensive information about chemical signals from hosts and their effect on parasite species can be found in other works (Smith *et al*., 1990; Yoneyama *et al*., 2008; Gomez-Roldan *et al*., 2008; Umehara *et al*., 2008). Finally, the availability of next generation sequencing technologies, metabolomics and its applications to produce continuous and massive information about parasitic weeds (Westwood *et al*., 2012; Pineda-Martos *et al*., 2014; Piednöel *et al*., 2012) must be exploited.
Some authors tried to find other methods for controlling this parasite, as modification of sunflower sowing date. Akhtouch et al. (2013) have showed that the modification of the SD affects differently the natural infection by *O. cumana* in susceptible and moderately resistant sunflower. Eizenberg et al. (2012) have validated a thermal time model based on the main role of temperature on the parasitism of *O. cumana* in susceptible irrigated sunflower. The effect of a shift of sowing date, on sunflower hybrids performance in fields infested by *Orobanche cumana* and under drought conditions as compared to irrigation might be investigated in the future.

CONCLUSIONS

Sunflower breeders and geneticists have been successful in responding to the rapid changes in the race composition of broomrape (*Orobanche cumana* Wallr). They found genes for resistance to this pathogen and incorporated them into elite lines of cultivated sunflower, making it possible to develop *Orobanche*-resistant hybrids. Research so far has shown that the genes for broomrape resistance are present in some wild species of the genus *Helianthus*.

Because information about pathogenic traits of the parasite is locally obtained, its validity is restricted to particular geographical areas within Europe. Comparisons of results about characterization of races at an international level, as well as effectiveness of resistance sources in international sunflower breeding programs, will be favoured by the universal adoption of the coded triplets system for nomenclature of *Orobanche cumana* races.
On the other hand, breeding sunflowers for resistance to the AHAS-inhibiting herbicides has appeared, as an alternative for the control of *O. cumana* together with other weeds of the

crop. This alternative for the control of *O. cumana* could be implemented together with genetic resistance to races of the parasite.
Physiological and molecular mechanisms governing the *Orobanche cumana* development and the establishment of the interaction with its hosts, for example the chemical stimulation of parasite seed germination, or the role of degrading enzymes on the parasite progression between host cells, have been studied and characterized. This will help to the identification of a specific targets for new control methods.

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Table 1. Proposal for standardized characterization and nomenclature of *Orobanche cumana* populations using the coded triplets system, which is based on the use of eight sunflower lines -termed differentials-, grouped into three sets. By: Molinero – Ruiz et al., 2015

	Group # 1		Group # 2		Group # 3	
Population of *O. cumana*	Differentials	Value if susceptible reaction	Differentials	Value if susceptible reaction	Differentials	Value if susceptible reaction
	AD66 (-)	1	Record (*Or3*)	1	LC1093 (*Or6*)	1
	K A-41 (*Or1*) [b]	2	S1358 (*Or4*)	2	P96 (*Or5, or6 or7*)	2
	J8281 (*Or2*)	4	P1380 (*Or5*)	4		
Code [a]:	Total in group # 1		Total in group # 2		Total in group # 3	

[a] Each population is identified by a code of three digits which are obtained by totals due to susceptible reactions of each of the lines into the set. Resistant reactions impart 0.
[b] Genetic resistance in each line according to Akhtouch *et al*. (2002), Pacureanu *et al.* (2004), Pérez-Vich *et al*. (2004) and Vrânceanu *et al.* (1980).

Table 2. Proposal for characterization of populations of *Orobanche cumana* using the coded triplets system, and its correspondence with the traditional method based on the use of consecutive capital letters (A, B, C, etc.) given in chronological order of identification of pathotypes (By: Molinero – Ruiz et al., 2015)

Line of sunflower	Coded races of *O. cumana*							
	100	300	700	710	730	770	771	773
AD66	S	S	S	S	S	S	S	S
K A-41	R	S	S	S	S	S	S	S
J8281	R	R	S	S	S	S	S	S
Record	R	R	R	S	S	S	S	S
S1358	R	R	R	R	S	S	S	S
P1380	R	R	R	R	R	S	S	S
LC1093	R	R	R	R	R	R	S	S
P96	R	R	R	R	R	R	R	S
Historical race	A	B	C	D	E	F	F or G?	F or G?

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INTEGRATED WEED MANAGEMENT IN SUNFLOWER: CHALLENGES AND OPPORTUNITIES

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ABSTRACT

The purpose of effective weed management is the inclusion of the best measures and strategies to make sustainable sunflower production, and unfavourable for weeds. Weed management strategy based on single approach, and use of only herbicide-tolerant sunflower hybrids, and application only post-emergent acetolactate synthase inhibiting (ALS)-herbicides, are not sustainable strategies. Application of pre-emergent herbicides in herbicide-tolerant sunflowers would protect the crop for the first four to five weeks of growth and should also provide flexibility for timing of post-emergent herbicides application. Moreover, over reliance on a single herbicide and herbicides with the same mode of action in herbicide-resistant sunflower, can lead to weed population shifts, spread of herbicide-resistant weeds, and herbicide-resistant volunteer plants in subsequent crops. The risk of transfer of the trait for herbicide tolerance into weeds belonging to related species is elevated. Herbicide-resistant weeds pose significant threats, and until we find a better solution to manage herbicide-resistant weeds, farmers will need to implement more diversity into weed management. Additional challenges are that no new herbicidal modes of action developed in the past 30 years, and some herbicides has been banned in many countries. Integrated weed management (IWM), is a sustainable approach to managing weeds by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks. Therefore, increasing concern over herbicide side effects on human health and the environment, herbicide resistant weeds, weed shifts, invasive weeds, and slow development of new herbicides are some reasons for urgent need of implementation of integrated weed management in sunflower production.

Key words: integrated weed management, herbicides, herbicide tolerance, sunflower, weed resistance

INTRODUCTION

Weeds continue to pose a huge challenge for the sustainable production of sunflower despite decades of implementation of contemporary methods in order of their control. The development of weeds resistant to herbicides and weed shifts indicate the inefficient of modern agro-technical measures. Integrated Weed Management (IWM) is a sustainable approach to the management of weeds by combining all available weed control techniques, including preventative measures, monitoring, crop rotations, tillage, crop competition, mechanical and physical control, herbicide rotation, herbicide mixtures, biological control, nutrition, irrigation, flaming, etc. in a way that minimizes economic, health, and environmental risks (Swanton et al., 2008). The objectives of IWM-based systems are to reduce the reliance on herbicides by adopting agronomic measures: (1) reduction of weed

seed banks in the soil (2) decrease of the density of weeds emerging in crops, (3) reduction of their relative competitive ability, and (4) control of emerged weeds using non-chemical techniques (Pardo et al., 2010).

As with all technologies, herbicides also face some challenges including safety and environmental issues, and the evolution of herbicide resistant weeds. To avoid or delay the development of herbicide resistant weeds, a diverse, integrated program of weed management practices is required to minimize reliance on herbicides with the same MOA. Weed management diversity must include chemical and nonchemical weed control strategies (Vencill et al., 2012). In the past two decades weed management has become a key issue for European agricultural practices due to following reasons: (1) frequent herbicide treatments in most crops throughout Europe, except, of course, in organic farming, (2) herbicides are the pesticide residues most frequently found when analyzing the quality of surface and ground-waters, (3) the development of weed populations resistant to the most frequently used herbicides has become a real threat to the sustainability of current chemical weed control strategies, (4) the increase in cost of chemical crop protection, due to the withdrawal of several old and cheaper herbicides (Ramesh, 2015). Therefore, these are key points for implementing innovative strategies which focus on lower pesticide inputs and combine all available weed control techniques within the IWM concept.

HERBICIDES DISCOVERY FOR SUNFLOWER: QUO VADIS?

Producers have fewer herbicide options for broadleaf weed control in sunflower compared to most other row crops. They traditionally relied on pre-emergence (PRE) herbicide, which require timely rainfall for activation (Kerr et al., 2004). On the other hand, the agricultural chemical industry has not brought any new herbicides with novel sites of action to market in last 30 years (Figure 1). In addition, tougher registration and environmental regulations on herbicides have resulted in a loss of some herbicides, particularly in Europe (Heap, 2014; Kraehmer et al., 2014). The demand for new resistance management solutions is rewarding the renewed focus on herbicide discovery. However, the regulatory requirements to develop and register new herbicides are ever increasing, especially in Europe. Consequently, the total cost for the discovery and development of one new herbicidal active ingredient is approaching 200 million euros (Phillips McDougall, 2012 cit. Kraehmer et al., 2014). In some sunflower regions pyroxasulfone is a new soil-applied herbicide which has the potential for use in sunflower (Olson et al., 2011).

The wide use of glyphosate in glyphosate-tolerant crops (Roundup Ready®) crops has slowed down herbicide discovery, and also resulted in a generation of farmers with little knowledge of weeds and weed-control techniques. The widespread appearance of glyphosate-resistant weeds, forewarned growers that the use of glyphosate alone for weed control was not sustainable. Growers began adding more diversity in their herbicide program, primarily through the addition of pre-emergence herbicides. Farmers will need reeducation in weed-control practices which may include diversification of cropping systems, the adoption of herbicide-resistance management strategies (Heap, 2014). This is a good example of the consequences of reliance on a single herbicide in weed management.

In order to restrict competition from generic herbicides, companies thus tend to modify their commercial formulations and/or offer stronger guarantees to farmers who use the herbicide-tolerant varieties in combination with their brand of the herbicide (Beckert et al., 2011). Examples of this can be seen in the market of herbicides in sunflower. Obviously, herbicides in sunflower are a non-renewable resource which should be protected.

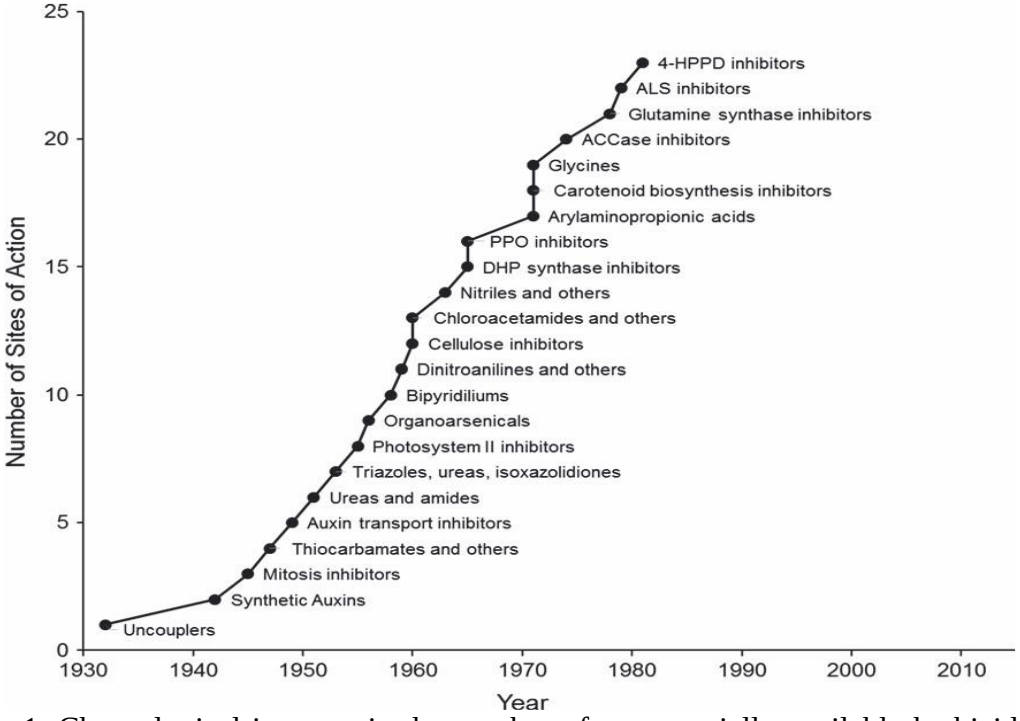

Figure 1. Chronological increase in the number of commercially available herbicide sites of action (Heap, 2014)

SUCCESSES AND CONCERNS WITH WEED MANAGEMENT SYSTEMS BASED ON THE USE OF HERBICIDE-TOLERANT SUNFLOWER HYBRIDS

Introduction of imidazolinone- and tribenuron-tolerant sunflower hybrids in practice is a revolutionary advancement in sunflower production. After launch in 2003, Clearfield® production system has been well adopted in sunflower growing countries (Table 1) due to a wide spectrum of weed and broomrape (*Orobanche cumana*) control, a high level of consistency, flexibility in the timing of herbicide applications, season-long weed control, and a low rate of herbicide application (Malidza et al., 2000, 2003 & 2012; Zollinger 2004; Nagy et al., 2006; Phening et al., 2008; Kukorelli et al., 2011; Kaya et al., 2012 & 2013). It is expected that the combination of improved imidazolinone formulation in new Clearfield Plus production system will provide a more efficient, flexible and reliable weed control system in sunflower, including more freedom in crop rotation. (Sala et al., 2012, Pfenning et al., 2012; Weston et al., 2012). Tribenuron-methyl contributes to weed control in sunflower by controlling annual broadleaf weeds and the perennial weed *Cirsium arvense* post-emergence, has excellent sunflower safety to tribenuron-tolerant hybrids, increasing the range of available herbicides in sunflower increasing no-till/conservation tillage practices, is non-transgenic, and has no restrictions in crop rotation (Zollinger 2004; Jocic et al., 2008 and 2011; Bozic et al., 2012). Sunflower hybrids tolerant to mentioned ALS-inhibiting herbicides, may be useful additional tools in cases of some difficult weed management situations or for diversification of weed-control strategies. However, their widespread and repeated use with associated herbicides, without regard to accompanying changes in weed flora, can rapidly make them ineffective strategy. Weed management strategy based on single approach, and use of only herbicide-tolerant sunflower hybrids, and application only post-emergent ALS inhibiting herbicides are not sustainable. Sustainable use of these sunflowers production systems can be caused by the compatibility of the weed-management objectives with current policies for the preservation of biodiversity within agricultural areas and the reduction of pesticide use. With herbicide-tolerant sunflower hybrids, the reduction of herbicide use is not necessarily and this

hybrids and associated herbicides may be properly used as a complementary strategy to classical pre-emergence chemical weed control to correct a specific weed management difficulties (Beckert et al., 2011; Nagy et al., 2006). Before commercialization of imidazolinone- and tribenuron-tolerant sunflower hybrids, soil-applied herbicides were especially important because there was no post-emergent herbicides for control of broadleaf weeds that could be applied. Despite available efficient post-emergence herbicides (imazamox, tribenuron), soil-applied herbicides are important as assurance that weeds will not emerge with the crop and be too large to control with the post-emergence herbicide application. Application of pre-emergent herbicides in imidazolinone-tolerant sunflowers would protect the crop for the first four to five weeks of growth and should also provide flexibility for timing of post-emergent herbicides application. Otherwise, weed control without pre-emergent herbicide must be initiated in the second to third week of crop growth. The information gained from this study could help producers of both IMI- and tribenuron-tolerant and conventional sunflower improve the cost effectiveness and efficacy of their weed management practices (Elezovic et al., 2012; Knezevic et al., 2011 & 2013). Experiences with Clearfield® production system for sunflower in Hungary indicate that the application of pre-emergent herbicides (dimethenamid-P + pendimethalin, fluorochloridon, etc.) with subsequent application of imazamox based products is an efficient and reliable practice (Nagy et al., 2006).

The French Technical Centre for Oilseed Crops and Industrial Hemp claims that the area planted to imidazolinone- and tribenuron-tolerant hybrids reached more than a million hectares in Europe (Spain, Turkey, Greece and eastern European countries) in 2009 and close to 2 million hectares in 2010. In France, BASF and DuPont estimate that imidazolinone- and tribenuron-tolerant sunflower hybrids accounted for 20,000 and 15,000 ha, respectively, in 2010, and 50,000 and 30,000 ha in 2011, or approximately 11% of the total area planted to sunflower in France for that year (Beckert et al., 2011).

Table 1. Year of introduction of Clearfield® production system for sunflower into various countries and total cultivated area with such system in Europe from 2007-2011

Countries	Year	Estimated total area in cultivation since 2007 in Europe (000 ha)
Turkey	2003	
Serbia, Spain	2004	
Bulgaria, Hungary, Romania, Slovakia	2005	
Moldova, Ukraine	2006	
Croatia, Italy	2007	240
Russia	2008	560
South Africa	2009	800
France, Kazakhstan	2010	1.450
Czech Republic	2011	2.850

Source:http://www.agro.basf.fr/agroportal/fr/fr/cultures/les_oleagineux/le_tournesol/les_herbi/dossier_clearfield.html

The new weed management strategy based on the use of sunflower hybrids tolerant to ALS-inhibiting herbicides is a efficient tool to control some invasive weeds, and to reduce concentrations of the plant's allergenic pollen in the air. A major key for the success of *Ambrosia artemisiifolia* control when using this technologies will be the management of resistance due to very frequent use of ALS-inhibiting herbicides and the control of volunteer

sunflowers in following crops (Kukorelli et al., 2011; Reisinger et al., 2013). However, there is strong probability of the development of resistance in some invasive weeds such *A. artemisiifolia*, which is already very abundant in sunflower production area, and which has become resistant to ALS-inhibiting herbicides in some parts of the world (Heap, 2016).

HERBICIDE-RESISTANT WEEDS AND RESISTANCE MANAGEMENT

Repeated exposure of a weed population to any herbicide in isolation may have two effects: (1) weed species that are not controlled by the herbicide will dominate the population (species shift), and (2) the pressure will be exerted on the population to select any resistant individuals that may be present (herbicide resistance). The development of both the species shift and herbicide resistance can be effectively managed by the practice of IWM (Beckie, 2014). Herbicide resistance threatens future agricultural productivity and needs to be better understood. Currently, more than 60% of the global herbicide market (value) is represented by products from only four mode of action, all of which actually have serious resistance issues (Kraehmer et al., 2014). Herbicide-resistant weed populations are evolving very fast as a natural response to the selection pressure imposed by the repeated use of herbicides with the same mode of action. The development of weed populations resistant to the most frequently used herbicides is a real threat to current weed control strategies in sunflower. There has never been a widely adopted technology that is not without disadvantages. Despite previous successes, strategy with over-reliance on herbicide-tolerant sunflower hybrids and accompanying herbicides are a double-edged sword and not sustainable. They can be a solution for the herbicide management, but on the other hand represent a risk for the development of resistance. The appearance and spread of herbicide-resistant weeds are not a specific result of the cultivation of herbicide-tolerant sunflower, but may be intensified by the conditions in which associated herbicides are used in such production systems. Herbicide resistance in weeds is a global problem and huge challenge no farmer can afford to ignore. Farmers are usually unwilling to use proactive management of weeds to prevent or delay the selection for herbicide resistance. The cost and effort of preventing/delaying resistance to many herbicides are widely perceived or estimated to be the same as that of managing herbicide resistant weeds, and therefore farmers often do not change their weed management program until resistance has occurred. The lack of proactive management of the evolution of herbicide- resistant weed populations may be due to farmers' primary interest in optimizing short-term economic returns, or inability to assess the economic risks associated with herbicide resistant weeds. There are currently 468 unique cases (species x site of action) of herbicide resistant weeds globally, with 249 species (144 dicots and 105 monocots). Weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 160 different herbicides. Herbicide resistant weeds have been reported in 86 crops in 66 countries (Heap, 2016). Now, there are more weed species that are resistant to ALS-inhibiting herbicides than to any other herbicide group. In addition, ALS-inhibiting herbicides are already widely used in cereal and other crops. The introduction of IMI-tolerant oilseed rape, IMI- and tribenuron-tolerant sunflower and within cereal-oilseed crop rotations will increase the selection pressure on weeds. In most cases, resistance to ALS-inhibiting herbicides is cross-resistance caused by an altered ALS enzyme. The frequent occurrence of weed populations resistant to ALS inhibitors can be attributed to the widespread usage of these herbicides (Tranel & Wright, 2002). A plant does not evolve resistance because herbicides cause a genetic change in the plant that makes it resistant. Rather, a few plants with natural resistance to the herbicide survive an application of the herbicide, and as those plants reproduce and each generation is exposed to the herbicide, the number of resistant plants in the population increases until they dominate the population of susceptible plants (Vencill et al., 2012). How to outsmart

herbicide-resistant weeds? Herbicide-resistant weed management practices most often recommended by weed scientists include: (1) using different herbicide MOAs in annual rotation, tank mixtures, and sequential applications; (2) adopting crop rotations that allow use of alternative MOAs or that change the balance of weeds in a field or both; (3) expanding the use of cultural control measures, such as increased seeding rates and altered planting dates; (4) using only labelled herbicide rates at labelled application timings; (5) preventing seed movement and using clean crop seed; (6) scouting fields; and (7) controlling weed escapes (Vencill et al.,2012).

Careful management of herbicides, including integrated use of crop rotation, cultural practices and rotated use of herbicides with different modes of action are critical to minimize the development of herbicide resistance. Diversifying weed management practices and using multiple herbicide mode of action need to be more widely implemented.

RISK OF GENE FLOW FROM HERBICIDE-TOLERANT SUNFLOWER CROP TO WEEDY SUNFLOWER

In addition to evolved weed resistance via herbicide selection pressure, resistance may also occur through gene flow. Weedy forms of cultivated sunflower (*Helianthus annuus*) are invasive species widely distributed in several regions of the world and are commonly controlled by applying ALS-inhibiting herbicides, such as imidazolinones or sulfonylureas. The widespread adoption of herbicide-resistant crops has exposed the weedy population to the high risk of crop-to-weedy gene flow. Due to high competitive ability, invasiveness and increasing area with herbicide-tolerant sunflower hybrids, problem with the weedy sunflower form had increased during the last decade. Weedy sunflower is also considered of major concern in the sunflower growing areas (Vischi et al., 2006; Ureta et al., 2008; Muller et al., 2009; Poverene & Cantamutto, 2010; Saulic et al., 2013). In addition, weedy sunflower causes decline in yield over 50% under more than 4 plants m^{-2} in sunflower crop (Muller et al., 2009). Risk of gene flow from sunflower hybrids to wild relatives was confirmed by some researchers (Marshall et al., 2001, Massinga et al., 2003, Bozic et al., 2015), who found that gene flow depends on distance. Development of strategies to avoid gene flow should focus on: isolation distances, pollen traps, male sterility and temporal reproductive barriers (Roumet et al., 2013). Herbicide resistant common sunflower populations have been reported (Bozic, 2010; White et al., 2002; Vrbnicanin et al., 2012). Differences in the level of herbicide-resistance could result in different fitness of weedy sunflower populations which could promote the invasiveness of these populations. To ensure sustainability and efficiency of weed management systems based on the herbicide-tolerant sunflower and associated ALS-inhibiting herbicides, crop rotation and herbicide usage with different modes of action should be considered. Except of herbicides use in sunflower crop with tolerance to imidazolinones and tribenuron-methyl, it is recommended to control weedy sunflower with mechanical measures as soon as the first weeds are detected on a field, and before they produce seeds and build up a big population (Muller et al., 2009; Presotto et al., 2012).

MANAGEMENT OF HERBICIDE-RESISTANT SUNFLOWER VOLUNTEERS

Control of common sunflower in many subsequent crops traditionally has been difficult. Because cross-resistance to selected imidazolinone, sulfonylurea, and triazolopyrimidine herbicides (Baumgartner et al., 1999), several herbicides are available to control imidazolinone - resistant common sunflower in maize, but in soybean options are very limited (All-Khatib et al., 2000). Our dose response experiments confirmed that the new tribenuron-tolerant hybrids has a higher tolerance to tribenuron-methyl and slightly cross-resistance to

imazamox. Similarly, tribenuron-tolerant hybrids were slightly resistant to imazamox (Malidza et al., 2012). By the contrary, the Clearfield Plus® trait confers high levels of tolerance to imidazolinones but complete susceptibility to sulfonylureas (Sala et al., 2008). Herbicides in Clearfield® production system are efficient in control of volunteer sunflower susceptible to imidazolinones, which also has a positive plant-health effect. With the wide-spread application of such technology, this advantage can turn to a disadvantage, as the possibilities of control imidazolinone-resistant volunteer sunflower decrease (Nagy et al., 2006).

CONCLUSION AND PATH FORWARD

IWM requires that weeds are managed with more than just herbicides. Higher level of complexity partly explains why IWM has not received the same attention as integrated management of other pests. Because of the diversity and flexibility of weed communities, weed management needs to be a continuous process. Adding to the complexity is the fact that most non-chemical tools are not as effective as herbicides, i.e. they cannot be considered as stand-alone methods, but has to be combined with other methods in a systematic way to provide sustainable and reliable weed control. It is difficult for weed researchers to provide credible IWM advice if they are conducting little or no real IWM research in sunflower. Finally, the challenge for weed scientists is to develop innovative, economical IWM systems that can be integrated into current and future cropping systems to bring a more diverse and integrated approach to weed management.

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SUNFLOWER CROP AND CLIMATE CHANGE IN EUROPE: VULNERABILITY, ADAPTATION, AND MITIGATION POTENTIAL

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ABSTRACT

Climate change is characterized by higher temperatures and CO_2 concentrations, extreme climatic hazards and less water available for agriculture. Sunflower, a summer crop often cultivated in drought-prone areas, could be more vulnerable to the direct effect of heat stress at anthesis and drought during its growing cycle, both factors resulting in severe yield loss, oil content decrease and fatty acid changes. Some adaptations through breeding, crop management, and cropping systems could be designed and enhanced to cope with these negative impacts. At the same time, new cultivation opportunities could be addressed in some parts of Europe where sunflower is not grown presently. In addition, sunflower crop could participate to the mitigation solution as a low GHG emitter compared to cereals.
Sunflower models should be revised to account for these emerging environmental factors in order to reduce the uncertainties in yield and oil predictions. The future of sunflower in Europe is probably related to its potential adaptation to climate change but also to its competitiveness and attractiveness for food and energy.

Key-Words: climate change, CO2, temperature, crop model, biotic stress, adaptation, mitigation

INTRODUCTION

In Europe, sunflower is mostly cultivated in Southern and Eastern regions. In 2013, Russia, Ukraine (both 49 %, 17.7 Mt) and UE-28 (19 %, 6.8 Mt) were the largest sunflower grain producers in the world accounting for 68 % of global volume. Sunflower crop is covering more than 4.5 M ha in UE-28: Romania, Spain, France, Bulgaria and Hungary being the main contributors (90 % of the UE-28 area). However, in most of these countries, there subsists major yield gaps (national yield between 1.1 and 2.4 $t.ha^{-1}$) and the slope of actual yield progress is rather flat in spite of the steadily genetic improvement (e.g. Salvi and Pouzet, 2010 for France). Climate change could be responsible for yield limitation as was observed for wheat (Brisson et al., 2010) although changes in cultural practices and land use could contribute as well.

The Intergovernmental Panel on Climate Change (IPCC) has predicted that the CO2 concentration may increase by 660–790 ppm from 2060 to 2090 (IPCC, 2007: IPCC 2014). This is expected to raise global temperatures due to the CO2 capacity to absorb infrared light and possibly change the precipitation patterns. In the period 1901-2005, the average annual temperature rose throughout Europe by 0.9 °C (Lotze-Campen, 2011); since the end of the

80s, the elevation of air temperature was clearly observed and the climatologists are speaking of climatic trend and not of interannual variability.

Global Climate Models (GCMs) indicate strongest warming over Eastern and Northern Europe during winter and over Western and Southern Europe during summer (IPCC 2007; 2014). Especially in the Southwestern parts such as France, Spain and Portugal, increase in average summer temperatures may exceed 6 °C by the end of the century. In addition, maximum temperatures could increase much more in Southern and Central Europe than in Northern Europe. However, precipitation trends should vary regionally (Lotze-Campen, 2011). In N. Europe and most of the Atlantic region, mean winter precipitation will increase contrary to the Mediterranean area (especially in the eastern part). Projections of seasonal precipitation patterns vary as well. It is likely that winter precipitation in Western, Northern and Central Europe will increase while it will decrease over the Mediterranean region. Summer precipitation will decrease substantially in Southern and Central Europe and to a smaller degree in Northern Europe. However, during spring and autumn, precipitation change should be marginal. Overall, the intensity of daily precipitation should increase substantially. Heat waves and droughts will occur more often (especially in the Mediterranean and much of Eastern Europe) due to the combined effect of warmer temperatures and less summer precipitation. In addition, droughts will start earlier and last longer.

Therefore, in its traditional production areas, sunflower crop will be exposed to major climate change and potentially impacted by water and temperature stresses. Sunflower is commonly viewed as a drought-tolerant crop and consequently as a possible solution for regions where water resources (used for irrigation) are decreasing and in situations where soil water deficit is expected to increase dramatically. When water is fully available, maize or soybean are preferred, and sunflower is often restricted to marginal areas or unirrigated farms. However if climate change is a threat for sunflower in southern and eastern regions, it could also offer new cropping opportunities in northern parts of Europe. As the only summer oilseed crop in Europe, it could break winter crop rotations where too much fertilizers and pesticides are currently used.

For the major crops (wheat, rice, and maize) in tropical and temperate regions, climate change without adaptation will negatively impact yields for local temperature increases of 2°C or more, although individual locations may benefit (Porter et al., 2014). No such evaluation was produced for sunflower crop in the last IPCC reports. This justified this preliminary review (i) of the impacts of climate change on sunflower grain and oil yields, (ii) of possible adaptation options, and (iii) of the contribution of sunflower to greenhouse gases (GHG) emissions.

CROP SUITABILITY

Sunflower cultivation is currently limited to Southern Europe and parts of Central / Eastern Europe for temperature reasons. A northward shift of the northern limits of crop suitability is likely to occur as temperature steadily raises (Olesen and Bindi, 2002). It is commonly admitted that the area suitable for crop growing may shift northward by 120-150 km per 1°C increase in annual mean temperature. In addition, sunflower could also become viable at higher altitudes than presently. In the Northern regions and in the continental part of Europe, warming will extend the length of the potential growing season allowing earlier planting and harvesting. Drier conditions can also increase the soil workability in spring.

Most of the crop suitability studies are based on thermal requirements (base temperature and growing degree days). Early studies still concluded to a possible migration of the crop northward with global warming (Carter et al., 1991). Tuck et al. (2006) used climate scenarios based on four IPCC SRES emission scenarios (A1FI, A2, B1 and B2) implemented

by four GCMs (HadCM3, CSIRO2, PCM and CGCM2) to predict the potential distribution of bioenergy crops in Europe under present and future climate. Their assumptions were that sunflower requires between 350 and 1500 mm of rain per year, with minimum and maximum monthly temperatures of 15 and 39 °C, respectively, between April and September. According to all models, sunflower will continue to be potentially grown in over 60% of southernmost Europe (35–44°N). The four models predicted very different potential distributions in Central Europe by the 2080s due to the different combined predictions of temperature increase, and change in precipitation among them: a 25% increase in 45–54°N by the 2080s due to increased summer temperatures (CGCM2 and HadCM3) vs. a decline of up to 25% in this latitude (CSIRO and PCMA). Sunflower should take advantage of the improved thermal regime (higher summer temperatures) at northern latitudes.

Some studies explicitly considered the extension of sunflower crop to southern England as a possible adaptation to climate change. The projections from UKCIP02 data indicate that the area suitable for sunflower production (using very early cultivars) will increase to approximately 79% of the land area of England by 2050 (Cook, 2009). However, when considering competition with other break crops at farm level, Gibbons and Ramsden (2008) concluded that sunflower area could increase from 0.3% in the baseline through 0.4% in the 2020s to 1.9% in the 2050s which looks quite minor. Hence, while the sunflower area is sensitive to the degree of climate change, there is little evidence of a 'tipping point' for a shift in break crops, within the range of climate outcomes modelled.

IMPACTS ON CROP YIELD

At southern latitudes, temperature increases, precipitation decreases as well as increases in climatic interannual variability, and a higher frequency of extreme events are to be expected (IPCC, 2014). These combined changes will lead to a shorter growing season (especially grain filling phase), increased water shortage and heat stress, which will reduce yields, lead to higher yield variability, and probably reduce the agricultural area of this traditional crop in regions as Spain, Portugal, Italy, and SW France.

To document these threats and be more accurate at regional level, several simulation-based studies were recently published where conclusions were given for sunflower. The most complete and recent one (AVEMAC project) was produced by JRC (EU) in 2012 (Donatelli et al., 2012; 2015). Two GCMs were used: Hadley CM3 (warm scenario) and ECHAM5 (cold); yield simulations were performed with the CropSyst model (Stöckle et al., 2003) at 2020 and 2030 horizons with or without technical adaptations. Both potential and water-limited yields were simulated for NUTS2 regions of EU-28. The average [CO2] in the atmosphere has been set to 355 ppm for 2000 (baseline), 400 ppm for 2020 and 420 ppm for 2030, in coherence with IPCC assumptions.

In terms of potential yield, yield improvement was simulated by 2020 compared to baseline time horizon in a magnitude of 5-10% or no change in whole Europe except decline in some places of Portugal, Romania and Bulgaria. Whereas, in 2030 time window, a detrimental effect of climate change by 5-20% was expected in southern parts of Europe (Spain, Italy, Hungary, Romania and Bulgaria) which might be due to the fact that high average "seasonal" temperatures can limit the photosynthetic rates and reduce light interception by accelerating phenological development. In contrary the yield gain in Northern France and Germany suggests that global warming may increase the length of the growing period and render suitable conditions for sunflower growing. From the warm 2030 scenario, a potential decrease in sunflower production of around 10% was simulated for all important Spanish regions. In France, potential decreases are estimated in sunflower production from 4% to 8% depending on the regions. Almost all regions in Hungary, Bulgaria and Romania

are estimated to be potentially affected by a significant decrease of 12-14 % in 2030. The analysis for the cold scenario anticipates to 2020 the variations foreseen in the warm scenario in 2030 for all most important Spanish regions. The 2030 cold scenario almost reflects the results obtained with the warm scenario except in France.

Considering water-limited yields (Figure 1), the results show an improvement (with HadCM3) in sunflower yield in Spain, Italy, Romania and Bulgaria (in general areas at southern latitudes) with some patches of decline in France and Germany in 2020, compared to the baseline time horizon. The improvements can be directly linked to the higher precipitation prediction compared to baseline. By 2030 the improvements get milder in Southern European countries, and countries in Eastern Europe see 10–30% yield decline. Higher evapotranspiration coupled with less rainfall compared to baseline period are expected with this scenario.

In conclusion, sunflower yield was simulated to potentially improve at northern latitudes, but with negative effects on yield at southern latitudes. In the warm scenario little to no potential changes are expected for sunflower by 2020; however, by 2030 the analysis indicates potential decreases in production in various areas, if adaptation to climate change is not taken into account.

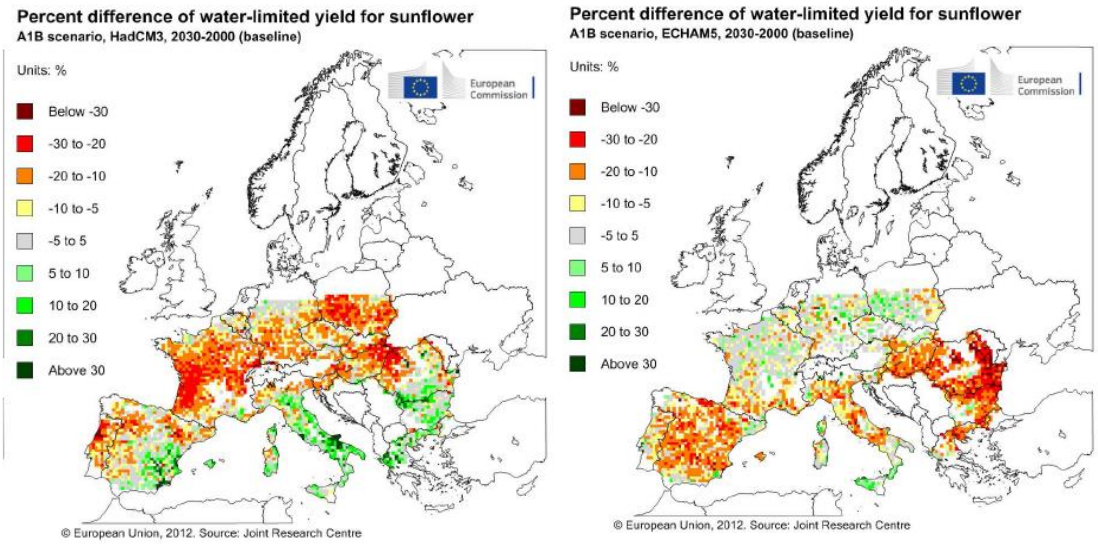

Figure 1 – Change in relative term of simulated water-limited sunflower yield for 2030 using the 'warm' (HadCM3) and the 'cold' (ECHAM5) realization of scenario A1B. No adaptation strategies are considered (Donatelli et al., 2012)

Since the pioneering study of Harrison and Butterfield (1996), several other studies have simulated the impact of future climate on sunflower yield at local, regional or national levels. Be careful that as crop models, GCMs, RCMs and GGE scenarios differed among studies as time is running, contradictory conclusions were often drawn.

Tubiello et al. (2000) investigated with CropSyst and two GCMs the potential effects of doubling the atmospheric [CO2] from 350 to 700 ppm on sunflower yields at two Italian locations. They concluded to limited changes for unirrigated sunflower as a consequence of soil water refillment during fallow period.

Guilioni et al. (2010) used both SUNFLO and STICS crop models (both including CO2 effects) to simulate baseline, next and far future climate. They concluded to minor changes for 12 locations in France, the positive effects of atmospheric CO2 compensating for negative effects of water stress. However they concluded to an increase of interannual variability during vegetative period. Crop duration will be reduced by 4 to 6 days per °C for

flowering time and by 7 to 12 days per °C for harvest date, as a function of RCM and genotype considered. The potential extension of sunflower crop northward in France was thus confirmed. The number of hot days (Tmax > 32 °C) during grain filling could increase from 8 (baseline) to 22 (far future) in Toulouse (SW France).

At European level, Moriondo et al. (2011) assessed the direct impact of extreme climate events (i.e. heat stress at anthesis stage) by using the outputs of HadCM3P regional climate model as drivers of a modified version of CropSyst model. They concluded that the increase in both mean temperatures and temperature extremes for the future period (2071–2100) under A2 and B2 scenarios resulted in: (a) a general advancement of the main phenological stages; (b) shortening of the growing season; (c) an increase in the frequency of heat stress during anthesis with respect to the baseline (1961–1990). The reductions in sunflower yields in the Mediterranean area changed on average from −14% to −34% (A2 and B2 scenarios), and the risk of low yields (i.e. below 1.8 t ha^{-1}) increased from 8% to 24%, where the highest differences were observed in the NE and SE regions and in the flat areas. In these regions, sunflower will be more prone to the direct effect of heat stress at anthesis and drought during its growing cycle if no adaptation is introduced.

CROP MODELS FOR EXPLORING THE IMPACTS OF CLIMATE CHANGE

Simple models have been used to map crop suitability based on growing degree days. Traditionally, yield estimation has been based on empirical data, simple evapotranspiration models and, lately, on process-based models (Garcia-Lopez et al., 2014). The impact of climate variability and climate change on grain yield and quality are now exclusively investigated using crop simulation models as recent developments and refinements have been done (formalisms, databases, climatic projections). Crop responses (development, growth and yield) are predicted by combining future climate conditions, obtained from GCMs and RCMs with the simulation of CO2 physiological effects, derived from crop experiments (e.g. FACE, see the review of Ainsworth and Long, 2005).

Crop models currently used for simulating sunflower yield in response to various environments differing by temperature and water are either:

- Generic: STICS (Brisson et al., 2003), CropSyst (Stöckle et al., 2003 ; Moriondo et al., 2011), EPIC/EPIC-Phase (Kiniry et al., 1992 ; Cabelguenne et al., 1999), AquaCrop (Todorovic et al., 1999), AqYield (Constantin et al., 2015), WOFOST (Todorovic et al., 1999)…
- Or specific: Oilcrop-Sun (Villalobos et al., 1996), QSUN (APSIM) (Chapman et al., 1993), SUNFLO (Casadebaig et al., 2011)

However, crop models should be still improved to reduce the uncertainty due to model structure when predicting yield in future environments. Only some of these models have been adapted to simulate crop response to increased [CO2] and high temperatures. The impact of extreme events should be included in crop modelling approaches, otherwise there is the risk of strongly underestimating crop yield losses (Moriondo et al., 2011). CropSyst and STICS which both include the effects of elevated [CO2] on crop photosynthesis and transpiration have been extensively used. As pointed out by Andrianasolo et al (2016), only a few models explicitly consider seed oil content.

The combined effects of elevated [CO2], high temperatures, drought and nutrient status as simulated by the models have to be compared as it was recently done for wheat and maize in the recent AgMip international initiative. More effort is still necessary to make these models operational tools for climate change impact assessment and adaptation design. Uncertainty has to be considered in model inputs and outputs. Ensemble crop simulation protocols have still to be developed for sunflower crop.

To be improved, models should integrate more physiological knowledge on the combined effects of CO2, drought and temperature on crop production.

PHYSIOLOGICAL IMPACTS OF CLIMATE CHANGE ON PRODUCTIVITY

CO2 fertilization effect

Rising atmospheric [CO2] can affect the growth and yield of C3 plants, mainly through enhancement in the rate of photosynthesis and carbon assimilation (Griffin and Seemann, 1996). Various studies have been conducted worldwide on the response of different crop species to [CO2] which confirmed higher rate of photosynthesis, plant growth and yield due to elevated [CO2] exposure (Ainsworth et al. 2008; Taub et al. 2008). In C3 plants as sunflower, radiation, water and N use efficiencies are all expected to increase with [CO2]. It is known that C3 crops plants produce more biomass and harvestable products under high CO2 environment compared with C4 due to the enhanced rate of photosynthesis (Long et al., 2006). There is also adequate evidence that the CO2 fertilization effect will continue for C3 plants at least until the [CO2] reaches 750 ppm (Seneweera and Norton, 2011). The extent of this increase will depend not only on the short-term stimulation of photosynthetic activity but also on longer-term acclimation responses (Sims et al., 1999). Most of the studies on plant response to elevated [CO2] have been conducted in cereal crops (e.g. wheat), and very few reports are available about the response of oilseed crops, especially sunflower. However, during the two last decades, some studies on sunflower confirmed the typical C3 response of sunflower to elevated [CO2].

Sims et al. (1999) grew sunflowers in large controlled-environment chambers receiving ambient and twice-ambient concentrations of atmospheric CO2. Exposure to 2 x [CO2] enhanced rates of net photosynthesis in individual upper-canopy sunflower leaves by approximately 50%. Cheng et al. (2000) using a whole-system gas exchange chamber and a 13C natural tracer method observed that total daily photosynthesis, net primary production, and respiration were consistently higher under the elevated [CO2] treatment than under the ambient [CO2] one. Luo et al. (2000) grew sunflowers in large environmentally-controlled chambers receiving atmospheric [CO2] of 400 and 750 ppm. They observed that elevated [CO2] increased canopy light utilization by 32% and carbon uptake by fully 53%. De la Mata et al. (2012) observed that photosynthetic CO2 fixation was boosted on young leaves growing under elevated [CO2]. The above findings all suggest that sunflowers should become more efficient at absorbing sunlight and using its energy to convert CO2 into carbohydrates as the [CO2] increases in the future. Consequently net photosynthetic rates and biomass production should increase as well.

De la Mata et al. (2012) also indicated that elevated [CO2] could promote early leaf senescence in sunflower plants by affecting the soluble sugar levels, the C/N ratio and the oxidative status during leaf ontogeny. Additionally, De la Mata et al. (2013) concluded that elevated [CO2] alter enzymes involved in N metabolism at the transcriptional and post-transcriptional levels, thereby boosting mobilization of N in leaves and triggering early senescence in sunflower plants.

There are very few reports on the impact of high [CO2] on the quality of sunflower seed oil. High [CO2] could affect nutritional quality of sunflower due to the dilution effect (Jablonski et al. 2002 ; Taub et al. 2008). Pal et al. (2014) reported the impact of high [CO2] exposure (550 ± 50 ppm) on oil percentage and quality of two sunflower genotypes raised inside open top chambers. Elevated [CO2] exposure significantly influenced the rate of photosynthesis and seed yield (61–68 % gain in biomass and 35–46 % increase in seed yield for two genotypes), but mineral nutrient and protein concentration decreased in the seeds (-13

%). However, oil content increased significantly in cv. DRSF 113 (15 %). Carbohydrate seed reserves increased with similar magnitudes (+13 %) in both the genotypes under high [CO2] treatment. Fatty acid composition in seed oil contained higher proportion of unsaturated fatty acids (oleic and linoleic acid) under elevated [CO2] treatment (Pal et al., 2014).

These findings conclude that rising atmospheric CO2 in changing future climate can enhance biomass production and seed yield in sunflower and alter their seed oil quality. However, the beneficial effects of high CO2 can be negated by other climate factors such as increase in atmospheric temperature and pattern of precipitation (Ainsworth et al., 2008).

Drought effects

Drought is the main environmental factor limiting sunflower plant growth in a wide range of environments. Sunflower, being a crop with medium water requirements, has the ability to tolerate a short period of drought. However, water stress may inhibit plant growth, decrease developmental activities of the cells and tissues and cause a variety of morphological, physiological and biochemical modifications (Ahmad et al., 2014). As water deficit should increase with climate change in southern environments, negative impacts on leaf expansion, biomass accumulation and oil production are all expected. These effects of drought on sunflower yield have been extensively studied and reviewed elsewhere in the literature (e.g. Connor and Hall, 1997; Chimenti et al., 2002; Ahmad et al., 2014). Negative impacts on oil concentration and oil quality are also expected (Andrianasolo et al., 2016).

High temperature

High temperature affects numerous biochemical and physiological traits in plants. In sunflower, compared to cereals, few efforts have been devoted to exploring the effects of heat stress, even though the crop can be damaged by high temperatures during specific sensitive stages of development (Connor and Hall, 1997).

After submitting sunflower plants to a day/night regime of 33/19 °C for 16 to 42 days, De la Haba et al. (2014) observed decreased leaf growth (lower specific leaf mass, reduced leaf area) and soluble protein content during leaf life span relatively to control plants (70% vs. 45%, respectively). They suggested that high temperatures promote soluble protein degradation in leaves. It also reduces net photosynthetic rate possibly by decreasing the content in photosynthetic pigments and the stomatal conductance. Early senescence observed at high temperature would result from the accumulation of soluble sugars and the associated decrease in starch levels.

In sunflower, constant high temperature decreases final grain weight and oil yield (Harris et al., 1978). Chimenti et al. (2001) applied constant temperatures (12 to 40°C) during grain filling which resulted in a curvilinear response of the rate of embryo filling with a peak at 25°C; embryo-filling duration had a minimum close to 34 °C, and embryo size continuously decreased with increasing temperature above 25 °C. Direct effects of brief periods of heat stress during grain filling were investigated by Rondadini et al. (2003). They exposed the capitulae of plants growing at 25°C to temperatures of ca. 35, 37 and 40°C for seven consecutive days during grain filling. Brief periods of heat stress resulted in a lower seed weight, a greater percentage of pericarp, a lower oil content and an altered fatty acid composition. In addition, the period from 12 to 19 days after anthesis (daa) showed the greatest sensitivity to heat stress regarding embryo and grain weight responses, whereas the period of greatest sensitivity for oil quality was from 19 to 26 daa (Rondanini et al., 2006).

Temperatures higher than 31 °C at anthesis stage were demonstrated to be detrimental for sunflower yield, inducing a reduced pollen and floret fertility (Chimenti and Hall, 2001).

Astiz and Hernandez (2013) showed that temperatures over 26 °C were supra-optimal for pollen production in sunflower, even under well-watered conditions.

Multiple abiotic stresses

Independently, the impact of increased atmospheric [CO2] and drought stress on crop growth and productivity was well documented, however the interaction between these two stresses are not well understood.

Vanaja et al. (2011) assessed the influence of enhanced [CO2] (700 ppm) under both well-watered and drought stress conditions on plant water status, gas exchange and various root and shoot parameters of sunflower crop plants grown in open top chambers. Sunflower responded significantly and positively with eCO2 under both water treatments for shoot:root ratio. Root volume showed a positive significant response with CO2 concentration enhanced over ambient level and the increment in root volume was 146 %. The leaf water potential, stomatal conductance and transpiration showed a decreasing trend with drought stress and eCO2 resulted in an ameliorative effect leading to higher net photosynthetic rates under drought stress. The beneficial effect of eCO2 in sunflower by ameliorating the adverse effects of drought stress was confirmed.

Conroy et al. (1988) observed that sunflower plants were more drought-tolerant when water was withheld under conditions that favor osmotic adjustment, namely after previous acclimation to drought, when water deficits are slowly imposed or when [CO2] was higher than 340 ppm. As water deficits increase, both leaf conductance to [CO2] and the capacity of the mesophyll to fix CO2 decline. Osmotic adjustment occurred during drought in expanded leaves which had been continuously exposed to 660 ppm or had been previously acclimated to drought. The effect was greatest when the treatments were combined and was negligible in non-acclimated plants grown at 340 ppm of CO2.

CLIMATE CHANGE AND PATHOGENS

Climate change could influence development of the pathogen, host resistance and host-pathogen interaction (Coakley et al., 1999). Direct or indirect impacts (via canopy change) of climate change on sunflower disease complex are expected. However, very few information has been produced for sunflower diseases (Debaeke et al., 2014).

Primary infection could be limited by the lack of precipitation and evapotranspiration increase. To infect the plants, downy mildew *(Plasmopara halstedii)* requires about 50 mm of free water during the 10 days surrounding planting date. Sclerotinia head rot (*Sclerotinia sclerotiorum*) needs 42 hours of free water for infecting florets. Phoma black stem (*Phoma macdonaldii*) requires free water at the trough level for significant stem infection. Phomopsis stem canker (*Phomopsis/Diaporthe helianthi*) will develop initial leaf lesions if relative moisture exceeds 90 % during 36 hours within canopy. High temperatures or elevated VPD could slow down or stop the growth of fungi in the tissues as their thermal optimum often ranges from 15 to 25 °C. Several successive days with Tmax > 32°C could be lethal for Phomopsis. At the same time some pathogens could be promoted by hotter and dryer conditions. *Macrophomina phaseolina* could be stimulated by low soil water content and temperatures within 28-30 °C range (Sarova et al., 2003). Premature ripening due to Phoma could be enhanced by dry conditions after flowering (Seassau et al., 2010).

The weakest vegetative growth of sunflower exposed to early soil water deficit could reduce the risk of primary infection by fungi that directly cause damage to leaves and stems (Debaeke et al., 2014). More precipitation in winter and elevated [CO2] could promote plant growth and favour the development of associated diseases.

If sunflower move northward to be grown in new environments that are free of inoculum, less attacks are expected in a first time especially if sunflower is grown less frequently as a break crop.

Ecological conditions in the future will be probably less prone to the diseases responsible of yield losses today. But some dominance changes may occur between pathogens (and pathotypes) according to their thermal pLITERATURE and their dependency to free water. Pathogens with long conservation forms in the soil (e.g sclerotia) could better tolerate unfavourable periods. The damage due to systemic pathogens could be reinforced if plants are suffering from water stress.

CLIMATE CHANGE AND POLLINATORS

Sunflower, as an allogamic plant, needs insects on flowering, especially the honey bees and bumble bees for seed production (De Grandi-Hoffman and Watkins, 2000 ; Oz et al., 2009). Breeding system of self-incompatibility and pollen not well adapted to the transport by wind hinder the process of pollination by anemophily. Numerous experiments have found that a seed set as low as 10-20% results when pollinators are absent and plants self-pollinate, compared to up to 90% seed set in flower heads accessible to pollinators. However, cultivars have different levels of self-fertility, and many modern sunflowers are fully self-fertile. Cross-pollination may still be preferred, as it appears to give higher yields and better quality in terms of oil content. At the same time, collecting nectar and pollen by honey bees in sunflower crops is also essential to apiculture (Delaplane and Mayer 2000). Unlike other insects, bees visit a great number of flowers to fulfill the needs of their colony assisting pollination by the way (Müller et al., 2006).

Temperature, precipitation and extreme events associated to climate change could modify the activity of pollinators (Kjøhl et al, 2011). Having different climatic requirements, pollinators and plants may therefore respond differently to changes in ambient temperature. For example, increased spring temperatures may postpone plant flowering time while pollinators might be unaffected. As stated before, pollen fertility may be greatly reduced at high temperatures (Astiz and Fernandez, 2013), which increases the importance of prompt pollination of self-pollinated varieties during hot weather. Water stress resulting from climate change may decrease flower numbers and nectar production. Extreme climate events might have detrimental effects on both crop plants and pollinator populations. High temperatures, long periods of heavy rain and late frost may affect pollinator activity either by reducing population sizes or by affecting insect activity patterns. Sunny days with low wind speed and intermediate temperature are optimal foraging conditions for pollinators.

There is still clear evidence of declines in both wild and domesticated pollinators (e.g. honey bees) (Potts et al., 2010). Pollination is under threat from different kinds of environmental pressures including habitat loss and fragmentation, agrochemicals, pathogens, alien species, climate change and the interactions between them (Potts et al., 2010). Pollinator declines can result in loss of pollination services which have important negative ecological and economic impact that could significantly affect crop production and food security (Gallai et al., 2009). Because of cross-pollination in sunflower, seed production activity (for hybrids) and commercial grain production could be both affected by decline associated to climate change and other causes.

CROP ADAPTATION TO CLIMATE CHANGE

Plant breeding is considered to be a substantial tool for adaptation strategies to climate change (Ceccarelli et al., 2010). Breeding for new varieties better adapted to thermal shocks (heat, cold) and drought is often suggested as the major long-term adaptation. The breeding

strategies aim at improved water efficiency, improved drought stress tolerance, and increased responsiveness to higher atmospheric [CO2] (Ceccarelli et al., 2010; Ziska et al., 2012). However, prospective results of plant breeding are unforeseeable and the impact assessment would strongly depend on the assumptions made on breeding advances (Grass et al., 2015).

Short-term strategies have been identified from current practices to take advantage of more favorable growing conditions or to offset negative impacts: shifting sowing dates, changing cultivars (earliness), revising soil management, fertilization and plant protection practices, introducing or expanding irrigation. Crop management still offers a range of opportunities to cope with drought-prone conditions (Debaeke and Aboudrare, 2004).

In sunflower, planting date could be anticipated to escape water stress at flowering and during grain filling. In some Mediterranean regions, sunflower can be planted in late autumn or winter with good results in water use efficiency and yield (Gimeno et al., 1989; Soriano et al., 2004). In northern parts, earlier sowing date in spring was attempted with sometimes unsuccessful results (Alline, 2009). Varieties adapted to early planting with increased vigor should be selected to take advantage of this practice. Without irrigation, the search and use of cultivars with lower base temperatures and shorter thermal times for emergence will become of great importance. The compensation of reduced crop duration with increasing temperature could be searched by using long cycle cultivars combined with early sowing date.

Crop models have been applied in given situations or at a regional scale to simulate impacts of climate change on yield as a preliminary task for simulating possible adaptations. Guilioni et al (2010) using STICS model recommended to use late-maturing cultivars and early planting with some perspectives to increase yield in France. Donatelli et al. (2015) simulated simple technical adaptions with CropSyst model. Sowing date was shifted by either bringing forward or delaying sowing by either 10 or 20 calendar days with respect to the baseline sowing date. The other factor was the length of the biological cycle as a proxy for simulating varieties from different maturity groups. Growing degree days was manipulated to get a realistic variation of flowering and physiological maturity. These authors concluded that adaptation for rainfed sunflower was not completely effective under the 2030 time horizon in a large belt from central France to the most eastern area of Europe. However, it must be pointed out that such results were obtained via simple adjustment of technical management without exploring possibly improved varieties. Also, more favorable patterns of winter rainfall may lead to increased availability of water, hence maintaining the feasibility of irrigation.

Undoubtedly, supplemental irrigation is an effective way to maintain or increase sunflower yield (and oil concentration) in dry conditions (Rinaldi, 2001; Demir et al., 2006; Klocke et al., 2013) but future water resources could be limited because of competition among users. More water in winter could however be stored for securing summer irrigation when possible.

Rainfed sunflower crop production in Mediterranean environments depends to a large extent on strategies that avoid the intense summer drought. The use efficiency of scarce water resources should be increased by promoting soil conservation techniques e.g. mulching in no-till systems for reducing soil runoff and evaporation as was attempted in semi-arid regions for sunflower (Aboudrare et al., 2006).

Crop diversification (at field, farm or territory level) could be recommended as a self-insurance measure to cope with more uncertain and fluctuant conditions and bring resilience to the system. Sunflower could be more present in the situations where water resources are scarce. Double cropping could benefit from the longer cropping duration on an annual basis (Grass et al., 2015). Very early sunflower varieties could be planted after oilseed rape, barley or pea completing their cycle in late spring. However irrigation will be absolutely required for crop establishment while summer water availability could be restricted in some areas.

Model-based tools and site-specific technology should be developed to optimize, support and secure farmer's decisions. Adaptation could range from tactical fine-tuning to deep changes in the nature of cropping systems with impacts downstream on land use and agricultural sector activity (machinery, inputs, market).

REDUCTION OF GHG EMISSIONS WITH SUNFLOWER CROPPING

On average, the total emission of greenhouse gas (GHG) of sunflower in France is about 900 kg CO_2-eq ha^{-1}, according to a calculation based on the average input applications in France (BIO IS, 2010), the emission factors for the production and transportation of inputs used in France and from the tier 1 method of IPCC to estimate direct and indirect nitrous oxide emissions (De Klein et al., 2006) (Table 1). The emissions of nitrous oxide (N_2O) account for almost half of the total GHG emissions, while the fuel consumption and the mineral N fertilizers are respectively responsible for 23 and 22 % of the total. Overall in sunflower, almost 70% of the GHG emissions arise from N applications because the tier 1 method calculates N_2O emissions as a percentage of the amount of N applied on the field. Hence, the reduction of GHG emissions in sunflower should focus on both the improvement of N efficiency, in order to decrease the amount of N application, and on the control of NO_3^- leakage and NH_3 emissions because those N leakages from the field result in indirect N_2O emissions. Factors that control some soil properties, especially soil humidity and pH, could also contribute to decrease GHG emissions because they have a major role on N_2O emissions (Granli et Bøckman, 1994), but they are not taken into account in the tier 1 method. The reduction of fuel consumption, which is mainly due to soil tillage, would also significantly contribute to decrease the total GHG emission of sunflower.

The same pattern of GHG emissions is also observed in the main other crops cultivated in France (Figure 2). However, the shares of N fertilizer and N_2O emissions are higher in other crops, compared to sunflower, because the amounts of N applications are greater: 38 kg N ha^{-1} in sunflower (Table 1) vs. 97 to 189 kg N ha^{-1} in other crops (data not shown). Hence, the total emissions per hectare of other crops are 3 to 3.6 fold greater than that calculated for sunflower. For this reason, cultivating sunflower is an effective way to produce oilseeds with low GHG emissions, even though its seed yield is relatively low: the seed yields taken into account in the calculations, which are representative of the average values in France, are 2.39 t ha^{-1} for sunflower and 3.28 t ha^{-1} for rapeseed, resulting respectively in 376 kg CO_2-eq / t of seeds and 812 kg CO_2-eq / t of seeds (data not shown).

CONCLUSIONS

- Sunflower yield was simulated to potentially improve at northern latitudes with climate change, but with negative effects on yield at southern latitudes
- In the next future (2050), the elevated [CO2] in the atmosphere could compensate for negative impacts of high temperatures, water stress and reduced crop duration; the CO2 fertilization effect will not prevent yield decrease at 2070-2100 horizon.
- A wide range of genetic and agronomic adaptations have to be evaluated and combined.
- As a low GHG emitter, more attention should be paid on sunflower in future cropping systems.

Table 1 – Inputs used for sunflower cultivation in France, nitrous oxide and GHG emissions

Inputs and nitrous oxide emissions		**GHG emissions** (kg éq. CO_2 ha^{-1})
Mineral N fertilizers	38 kg N ha^{-1}	200.6
Mineral P fertilizers	29 kg P_2O_5 ha^{-1}	16.6
Mineral K fertilizers	22 kg K_20 ha^{-1}	11.6
Pesticides	3 kg ha^{-1}	23.1
Seeds	4 kg ha^{-1}	8.1
Fuel	67 l ha^{-1}	206.0
Seed drying	354 MJ ha^{-1}	9.3
Nitrous oxide	0.91 kg N-N_2O ha^{-1}	422.2
TOTAL		**897.6**

GHG emissions were calculated from mean input applications in France (BIO IS, 2010), the emission factors for the production and transportation of inputs used in France and from the tier 1 method of IPCC to estimate nitrous oxide emissions (De Klein et al., 2006).

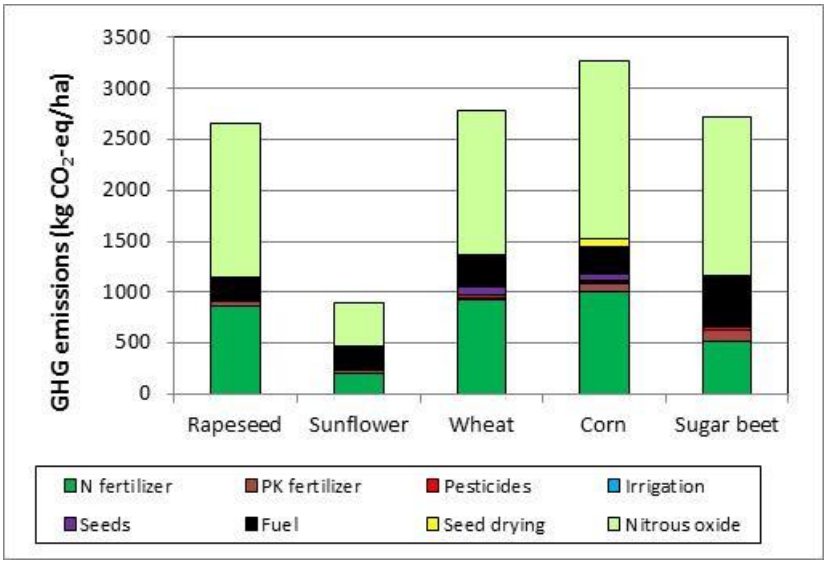

Figure 2 – GHG emissions of sunflower compared to other crops

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SUNFLOWER SEED OIL: A PREMIUM OIL FOR FOOD APPLICATIONS

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ABSTRACT

Sunflower seed oil is considered a premium oil owing to its light color, mild flavor and good oxidative stability. Conventional, also referred to as regular, sunflower seed oil is rich in unsaturated fatty acids (about 58% linoleic, an essential polyunsaturated fatty acid, and 29% oleic acid) and low in saturated fatty acids. High oleic (90% oleic acid), high-stearic/high-oleic (24% stearic and 62% oleic), high- stearic/high-linoleic (30% stearic and 50% linoleic), high-palmitic/high-linoleic (32% palmitic and 46% linoleic) and high-palmitic/high-oleic (29% palmitic and 56% oleic) sunflower seed oils have also been developed. Sunflower seed oil is one of the most desirable oils in the world and it is preferred over other vegetable oils such as soybean, cottonseed, and canola oils in many countries. It has typically been one of the oils that is marketed for retailing and domestic consumption either as pure sunflower seed oil or in vegetable oil blends used for cooking. In industry, sunflower seed oil has been widely used for frying, especially for frying snack foods. Emulsions, sauces and margarines are formulated with sunflower seed oil as well. Due to its bland taste, sunflower seed oil is an excellent flavor carrier and used for encapsulation of volatile oils and flavor compounds. The high stearic/high oleic sunflower oils can be fractionated to obtain products with high levels of solids and different melting profiles that can be used in wide variety of food formulations, including fillings, spreads, coatings, and confectionary products. This presentation will also highlight recent research on new food applications including oleogels and edible films and potential uses of oil bodies isolated from sunflower seeds as natural emulsifiers for edible formulations.

Key Words : Sunflower oil, quality, oxidative stability, fatty acids, other uses

SOURCE AND SINK AFFECT PHYTOSTEROL CONCENTRATION AND COMPOSITION OF SUNFLOWER OIL

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ABSTRACT

Phytosterols are oil compounds that help to reduce serum cholesterol. It is unknown if variations in source or sink during grain filling affect these compounds as in other quality traits (tocopherol concentration, fatty acid composition, etc.). The aim of this study was to evaluate the effect of variations in source or sink on phytosterols concentration and composition of the oil in sunflower genotypes with different fatty acid composition. Two field experiments were performed using a traditional, a high oleic (HO) and a high stearic-high oleic (HSHO) hybrid. At the beginning of grain filling treatments were applied to modify the source or sink: 75-80% leaf removal, 50% of grains removal and control. Oil phytosterols concentration and its composition were determined by GLC. In all hybrids, defoliation treatments increased phytosterols concentration in the oil, but reduced the content per grain, compared to the control. Removing grains increased the content of phytosterols per grain but it did not affect its oil concentration because oil per grain was also increased. The most abundant phytosterol was β−sitosterol, followed by Δ^7−stigmastenol, campesterol, stigmasterol and Δ^7−avenasterol. Variations in source or sink only in few cases modified phytosterol composition. In both experiments, the HSHO hybrid had a higher proportion of campesterol and stigmasterol and lower of β−sitosterol than traditional and HO. These results confirm that crop management is important not only for maximizing yield but also for obtaining a good oil quality.

INTRODUCTION

Bioactive compounds are molecules present in several foods that have benefits for health, like tocopherols and phytosterols, among the most important. In plants, phytosterols play an important role in the regulation of membrane fluidity and permeability (Schaller, 2003), embryogenesis (Clouse, 1996), and as precursors of brassinosteroid hormones involved in plant growth and development (Lindsey et al., 2003; Merah et al., 2012). In humans, they have properties as anti−cancer, anti−inflammatory, anti−oxidation activities and prevention of cardiovascular diseases (Hansel et al., 2011; Valerio and Awad, 2011). However, the reduction of total plasma cholesterol and low−density lipoprotein cholesterol (LDL) levels in humans is the best characterized role of phytosterols (Brufau et al., 2008). A meta−analysis of 41 trials showed that a phytosterol intake of 2 g/day reduced LDL cholesterol by 10% (Schwartz et al., 2008). Therefore these compounds help to control plasma cholesterol and prevent cardiovascular diseases (Brufau et al., 2008; Ostlund Jr, 2007; Palou et al., 2005; Roche et al., 2010a).

There is intra− and inter−specific variability in the amount and concentration of phytosterols in oils (Fernández-Cuesta et al., 2014; Fernández-Cuesta et al., 2011; Roche et al., 2010b). Among the vegetable oils, sunflower is characterized for presenting high concentration and good composition of antioxidants. In this species, a variation in oil phytosterols concentration between 3513 and 4936 mg/kg was found due to the environment (Nolasco et al., 2010). Differences up to 118 mg of phytosterols/100 g seed due to changes in sowing date were observed by Roche et al. (2010b). It is known that the amount of source or sink in sunflower partially explains variations in the amount of oil, the fatty acids concentration, and other grain components such as tocopherols (Izquierdo et al., 2008; Izquierdo et al., 2011; Ruiz and Maddonni, 2006). However it is unknown whether they also influence the synthesis of phytosterols, determining their concentration and final composition. So, the objective of this work was to investigate the effect of source or sink on the concentration and composition of phytosterols in sunflower oil. Understanding these effects is important because those management practices that affect the amount of source or sink of the crop (e.g. hybrid choice, sowing date, plant density, etc.) could not only affect yield but also the quality of the oil produced.

MATERIALS AND METHODS

Two field experiments were carried out in Balcarce (37ºS, 58ºO Argentina) during 2012/2013 (Exp 1) and 2014/2015 (Exp 2) growing seasons. A traditional (Macon), a high oleic (Olisun 2) and a high stearic−high oleic (HS05) genotype were used in Exp 1. The traditional and the high stearic−high oleic genotypes were used in Exp 2. The experiments were carried out with a split-plot design with three blocks, where the genotype was assigned to main plots and treatment to modify the amount of source or sink of the plant (F−D) to subplots. Treatments to modify the source or sink consisted of removing leaves or grains. The size of the subplots was six rows 0.70 m apart and 9 m long, at densities of 7 pl/m^2. Treatments were applied in early grain filling (R_6). The sowing date and applied treatments of each trail are present in Table 1.

Table 1: Sowing date and applied treatments of each trail.

	Sowing date	Treatments
Exp 1	30−oct	75% leaf removal ($D_{75\%}$),
		50% grain removal ($R_{50\%}$)
		Control (T)
Exp 2	22−oct	80% leaf removal ($D_{80\%}$),
		Control (T)

In both experiments, weed and pest were controlled and water and nutritional stress were prevented by irrigation and fertilization. Phenology was recorded as Schneiter and Miller (1981). Before flowering, capitula were covered with nylon pollination bags to prevent cross−pollination to preserve the fatty acid composition of each genotype. Plants were harvested after physiological maturity. Oil content was determined by nuclear magnetic resonance according to Robertson and Morrison (1979). Oil was extracted from grounded grain using n−hexane as solvent by percolation−immersion for 3 h at room temperature and 3h at 80ºC (Izquierdo et al., 2011). The amount and type of phytosterol were analyzed by gas chromatography (Fernández-Cuesta et al., 2012). The comparison of phytosterols

concentration, amount per grain and composition between genotypes and F−D treatments were performed by analysis of variance using R package (R CORE TEAM, 2012).

RESULTS AND DISCUSSION

Total phytosterols concentration

Total phytosterols concentration varied between 3157−5139 µg/g of oil, among experiments, genotypes and F−D treatments. These concentrations are higher than those observed by Nolasco et al. (2010), but were in the same range reported by CODEX STAN 210 (1999), that was between 1700−5300 µg/g of oil for traditional and high oleic sunflower. In both experiments, the interaction between genotype and F−D treatment on total phytosterols concentration were no significant ($p>0.35$).

Total phytosterols concentration was increased by leaf removal treatment ($D_{75\%}$ and $D_{80\%}$) compared to controls ($p<0.0022$,

Figure **1**). In Exp 1 grain removal did not affect total phytosterols concentration. Among the three genotypes, Macon presented the lowest phytosterols concentration (3674 vs >3900 µg/g). No significant variations were observed between hybrids in Exp 2. There are no reports in the literature related to the effect of changes in source and sink on phytosterols concentration. An increase of total phytosterols concentration with a decrease in available water was observed by Anastasi et al. (2010). But it is unknown whether the results reported by these authors are mediated by direct effects of water, or an effect on the source or sink of the plant.

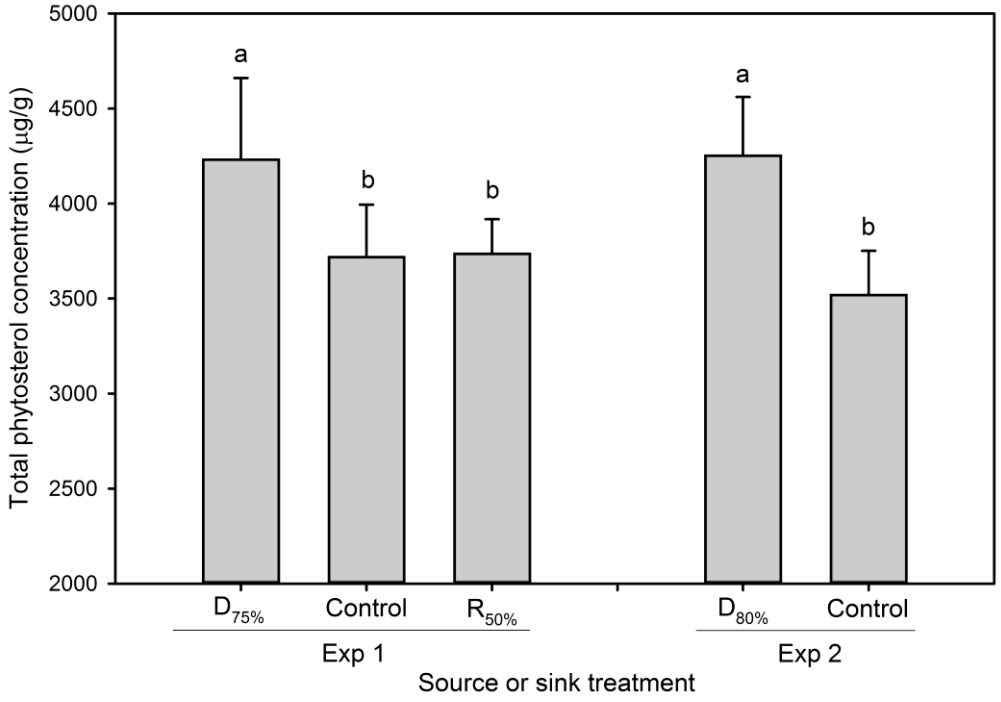

Figure 1: Total phytosterol concentration for each F−D treatment for Exp 1 and Exp 2. Means with the same letter are not significantly different in each experiment.

Total phytosterols per grain

The amount of phytosterols per grain varied between 57 and 118 µg/grain. Those values are similar to those reported by other authors (Anastasi et al., 2010; Fernández-Cuesta et al., 2014; Roche et al., 2010b). There was no significant variation between genotypes and F−D treatments in Exp 1 ($p>0.07$). $D_{75\%}$ presented the lowest value of total phytosterols per grain, followed by control and $R_{50\%}$ (

Figure **2**). Macon and Olisun 2 presented the greatest differences between F−D treatments (data not shown). In Exp 2, total phytosterols per grain were not modified by F−D treatments ($p>0.2688$,

Figure **2**). Higher total phytosterols per grain were observed in a high oleic genotype than traditional ones by Anastasi et al. (2010). However, traditional and high oleic genotypes presented similar total phytosterols per grain in our experiments. In both trials, the amount of phytosterols per grain was directly related to the weight of the grains. Thus, HS05 presented less total phytosterols per grain than Macon and Olisun 2 (63 vs >93 µg/ grain Exp 1, $p<0.0010$), due to lower grain weight.

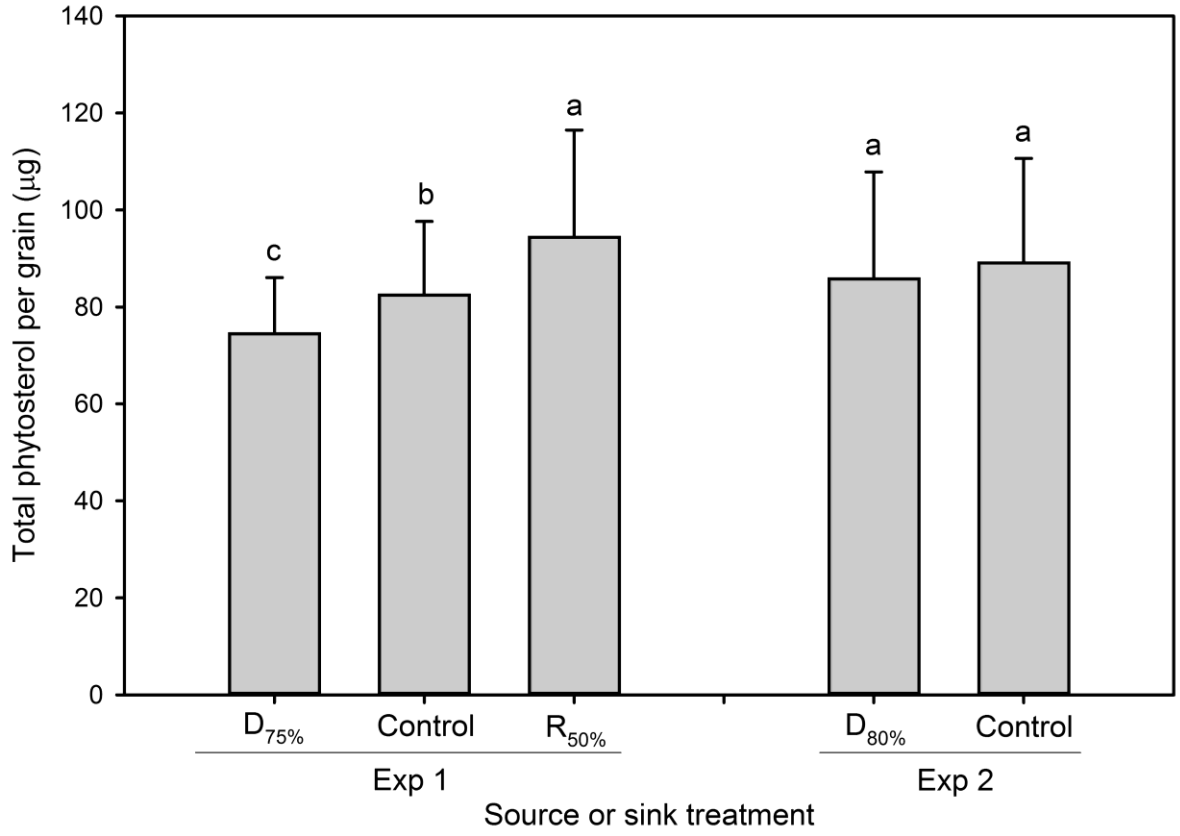

Figure 2: Total phytosterols per grain for each F−D treatment for Exp 1 and Exp 2. Means with the same letter are not significantly different in each experiment.

Phytosterols composition

The most abundant phytosterol was □−sitosterol (57% in average), followed by campesterol, □^{7}−stigmastenol and stigmasterol representing around 10% each of total phyosterols (

Figure **3**). The Δ7−avenasterol was found in concentrations close to 3% of total phytosterols identified. This composition of phytosterols is similar to that reported by other authors for the same species (A.O.C.S., 2014; Velasco et al., 2013).

Genetic variability for phytosterol composition was reported in sunflower (Merah et al., 2012; Roche et al., 2010b; Velasco et al., 2013). This variability was observed in the three hybrids used in our experiments. In both experiment, HS05 presented a lower β−sitoesterol and higher stigmasterol proportion than Macon. No significant variation in phytosterol composition was observed between Macon and Olisun 2.

The proportion of β−sitoesterol was not modified by F−D treatments ($p>0.0551$). $R_{50\%}$ increased 1.1 percentage points the campesterol proportion compared to control and $D_{75\%}$ in Exp 1. $D_{80\%}$ decreased 1.6 percentage points the proportion of campesterol compared to control in the HS05 hybrid, while this percentage did not change in Macon, in Exp 2.

Stigmasterol proportion increased in the order $D_{75\%}$, control and $R_{50\%}$ in Exp 1, whereas there was no difference between treatments in Exp 2. Interaction between genotype and F−D treatment was found for Δ7−stigmastenol and Δ7−avensterol proportion. $R_{50\%}$ increased Δ7−stigmastenol proportion than control and $D_{75\%}$ for Macon, whereas the opposite effects for Olisun 2 were observed. No significant variation in Δ7−stigmastenol and Δ7−avensterol proportion between F−D treatments in HS05 was observed. $R_{50\%}$ and $D_{75\%}$ were those with the highest and lowest Δ7−avensterol proportion, respectively in Macon, whereas the opposite effect was found in Olisun 2.

Anastasi et al. (2010) observed increases in the proportion of β−sitoesterol or campesterol when water availability was increased. On the other hand, Roche et al. (2010b) reported variations in phytosterols composition when sowing date was delayed, effect attributed to the variations in temperature during grain filling. Variations in phytosterols composition observed in our work cannot be attributed to water availability or temperature since all treatments were conducted under similar conditions and sowing date. So, these effects are explained by the variations in source or sink during grain filling.

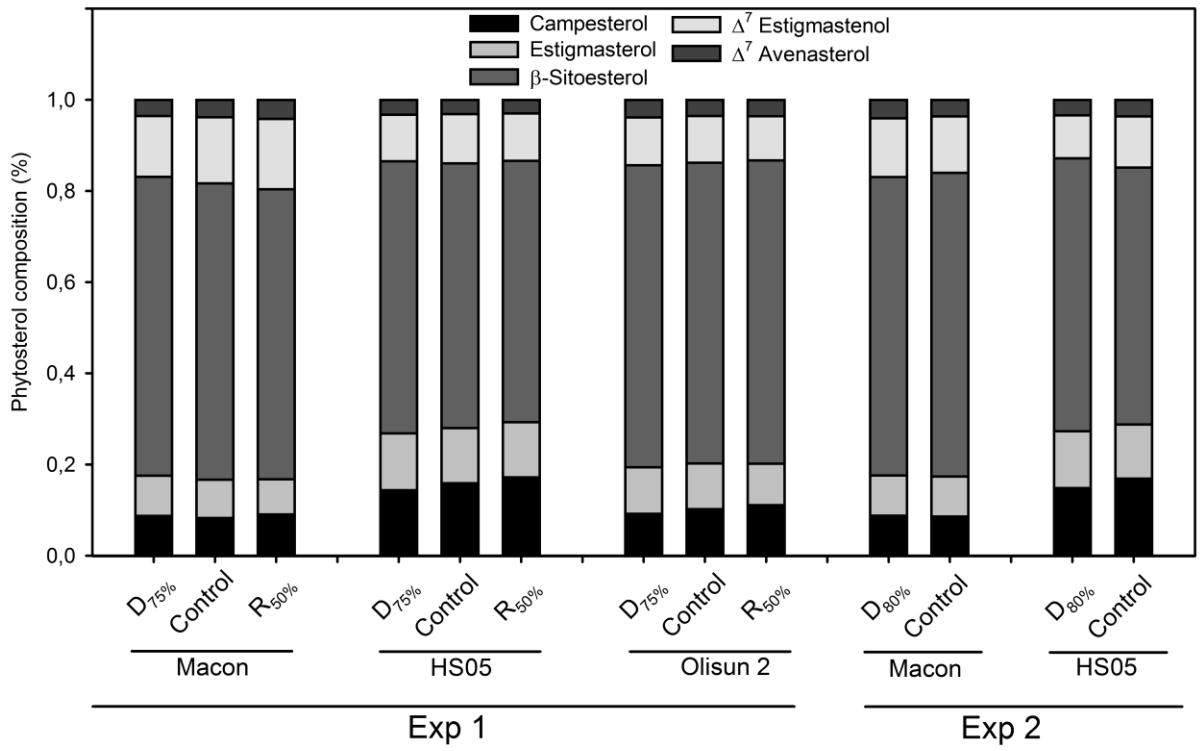

Figure 3: Phytosterol composition for each F−D treatment and genotype in Exp 1 and Exp 2.

CONCLUSIONS

In this work we observed that reductions in source of plants reduce the amount of phytosterols per grain and increase its concentration in the oil. These effects were similar in genotypes with different oil fatty acid composition. These results agree with those reported for other minor oil constituents such as tocopherols. More research is needed to understand the relation between minor oil constituents and oil biosynthesis and how their final concentration in the oil is determined in sunflower crops grown under different source or sink conditions.

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PHYSIOLOGY

DO CELL WALL PROTEINS AFFECT THE SETTING OF GRAINS AND THEIR POTENTIAL WEIGHT IN SUNFLOWER?

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ABSTRACT

Physiological bases of grain setting and potential grain weight are still partially understood in sunflower. There are evidences that grain number (GN) and weight (GW) are sensitive to environmental conditions immediately before flowering (R5) and during grain filling. Additionally, it has been pointed out that a better knowledge about the growth of maternal tissues of grains (ovary/pericarp) will improve the understanding of GN and potential GW settings, highlighting the key role of expansins (proteins controlling plant cell wall loosening). This study aimed to evaluate the impact of ectopic applications of cell wall proteins, including expansins, on GN and GW in sunflower. Two contrasting grain weight genotypes were sown in a split plot design with three replicates at the Agricultural Research Station (UACh), Chile. Cell wall proteins were extracted from sunflower seedlings and they were applied on the capitula at R4 or after 10 days of flowering (R5). Two control treatments (without proteins and only buffer applications) were also assessed. Extracts of proteins were assessed by SDS-PAGE and by mapping and database searches. Fresh and dry weight of ovaries and grains (dissecting pericarp and embryo) were recorded from R3 to physiological maturity. At harvest, GN, GW and oil concentration were measured. Proteins applied at R4 increased ($P<0.05$) GN (20%) and GW (30%) in both genotypes. Lower impact was found under applications at 10 days after flowering. Remarkably, oil concentration of grains was not affected ($P>0.05$). These results support that the growth of maternal tissues before R5 affects GN and potential GW in sunflower highlighting the likely key role of expansins.

Key Words : expansins, ovary, kernel, grain yield

THE GENETICS AND EVOLUTION OF SOLAR TRACKING

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ABSTRACT

The heliotropic movement of sunflower shoots, also known as solar tracking, is a dramatic example of a diurnal rhythm in plant growth. The shoot apex continuously tracks the sun's position in the sky as it changes from east at dawn to west at dusk over the course of the day. At night, the apex reorients back to an eastward orientation. As a sunflower reaches reproductive maturity, these cycles dampen, and disks predominantly maintain an eastward orientation at anthesis. Though these phenomena have long been observed, the developmental and molecular mechanisms by which external cues and internal rhythms are integrated to produce these diurnal patterns of growth are largely unknown. We have taken developmental and natural variation approaches at multiple evolutionary scales to understand the physiology, genetics, and diversity of these traits. Manipulative studies implicate the circadian clock as a driver of nocturnal reorientation and as a regulator of mature head orientation. Through phenotyping an association mapping panel of 280 cultivated sunflower lines with time-lapse imaging in the field, we have described ample diversity in the mean and variance of the diurnal phase of solar tracking movements and the orientation of mature disks, and we have identified several SNPs significantly associated with multiple solar tracking parameters. Finally, a survey of other diploid *Helianthus* species reveals that solar tracking is common among annuals and perennials with broad distributions but not found in basal rosette perennials of the southeastern US, suggesting this trait likely evolves as a component of a resource-acquisitive ecophysiological syndrome.

Key words: heliotropism, movement, circadian clock, phototropism, natural variation, association mapping

INTRODUCTION

Plants experience daily predictable cycles in the availability of resources and in the occurrence of environmental stresses. To cope with these oscillating environmental conditions, many aspects of plant growth, development, and physiology are adapted occur with diurnal rhythms such that peak activity coincides with the most favorable portion of a 24-h period. Although fluctuations of external cues like light or temperature may be the sole drivers of these diurnal plant traits, more often internal rhythms driven by the endogenous circadian clock also play an essential role in jointly coordinating these biological cycles (Alabadi and Blazquez, 2009; Harmer, 2009). Clock regulation is especially important for activities that must anticipate the availability of resources or the onset of environmental pressures, as waiting to directly experience these factors as cues may leave plants with insufficient time to mount fully effective responses, (e.g., activating metabolic or physiological defenses against diurnally active herbivores and pathogens; Wang et al., 2011).

Solar tracking, or heliotropism, of the growing stems of the common sunflower, *Helianthus annuus*, is perhaps the most conspicuous example of a diurnal growth trait in the plant kingdom (Vandenbrink et al., 2014; Kutschera and Briggs, 2016). During the day, the stem grows such that the shoot apex continuously reorients to remain normal to incident sunlight throughout the day, thus tracing a path from facing east at dawn to facing west at dusk (Fig. 1). The stem also reorients at night such that the shoot apex once again faces east in anticipation of dawn (Fig 1). Both movements appears to be largely driven by growth through irreversible cell expansion, as sunflower lacks specialized motor organs known as pulvini that promote reversible, turgor-driven heliotropism of leaves in other systems (Koller, 2001).

Heliotropic movement begins soon after sunflower seedlings begin expanding their true leaves but then slows as plants approach anthesis, at which point the plants stop tracking and maintain an easterly orientation until senescence (Shibaoka and Yamaki, 1959; Lang and Begg, 1979). This final point has been subject to a long-running misconception. For centuries, many authors have erroneously stated that mature heads do track the sun (e.g., Gerarde, 1597; Kircher, 1667; Koller, 2011), leading those who have then failed to observe floral heliotropism to dismiss the phenomenon entirely (Gerarde, 1597; Meehan, 1884; Kellerman, 1889). However, seminal studies corrected the literature by publishing photographic evidence of the daily movements of young plants (Schaffner, 1898, 1900).

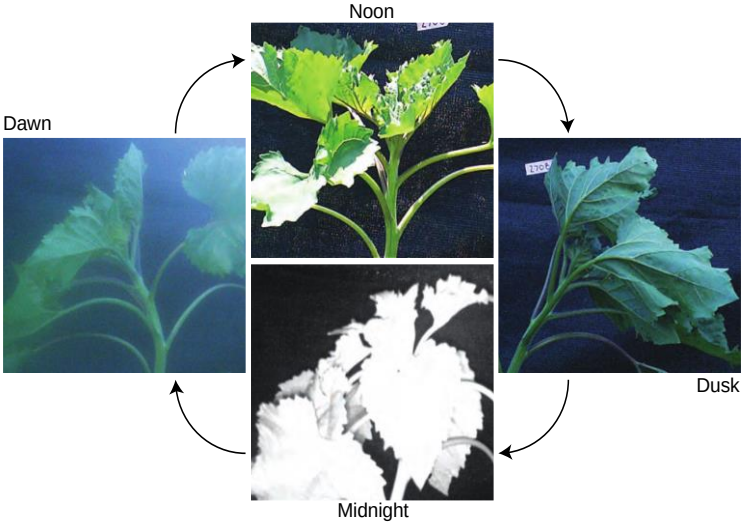

Fig. 4: Solar tracking and noctural reorientation of the sunflower stem. East was to the left and west to the right of the plant filmed in this series. Midnight photo taken with infrared LED flash built into camera.

Although the solar tracking of sunflower stems has been observed for centuries, the biological mechanisms that govern this behavior and the evolutionary history of the trait have received little attention (Shibaoka and Yamaki, 1959; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). For instance, while we know a moving light source is a critical driver of heliotropic bending, how this signal from the changing relative position of the sun is perceived and how it leads to differential growth of lateral stem segments paced to the sun's east-to-west trajectory are largely unknown. Moreover, although an endogenous rhythm has been implicated in the regulation of solar tracking because plants rotated 180º take several days to fully match their growth to the new orientation (Shibaoka and Yamaki, 1959), the nature of this rhythm and its interactions with environmental signals are also not understood. Finally, the evolutionary history of solar tracking, the abundance of natural variation in this trait, as well as the ecological functions of heliotropism and the eastward orientation of mature disks have been little explored.

Here, we discuss what is known with respect to the first two physiological questions and also report several aspects of our work in progress that aims to address the final evolutionary question using a variety of approaches. First, we review previous studies on the regulation of solar tracking. Then, we report an initial assessment of natural variation in the timing of nocturnal reorientation using a recently generated association mapping panel of cultivated

sunflower. Finally, we discuss how our preliminary survey of diversity in solar tracking within the genus indicates how solar tracking may serve as part of a larger ecophysiological syndrome adapted for resource acquisition.

POSSIBLE MECHANISMS REGULATING SOLAR TRACKING

Surprisingly little has been published on the physiological mechanisms underlying solar tracking despite the long period over which this trait has been recognized (Schaffner, 1898, 1900; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). Given that sunflowers do not have pulvini, it is very likely that the movements of solar tracking stems are due to asymmetric growth on the two sides of the stem, as has been reported for the petioles of leaves undergoing rhythmic 'sleep movements' (Pfeffer, 1903). The coincident timing with which solar tracking and leaf cell expansion cease at anthesis has also led several authors to infer that solar tracking is a growth mediated process (Lang and Begg, 1979; Koller, 2001). However, unlike rhythmic leaf movements, the initiation of solar tracking requires cues from the environment. Strongly directional light is clearly required to drive stem movements during the day. Plants grown under stationary overhead light in greenhouses or growth chambers do not track (Shell and Lang, 1976; B. Blackman, S. Harmer, personal observation), and several investigators have reported instances in which young plants have failed to track on cloudy or rainy days (Schaffner, 1898; Shibaoka and Yamaki, 1959). It is very likely that the daily east-to-west movements of sunflower plants is auxin-mediated and is initiated by the well-studied phototropin signaling pathway (Fankhauser and Christie, 2015).

However, no strong directional light source exists in nature that can explain the stereotyped west-to-east nocturnal reorientation of sunflower stems. We suggest that this directional movement at night in anticipation of dawn may be generated by circadian regulation of growth pathways. Several lines of evidence support this possibility. For instance, resetting of solar tracking movements takes several days when plants are experimentally rotated 180º during the night (Shibaoka and Yamaki, 1959). In addition, under long day photoperiods, the speed of stem movement must be and is substantially more rapid at night than during the day for the shoot apex to face east by dawn (Schaffner, 1898, 1900; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). Finally, in some instances developing buds have been observed to achieve their eastward orientation well ahead of dawn (Shell and Lang, 1975; B. Blackman, personal observation). These observations all suggest involvement of an endogenous mechanism in solar tracking.

We therefore predict that the circadian clock provides the mechanistic basis for the endogenous rhythms that interact with directional light signaling and other environmental cues to drive solar tracking and nocturnal reorientation. In particular, we expect that the circadian clock drives diurnal rhythms in the abundance or activity of light signaling components and hormones that drive differential stem growth (Foster and Morgan, 1995; Millar and Kay, 1996; Jouve et al., 1999; Covington et al., 2008). The circadian clock may also gate how responsive plants are to these stimuli at particular times of day, following a paradigm that has been developed through the study of plant growth and organ expansion in controlled environmental conditions (Covington and Harmer, 2007; Nozue et al., 2007; Arana et al., 2011). We are currently conducting organismal and molecular experiments that will allow us to better understand the physiological mechanisms underlying solar tracking under naturally fluctuating field conditions and, in doing so, to determine whether the circadian clock does in fact play an instrumental role in governing one or more aspects of this fascinating plant growth behavior.

ASSOCIATION MAPPING IDENTIFIES NATURAL VARIANTS ASSOCIATED WITH SOLAR TRACKING

Natural variation can also provide a useful entry point to begin connecting genotype to phenotype and thus to understand the molecular basis of particular traits. We have complemented our ongoing developmental studies by taking an association mapping approach to further characterize the molecular mechanisms that regulate solar tracking. Concerted efforts by the Compositae Genome Project and the Sunflower Genome Consortium over the past decade have produced a panel of 288 lines that harbor ~90% of the common alleles segregating in cultivated sunflower (Kane et al., 2011; Mandel et al., 2011, 2013; Bachlava et al., 2012; Bowers et al., 2012). This panel is a tremendous resource. Because the genotypes are known and the lines are largely inbred and homozygous, any phenotype that can be scored on the panel can be quickly associated with single nucleotide polymorphisms (SNPs). Moreover, because this panel has been thoroughly genotyped by a succession of genomic methods over time with release of whole-genome resequencing data for the whole panel imminent, the genotypic resolution for association mapping is becoming comprehensive and high-resolution (Mandel et al., 2013; Nambeesan et al., 2015).

We have phenotyped the sunflower association mapping panel for solar tracking at a field plot at Morven Farm, VA, a property owned by the University of Virginia Foundation. Because filming all lines concurrently was prohibitively costly and difficult, we planted three replicates per line across a series of fifteen staged plants. Replicates were evenly distributed such that each accession had one replicate grown in the first third of the plantings, one in the middle third of the plantings, and one in the final third of the plantings. For a given replicate, three seeds were sown in a five-gallon paint bucket containing local soil mixed with 10% compost and with several holes drilled in the bottom for drainage. Plants were watered once or twice daily dependent on local conditions and plant size, and thinning was performed two weeks after germination.

Plants were filmed ~5 weeks on average after sowing, during the developmental period after budding but well before anthesis for most accessions. For filming, the buckets were placed in front of a matte black backdrop, and we used Bushnell X-8 trail cameras to capture images every 10 min for 48 to 72 h. The resulting time-lapse videos were visually evaluated for several traits, including the timing of nocturnal reorientation (i.e., the time relative to dusk when the stem first appears to move eastward instead of westward). The compass orientation of heads at anthesis was also scored on all plants. Association mapping was conducted for the means and coefficients of variation for each trait using a mixed-linear model that controlled for population structure and kinship in TASSEL v3.0 (Bradbury et al., 2007; Zhang et al., 2010). Genotypic data for the panel consisted of ~5.8K SNPs previously scored using an Illumina Infinium SNP array (Mandel et al., 2013).

We observed abundant variability in the timing of nocturnal reorientation in the association mapping panel. While the majority of lines began nocturnal reorientation within 30 minutes before or after dusk (mean = -6.2 ± 2.5 min), a notable number of lines began nocturnal reorientation over an hour earlier or later than dusk (Fig. 2A). The variability of this trait within lines also varied among lines. That is, for lines where three replicates were scored, we observed that the standard deviation in the timing of nocturnal reorientation ranged from 2 min to 2 h.

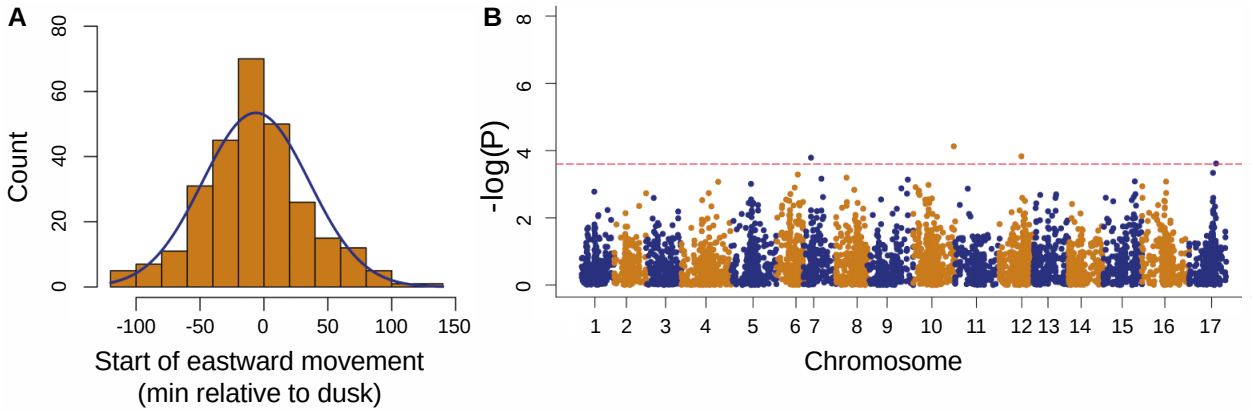

Figure 5: Phentoypic and genetic variation in timing of nocturnal reorientation. (A) Distribution of 288 cultivated sunflower lines scored by time-lapse photography for the time relative to dusk when the stem begins to reorient toward East. (B) Manhattan plot illustrating the significance level of associations tests for ~5.8K SNPs with the timing of nocturnal reorientation. Dashed red line indicates significance threshold after correction for multiple tests.

Association mapping yielded several SNPs significantly associated with variation in the mean timing of nocturnal reorientation (Fig. 2B). The significantly associated SNPs are located in annotated transcripts homologous to a mitochondrial ATP synthase G subunit family protein, NAD(P)H-quinon oxidoreductase subunit L, a DnaJ domain transcription factor, and a DTW domain-containing protein. We also detected several SNPs associated with variability in mature head orientation, including a homolog of the core circadian clock component *LATE ELONGATED HYPOCOTYL* (*LHY*), possibly corroborating a role for the clock in solar tracking traits.

The limited number of significant SNPs observed may reflect the genetic architecture of intraspecific variation in this trait. Traits largely governed by many rare alleles and/or common alleles of moderate effect typically show similar patterns. However, these findings may also reflect the limited sampling of genomic space provided by the current genotypic dataset. We expect the strength of our approach to improve as the full resequencing dataset for the association mapping panel becomes available. That data will be very helpful for determining whether these genes or closely linked genes are best associated with the trait and thus most likely to have a causal influence. Moreover, we expect a sizable portion of the genome is not in strong linkage disequilibrium with any of the SNPs in the current sample, and thus there may be ample potential to detect additional significantly associated polymorphisms.

SOLAR TRACKING: A RESOURCE-ACQUISITIVE ECOPHYSIOLOGICAL SYNDROME TRAIT?

Solar tracking has been most remarked upon and studied in wild and cultivated populations of the common sunflower, *Helianthus annuus*. However, Schaffner also observed

solar tracking of the stems of two other wild *Helianthus* species over 100 years ago (Schaffner, 1898, 1900). These old observations raise several questions. How far back in the sunflower lineage did this behavior evolve? Is solar tracking evolutionarily labile? Does solar tracking demonstrate correlated evolution with other characters as part of a broader ecophysiological syndrome?

To address these questions, we filmed a subset of the diploid *Helianthus* species during the summers of 2014 and 2015 at our field site at Morven Farm outside of Charlottesville, VA, USA. Seeds were scarified and germinated on moist Whatman paper in Petri dishes in the dark for up to 7 days. After one day of light exposure, the seedlings were transplanted into cell packs containing a 1:1 mixture by weight of Fafard 3B soil and calcined clay. Seedlings were raised for up to four weeks in the University of Virginia Greenhouses under 16 h days before transplantation into the ground or into buckets filled with soil at our field site. Stems were filmed for 72 to 96 h during the developmental period after budding but before anthesis. Images captured every 5 or 10 min, and the resulting time-lapse videos were visually evaluated for evidence of tracking.

A revised, generally well resolved phylogeny of diploid *Helianthus* developed through sequencing and analysis of 170 nuclear genes was recently published (Fig. 3; Stephens et al., 2015). When considered on this tree, our preliminary findings show a striking pattern of character evolution for solar tracking. The phylogeny resolves the genus into three majors clades: annuals, erect perennials with widespread distributions in North America, and perennials mostly endemic to the southeastern United States that often grow as basal rosettes. In our diversity survey, we observed solar tracking for all members sampled from both the annual and widespread perennial clades (Fig. 3). We also observed solar tracking for another member of the widespread perennial clade not included in the diploid tree because the species consists of both diploid and polyploid populations, *H. decapetalus*, and Schaffner reported tracking of the polyploid *H. pauciflorus*, which belongs to this clade as well (Schaffner, 1898). In contrast, we did not observe solar tracking for any of the members of the southeastern perennials sampled or for additional closely related but poorly resolved perennial species (Fig. 3). Although some of these taxa do grow as basal rosettes (*H. atrorubens*, *H. radula*, *H. occidentalis*), others do not (*H. floridanus*, *H. mollis*). Thus the pattern we observe cannot be explained solely by constraints on internode elongation during the period of active leaf expansion.

Notably, a recent macroevolutionary analysis reported similar phylogenetic patterns for many leaf economics spectrum and resource use traits (Mason and Donovan, 2015). That is, correlated patterns of evolution were observed such that the annual and widespread perennial clades appear to evolve a correlated syndrome of resource-acquisitive trait values (e.g., deltoid leaves, greater vein length per unit area, higher stomatal conductance). In contrast, the southeastern perennial clade appears to evolve toward a syndrome of resource-conservative trait values (i.e., lanceolate or acuminate leaves, lower vein length per unit area, lower stomatal conductance). If more comprehensive sampling confirms the similar preliminary pattern we observe for solar tracking, then these findings would corroborate the hypothesis that solar tracking serves a critical function in enhancing resource acquisition, a longstanding idea that has been difficult to test empirically. Because we have not been able to grow and film an outgroup to the genus and yet observe tracking of *H. porteri*, the most basally diverging taxon with in the genus, the important question of when and in what lineage solar tracking first evolved remains unresolved. In addition, due to poor resolution of branching events ancestral to the southeastern perennial clade, some uncertainty remains about how

strictly congruent the transition to a resource-conservative ecophysiological syndrome is with the evolutionary loss of solar tracking.

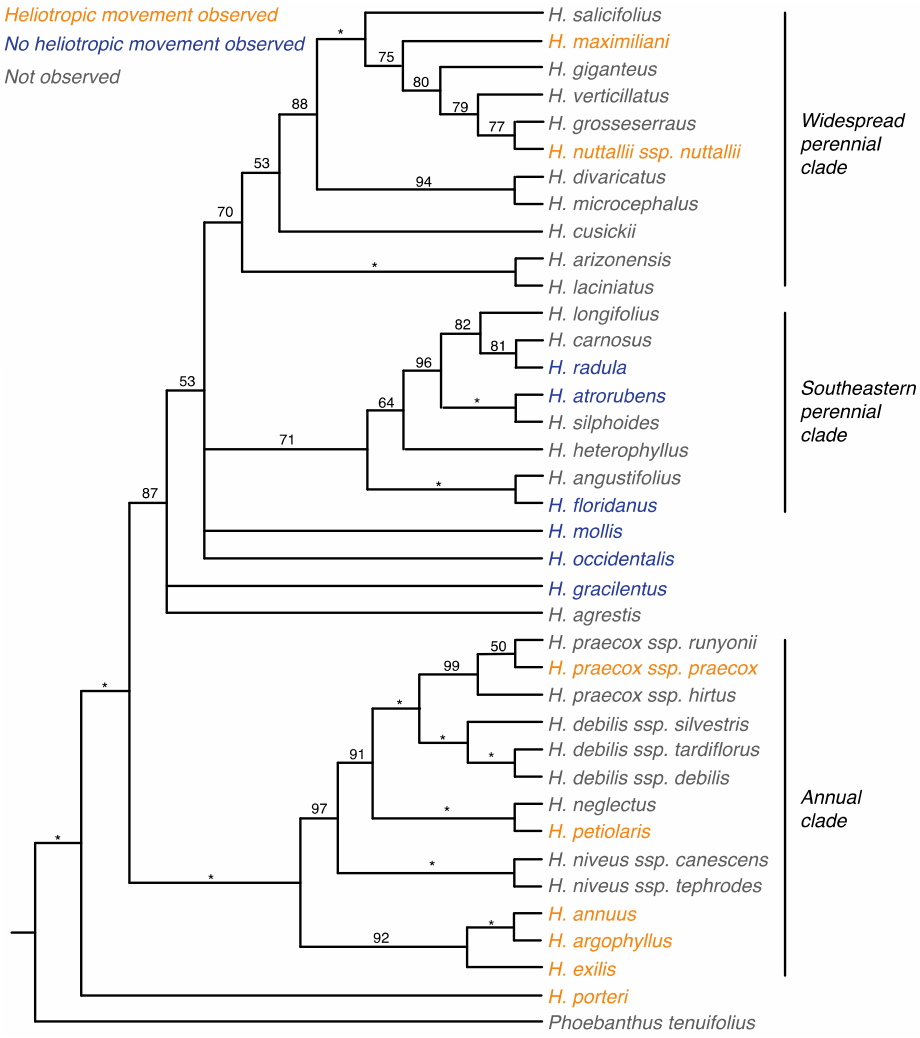

Figure 6: Phylogenetic survey of solar tracking. Species names are colored by trait status (see inset). Figure adapted from Stephens *et al.* 2015. We also observed that a diploid accession of *H. decapetalus*, a member of the widespread perennial clade not included in the species tree does exhibit solar tracking. The species tree was constructed with Maximum Pseudo-likelihood Estimation of the Species Tree v1.4 (MP-EST; Liu *et al.* 2010). Boostrap support provided for nodes, asterisks indicate bootstrap support = 100. Nodes with <50 bootstrap support collapsed.

CONCLUSIONS AND FUTURE DIRECTIONS

It has been extensively shown in several systems under controlled conditions that the interaction of the circadian clock with external signals drives diurnal cycles of light signaling components and hormones that play essential roles in directional plant growth. By focusing on solar tracking as a model system, we are working to determine whether this paradigm also holds true for a growth trait that impacts plant fitness in changing natural environments. Natural variation shows great promise as an experimental means of learning about these underlying mechanisms, and we expect the release of whole genome resequencing data for the cultivated sunflower association mapping panel to enhance these efforts dramatically. In

addition, the diversity in solar tracking that we have observed among *Helianthus* species appears to provide insight into the function of solar tracking as part of an ecophysiological syndrome of evolutionary correlated traits that enhance resource acquisition. Comparative developmental and transcriptomic studies across species that do and do not track may also prove a fruitful means of gaining understanding into the mechanisms that regulate this fascinating plant growth trait.

ACKNOWLEDGEMENTS

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EVALUATION OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) SINGLE CROSS HYBRIDS UNDER HEAT STRESS CONDITION

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ABSTRACT

Sunflower is an important oilseed crop which shows susceptibility to heat stress. In present study, 63 single cross hybrids were evaluated under heat stress condition for two years and compared with the two commercial hybrids. Genotype and genotype × environment (GGE) was used to differentiate single cross hybrids on the basis of multiple traits. GGE biplot showed that several single cross hybrids had higher seed yield potential than standard checks. Moreover, SYP was related with pollen viability showing that achene yield was product of high gametophytic fertility under heat stress. Hybrids having high seed yield potential under heat stress had lower cell membrane injury (CMI) showing that potential hybrids could be selected on the basis of CMI during seedling stages. GGE biplot for SYP and its components showed that single cross hybrids were characterized into two major groups. Group I was further characterized into two sub group. Group Ia included hybrids with high 100-SW, while group Ib had the hybrids with high number of achenes head^{-1} and head diameter. Group II had the hybrids with high kernel weight and kernel to achene ratio. The hybrids could be recommended according to their potential utilization in the seed industry.

Key words: Achene, gametes, kernel, cell membrane injury, heterosis, biplot

INTRODUCTION

Heat stress is a major production constraint in summer crops which causes significant repressing effects on grain yield and quality (Kalyar et al. 2014; Niazi et al. 2014). Sunflower reproductive cycle has also been subjected to heat stress which reduces the achene and oil yield in various parts of the world (Rondanini et al. 2003; Rondanini et al. 2006; Kalyar et al. 2014; Van der Merwe et al. 2015). High temperature induced fine tuning of canopy architecture such as leaf and petioles angles (Kalyar et al. 2013b). Moreover heat stress reduces the pollen viability, seed germination, leaf area, reproductive biomass and increased cell membrane injuries when sunflower breeding populations were subjected to the reproductive heat stress (Kalyar et al. 2014). It was observed that heat stress accelerate the heat unit accumulation and thus reduce the growth period (Rondanini et al. 2003). The brief exposures of capitula to the heat stress > 35°C decreased the grain weight and oil contents by 40% and 30% respectively due to reduction in growth period (Rondanini et al. 2003). Exposure of temperature greater than 29°C at 10-12 days after anthesis for period of 7 days reduced grain yield by 6% (Rondanini et al. 2006). Corbineau et al. (2002) noted that exposure of the sunflower seed to high temperature of 45°C inhibits its germination and it also increased the electrolyte leakage in the incubation medium. High temperature was also

known to modify oil quality and oleic acid has been shown to increase at the expense of linoleic acid (Flagella et al. 2002; Rondanini et al. 2006).

Sunflower segregating and advanced germplasm has been screened with various selection criteria for the introgression of heat resistance in inbred lines. Kalyar et al. (2013a) selected segregating plants maintaining medium leaf temperature (T_{leaf}) under heat stress. The plants which maintained medium T_{leaf} had high values of leaf gas exchange traits. The usefulness of this trait was also depicted from high heritability, selection gains and its positive relationship with reproductive biomass. Similarly, Kalyar et al. (2013b) identified differences within F_2 population with respect to leaf inclination and upward leaf inclination was found useful to avoid high post noon temperature and cell membrane injury. Differences between pre and post noon leaf temperature (Δ) was useful criteria for selection of heat resistant plants. Δ was also found useful due to presence of high realized heritability and provided sustainability to achene and oil yield under heat stress when plants differing for Δ were compared in F_4 generation (Kalyar 2015). On the basis of these grounds, progenies obtained from the selection of high Δ were advanced to derive 19 CMS lines which were crossed with 5 different restorer lines to develop 63 single cross hybrids. These single cross hybrids were then tested under heat stress condition to select prominent hybrids under prevailing conditions.

MATERIAL AND METHODS

The studies were carried out in the research field of the Department of Plant Breeding & Genetics, University College of Agriculture, Sargodha during the year 2014-15.

DEVELOPMENT OF PLANT MATERIAL

Development of heat stress resistant plant material was started in 2008. Heat resistant breeding material was selected in promising F_2 crosses. Selection was carried out on the basis of plant ability to maintain low post noon temperature as compared to pre noon leaf temperature (Δ). Effectiveness of the selection was further tested in selected progenies on the basis of genetic gain and realized heritability. Promising plants were evaluated for general combining ability. The superior lines were then converted to cytoplasmic lines in back cross scheme. Sixty three single cross hybrids were developed by using 19 cytoplasmic male sterile line and 5 male restorer lines in all possible combinations i.e. each of female line with each of male sterile lines. The lines were selected on the basis of their relative heat tolerance. 63 combinations were obtained from the controlled mating of these lines. In order to obtain cross combinations, male and female lines were planted in experimental field during the crop season February 2013 and 2014. Each line was grown in single row of 4.5 meter containing 14 plants. All female lines floral heads were covered with net bags to avoid pollen contaminations due to insect pollinators. Pollen from each male restorer lines was collected in early morning and dusted over the female line with the help of the camel brush. Pollination was continued until all the stigmas withered on floral head. The developed seed from each cross combination was harvested separately from mature heads, dried and stored in cool place for cultivation in next crop season.

EVALUATION OF PLANT MATERIALS

63 single cross hybrids along with check variety HYSUN-33 and S-278 were evaluated for heat stress tolerance in randomized complete block design with three replications at field research area of University College of Agriculture, University of Sargodha during the cropping season 2014-2015. Sowing was done on raised bed of 75 cm consecutively for two years on March 15, 2014 and March 18, 2015 to expose the hybrids reproductive growth cycle to the high temperature of May-June (Figure 1).

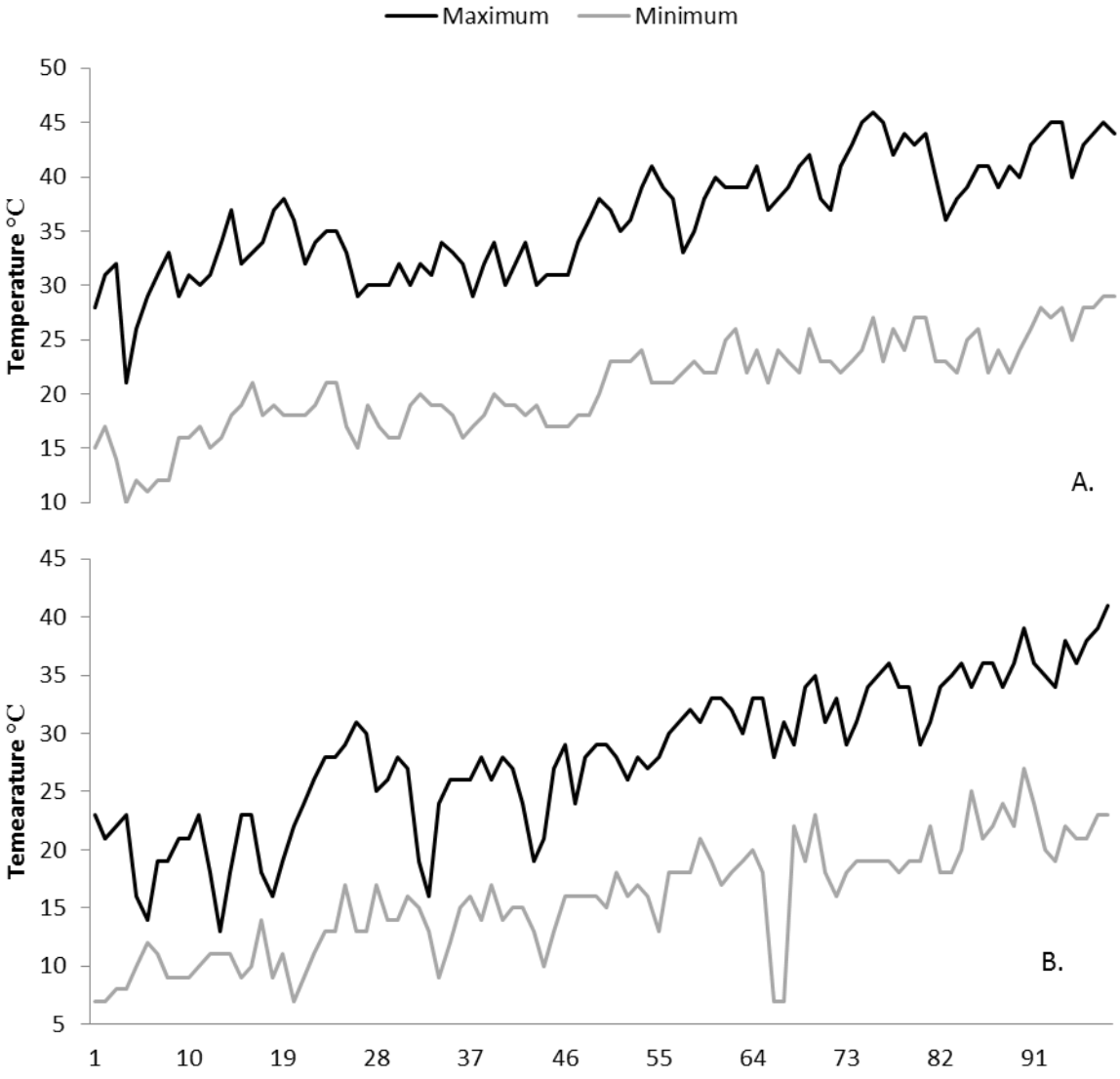

Figure 1. Mean maximum and minimum temperature during the crop season year 2014 and 2015

Soil texture and minerals analysis showed a sandy loam type of soil with EC=2.91±0.19; EC=2.84±0.33; pH=7.16±0.11 pH=7.07±0.14; available potassium was 226±10.34 and 187 ±7.52 and available phosphorous was 17.34±3.69 and 21.58±5.92 during the year 2014-15 at the time of sowing. Each hybrid was sown in three rows each of 4.5 meter with plant to plant distance of 30 cm. The crop was irrigated from canal water to avoid the water stress condition when soil moisture contents were below the field capacity. The field capacity of the soil was 14% by weight measured through gravimetric method. The fertility of

soil was raised by using inorganic urea and diammonium phosphorus fertilizer at the rate of 70 kg acre^{-1} of nitrogen and 45 kg acre^{-1} of phosphorous. Pest scouting was carried out at regular interval and insecticide was sprayed to control the attack of armyworm (200 mL lufenuron, Match ® Syngenta) and red pumpkin beetles (250 / 100 mL dichlorovos, Diptrex) before they exceed threshold level during the year 2014 and 2015 respectively. Herbicide S-metachlor, Dual gold ® Syngenta was used to control pre-emergent weeds after sowing. The crop was evaluated for various plant traits. Pollen viability was measured at the time of anthesis while yield and yield components were determined at the time of maturity.

POLLEN VIABILITY

Pollen viability was estimated by 2% tri-phenyl tetrazolium chloride stain (Prasad et al. 2006). Disc florets were obtained from each head rows during early in the morning (08:00 h) from each plant. Anthers were sequezzed to obtain pollen grains. The sequzed pollen were collected on clean slides. Pollen were stained by adding a drop of stain. The stained pollen with reddish purple color were considered alive due to formation of insoluble red formazan. The reading was noted 30 min 30 min after staining under (10x) light microscope. Pollen viability was noted as the ratio of stained redish pollen to the total pollen.

MORPHOLOGICAL TRAITS

Seed yield was determined by harvesting five heads from each middle row of each hybrid from each block. Heads were manually threshed and seed was dried to uniform moisture content (12%). Dried seed harvested from each head in a row was weight over digital balance to calculate seed yield head^{-1}. Kernel mass was measured by removing the seed coat of 100 seeds. Kernels mass were measured over the analytical balance. Dead seed was determined by carefully examining 100-kernels and all the black kernels or achenes without any kernels were considered as dead. Dead seed (%) was calculated by dividing no. of dead seed to the total no. of seeds observed. Random sample of 100 seeds was counted manually and mass was measured over digital balance. No. of achene head-^{1} was counted through seed counter. Plant height was measured with measuring tape from stem base to the attachment of head. Head diameter was also measured with scale. Oil contents were estimated through petroleum ether extraction on Soxhlet apparatus. Achene size was determined through vernier caliper.

CELL MEMBRANE INJURY

Cell membrane injuries (CMI) were determined through electrolyte leakage in the leaf disc in the department of Agronomy, University of Agriculture, Faisalabad. In order to determine the cell membrane injuries in sunflower single cross hybrids, seedlings were raised in small pots of 15 × 4 cm. Two seeds were sown in each pot which was thinned to single seedling after germination (DAE). First true leaves were tagged on seedling and leaves of similar age (15 DAE) were used to determine the cell membrane injury. Temperature was maintained at 25±2 and humidity was 45%. Photon flux density was 650 µmol m^{-2} s^{-1}. Experiment was laid out in completely randomized design with six replications. Four leaf discs of 5mm in size were dissected from each leaf. Leaf disc were put in glass vial. Two set of leaf disc having three vials for each hybrid was created by treating the leaf disc with two temperature regime. One set was kept at room temperature 25 °C and other set received a

treatment of high temperature 42-50°C for one hour with 2 °C increment after every 10 minutes. 20 mL of deionized water was added to each vial after two temperature treatment. Vials were incubated at 10°C for 12 hours, afterward electric conductivity of both treatments was determined. All vials were autoclaved at 121 °C and final electric conductivity was measured. Cell membrane injury was determined using following formula:

CMI%=(1-(T1/T2))/(1-(C1/C2)) ×100

%injury= (100-CMS)

where T1 and T2 are treatment conductivities before and after autoclaving and C1 and C2 are the respective control conductivities.

BIOMETRICAL PROCEDURES

Data obtained was subjected to the analyses of variance under factorial arrangement where hybrids and years were considered as factors. Biometrical parameters such as genotypic, phenotypic, GCV% and heritability over year were measured as outlined by Allard (1960) where $\sigma^2 g$ (genotypic) = (MSg-MSgl)/rY where MSg is the mean sum of square due to hybrids, MSgl is mean sum of square due to interaction of hybrids × year and r= replication and Y was year). $\sigma^2 p$ (phenotypic)= $\sigma^2 g + \sigma^2 gl + \sigma^2 e$. Heriatbility = ($\sigma^2 g / \sigma^2 p$) x 100 ($\sigma^2 g$ = genotypic variance/σ^2 p=phenotypic variance). Genotypic coefficient of variation (GC%) = ($\sigma^2 g$ /X) × 100. A genotype plus genotype by environment (GGE) analysis (Yan & Kang 2003) was carried out to analyze the heat tolerance traits and yield in order to select promising single cross hybrids under heat stress. Another biplot was developed to study the relationship of yield components and select promising hybrids on the basis of multiple yield contributing traits. The traits were standardized before the analysis in accordance with different scales of the chosen variables. The biplot calculations were made using the 'scale' and 'svd' procedures of the R software (R 2013).

RESULTS

Analysis of variance showed significant variation ($P \leq 0.01$) for single cross hybrids and years for all traits under study. However, interaction due to single cross hybrids and year was significant ($P \leq 0.05$) for traits such as seed yield plant^{-1} (SYP), head diameter (HD), leaf area (LA), dead seed (DS%), kernel weight (KW) and kernel to seed ratio (K to S). Significant ($P \geq 0.05$) interaction showed that single cross hybrids changed their relative ranking for these traits. Hertiability estimates for various yield and its components was moderate to low. Heritability estimates for SYP was moderate which showed that high yielding hybrids may be directly selected through SYP per se under heat stress environment. Traits such as 100-SW, PH, DS%, KW and K to S had moderate heritability while traits such as HD, PV and LA had low heritability (Table 1-2). Among the traits, the highest heritability was shown by SPH. Thus, SPH could also be used along with SYP to determine the yield potential of hybrids under heat stress environment. Among the traits the highest phenotypic variation was shown by leaf area and seed yield while SYP showed the highest genotypic variation among single cross hybrids.

GGE biplot analysis was carried out to characterize single cross hybrids on the basis of multiple traits relevant to SYP and adaptability under heat stress. GGE biplot analysis characterized the germplasm into two groups on the basis of four selected traits (Fig. 2.). Group 1 included the hybrids with high yield and pollen viability while group II included single cross hybrids with high CMI and DS%. The traits such as PV and SYP were close to each other showing positive relationship between the two traits during the year 2015. It was

also concluded that SYP could also be dependent over high PV under heat stress. Therefore, simultaneous selection for PV and SYP could also be practiced. Hybrids such as H-32 and H-21 had high SYP with good PV during the year 2014 (Fig. 2a) while H-35 had high SYP and PV during the year 2015 (Fig. 2b). Hysun-33 had low SYP with high DS% under heat stress. There was also relationship between CMI and DS%. Hybrid H-51, H-48 and H-32 had the highest DS (ratio) while H-7 and H-26 had high CMI and DS during the year 2014 and respectively 2015.

GGE-biplot yield and its components have been shown in Fig. 4a and 4b. GGE-biplot showed that all chosen traits had positive relationship with yield. Traits characterized the single cross hybrids into two groups. The single cross hybrids were grouped on the basis of HD, SYP, SPH and 100-SW in group I. The yield components such as HD, SPH and 100-SW were found close to SYP and had positive relationship with SYP. H-35 showed the highest HD and 100-SW during the year 2014, and thus seed yield was dependent over seed size in this hybrid (Fig. 4a). H-27 and H-29 had high SYP and NSH during the year 2014 while the same hybrid had the highest SYP, NSH, 100-SW and HD during the year 2015 (Fig. 4b). During the year 2015, H-35 was also characterized as high yielder but its yield was not dependent over higher values of morophological traits. Group II included hybrids with high KW% and K to S ratio. Hybrids with high KW and K to S ratio were good for industrial exploitation. Hybrids such as H-58 and H-40 had the highest KW and K to S ratio in both years (Fig. 4a and 4b).

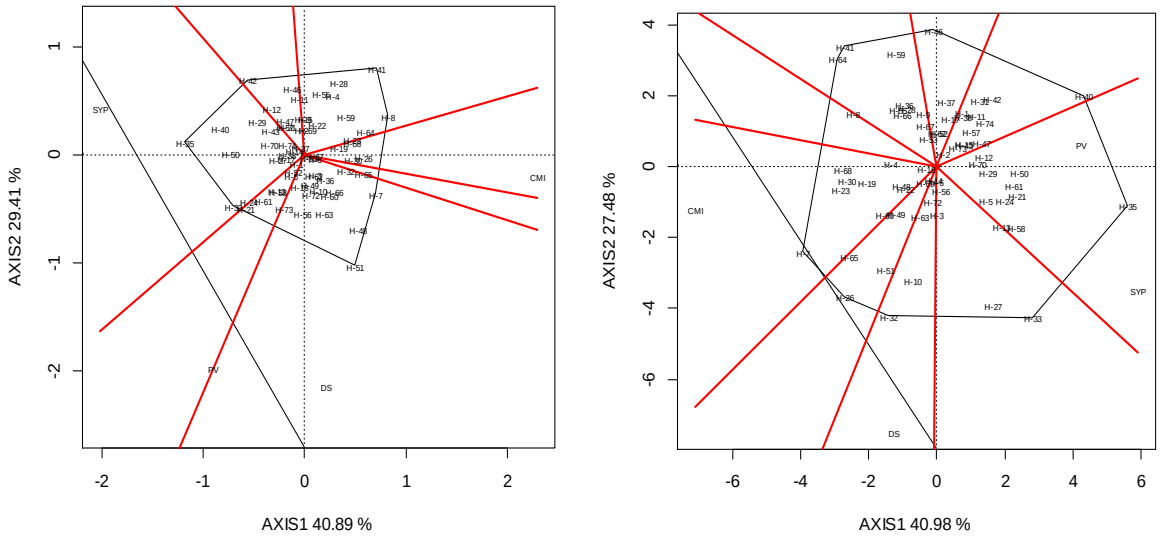

Figure 2. GGE biplot for standardized seed yield plant^{-1}(SYP), pollen viability (PV), cell membrane injury (CMI) and dead seed ratio (DS) for sixty five single cross hybrids under heat stress for year 2014 (a) and 2015 (b). All traits were averaged across replications for each combination of Genotype-by-Trait. The first principal component axis (PC1) retains 49% and the second 29% and 27% of sum of squares for year 2014 and 2015 respectively.

GGE-biplot yield and its components have been shown in Fig. 3a and 3b. GGE-biplot showed that all chosen traits had positive relationship with yield. Traits characterized the single cross hybrids into two groups. The single cross hybrids were grouped on the basis of HD, SYP, SPH and 100-SW in group I. The yield components such as HD, SPH and 100-SW were found close to SYP and had positive relationship with SYP. H-35 showed the highest HD and 100-SW during the year 2014, and thus seed yield was dependent over seed size in

this hybrid (Fig. 4a). H-27 and H-29 had high SYP and NSH during the year 2014 while the same hybrid had the highest SYP, NSH, 100-SW and HD during the year 2015 (Fig. 4b). During the year 2015, H-35 was also characterized as high yielder but its yield was not dependent over higher values of morophological traits. Group II included hybrids with high KW% and K to S ratio. Hybrids with high KW and K to S ratio were good for industrial exploitation. Hybrids such as H-58 and H-40 had the highest KW and K to S ratio in both years (Fig. 3a and 3b).

Mean values of promising hybrids have been shown in Table 1. Results showed that commercial hybrid S-278 had the highest oil contents% followed by the H-58 and H-29. Other commercial hybrid HYSUN-33 had significant lower oil content% than promising hybrid H-58 and H-29. Commercial hybrid S-278 had the lowest oil yield (Table 3). Hybrid H-35 also had higher oil yield than commercial hybrids but had very low oil contents and could be considered as non-oil seed type. On the other hand hybrids H-58 and H-29 could be regarded as oilseed type hybrids for cultivation under heat stress condition. Achene size was estimated on the basis of achene length, width and area. H-35 had the highest while commercial hybrids S-278 had the lowest achene size (Table 1). H-58 had lower achene width than commercial hybrids HYSUN-33.

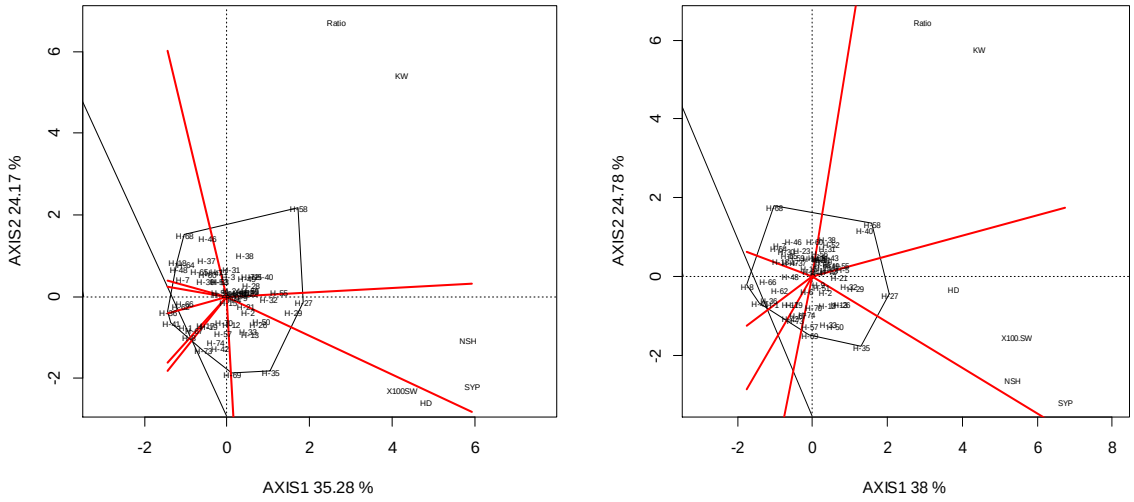

Figure 3. GGE biplot for standardized head diameter (HD), seed head^{-1} (SPH), seed yield (SY) and 100-seed weight (100-SW), kernel weight (KW), kernel to seed ratio (ratio) for sixty five single cross hybrids under heat stress for year 2014 (a) and 2015(b). HD, SPH, SY, KW and ratio were averaged across replications and years for each combination of Genotype-by-Trait. The first principal component axis (PC1) retains 35%, 39% and the second 24% and 25% of sum of squares for year 2014 and 2015 respectively.

Table 1. Variation in mean performance of promising single cross hybrids along with standard hybrids for traits related achene and oil contents.

Hybrid	Year	Oil Content%	Oil Yield (g)	Achene		
				Length	Width	Area
H-58	1	35.32±1.15	27.53±3.47	10.81±0.07	5.27±0.10	56.97±2.38
	2	38.27±2.19	34.83±2.91	9.59±0.05	5.53±0.06	53.03±3.16
	Average	36.79b	31.18a	10.20d	5.21e	53.14d
H-29	1	35.71±2.08	29.85±1.98	11.01±0.05	5.85±0.04	64.41±2.64
	2	36.12±1.52	34.31±2.07	10.52±0.06	5.19±0.08	54.60±3.18
	Average	35.92b	32.08a	10.77c	5.52d	59.42c
H-35	1	24.37±2.65	30.46±3.42	12.85±0.08	7.27±0.05	93.42±1.89
	2	26.48±1.69	35.22±2.77	13.68±0.04	7.85±0.02	107.39±2.38
	Average	25.43d	32.84a	13.27a	7.56a	100.28a
H-27	1	25.29±2.39	29.84±3.41	10.84±0.05	6.48±0.06	70.24±1.93
	2	27.12±1.71	26.04±2.82	11.15±0.06	7.10±0.08	79.17±2.12
	Average	26.21d	27.94b	11.00b	6.79b	74.66b
S-278	1	42.13±1.92	10.95±3.19	9.65±0.08	4.47±0.09	43.14±2.26
	2	39.48±2.19	9.08±2.64	10.52±0.03	5.16±0.06	54.28±2.67
	Average	40.81a	10.02d	10.09e	4.82f	48.56e
Hysun-33	1	32.72±1.09	19.30±1.97	10.70±0.09	5.82±0.08	62.27±3.42
	2	34.16±1.28	19.81±2.14	9.83±0.06	5.63±0.03	55.34±2.34
	Average	33.44c	19.56c	10.27d	5.73c	58.77c

DISCUSSION

Heat stress was one of major production constraints in sunflower which caused significant yield losses of sunflower (Kalyar et al. 2014; Rondanini et al. 2006; Kalyar et al. 2014; Van der Merwe et al. 2015). It was observed that heat stress accelerate the heat unit accumulation and thus reduce the growth period (Rondanini et al. 2003). The brief exposures of capitula to the heat stress > 35°C decreased the grain weight and oil contents by 40% and 30% respectively due to reduction in growth period (Rondanini et al. 2003). Exposure of temperature greater than 29°C at 10-12 days after anthesis for period of 7 days reduced grain yield by 6% (Rondanini et al. 2006). Corbineau et al. (2002) noted that exposure of the sunflower seed to high temperature of 45°C inhibits its germination and it also increased the electrolyte leakage in the incubation medium.

In this study, plant material was originally selected for heat resistance on the basis of (Δ) in the initial segregating generation (Kalyar 2015). Δ was also found useful due to presence of high realized heritability and provided sustainability to achene and oil yield under heat stress when plants differing for Δ were compared in F_4 generation (Kalyar 2015). In comparison to the commercial hybrids, selected progenies showed an advantage of 5%, 47%, 5% and 45% for oil contents (OC%), 100-SW, HD and seedling survival % respectively while 62% and 75% lower unfilled grain% and pollen sterility% over commercial hybrids. Promising progenies were converted to the cytoplasm male sterile lines and mated to the diverse restorer lines to generate single cross hybrids. These hybrids showed substantial genetic variation and moderate heritability for seed yield under heat stress. The variation for seed yield appears due to differences in pollen viability, cell membrane injury and dead

seed%. High yielding hybrids had higher pollen viability showing high seed yield was function of higher gametophytic tolerance (Coast et al. 2015; Das et al. 2014). It has been noticed earlier that heat stress reduces the pollen viability in various species and pollen viability was used as marker to differentiate heat tolerant genotypes (Coast et al. 2015; Das et al. 2014; Kalyar 2015). Hybrids showing high pollen viability were negatively related with cell membrane injury (r^2=-0.43). Therefore, Cell membrane injury could be used to discriminate sunflower hybrids for heat resistance during seedling. Presence of relationship between the pollen viability show that seedling stage heat resistance was also depicted in adult phase heat resistance (Fokar et al. 1998). Lowered cell membrane injuries also tend to reduce the dead seed% (r^2=0.35). High dead seed% affects the oil content to greater extent and thus selection for lower CMI tends to reduce oil yield losses under heat stress (Kalyar et al. 2014).

GGE biplot analysis also partitioned the single crosses hybrids on the basis of various yield components. Generally, GGE biplot partitioned hybrids into two major groups on the basis of yield component. Group I included hybrid on the basis of high 100-SW, seed head^{-1} and head diameter. These traits were significant contributor for seed yield. This group contained two type of hybrids i.e. high seed yield due to higher 100-SW. Thus 100-SW was an important yield component and indicated the importance of greater grain filling or greater seed size for high seed yield. Increased grain filling% has been an important contributor of seed yield under heat stress and indicative of better photosynthates mobilization and food reserve mobilization (Kalyar et al. 2014). However, high 100-SW also tends to reduce seed per head and increase seed size which may reduce the oil yield potential of hybrids. However, our study indicated in-significant ($P \geq 0.05$) relationship between seed per head and 100-SW (r^2=0.15) showing mixed response of hybrids to the increased 100-SW. Another sub group included hybrids with high seed yield head^{-1} and head diameter. Thus photosynthetic process was used to maximize the number of seed per head through high head diameter (Rauf & Sadaqat 2008). Group II included hybrids with high kernel weight and kernel to seed ratio. Both traits are positive contributors to oil yield extraction and thus hybrids with these traits were considered superior for oil yield potential.

It is concluded from the above results that hybrid H-29 and H-58 could be regarded as potential high oil yielding hybrid with good achene yield potential and moderately high heat tolerance. On the other hand, H-35 was promising non-oil seed hybrid with high heat resistance.

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EXPLORING DROUGHT TOLERANCE RELATED TRAITS IN (*HELIANTHUS ARGOPHYLLUS, HELIANTHUS ANNUUS*) AND THEIR HYBRIDS

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ABSTRACT

Drought is major constraint for sunflower (*Helianthus annuus*) production worldwide. Drought tolerance traits have been identified in the related wild species *Helianthus argophyllus*. A study was initiated to develop sunflower drought tolerant germplasm by crossing cultivated sunflower with this species and analyze drought tolerance traits in the interspecific plant material. *H. annuus* and *H. argophyllus* populations, *H.annuus* intraspecific hybrids and *H. annuus* × *H. argophyllus* interspecific hybrids were grown along with the commercial hybrid Hysun-33 under three stress regimes induced by PEG-6000 (T_3) and application of abscisic acid through foliar spraying (T_1) or irrigation (T_2), along with a control (T_0). Morpho physiological traits and growth regulators contents were assessed. Exogenous application of ABA, both by foliar spray and irrigation, had a negative impact on leaf area, stomatal conductance, transpiration and photosynthesis. Across the different groups of germplasm, there was a negative association between leaf area and stomatal conductance and a positive association between excised leaf water content, stomatal conductance and cuticular wax content. *H. argophyllus* populations had a significantly lower leaf area and higher water use efficiency, leaf cuticular wax content under all treatments and maintained higher net photosynthetic rate and stomatal conductance under osmotic stress. Small leaf area and high cuticular waxes content of the wild species were however not inherited in interspecific hybrids, suggesting to rather select transgressive segregants in F2 for these traits. The higher accumulation of indole-acetic acid observed under stress in *H. argophyllus* could contribute for a more developed root system. *H. argophyllus* lines accumulated less zeatin and gibberellic acid and more abscisic acid than the other groups of germplasm, but were able to maintain a higher zeatin content under stress that could result in maintaining growth and biomass. *H. argophyllus* populations and interspecific hybrids showed significant lower accumulation of silicium in all stress environments, reflecting reduced transpiration. These results are discussed with a view of using *H. argophyllus* to improve drought tolerance in cultivated sunflower.

Key words: cuticular wax, growth regulators, interspecific crosses, leaf area, leaf gas exchange parameters, silicium

INTRODUCTION

Drought is a major yield limiting factor for sunflower (*Helianthus annuus* L.) production in semi-arid regions (Ravishankar and others 1991, Rauf 2008, Rauf and others 2015). Oil and achene yield losses due to drought stress were reported in various parts of the

world (Jasinkas 1999; Kazi and others 2002; Hussain and others 2010; Woli and others 2014; Yin and others 2014). Water shortage induces significant modifications in physiological and biochemical processes involved in biomass production and modifies dry matter partitioning (Rauf and Sadaqat 2007; Rauf and Sadaqat 2008a Baloğlu and others 2012; Fernández and others 2012). Breeding for drought tolerance is consequently essential to reduce yield losses in sunflower in drought-prone areas (Adiredjo and others 2014; Rauf and others 2015). In order to identify possible sources of drought tolerance, cultivated sunflower germplasm from diverse sources was evaluated on the basis of relative performance under drought stress (Rauf and Sadaqat 2008b). However, the narrow genetic base of cultivated germplasm has limited the scope of these studies.

Some sunflower wild related species have been reported as drought tolerant and the introgression of traits from these species is expected to increase drought tolerance in the cultivated germplasm (Jan and others 2014). Among the related species, *Helianthus argophyllus* L. was identified as particularly drought tolerant (Jan and others 2014). Based on this information an experiment was carried out to identify possible traits of drought tolerance in *H. annuus*, *H. argophyllus* and their interspecific progenies, by using abscisic acid to induce drought like symptoms and by irrigating plants with a solution of polyethylene glycol (PEG-6000) to create osmotic stress. Genotypic variations were previously observed in sunflower plants submitted to exogenous application of ABA for production of abscisic acid (Ouvrard and others 1996) and maintenance of relative water content and yield (Hussain and others 2010) while the use of PEG has proven to be efficient to screen for tolerance to osmotic stress in this crop (Khalil and others 2015). In the present study, the traits assessed included morphological traits (leaf area, plant height, biomass), physiological characters (net photosynthetic rate and stomatal conductance, excised leaf water loss, epicuticular wax content, leaf silicium content), and plant regulators (gibberellic, indol acetic and abscisic acids and zeatin) contents. These traits are relevant to the drought tolerance breeding and therefore could indicate the type of resistance in sunflower genotypes.

MATERIALS AND METHODS

Plant Material

Three *H. argophyllus* accessions (ARG-1805, ARG-1802 and ARG-1806), introduced from the USDA germplasm collection, and two CMS lines of cultivated sunflower (*H. annuus*) were used in this study. The two lines (CMS-14 and CMS-20) were crossed with *H. argophyllus* accessions to develop six interspecific hybrids. The commercial drought tolerant hybrid Hysun-33 was used as a check. Details of plant material have been shown in Table 1.

Seeds were germinated in large polythene bags containing equal volume of sand, silt and field soil. After thirty days *H. argophyllus* plants, sensible to the photoperiod, were transferred to the field for induction of flowering. At 120 days after emergence, plants were covered from 15:00 to 7:00 during 45 days with black cloth to provide 16 hour dark period. On the other hand, *H. annuus* lines were sown after the start of black cloth treatment to the *H. argophyllus* populations to synchronize flowering. Pollen of the *H. argophyllus* plants was collected at 7:00 in the morning and deposited with the help of a camel brush over the stigma of the CMS lines. Meanwhile, heads of CMS lines were covered with white cloth bags to avoid foreign pollen contamination. Several rounds of pollination were carried out until stigmas completely wither. Interspecific hybrid seeds, as well as seeds of *H. argophyllus* and CMS lines were collected. CMS lines were maintained by tying the floral buds and pollinating the same plants with maintainer lines. Four hybrids were created by crossing *H. annuus* L. female lines CMS-14 and CMS-20 with male fertile lines R-12 and R-18.

Experimental conditions

All the plant material including parental lines and hybrids were evaluated in growth chamber, at the Plant Breeding & Genetics Department, College of Agriculture, University of Sargodha, Pakistan. Two seeds of each genotype were germinated in boxes (10 × 9cm) containing 8.5 kg of field soil: sand: silt. There were three boxes genotypes^{-1}. Temperature was maintained at 25±2°C while humidity was maintained at 40% and light intensity at 650 μmolm^{-2}s^{-1} in all treatments. Treatments included a control (treatment T_0) and three stress treatments. Drought like symptoms were induced through foliar (treatment T_1) and irrigational application (treatment T_2) of 8 μ mol of abscisic acid from germination and at 4 days intervals according to Shinozaki and others (2000), Tuteja (2007) and Fujita and others (2011). Osmotic stress was generated by irrigating plants with a solution containing 50 g L^{-1} of polyethylene glycol (PEG-6000) according to Khalil and others (2015) (treatment T_3).

Plant measurements

Leaf area was assessed using a CI-302 leaf area meter (CID-Bioscience, Camas, USA). Plant height was measured from the base to the top of canopy. Biomass was measured on digital balance. Leaf gas exchange parameters were determined on 26 days old leaves from the top of canopy by using a photosystem CI-340 (CID-Bioscience Camas, USA). Temperature was maintained at 25±2°C while humidity was maintained at 40% and light intensity at 650 μ mol m^{-2}s^{-1} in all treatments. Water use efficiency was ratio of net photosynthesis rate to transpiration (Pn E^{-1})

Excised leaf water loss was assessed according to Dhanda and others (1989), 45 days after emergence, on fully expanded leaves (ie, fifteen days old leaves) at the 2nd node from the top of canopy. Leaves were removed from the plants and their fresh mass was measured immediately on an analytical balance. Leaves were kept at 25°C for six hours to determine wilting leaf mass. Finally leaves were oven dried at 70°C for 24 hours to determine dry mass. Excised leaf water loss was calculated as (fresh leaf mass – wilted leaf mass)/(fresh leaf mass – dry leaf mass).

Epicuticular waxes were determined by the method of Ebercon and others (1977) at 45 days after emergence, on expanded (15 days old) leaves from the top of canopy. Leaf disc of known size (30 cm^2) were dipped in 15 ml pre-distilled chloroform at 25°C for one hour. The extract was filtered, chloroform was evaporated and 5ml of reagent was added to each sample. The reagent was prepared by dissolving 20 g potassium dichromate in 40 ml distilled water. The solution was mixed and further heated for 30 minutes in a concentrated 1 liter H_2SO_4 to make it colorless. Samples were cooled and 12 ml of distilled water was added to each sample. The samples were kept at room and color change was awaited. Optical density (590 nm) of the sample was measured using a spectrophotometer. The standards were prepared by obtaining cuticular waxes from large samples of wild sunflower leaves dipped in chloroform. Obtained waxes (300mg) were mixed in three replicates with the 5ml reagent to each of the replicates and heated until the standard became colorless. The standards were further prepared by mixing stock solution of 0.1 ml, 0.5ml, 1ml, 2ml, 3ml and 5ml to get final concentration of 1μg ml^{-1}, 5 μg ml^{-1}, 10 μg ml^{-1}, 20 μg ml^{-1}, 30 μg ml^{-1} and 50 μg ml^{-1} of standard solution.

Leaf silicium content (μg L^{-1}) was determined according to Dai and others (2005). Leaves samples of each line and crosses were oven dried at 60 °C for at least 7 days. Dried samples were grounded and passed through sieve of 60-mesh. The samples were again dried at 60 °C for 48 hours. 100-mg of sample was poured into polyethylene tubes and 3ml of 50% NaOH was added to each sample. All tubes were covered with loose plastic caps. Tubes were vortex and afterward autoclaved at 121°C for 20 minutes. Volume of plastic tubes was adjusted to

50ml through ddH_2O. 1 ml sample was added to volumetric flask which was further added up by 30 ml of 20% acetic acid and 10 ml of ammonium molybdate (54g L^{-1}, pH 7.0). 5 mL of 20% tartaric acid was added to the tube after 5 min interval followed by 1 ml reducing solution. Volume was adjusted at 50 ml by 20% acetic acid. Measurement was done at 650 nm on spectrophotometer (UV 2600).Standards were prepared by taking 1g ultrapure SiO_2 and slowly heating it to 1000°C in muffle furnace. Temperature was stabilized at 1000°C for 1 hour. 0.1g of treated SiO_2 was further transferred to nickel crucible and slowly heated to 1000°C after adding 2g of Na_2CO_3 to form a lucent melt. Crucible was taken out from the furnace and 5mL of boiling ddH_2O was added in the crucible. The melt was transferred to plastic bottle which was further dissolved by adding 150 mL of ddH_2O. Finally the volume was raised to 1000 ml in volumetric flask and solution was transferred to plastic bottle. Bottle contained stock solution of 0.1 mg mL^{-1} of SiO_2.

Plant growth regulators were determined according to Ergün and others (2002). Each plant sample (leaves) (2g) was grounded in a 60 ml solution (methanol: chloroform: 2N ammonium hydroxide, 12:5:3 v/v/v). The obtained plant extract was treated with 25 ml distilled water. Upper whitish phase was aspirated and chloroform phase discarded. Whitish phase (water-methanol) was further used to evaporate the methanol in extract through rotary evaporator. Obtained water phase was treated with 15 ml ethyl acetate at 2.5, 7 to obtain free form of plant growth regulator. The extract was adjusted at 11 pH and hydrolyzed at 70°C for one hour and plant extraction was done at 2.5, 7 pH to get bound form of plant growth regulators. Ethyl acetate in all plant extract was evaporated at 45°C through rotary evaporator to minimum level to have concentrated sample. The concentrated samples were treated with 1 ml methanol and were run on TLC plates (Silica Gel, 254, Merck Chemicals Germany) to separate gibberellic acid(GA_3), indole-3-acetic acid (IAA) and abscisic acid (ABA) for the samples obtained at pH 2.5 and zeatin at pH 7. Plant growth regulators were extracted through glass plaque with reference to RF value of synthetic plant growth regulators (ABA, zeatin, GA_3 and IAA). Obtained samples were treated with the 1.5 ml methanol and filtered. The samples were then analyzed on spectrophotometer (UV 2600) to determine the optical density along with standards. Optical density was determined at 280 nmfor IAA, 254 nm for GA3, 263 nm for ABA and 269 nm for zeatin.

RESULTS

Plant biomass was reduced 12%, 8% and 34% in the treatments T_1, T_2 and T_3 respectively, compared to the control T_0 (Table 2). Hysun-33 showed the highest plant biomass and *H. argophyllus* the lowest in all treatments. Interspecific crosses had similar plant biomass as Hysun-33 in T_o, T_1 and T_2 but a significantly higher biomass than the commercial hybrid in T_3. Plant height decreased by 26%, 29% and 29% in T_1, T_2 and T_3, compared to T_o (Table 2). Hysun-33 had the highest plant height and *H. argophyllus* the lowest in all treatments. Plant height of interspecific crosses was close to Hysun-33 in T_o and T_1, but significantly lower in T_2 and T_3.Plant height was significantly higher in interspecific hybrids that in intraspecific hybrids in both T_1 and T_2.

Excised leaf water loss (LW) increased with ABA and osmotic stress treatments in all sets of germplasm (Table 3). Averaged over the species and their crosses, the highest LW was noted in ABA foliar treatments. *H. argophyllus* populations maintained the highest LW while interspecific hybrids showed the lowest values in all four treatments. The commercial hybrid Hysun-33 also showed the lowest LW in control treatment. Leaf area decreased by 24%, 11% and 24% T_1, T_2 and T_3, respectively, compared to T_o (Table 3). Hysun-33 had the highest leaf area in this treatment. Leaf area of the commercial hybrid remained unaffected in the three

treatments. Leaf area of interspecific hybrids was similar to this of the commercial hybrid in T_o and T_1, but significantly decreased in $T_2$and T_3.It was higher than this of intraspecific hybrids in T_o and T_1, but not in T_2 and T_3. *H. argophyllus* showed the lowest leaf area in all treatments.

There was a significant decrease in P_N of all genotypes due to stress treatments (Table 4). P_N experienced a decrease of 30%, 57% and 52% in to T_1, T_2 and T_3, respectively. Hysun-33showed the highest P_N in T_o. Net photosynthesis of this hybrid however drastically decreased in T_1, T_2 and T_3. *H. argophyllus* populations along with interspecific hybrids and Hysun-33 showed the highest P_N in T_2. *H. argophyllus* population and interspecific hybrids tend to maintain P_N in all treatments. Interspecific hybrids showed the highest P_N in T_2 and T_3 and *H. argophyllus* populations the highest P_N in T_1. Stomatal conductance was reduced by 71%, 78% and 81% in T_1, T_2 and T_3 treatments (Table 4). In T_0, Hysun-33 showed the highest stomatal conductance and *H. argophyllus* the lowest. *H. argophyllus* had the highest stomatal conductance in T_1 and *H. annuus* in T_2. In T_3, *H. argophyllus* populations and interspecific hybrids showed the highest stomatal conductance.

There was decrease of 58%, 69% and 62% in transpiration rate (E) due to treatments i.e. T_1, T_2 and T_3 (Table 5). Hysun-33 showed the highest E in T_0 and T_1 while crosses (both types) in T_2 and interspecific crosses in T_3. Water use efficiency (WUE) increased by 13%, 17% and 13% in T_1, T_2 and T_3 (Table 5). *H. argophyllus* showed the highest WUE in all treatments.

Silicium content increased by 124%, 90% and 96% in T_1, T_2 and T_3, respectively, compared to T_0 (Table 6). Hysun-33 had the highest silicium content in T_o, T_2 and T_3. Silicium content was similar in the commercial hybrid and intraspecific hybrids inT_2. *H. argophyllus* and interspecific crosses showed the lowest silicium content in all treatments. Leaf cuticular waxes decreased as a result of the ABA treatments and osmotic stress. There was decline of 46%, 53% and 51% in T_1, T_2 and T_3 respectively when compared to T_o (Table 6). *H. argophyllus* showed the highest leaf cuticular waxes in all treatments. Interspecific hybrids showed significantly lower leaf cuticular waxes than *H.argophyllus*.Hysun-33 showed the lowest cuticular waxes content in all treatments.

Abscisic acid (ABA) content increased by 67%, 133% and 47% in T_1, T_2 and T_3, compared to T_o (Table 7). The highest ABA content was noted in *H.argophyllus* in T_1 and $T_3$and in Hysun-33 in T_2. Interspecific hybrids showed higher ABA contents than intraspecific hybrids in T_2.GA_3 content decreased by 33%, 31% and 15% in T_1, T_2 and T_3 (Table 7). The interspecific hybrids had the highest GA3 contents in T_o and Hysun-33 in T_1, T_2 and T_3. GA_3 content increased under stress in Hysun-33 and decreased in the other groups of germplam. *H. argophyllus* showed the lowest GA3 content in all treatments.

Zeatin content decreased by 12%, 28% and 26% in T_1, T_2 and T_3, compared to T_o (Table 8). Hysun-33 showed the highest zeatin content in all treatments. In T_1 and T_2, *H. argophyllus* and interspecific hybrids had higher zeatin content than *H. annuus* and the intraspecific hybrids. IAA increased by 28%, 17% and 19% in T_1, T_2 and T_3 (Table 8). In all these treatments, *H. argophyllus* and interspecific hybrids had a higher IAA content than *H. annuus* and intraspecific hybrids, respectively. Hysun-33 showed the lowest IAA in all treatments.

In the GGE biplot analysis carried out on physiological traits, the two first components depicted 90.65% of the variation (Fig. 1). In T2, cuticular waxes content was positively related with stomatal conductance. In T_3 stomatal conductance was negatively related with leaf area. In both T_1 and T_2, excised leaf water content was positively related to stomatal conductance. In the GGE biplot analysis carried out on plant morphological traits and growth

regulators, the two first components depicted 71.65% of the variation (Fig. 2). Plant biomass was positively related with zeatin contents in all treatments. ABA content was negatively related with plant biomass in T_1 and T_3, stomatal conductance in T_0 and T_2, zeatin contents in T_3, and net photosynthetic rate in T_1.

CONCLUSION

The present study confirmed the value of *H. argophyllus* to improve drought tolerance in cultivated sunflower, previously reported by different authors. Additional traits of potential interest were detected in this species, like smaller leaves with higher cuticular wax content (which allow reducing evapo-transpiration losses) and capacity to maintain net photosynthetic rate and accumulate more ABA, IAA and zeatin under osmotic stress. Some of these traits, as smaller leaves have to be considered carefully as they could be counter-productive under mild stress or optimal conditions. On the other hand, the effects of growth regulators on final yield under different environmental conditions are not yet fully elucidated. Finally, the inheritance of those traits has to be further investigated. The high excised leaf water content and leaf cuticular wax content under stress of the wild species was not maintained in the interspecific hybrids.

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EFFECTS OF HERBICIDE AND SALINITY STRESSES ON SOME DEFENSE RESPONSES OF SUNFLOWER PLANT

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ABSTRACT

Plants in nature are usually simultaneously subject to various abiotic stress factors such as temperature, drought, salinity and pesticides. Pendimethalin is a herbicide used commonly for fight against weeds with narrow and broad leaves. This herbicide is used commonly in our country especially in areas where cotton, sunflower and vegetables are grown. In this study, defense responses of sunflower plant subjected both separately and simultaneously to herbicide and salinity stresses are investigated. It was found that application of these two stress factors both separately and simultaneously on sunflower leaves caused changes in pigment content, lipid peroxidation level and antioxidant enzyme activities.

Key Words : Sunflower, pigment, lipid peroxidation, antioxidant

IMPACT OF EXOGENOUSLY APPLIED GLYCINE BETAINE ON PHYSIOLOGICAL ATTRIBUTES OF SUNFLOWER UNDER DROUGHT STRESS

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ABSTRACT

Scarcity of water causes physiological, biochemical & oxidative damages in sunflower (*Helianthus annuus* L). Effect of exogenous glycine betaine (GB) application through foliar and Hoagland's nutrient solution in amelioration of water stress of sunflower hybrids was studied. Appropriate time, mode and doze of GB application were tested. Three levels of irrigation were used normal irrigation, no irrigation at vegetative and reproductive stages. Three levels of glycine betaine i.e. 0, 75 mM and 125 mM were applied by means of foliar application at vegetative and flowering stage. Morphological parameters such as root and shoot length, fresh and dry weights were recorded three weeks after the GB application. Physiological and biochemical parameters including leaf water and osmotic potential, turgor potential, relative water content, photosynthetic rate, chlorophyll content, protein, soluble sugars and amino acid content were recorded. Nevertheless exogenous GB improved these attributes. However, the irrigated mode of exogenous GB application was more effective than foliar application, among the doses applied 125mM proved more effective. Role of exogenously applied glycine betaine was more pronounced at flowering stage than vegetative stage.

Keywords: Drought, Glycine betaine, Sunflower, Exogenous.

INTRODUCTION

Water stress is being considered the primary factor in limiting crop production (Ashraf and Harris, 2013) and affect plant growth and productivity (Chaves *et al*., 2009). Glycinebetaine (GB) is an amino acid derivative and many scientists hypothesized that GB in low concentration can improve stress tolerance. GB reduces lipid peroxidation of the cell membranes and prevents deterioration of photosynthetic protein complexes (Holmstrom *et al*., 2000; Iqbal and Ashraf, 2008). Endogenous glycinebetaine concentrations show variation in plants, with some taxa accumulating the compound naturally, whiles not others (Bluden *et al*., 2001).

Sunflower (*Helianthus annuus*) is important oil seed crop and its yield is adversely affected by water stress. Water stress affects sunflower plant growth and biomass production (Tahir and Mehdi, 2001). Water stress at vegetative and reproductive growth phase in sunflower may result in 61% and 40% yield reduction, respectively (Bluden *et al*., 2001; Iqbal, 2004). Osmolytes production in water stress condition is an important physiological adaptation to minimize the detrimental effect of stress and achieved by the accumulation of osmolytes such as proline and glycinebetaine (Holmstrom *et al*., 2000). GB accumulation in plants helps to reduce adverse effect of water stress (Iqbal, 2004; Yang and Lu, 2005). Exogenous GB may have also enhanced the ability of cells to retain water without disturbing the cellular functions. Limited knowledge is available about, effective concentrations of GB,

timings and frequency of exogenous GB application. This is prerequisite for GB role in crop stress tolerance. Therefore, the present study is a step towards determining appropriate doze, mode and time of application of GB that could be more beneficial to alleviate the water deficit condition.

MATERIALS AND METHODS

This study was carried out at glass house of department of botany PMAS Arid Agriculture University, Rawalpindi. Seeds of both hybrids (hybrids Hyoleic-41 and Hysun-33) were brought from crop science, NARC Islamabad. Sodium hypochlorite 5% solution was used for surface sterilization. Uniform size seedlings were transplanted to earthen pots containing 10 kg soil and having 40 cm diameter. Soil used: compost, sand, farmyard in a ratio of 2:1:1. Drought stress was imposed by obstructing the water for 9 days at two stages of plant that is vegetative and reproductive stages. Three treatments of glycine betaine were applied with the onset of drought stress at both the stages of sunflower hybrids. Two differnet mode of Glycine betain application were applied wiz irrigation of plant with GB dissolved in Hogland nutrient medium; and through foliar application by hogland solution subsequently.

Morphological Attributes:

Total number of leaves per plant were counted for vegetative growth features after application of GB for three weeks. Root and shoot fresh weight was determined after harvesting. For dry weight of plants the root and shoots were dried at 65 ^{0}C in oven.

Physiological Attributes:

For the determination of relative water content, Unyayar *et al*. (2005) method was adopted. Leaf water potential was calculated by Scholander pressure chamber (Scholander *et al*., 1965). Freezing point osmometer was used for calculation of osmotic potential of flag leaf, turgor pressure was calculated as well (Garnier and Berger, 1985). For each treatment photosynthetic rate was measured using a portable photosynthesis system (Infrared Gas Analyzer. ADC-LCA-4). Leaf chlorophyll content was analyzed by method of Hiscox and Israelstam (1976)

Biochemical Attributes:

A sample extract was used to determine spectrophotometrically by Bradford method for the determination of protein (1976), Ninhydrin method was used for the determination of amino acids in flag leaf extarct (Hamilton and Van Slyke, 1943). Soluble sugars were estimated by Dubois *et al*. (1951) method. Bates *et al*. (1973) method was used to calculate proline content of plants.

Statistical Analysis

Data were statistically analyzed using Statistics 8.1 program by comparing means by LSD at significane level $P \leq 0.05$.

RESULTS

Morphological Parameters

Water stress is one of major abiotic stresses that drastically reduce plant growth and productivity. Water stress decreased shoot fresh and dry weight at both vegetative and reproductive stages. When shoot fresh weight data was subjected to ANOVA ($p \leq 0.05$) significant difference was observed. In stress conditions shoot fresh weight was comparatively higher at reproductive stage 125 mM Gb via foliar application as compared to irrigation GB application (Fig. 1).Among the two varieties v2 (Hyoleic-41) grew better under

stress condition. Hyoleic-41 was responeded more efficiently as compared to Hysun-33 under water stress condition Results revealed that maximum value of shoot fresh weight was observe at T8 and minimum value was observed at T3. Shoot fresh weight of plants with foliar 125 mM GB application was highest with increase of 27% as compared to other GB concentrations. Shoot dry weight when subjected to ANOVA ($p \leq 0.05$) showed considerable difference was observed among all treatments. The most effective treatment was 125 mM GB foliar application at reproductive stage (Fig. 2).GB foliar application at reproductive stage show 29% increase in shoot dry weight and 25% increase due to GB application via irrigation at reproductive stage. The highest shoot dry weight was recorded at T8 (foliar 125 mM Gb application @ reproductive stage) Sunflower shoot and root fresh and dry weight considerably decline under water stress condition. For root fresh and dry weight of sunflower plant under water stress condition subjected to ANOVA ($p \leq 0.05$) a considerable difference was recorded among all treatments. Results of root fresh weight depict that highest root fresh weight was observed at 75 mM GB applied through irrigation at reproductive stage (Fig. 3 & 4). The most effective treatment was T11 for root fresh and dry weight followed by T10 (125 mM GB applied via irrigation at vegetative stage). In root fresh and dry weight GB applied via irrigation was more effective as compared to foliar GB application. Root and shoot fresh weight was considerably increased in water stress condition due to GB application. GB act as growth regulator and enhance root length under stress condition. Root length of Hysun-33 and Hyoleic41 was expressively increased at T8 (foliar applied 125 mM GB @ reproductive stage) (Fig. 5). Root length was recorded at T8 and T 12 with an increase of 13% and 12%. ANOVA analysis of number of leaves data showed significant difference among treatments and cultivars. Gb application via foliar or irrigation both methods were effective for number of leaves. Highest number of leaves were observed at T12 with an increase of 46% followed by T8 with an increase of 35% (Fig 6). Shoot growth is severely affected due to water shortage and shoot length was drastically reduced in water stress condition. Statistical analysis of shoot length data was showing significant difference at ($p \leq 0.05$). GB application via foliar spray was seems to more effective in case of shoot length as compared to GB application through irrigation (Fig 7). Hyoleic41 was observed with maximum shoot length as compared to Hysun-33. Shoot length maximum value at T7 & T8 with an increase of 27% and 22%, respectively.

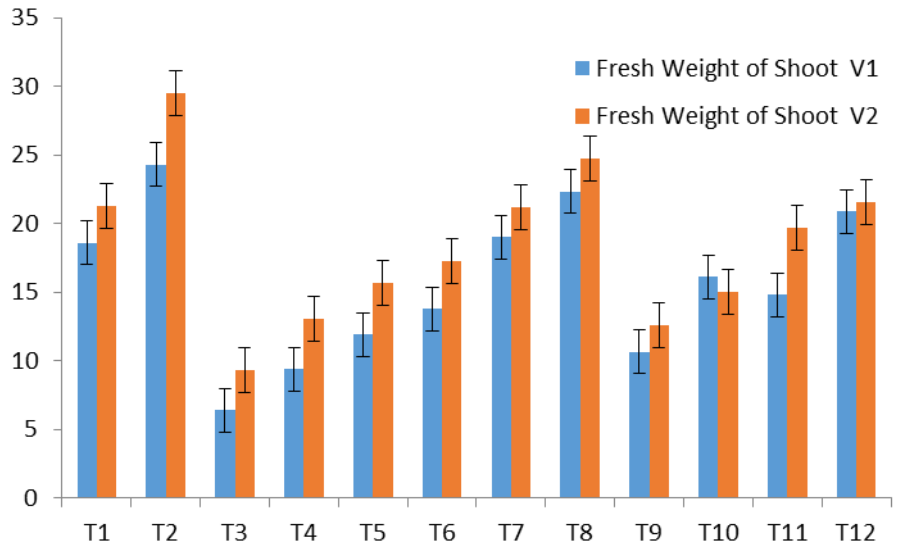

Figure 1: Impact of GB application on shoot fresh weight (gm) of sunflower

T1=Normal irrigation(vegetative), T2= Normal irrigation(reproductive), T3 = stress at vegetative stage, T4= Stress at reproductive stage, T5= Foliar GB application @75mM at vegetative stage, t6= Foliar GB application GB @125mM at vegetative stage, T7= Foliar GB application GB @75mM at reproductive stage, T8= Foliar GB application GB @125mM at reproductive stage, T9= Irrigation of GB @75mM at vegetative stage T10= Irrigation of GB @125mM at vegetative stage, T11= Irrigation of GB @75mM at reproductive stage, T12= Irrigation of GB @125mM at reproductive stage.

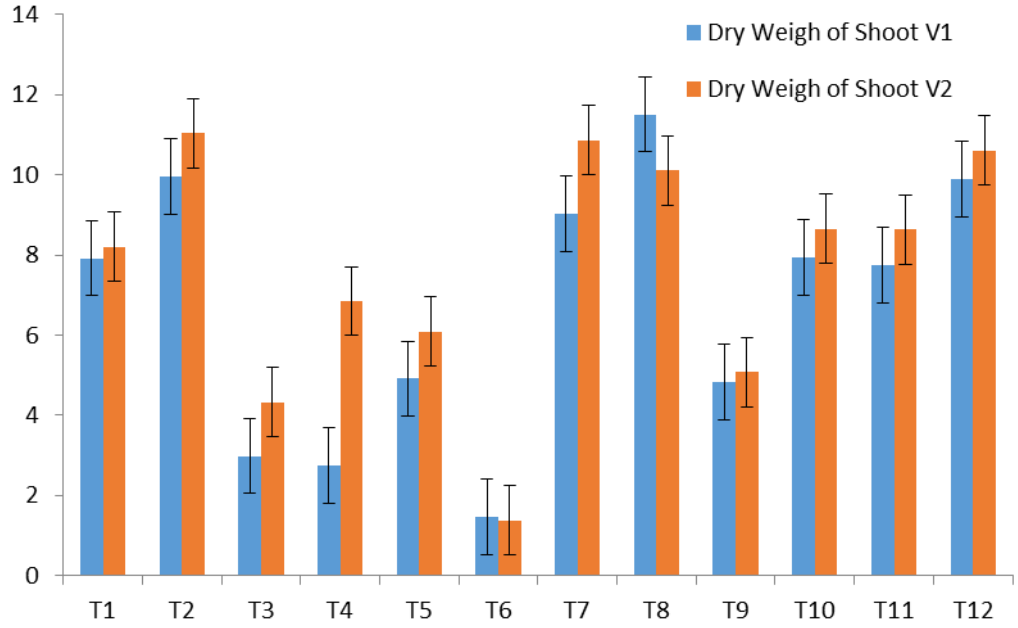

Figure 2: Impact of GB application on shoot dry weight (gm) of sunflower

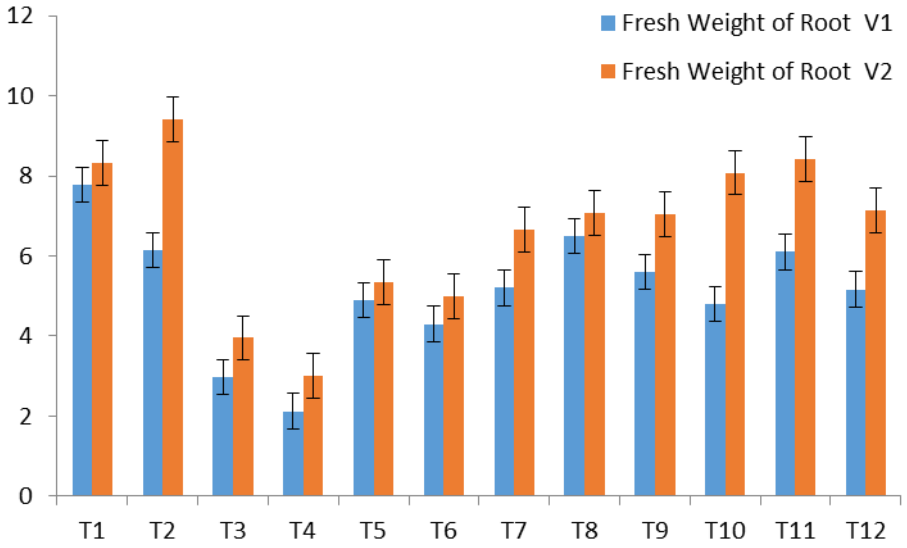

Figure 3: Impact of GB application on root fresh weight (gm) of sunflower

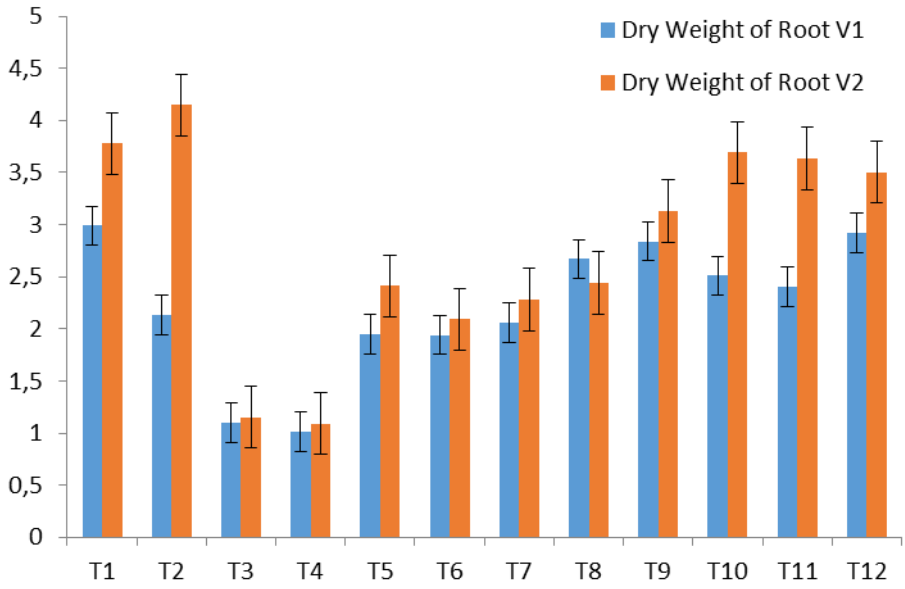

Figure 4: Impact of GB application on root dry weight (gm) of sunflower

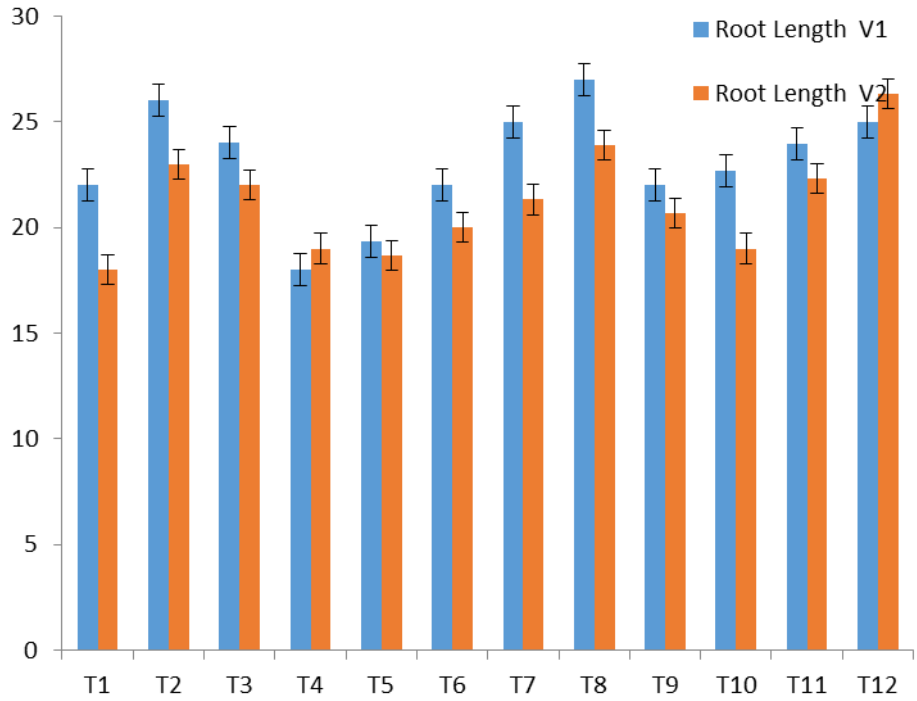

Figure 5: Impact of GB application on root length (cm) of sunflower

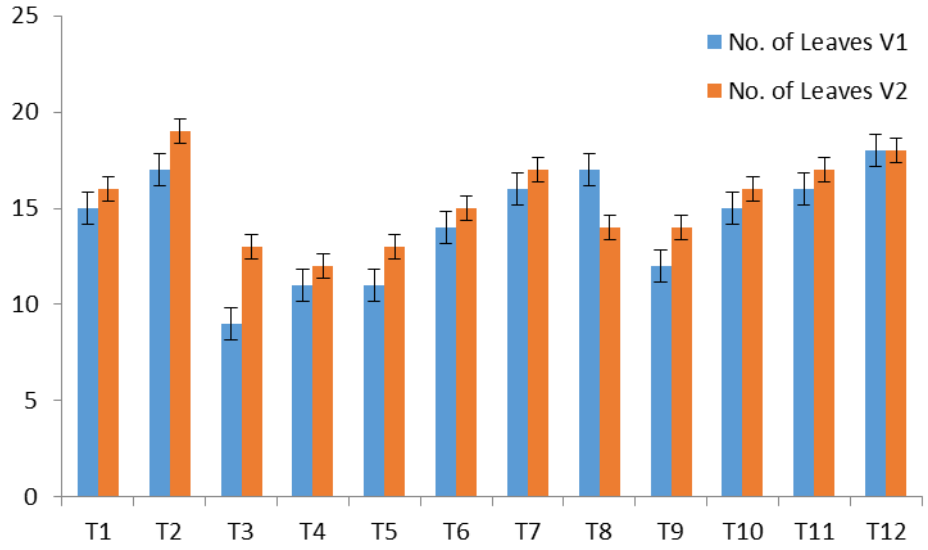

Figure 6: Impact of GB application on number of leaves of sunflower

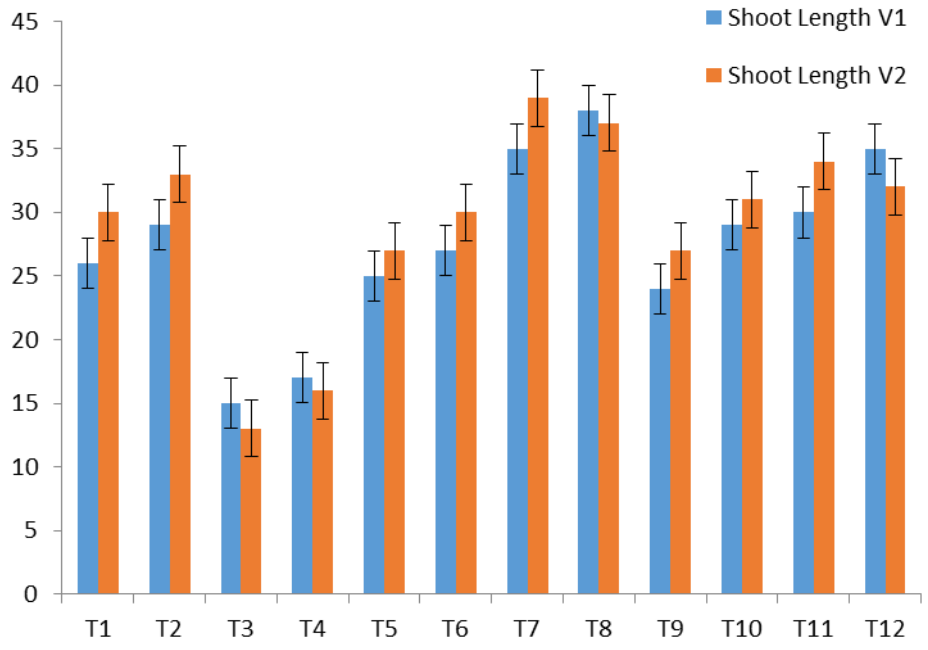

Figure 7: Impact of GB application on shoot length (cm) of sunflower

Physiological Parameters

Drought condition has prominent effect on physiological processes of plant and drastically effect photosynthetic machinery of plant. A considerable decline in total chlorophyll content of plant was observed under water stress condition as compared to control condition. Total chlorophyll content data were subjected to ANOVA ($p \leq 0.05$) and significant difference was detected among all treatments and sunflower cultivars (Fig 8). The most effective treatment for total chlorophyll content in foliar application was T6 (125 mM GB application @vegetative stage) and T8 (125 mM GB application @vegetative stage). Total chlorophyll content showed an increase of 15% and 11% due to GB application via

foliar and irrigation as compared to control condition. Chlorophyll a and chlorophyll b show similar trend (Fig 9 & 10). Treatment effects of chlorophyll a (foliar & irrigated application of 125 mM GB) were more significant at reproductive stage with an increase of 10 and 8%, respectively. Chlorophyll b was negatively influenced by water stress and effective treatment for chlorophyll b was T8 with an increase of 15%. Rate of photosynthesis also had a pattern analogous to transpiration rate in normally watered plants. Photosynthetic rate data were subjected to ANOVA ($p \leq 0.05$) and obvious difference was recorded. GB application via foliar spray was effective for photosynthesis rate (Fig 11). Photosynthetic rate were increased 15% because of GB application as compared to respective control. The present study findings confirmed that GB application at vegetative and reproductive stage are effective to enhance photosynthesis. Plant water relation drastically influenced by water stress. Relative water content results were subjected to ANOVA ($p \leq 0.05$) and showed considerable difference. Relative water content of GB treated plants show an increase of 19% as compared to respective control (Fig 12). The most effective treatment was foliar GB application at reproductive stage with an increase of 17%. Results of water potential were subjected to ANOVA ($p \leq 0.05$) and noteworthy difference was observed (Fig 13). In drought condition without GB application 30% reduction in water potential was observed. The most effective treatments for water potential T6 (foliar 125 mM Gb applied @vegitative stage) and T10 (125 mM Gb applied via irrigation @ vegetative stage). Highest values of water potential were observed in plants treated with GB foliar spray. ANOVA analysis of osmotic potential results showed significant difference at ($p \leq 0.05$) (Fig 14). Osmotic potential of plant without GB treatment under water stress with an increase of 17 and 20 % at vegetative and reproductive stages, respectively. Turgor potential play an important role to maintain turgidity of cell. Turgor potential was recorded and a considerable difference was found (Fig 15).

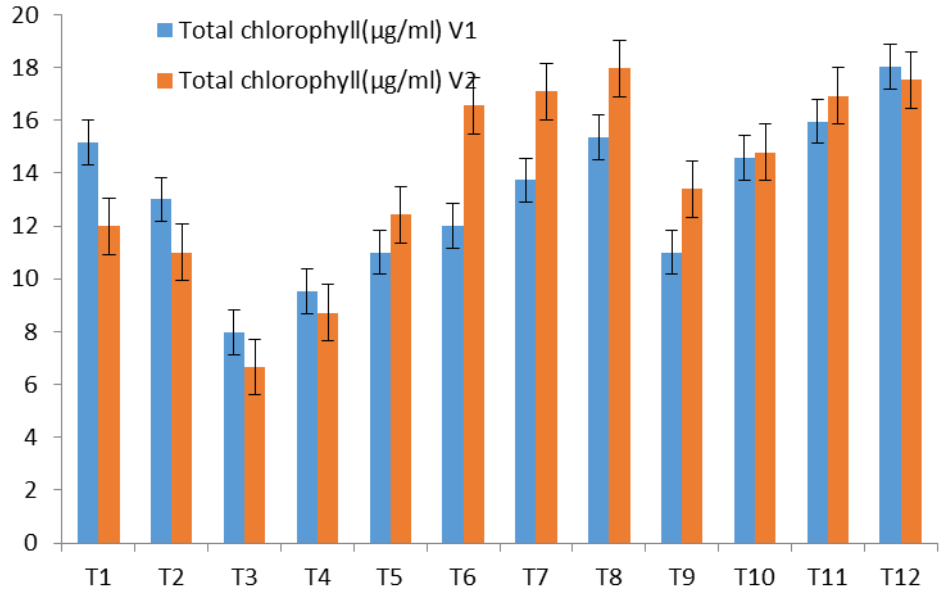

Figure 8: Impact of GB application on total chlorophyll of sunflower

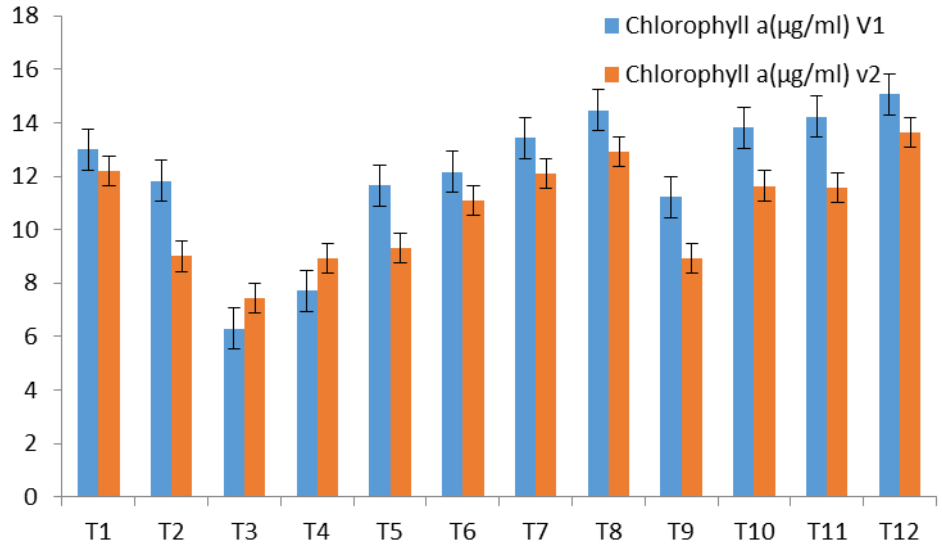

Figure 9: Impact of GB application on chlorophyll a of sunflower

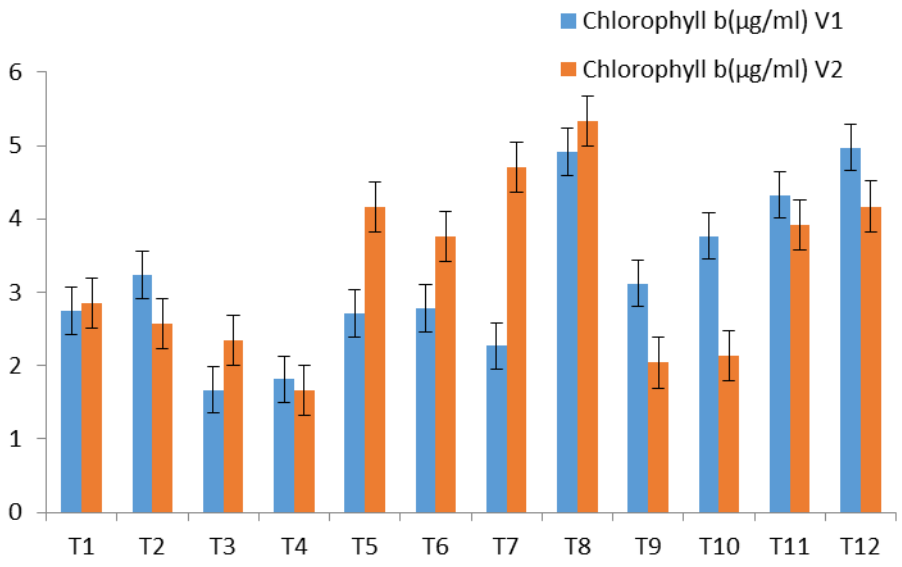

Figure 10: Impact of GB application on chlorophyll b of sunflower

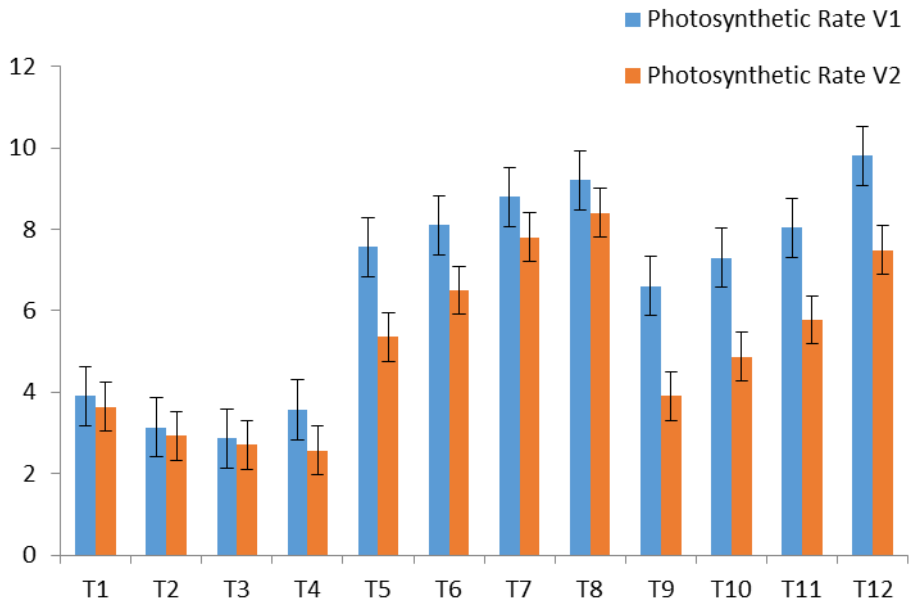

Figure 11: Impact of GB application on Photosynthetic rate of sunflower

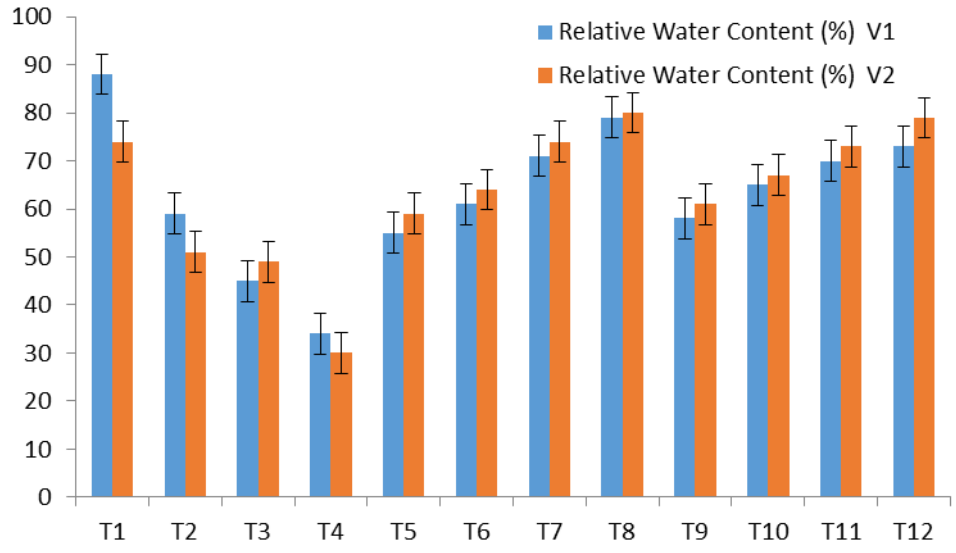

Figure 12: Impact of GB application on relative water content of sunflower

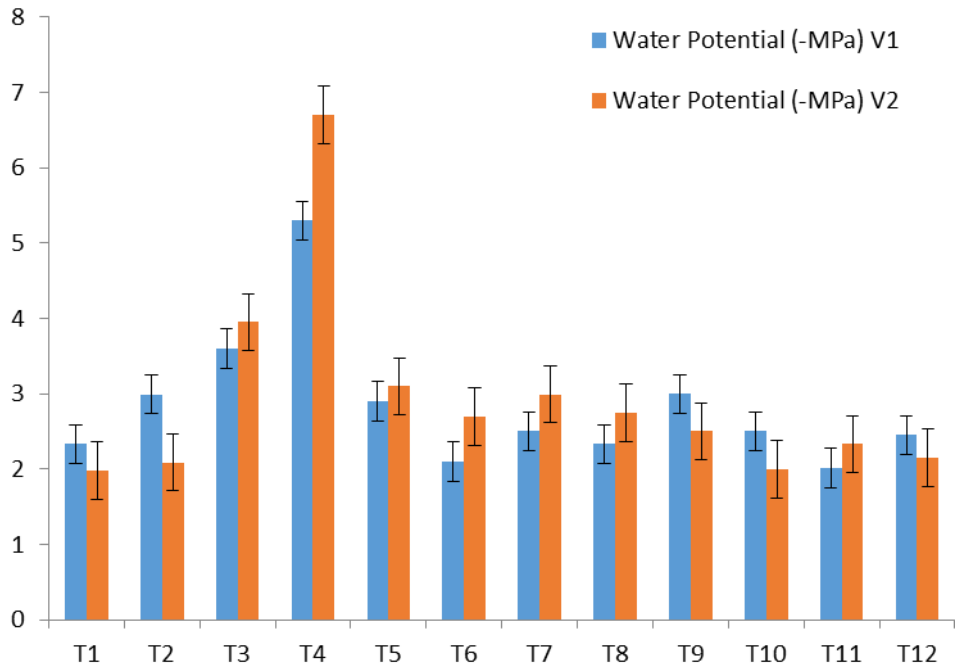

Figure 13: Impact of GB application on Water potential of sunflower

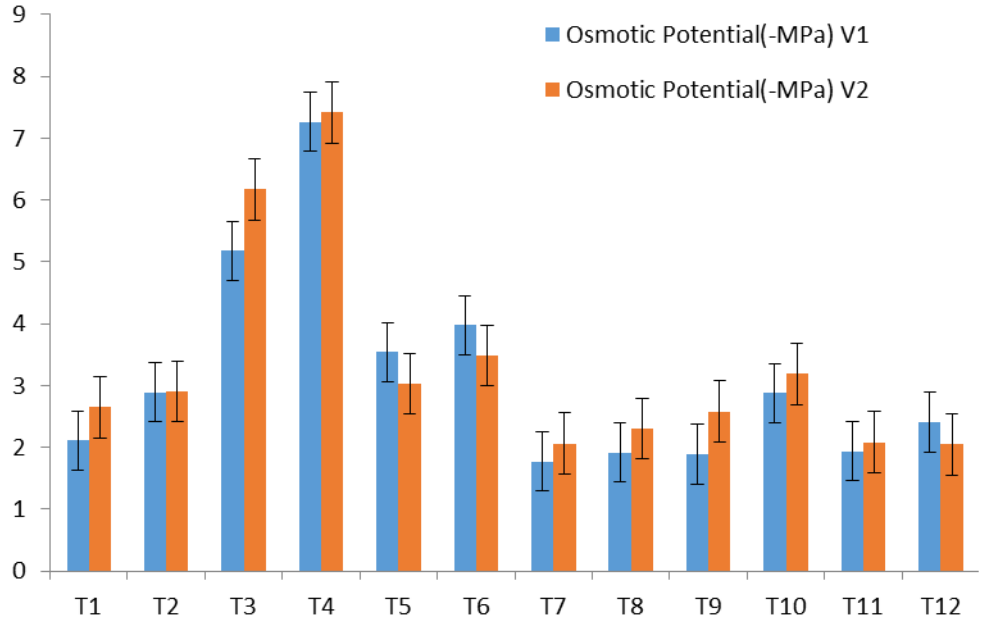

Figure 14: Impact of GB application on osmotic potential of sunflower

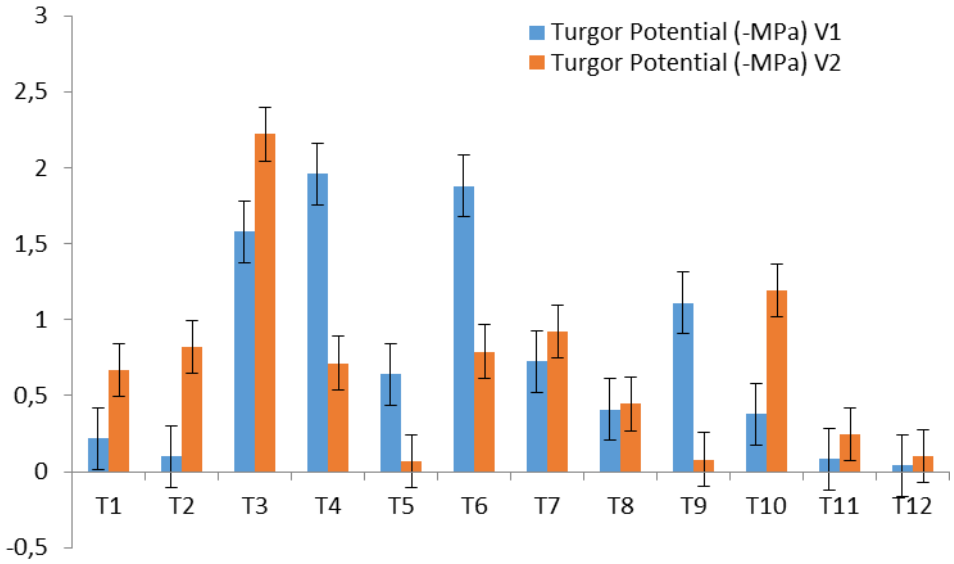

Figure 15: Impact of GB application on Turgor potential of sunflower

Biochemical Parameter

When plants are exposed to abiotic stress condition plant produces different types of osmo protectants that helps plants to tolerate unfavorable condition. Results of protein content were showing significant difference when subjected to ANOVA ($p \leq 0.05$). An increase of 13% of protein content in plants was recorded under water stress (Fig 16). The most effective treatment for GB treated plants were T6 (foliar 125 mM GB application at vegetative stage) and T7 (foliar 125 mM GB application at productive stage). In drought stress condition, it was observed that 21 % increase in free amino acid due to GB treatment via irrigation (Fig 17). The most effective Gb concentration for free amino acid was 125 mM at reproductive stage. Similarly higher content of proline content was observed in water stress condition. Results of proline were subjected to ANOVA and significant difference was observed among treatments (Fig 18). Maximum proline production was observed in GB treated plants with an increase of 23% as compared to respective control. High amount of total soluble sugar was found in water stress (Fig 19). Because in less amount of water, soluble sugar accumulate to combat stress condition. Under water stress without GB treatment an increase of 14% soluble sugar content was observed. Results of soluble sugar content was show significant difference among treatments ($p \leq 0.05$).

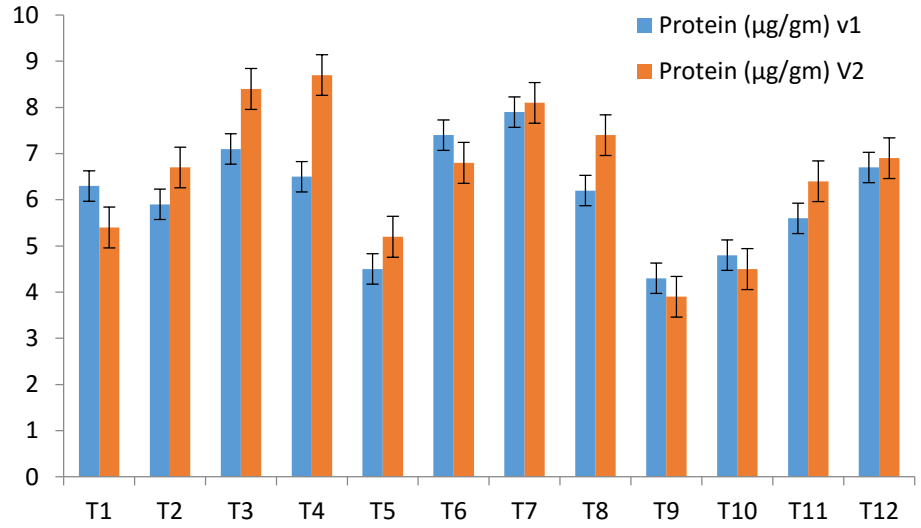

Figure 16: Impact of GB application on protein content of sunflower

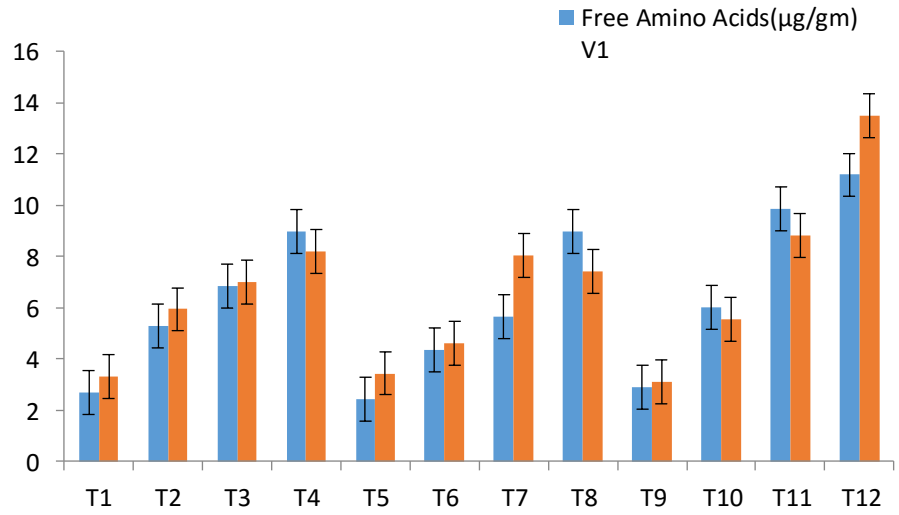

Figure 17: Impact of GB application on free amino acids of sunflower

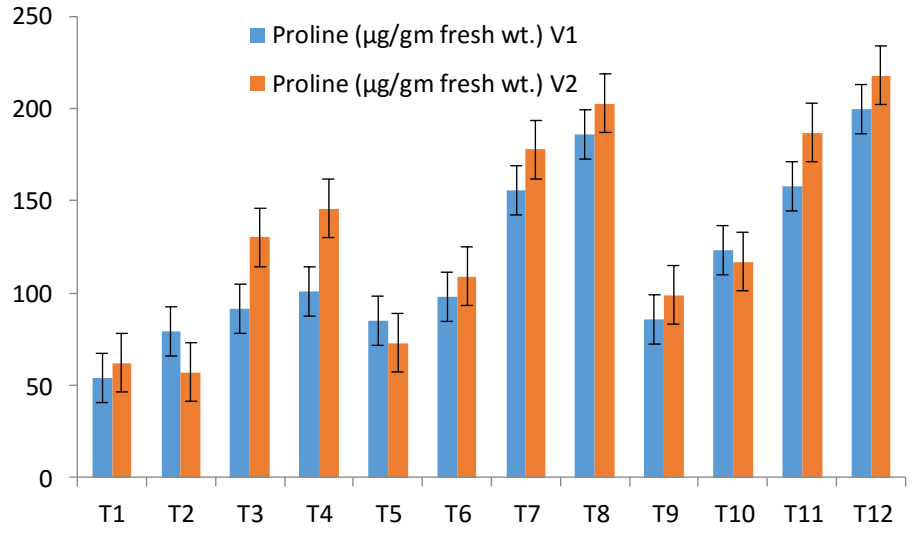

Figure 18: Impact of GB application on proline content of sunflower

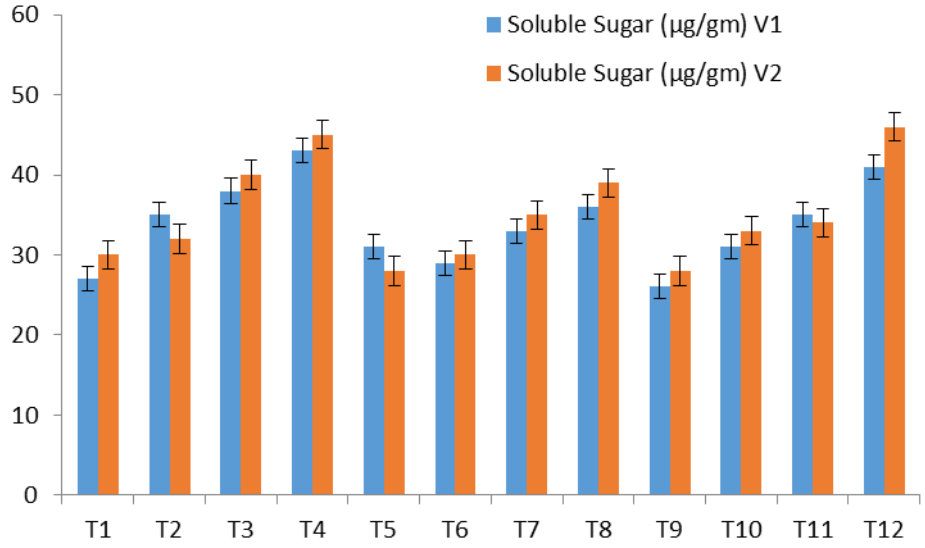

Figure 19: Impact of GB application on soluble sugar of sunflower

DISCUSSION

Drastic decrease in fresh weight of both hybrid plants was recorded under water deficit environment. As water stress considerably reduced the fresh weight of wheat crop (Rane *et al*., 2001), *Abelmoschus esculentum* (Rane *et al*., 2001) and pearl millet (Kusaka *et al*., 2005). The upshot for suppression cell growth and cell expansion due to the lower turgor pressure may results in such drastic decrease. Exogenous application of GB mitigates the stress effect and increased the fresh weight of sunflower hybrids at both the growth stages. Both hybrids have shown better results under irrigated mode of GB application as compared to foliar application.

The effect of irrigation levels was significant for dry weight of both the hybrid varieties under drought condition as water scarcity drastically decreased the dry weight of plants. This decrease in dry weight might be due to reduced photosynthetic activity, leaf senescence and plant growth (Rane *et al*., 2001; Kusaka *et al*., 2005).

Endogenously synthesized glycinebetaine improves the growth of above ground biomass following foliar application of GB. GB stabilize the turgor pressure and enzymes involved in amino acid metabolism, even at leaf concentrations upto 500 milliMole (Sankar *et al*., 2007). Laurie and Stewart (1990) reported that exogenous application of GB improved the growth in sunflower under drought and our results are also in accordance with above mentioned study. Several other reports clearly demonstrated the pronounced impact of exogenous application of GB on plant vegetative and reproductive stages in several crops e.g. maize (Agboma *et al*., 1997a) and soybean (*Glycine max*) (Hussain *et al*., 2008).

Better root system is fundamental adaptive mechanism that improves the ability of a plant to capture water towards drought stress. According to Agboma *et al*. (1997b) the root surface area in *Populus* species decreased in drought stress in the similar way the stem length in *Albizzia* decreased under water shortage (Agboma *et al*., 1997c). Lower turgor pressure reduce the plant height, this reduction in plant height might be associated with decreased cell enlargement and cell growth under stress (Sundaravalli *et al*., 2005).

The economic yield of crops can be estimated by the rate of photosynthesis. Drought stress decreases the photosynthetic activity. Similar results were obtained in present study. However, inhibitory effect of drought stress was ameliorated by foliar application of compatible solute (GB). Exogenous application of compatible solutes increased the net photosynthetic rate and also has been reported in maize (Yang and Lu, 2005) and tomato (Munns and Tester, 2008). Under drought or salt stress, GB not only control the stomatal conductance but also maintain Rubisco activity and chloroplast ultrastructure (Lopez *et al*., 2002).

There was a considerable decline observed for chlorophyll content of both hybrid under drought stress. Chl *a*, Chl *b* and Chl *a* + *b* contents decrease under progressive drought stress in maize (Nawaz and Ashraf, 2007). Exogenous application of GB improved photosynthetic pigments and thus enhanced photosynthetic capacity in various crops and vegetables e.g., tomato (Raza *et al*., 2006) and wheat (Anjum *et al*., 2011).

All water relations were disturbed due to water shortage including, turgor potential leaf water potential, osmotic potential and relative water contents at both growth stages of sunflower (Anjum *et al*., 2011). RWC decline depicts loss of turgor that results in limited water availability resulting in loss of turgidity which stops or decrease the cell expansion process in crop plants. In present study decrease in RWC was observed under drought conditions. Exogenous application of GB reversed the effect of water stress on RWC. This is in accordance with previous finding (Iqbal, 2004; Farooq *et al*., 2008) who reported an increase in RWC in kidney beans by foliar application of GB under abiotic stress. Active lowering of osmotic potential is generally considered as an adaptation under drought to maintain turgor (Iqbal, 2004). A beneficial drought resistance character is osmotic adjustment which is adopted by green plants (Meek *et al*., 2003) at lower leaf water potentials (Iqbal, 2004). To cope up with the stress effect, foliar application of GB is used as an important tool and has strong potential to reverse the stress effects. GB increased the turgor potential of the plant cells by osmotic adjustment (Iqbal *et al*., 2008).

Reduction in protein synthesis and proline accumulation in many crop plants have been widely studied (Iqbal *et al*., 2008) and are of the several biochemical indices of water deficit injury. Reduction in protein contents was observed in our study during exposure of drought stress. In leguminous plants, soluble protein content in both leaves and nodules decreased as drought progressed with more drastic decline in nodule tissues. Drought stress produced drastic effect on the soluble protein contents of leguminous plants in both leaves and nodules but the effect was more severe in nodule tissues. Amino acids accumulation in cell sap is one of the adaptations under water scarcity. Amino acid contents showed an uplift in sorghum plants when exposed to moisture stress conditions (Yaday *et al*., 2005). Similar results were observed in present study when sunflower plants were exposed to water stress. Osmotic adjustment alleviates some of the hazardous water stress effects. Lv *et al*. (2007) reported the accumulation of GB for drought stress tolerance in plants and the accumulation was more effective in transgenic plants containing enhanced activity of GB accumulation as compared to wild plants.

Accumulation of proline is chief indicator of drought stress tolerance in higher plants (Iqbal, 2004). The proline contents of leaf was increased in both sunflower hybrids at both stages. Accumulation of proline in various parts of plants is reported earlier particularly in wheat crop. Elevated level of proline was also observed in vegetable crop *Abelmoschus esculentus* (L.) under drought (Sankar *et al*., 2007). Glycine betaine application under drought condition ameliorated the stress effect at both the stages by accumulating proline in leaves. Exogenous application of GB increased the soluble sugars contents in sunflower at both growth stages (Lin *et al*., 2002; Lv *et al*., 2007) similar results were observed in present study.

CONCLUSION

Exogenous GB application increases chlorophyll content and photosynthetic rate of sunflower under water stress. According to this study, foliar application proved to be more effective among the two modes of application that have been investigated in the present study. The concentration of 125 mM GB were more operative as compared to 75 mM GB . Growth of both sunflower hybrids was effected by drought stress and had shown improvement towards application of GB but the reproductive stage was more sensitive as compared to the vegetative stage. It is concluded that 125mM GB concentration could be applied exogenously at reproductive stage to ameliorate the adverse effects of water shortage.

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BIOACTIVITY AND PHYTOCHEMICAL EVALUATION OF SUNFLOWER (HELIANTHUS ANNUUS L.) LEAF EXTRACT

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is a major oil yielding crop globally. Besides plant species has been traditionally used against fever, cuts, wounds, pulmonary infections and coughs. The experiment was conducted to validate its medicinal valve. Sunflower seeds were sown in pots and plants growth was maintained under greenhouse conditions. At flowering stage leaves were collected, shade dried and ground to fine powder. Sunflower leaf extract was prepared by cold maceration technique. Methanol extract was tested for antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl method (DPPH assay). Cytotoxicity was carried out by using Brine Shrimp Lethality assay and phytotoxicity by Radish Seed Germination assay. Extract exhibited significant antioxidant activity with IC50 value 0.89 mg/ml. A mild cytotoxic while a strong phytotoxic effect was observed with LD50 value 15 mg/ml and 0.4 mg/ml respectively. Phytochemical analysis revealed alkaloids, flavonoids, total phenolics and sterols in extract. In conclusion Sunflower extracts is a good candidate for drug development and isolation studies should be carried out to separate the bioactive components.

Key Words : Sunflower, cytotoxicity, allelopathy, antioxidant, pulmonary infections

THE ESTIMATING DROUGHT STRESS TOLERANCES OF SUNFLOWER INBRED LINES UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

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ABSTRACT

Drought is the most severe factor reducing yield in sunflower. Sunflower plants responses to drought with some processes and changes at morphological, anatomical and molecular levels mostly with decreasing photosynthesis. In this study, 9 inbred lines of sunflower were investigated to get information about drought responses of them. With this purpose forty-day old 9 inbred lines growth at controlled well watered conditions were exposed to drought for 10 days, recovery of 5 days followed. Fast chlorophyll a fluorescence kinetics was determined and analysed using JIP test. Pigment content (chlorophyll *a*+*b*), relative water content (RWC) and membrane damage (ELC) were measured for both drought and recovery treatments. The adverse effects of drought stress were observed on photosynthetic efficiency that obtained by photosynthetic performance index (PItotal), RWC and ELC of leaves. However, following rewatering, recovery was observed for all inbred lines at different level. 9 sunflower inbred lines were classified into three groups; tolerant, less tolerant and sensitive, according to the injury index.

Key Words : sunflower, drought tolerance, inbred lines, controlled environments

EFFECTS OF NAPHTHALENEACETIC ACID AND N6-BENZYLADENINE ON ANDROGENESIS IN *HELIANTHUS ANNUUS* L.

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ABSTRACT

The aim of this study was to investigate the effects of NAA and BA on androgenesis in sunflower anthers. Hybrid sunflower breeding line taken from Trakya Agricultural Research Institute was used as a material. 70 days old capitula were sterilized and then anthers obtained from different sized capitula and flowers were transferred to the MS medium including four different concentrations (0, 0.5, 1 and 2 mg/l) of NAA and/or BA. Anther cultures were incubated at photoperiod or continuous dark conditions. Observations showed that uninucleate microspores were available in flowers 3-4 mm in length. While maximum callus induction for photoperiod condition was 87% when MS medium including 2 mg/l NAA and 1 mg/l BA was used, it was 90% for continuous dark condition at the same medium. Both plant growth regulators had no effect on androgenesis when used alone. But the androgenetic stimulation was gradually rose when they used together with increasing concentrations. When only light effect on androgenesis taken into account, callusing was 26% and 12% for photoperiod and continuous dark conditions respectively. There was no regeneration when anthers were transferred to regeneration medium. Investigation on anther-derived callus showed that there were both haploid and diploid cells too in it. According to these results, it was proposed that callus were formed from microspores. Finally, it was determined that NAA, BA and light had significant effects on stimulating androgenesis in sunflower.

Keywords: *in vitro,* Sunflower, Anther Culture, Haploid Production

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important source of vegetable oil in the world and in Turkey. In Turkey, sunflower oil is often preferred as vegetable oil consumption. Therefore, the importance of sunflower is increasing in recent years. Hybrid varieties are generally used in sunflower agriculture. However, due to the use of the same gene source, it is approaching the upper limit of capacity of genetic productivity in varieties obtained using traditional breeding methods. It is only possible through the use of biotechnological methods to overcome this problem. Therefore, the studies in this context in recent years are getting important every day and new achievements are obtained every year.

In this study, anther culture which is one of the biotechnological methods for obtaining haploid plant has been studied. As known, while breeding process with traditional methods lasts 7-8 generation, it can be reduced to a single generation using double-haploid technology. In this context, with the aim of promoting androgenesis in the anthers of sunflower (Helianthus *annuus* L.), the effects of different hormone combinations have been investigated. And, cytological observations were performed to determine the ploidy level of obtained calli.

Choi (1991), argued that optimal levels of hormones are needed according to the physiological status of anther tissue for androgenesis to reach success. Several specific plant growth regulators are used in anther culture. Generally, auxins such as 2,4-D, IAA, IBA, NAA, or alternatively, cytokinins such as kinetin, zeatin, and riboside were used in initiation and regeneration medium (Luckett and Darvey 1992). Vijaya Priya et al. (2003) has argued that for callus induction in wild sunflower species the presence of 2,4-D with auxin and cytokinin at low concentrations is sufficient but additional auxin and cytokinin is not necessary to increase the amount of callus. Similarly, despite the best callusing rates are obtained with the use of 0,5 mg/L NAA and BA in the culture of anthers derived from interspecific hybrids of the sunflower, when the concentration was increased to 1 mg/L, there was no significant changes in the rate of callusing (Nurhidayah 1996). On the other hand, Gurel and Kazan (1998) reported that increasing BAP concentration for all NAA concentrations was constantly increased the rate of callusing from different genotype of sunflower explants taken from different somatic tissues. Based on these information, the effect of NAA and/or BA (0 - 0,5 - 1 and 2 mg/L) on sunflower anthers for callus induction were studied. In Turkey, haploid culture studies on sunflower are less common and this study has been made in terms of creating a basis for these studies.

MATERIALS AND METHODS

In this study, hybrid sunflower breeding lines which has resistance gene to the orobanche and downy mildew was used. Seeds were obtained from Trakya Agricultural Research Institute. Sunflower capitula (figure 1) were collected from the plants of 70 days old. Flower buds (figure 2) 3, 4, 5 and 6 mm in length were isolated from capitulum of 3, 4, 5 and 6 cm diameter. The anthers removed from these flowers were analyzed by the asetocarmine squash method under Olympus photomicroscope. For sterilization Anthers were shaken in 15% commercial bleach for 20 minutes and for 2 minutes in 70% alcohol solution then rinsed with sterile distilled water three times. The sterile anthers were transferred to culture medium at photoperiod (16/8 light/dark) or continuously dark conditions. MS basic medium was combined with four different concentrations of NAA and/or BA (0-0,5-1-2 mg/L). 0,1% and 0,5% PVP added to the medium to prevent browning observed in Anther. The calli were transferred to MS medium which includes 0,5 to 1 or 2 mg/L BA and/or 0,1 mg/L NAA for regeneration experiments. Some calli are reserved for cytological examination in order to determine ploidy level of the cell. Fresh calli were analyzed by the asetocarmine squash method under Olympus photomicroscope. The differences among the averages of all experimental groups were tested by one-way ANOVA. In this test, the differences were compared with Tukey test at 0,01 significance level.

RESULTS AND DISCUSSION

Microscopic examination on the anthers obtained from 3, 4, 5 and 6 mm length of flowers was made with the aim of determining the appropriate microspores for successful anther culture (figure 3). It was observed that uninuclear microspores were seen in flowers of 3 and 4 mm length. Meriç (2002) reported that 3, 4 and 4.5 mm in length sunflower flowers are in the consistent with our preliminary investigations. Therefore, in this study with the aim to obtain microspores in the uninucleate stage, anthers were provided from flower of 3 and 4 mm in length. Additionally, according to our study, it was observed that the flowers of 3-4 mm in length located in the uninucleate stage, but 5.5 cm and up in length are in the binucleate stage. The literature is capitulum of 3 to 4 cm in diameter.

Figure 1. **Sunflower capitula**

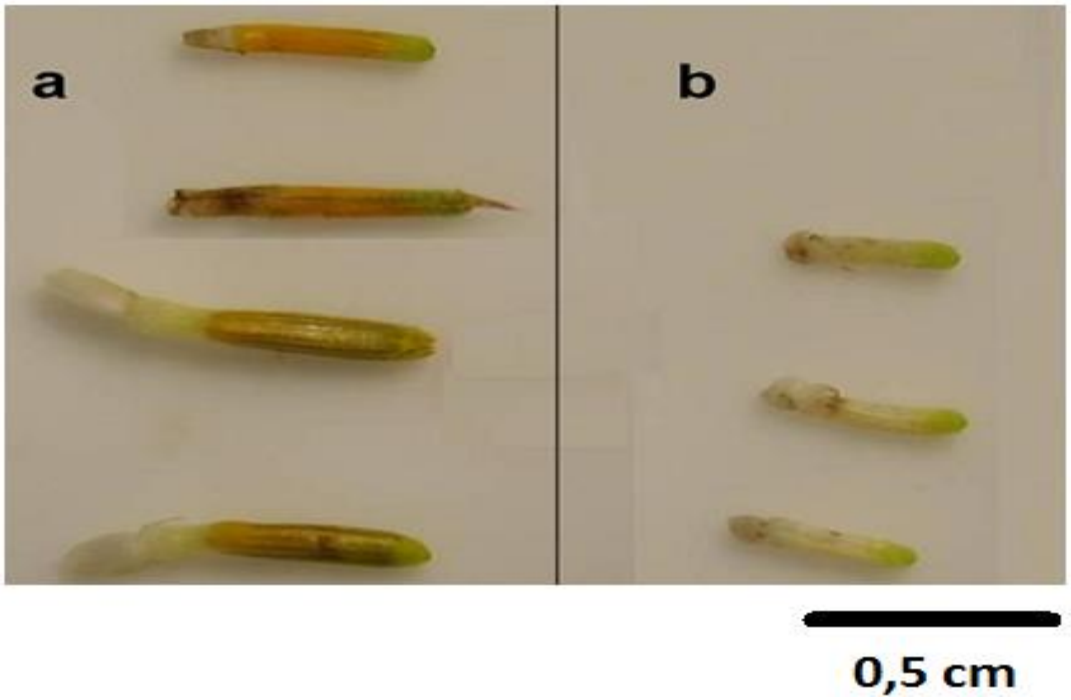


Figure 2. Sunflower tubular flowers. a) 5-6 mm in length, b) 3-4 mm in length

One of the most evident result of our study, any micspor response has not occurred in hormone-free basic MS medium (Table 1). All anthers in this medium browned and lost their vitality after 2 weeks even they showed some swelling at first. Vijaya Priya et al. (2003) studied on six different sunflower genotypes and reported that callus formation was not observed in hormone-free MS basic medium as similar to our findings. They explained this case by idea of the endogenous hormone levels in the anthers were not enough the promote callusing. We agree with this idea because of androgenic response does not occur in hormone - free MS medium in our study.

Table 1. Effect of NAA and BA on Callusing.

Light Regime	NAA mg/L	BA mg/L	Callusing %*
Photoperiod	0	0	0 a
"	0	0,5	0 a
"	0	1	0 a
"	0	2	0 a
"	0,5	0	0 a
"	0,5	0,5	7 a
"	0,5	1	27 abcd
"	0,5	2	47 cde
"	1	0	0 a
"	1	0,5	0 a
"	1	1	47 cde
"	1	2	50 def
"	2	0	0 a
"	2	0,5	73 efg
"	2	1	87 g
"	2	2	77 fg
Continuously Dark	0	0	0 a
"	0	0,5	0 a
"	0	1	0 a
"	0	2	0 a
"	0,5	0	0 a
"	0,5	0,5	0 a
"	0,5	1	20 abc
"	0,5	2	20 abc
"	1	0	0 a
"	1	0,5	0 a
"	1	1	0 a
"	1	2	7 a
"	2	0	0 a
"	2	0,5	40 bcd
"	2	1	90g
"	2	2	13 ab

*Values within each column followed by the same letters are not significantly different by the Tukey test at 0.01% probability level.

Another important result is that when NAA or BA used alone, there was no callus formation from anther (Table 1). On the other hand, when NAA and BA used together callus formation was observed in anther after two weeks (figure 4). Moreover, when NAA and BA concentration increased, the rate of callus formation is increased significantly. The best callus formation ratio (90%) was obtained on MS basic medium consisting of 2 mg/L NAA and 1 mg/L BA after 6 weeks (figure 5). Vijaya Priya et al. (2003), in their study, when 0.1 mg/L

NAA and 0.2 mg/L BA is added on the MS medium of, callus formation was observed depending on genotype ranged from 77% to 97%. When the rate of growth regulators are increased as 2.0 mg/L NAA and 1.0 mg/L BA, callus formation rates ranging still from 90% to 74% depending on the genotype was observed. They reported that the increasing concentrations of auxin and cytokinin (NAA and BAP) do not have a significant effect on the callusing rate. Similarly, Nurhidayah (1996) reported in his study that, the increasing concentrations of NAA and BA do not cause a significant increase in callus formation. These findings contradict with our findings. On the other hand, Gurel and Kazan (1998) reported that increasing BAP concentration for all NAA concentrations was constantly increased the rate of callusing from different genotype of sunflower explants taken from different somatic tissues. Although Vijaya Priya et al. (2003) has been reported that optimum doses of growth regulators are enough to callus induction and the increase of the dose does not influence the callusing rate, we claim that increasing the concentration of NAA and BA, increases callus formation rate based on the sunflower genotypes we used. As the different genotypes are used in each different study, this is believed to be the cause of these conflicts. Different genotypes and incubation conditions (constant light-dark-photoperiod) have various effects on callus formation. Considering the interaction between these factors, if the target is androgenetic stimulations in anthers of sunflower, our proposal is that concentration and combination of growth regulators must be optimized separately for each genotype and incubation condition.

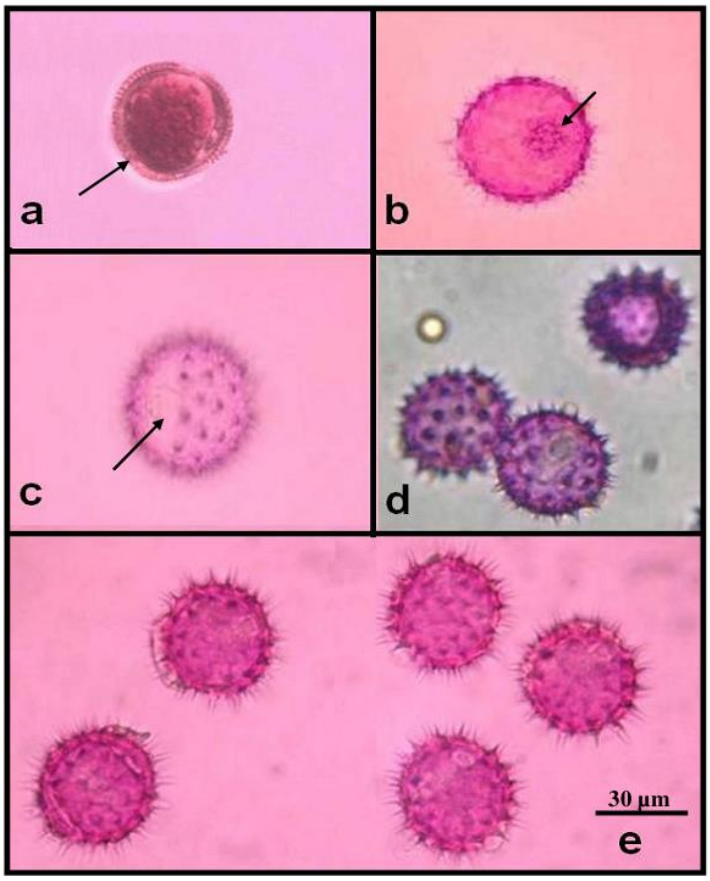


Figure 3. Sunflower microspores and pollens. a) early-uninucleate microspore stage b-c) late-uninucleate microspore stage d-e) binucleate pollens

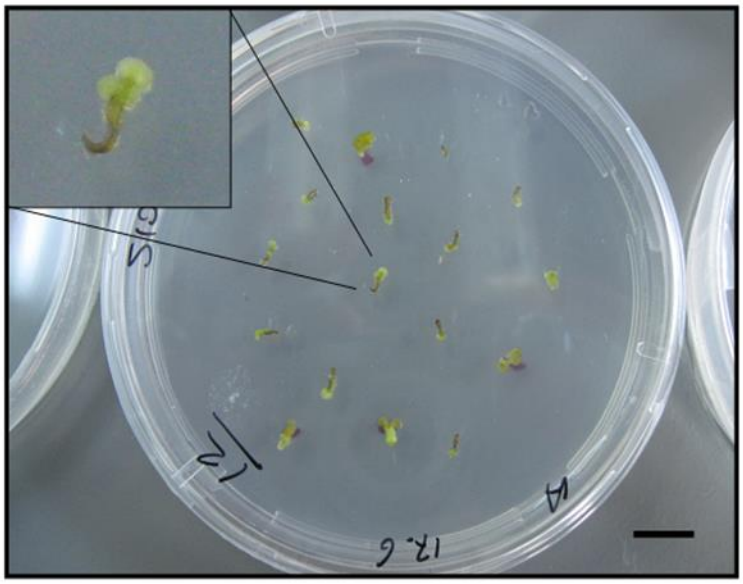


Figure 4. Second week of anther culture. MS medium includes 2 mg/L NAA and 1 mg/L BA. Bar=1 cm.

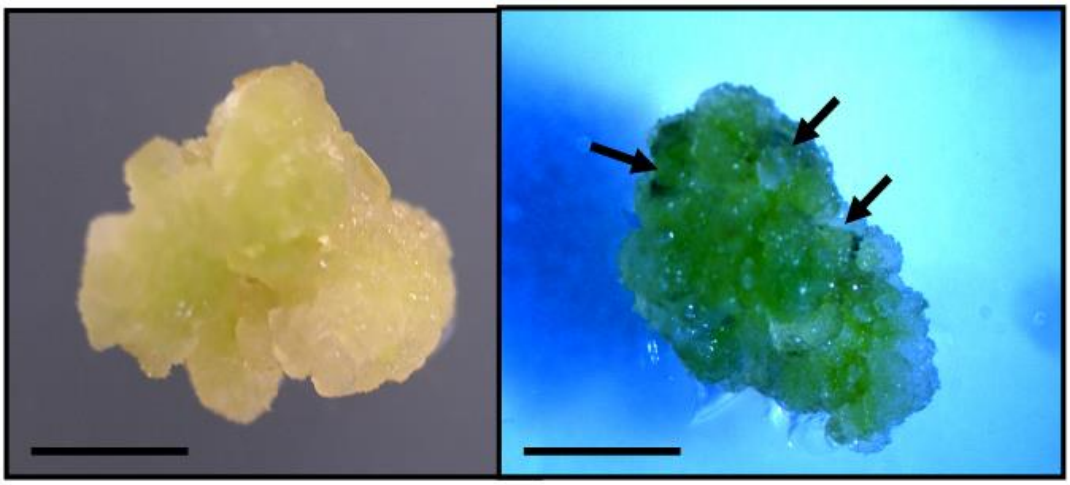
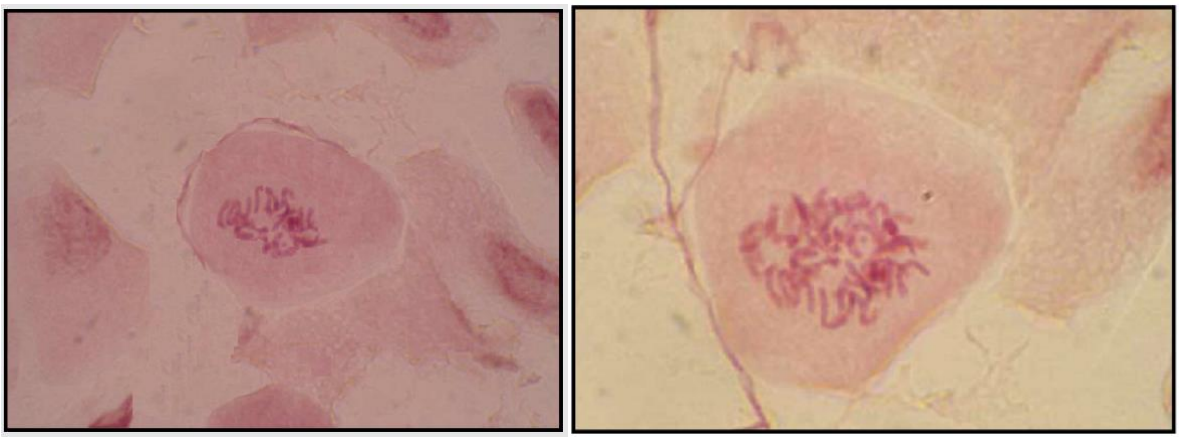


Figure 5. Sixth weeks old calli on MS medium includes 2 mg/L NAA and 1 mg/L BA. Yellowish callus occurred in dark condition on left, and green callus occurred in photoperiod condition on right. Bar=1 cm.

Previous studies have reported that by adding antioxidants such as PVP to the medium prevent browning (Roy and Sarkar 1991; Sudripta et al. 1999). In this study four different concentrations of PVP were tested with the reason of overcome the browning problem occurred in anther, but there was no statistically significant effect in terms of PVP. There was no shoot regeneration from callus in any experiment group. It is necessary to keep in mind that the low productivity is common in shoot regeneration from anther culture (Thengane et al. 1994). When the literature is studied it is outstanding that this case is common. Bohorova et al. (1985) has achieved anther callus around 70-100%, but failed to obtain shoot regeneration. Gürel et al. (1991a) reported low frequency direct embryo formation and shoot regeneration in the sunflower anther culture, but a whole plant could not be obtaioned. Gürel et al. (1991b) and Coumans and Zhong (1995) tested the isolated microspore culture and even they obtained continuous cell division and microcallus formation, they could not achieve shoot regeneration. A total of 200 callus have been examined, but chromosomes could be counted in small number of cells. However, both haploid and diploid cells were also observed in the callus (figure 6). The observation of haploid cells indicates that calli are microspore-derived. It is believed that diploid cells are spontaneous double-haploids as well.

Figure 6. Microscopy of sunflower callus. Hapliod (on left) and diploid (on Right) cells.



As a result, in this study it was found that the NAA should be used together with the BA to stimulate androgenesis from sunflower anther derived from flowers 3-4 mm in length. It is essential to product double-haploid plants originated from anther to contribute the sunflower breeding work in Turkey. Therefore, we hope the results of this study will be a guide for future studies.

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CYTOKININS: THE KEY TO DIFFERENCES IN PATTERNS OF CANOPY SENESCENCE IN STAY-GREEN AND FAST DRY-DOWN SUNFLOWER HYBRIDS

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ABSTRACT

This study documents the dynamics of cytokinin levels in leaves and their association with root functionality and leaf senescence in irrigated crops of two sunflower hybrids with different patterns of leaf senescence (stay-green[SG] and fast dry-down[FDD]) grown in two years. During the grain filling-phase, green leaf area index (GLAI) and live root length density (LRLD) were followed, together with total chlorophyll content (CT), fluorescence (Fv/Fm), net photosynthesis (Pn) and trans-zeatin (ZT) levels in leaves of positions H17, H20, H22 and H24. In all positions, hybrids and years the beginning of leaf senescence was firstly associated with decreases in CT, followed by falls in Fv/Fm and Pn. Root senescence differed ($p<0.05$) between hybrids, where FDD always started first, and changes in LRLD preceding those of GLAI. ZT levels in leaves decreased ($p<0.05$) between active-phase and those in senescence-phase. At all positions, the beginning of decrease was later ($p<0.05$) and initial ZT levels were higher ($p<0.05$) in SG: 2.34(H17), 3.03(H20), 4.14(H22) and 7.96(H24) times higher than FDD leaves positions. The decrease per degree-day was 1.11%(H17), 0.63%(H20), 0.39%(H22), 0.58%(H24) of initial values in FDD and 0.79%(H17), 0.72%(H20), 0.27%(H22), 0.64%(H24) in SG. Differences in leaf senescence between SG and FDD were mainly associated with initial ZT levels in leaves. These results are the first to describe variations of leaf cytokinin levels during leaf senescence in sunflower (and other cultivated species), suggest that beginning of leaf senescence is inversely related to leaf ZT levels, and demonstrate that root senescence precedes that of leaves.

Key Words : Canopy senescence, Chlorophyll content, Cytokinins, Leaf senescence, Root senescence, Sunflower, Trans-zeatin levels

PHYSIOLOGICAL BASIS AND ANTIOXIDANT ACTIVITY IN COLD STRESS RECOVER IN SUNFLOWER (HELIANTHUS ANNUS L.)

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ABSTRACT

Tolerance to low temperatures is an important trait, considering that the sunflower production area is expanding to marginal regions with suboptimal growing conditions, and there is an increasing requirement of early sowing to maximize the growing season over Mediterranean areas in countries such as the United States of America, India and Argentina. The present study of the response of sunflower to low temperatures focused on the primary responses on young plants after 96 h under cold treatment at 5°C with the aim of detecting regulatory mechanisms induced at this early stage. Studying the antioxidant activity and physiological bases involved in recovery from cold stress in sunflower seedlings may allow these characteristics to be used in breeding programs aimed at selecting varieties of sunflower adapted to stress from sub-optimal temperatures. The purpose of this research was to establish the recovery from cold stress in terms of the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and its relationship with the physiological response of two sunflower hybrids to the contrasting response to cold stress. Prior studies in the Plant Physiology Laboratory identified two sunflower hybrids with contrasting response to cold at the germination stage: sensitive the hybrid *Pampero*(PM) and tolerant the hybrid *Sierra Alto Oleico* (SA). Ten day old seedlings of commercial hybrids PM and SA were placed in cold storage for 96 hours at 5°C, and cold stress recovery was assessed in terms of the following variables: Level of Damage to Cell Membranes through the content of malondialdehyde (MDA), Antioxidant Enzyme Activity: Superoxide Dismutase (SOD) and Catalase (CAT), and Chlorophyll Content at 0, 24, 48 and 72 hours after exposure to cold. In addition, Total Plant Dry Mass and Leaf area were determined per plant. The response to cold stress was greater in the SA than the PM hybrid, suggesting that the former possesses repair mechanisms at the cell level which are activated more quickly in response to low temperature. This coordination and activity levels of the enzymes SOD and CAT found in this study in SA are in accordance with the lower level of cell damage (observed in lower MDA levels), as compared to PM. Higher antioxidant activity and lower MDA levels allow sunflower plants to maintain their photosynthesizing apparatus active, maintaining the functionality of chlorophyll for dry matter production, and leaf area during the early stages of growth, after exposure to cold stress. All the variables described here may be used as criteria for screening cold-stress tolerant sunflower genotypes.

Key Words : sunflowers genotypes, cold stress recover, abiotic stress, physiological traits, oxidative stress, antioxidant defence.

EXPRESSION OF DEFENSE RELATED GENES IN LEAVES OF TWO SUNFLOWER LINES AFTER INFECTION WITH SPORES OF PLASMOPARA HALSTEDII

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ABSTRACT

Two sunflower lines, susceptible and resistant to downy mildew race 730 were used in this study. Susceptible line was Ha-26 and resistant line was its backcross BC/8 analogue, containing Pl6 gene for downy mildew resistance introduced from initial cross with Ha336. Inoculation with the suspension of *P. halstedii* zoospores, was done on the plants in the phase of first pair of leaves. Twelve days after infection susceptible line developed typical disease symptoms, i.e. leaf chlorosys with or without sporulation, which was not the case with resistant line. In the time period of 2 to 96 hours after treatment leaves were harvested and immediately frozen in liquid nitrogen. Total RNA was isolated by RNAeasy kit (Quiagen). cDNA synthetised by RevertAid First Stand cDNA Synthesis Kit (Fermentas), was used as template in PCR to examine the expression pattern of several defense related genes. The expression of genes for enzymes involved in H2O2 production (Caox; Oxox) was constitutive but significantly higher in resistant line, already 2 h after infection. Similar results were obtained for SODc gene. Higher accumulation of SODp and chitinase transcripts was observed up to 4h after infection in resistant line. PR5 transcript was upregulated in early phases after infection only in resistant line. Our results indicate that the early response to secondary downy mildew infection resembles to hypersensitive-like reaction and is partly responsible for the resistance conferred by Pl6 gene.

Key Words : downy mildew, Pl6, gene expression

A SOURCE-SINK BASED DYNAMIC MODEL FOR SIMULATING OIL AND PROTEIN ACCUMULATION IN SUNFLOWER ACHENES

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ABSTRACT

The potential interest of a dynamic crop model is to provide reliable predictions of oil concentration (OC) soon before harvest as well as helping to understand at which time oil dynamics was affected by environmental stresses or management. For that purpose, we proposed a "source-sink" based dynamic model describing on a daily step nitrogen and carbon assimilations and remobilizations during sunflower grain filling. Priority rules were established for C and N depletion from "source" organs, as well as for their allocation into "sink" organs. Photosynthesis was simulated using the radiation use efficiency approach and nitrogen uptake according to Pan *et al.* (2006) formalisms. Water and N stresses were computed by SUNFLO crop model from climatic and soil data and genotype characteristics. The "source" and "sink" variables were initialized at flowering and main outputs were oil and protein concentrations and weights per m². The model was calibrated on 24 crop situations in 2012 and evaluated independently on 50 other situations (3 years) with contrasted genotypes and environments. Global trends were well reproduced for all "source" and "sink" components but most variables tended to be overestimated. The main indicators of model quality for predicting OC were: RMSE = 6.1 (%), efficiency = 0.97, R^2 = 0.94 and Bias = -0.06 (%). A sensitivity analysis suggested us to reduce the number of parameters, better describe photosynthesis and N uptake processes, and improve the parameterization of genotype and nitrogen effects in order to decrease the prediction error and provide a relevant tool for OC prediction.

Key Words : source-sink model, seed oil content, seed protein content, C-N remobilization, C-N assimilation

MORPHOANATOMY OF INCOMPLETELY DEVELOPED FRUITS IN THE SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

The occurrence of fruits with poorly developed embryo or absent, usually defined as empty, seedless or incompletely developed fruits (IDF), significantly reduces the sunflower yield. The morphology and anatomy of ovaries and fruits sampled 10 and 20 days after anthesis (DAA) and at physiological maturity of Dekasol 3940 were analyzed. Samples were taken on different positions around the capitulum close to the midpoint of the radius. The percentage and size of IDF were similar between sampling dates and capitulum positions. The dimensions of the IDF with black pericarp (IDFBP) and filled fruits (FF) were similar, indicating that pericarp development had finished 10DAA. The width (20%) and thickness (46-57%) of IDF with white pericarp (IDFWP) were lower than IDFBP. 10DAA, 17% of IDFWP presented embryos while the remaining enlargement of the embryo sac and proliferation of integumentary tapetum. The IDFBP presented a developed endosperm with (78%) or without (22%) embryo. At 20DAA, the embryo width and length in IDF did not exceed that of the FF at 10 DAA. The results indicated that IDF were generated by embryo abortion within 10DAA and not from any kind of fertilization failure. The percentage of IDF correlated with FF weight (r = -0.84 **) and capitulum diameter (r = -0.76 **).

Key Words : Sunflower, Empty Fruits, Ovary and fruits anatomy.

LIGHT DEPENDANT BIOSYNTHESIS OF SESQUITERPENE LACTONES IN SUNFLOWER

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ABSTRACT

Asteraceae are rich in sesquiterpene lactones (STL), compounds known to elicit various bioactivities and to serve particularly as protectants against herbivory. In sunflower, STL primarily occur in capitate glandular trichomes (CGT) from where enzymes of their biosynthesis were characterized. In inner tissues, STL are ca. 10.000-fold less concentrated and have completely different function. They probably are involved in light dependent growth reactions. Here we report on the CGT development and STL biosynthesis on leaflets under different light qualities related to cryptochrome and phytochrome. CGT cell division took place between 48 and 72h after seedling germination. Afterwards, STL were produced and secreted into an external cuticular globe. Trichome formation was independent from light quality or intensity applied during germination. However, STL synthesis and expansion of the cuticular globe was triggered by near red (660nm) and blue light (465nm) irradiation during cultivation, whereas far red light (730nm) and darkness did not lead to significant STL accumulations. Short pulses of near red light versus continuous irradiation lead to similar amounts of STL. Moreover, far red light pulses extinguished effects of near red light pulses, thus indicating phytochrome regulation of STL in trichomes. Near red light also influenced the synthesis of the STL 8-epixanthatin in inner sunflower tissues. Compared with seedlings grown in darkness or far red light, near red light increased the STL amount in roots of seedlings 5-fold or more. Internal STL seem to be involved in tropisms. Moreover, they leak into the rhizosphere where they induce germination of broomrape.

Key Words: Trichomes, terpene synthesis, herbivory, phototropism, phytochrome, cryptochrome, sunflower broomrape

LEAF SENESCENCE IN SUNFLOWER WAS ADVANCED OR DELAYED DEPENDING ON CHANGES IN THE SOURCE-SINK RATIO DURING THE GRAIN FILLING PERIOD

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ABSTRACT

Lifespan of leaves is usually associated to grain yield in sunflower. During the grain filling period, demand of grains converges with a decreasing green coverage. The source-sink ratio (SSR) allows estimating a carbon budget between the organs vegetative and reproductive of the plant. The aim of this work was to study the effect of the source-sink ratio during the grain filling period on the leaf senescence in sunflower. Sunflower hybrid VDH 487 was sown in a field trial, and maintained in good conditions of water and nutrition. The SSR was increased in three moments (head removal), and decreased in one (shading), during the grain filling period. Senescence was evaluated from the decrease in the levels of chlorophyll, fluorescence, carbohydrates and dry matter, in leaves 15, 20 and 25 (from the bottom). The progress of all studied variables was advanced about 175°Cd in leaf 15 in comparison with leaves 20 and 25 (p<0.05). The increase in the SSR delayed leaf senescence measured trough all variables studied in this work up to 400°Cd, depending on the leaf number and the variable itself. In general terms, delay was greater when plants were submitted to longest periods of high SSR. Conversely, a decrease in SSR advanced senescence. Depending on the leaf and the studied variable, this advancement attained up to 256°Cd (for the decrease in carbohydrates content). In this work, we verified, by manipulating SSR, that leaf senescence is not fixe in time. As in most monocarpics, grain filling played an essential role in senescence.

Key words: Sunflower, Senescence, Source sink ratio, Grain filling period

INTRODUCTION

Lifespan of leaves is usually associated to grain yield in sunflower. Senescence is a genetically programmed process which produces cell disruption and finally its death after nutrient recycle to younger or storage tissues (Gan and Amasino, 1997; Buchanan-Wollaston et al., 2003). It is influenced for both genetics and environmental variables. As it generally occurs at the end of the lifespan it is associated to a decrease in photosynthetic activity, macromolecules degradation, loss of chlorophyll, decrease in nitrogen content, etc. (Smart, 1994; Buchanan-Wollaston, 1997). Some, or all, of these processes can be used as traces of senescence progress (Moschen et al., 2012). Loss of chlorophyll is probably the feature the most used to characterize leaf senescence, but carbohydrates and photosynthesis are also mentioned in literature (Crafts-Brandner et al., 1984; Sadras et al., 2000).

Reproductive growth in annuals is related to leaf senescence since senescent leaves increase after flowering (Thomas and Stoddart, 1980; Gan and Amasino, 1997; Yoshida, 2003). Thus, demand of grains converges with a decreasing green coverage during the grain filling period.

The source-sink ratio (SSR) allows estimating a carbon budget between the organs vegetative and reproductive of the plant. Imbalances of SSR can accelerate leaf senescence (Rajcan and Tollenaar, 1999). In oilseeds species, including sunflower, experiments preventing grain formation showed a delay in leaf senescence (Lindoo and Nooden, 1977; Ho et al., 1987). In maize, there are contradictory findings in literature. While in most works an increase in the SSR resulted in advanced leaf senescence (Rajcan and Tollenaar, 1999; Allison and Weinmann, 1970), some others showed an advance or a delay in leaf senescence (Thomas and Smart, 1993). In all of these cases, carbohydrates metabolism plays a principal role (Wingler et al., 2006).

During the grain filling period both the source and the sink adjust their relative magnitude in response to environmental and own of the plant factors. Thus, not only SSR could change as it progress through the period, but we can hypothesize that it effect on leaf senescence could also be modified. No reports were found about the effect of the changes in SSR in different moments of the grain filling period on leaf senescence.

The aim of this work was to study the effect of the source-sink ratio during the grain filling period on the leaf senescence in sunflower.

MATERIALS AND METHODS

The experiment was conducted at the INTA Balcarce Experimental Station, Argentina (37° 45´S, 58° 18´W) during the 2012/13 season. Hybrid VDH 487 (Advanta) was sown the 22 Oct. Emergence occurred 9 days after sowing. Plant density was 5.7 plants m-2. The experiment was conducted under good nutritional and water conditions. Weeds and insects were controlled. Phenological stages were recorded according Schneiter and Miller (1981). Flowering occurred the 9 Jan. and physiological maturity the 12 and 16 Feb. in control and shading treatments, respectively. Time was expressed on a thermal time basis by daily integration of air temperature with a threshold temperature of 6 ◦C (Kiniry et al., 1992). The following treatments were applied in a factorial combination of different levels of source-sink ratio (SSR) in a randomized block design with three replicates: (i) head removal in stages R6, R7 and R8, (ii) 50% reduction of solar radiation in R7 stage by shading, and (iii) untreated control. Each plot consisted of four 10m long rows spaced at 0.7m. Daily mean temperature and solar radiation and rainfall were measured in a weather station located 400 m from the experiment. Target leaves (15, 20 and 25 from the bottom) were selected based on the representativeness of the profile of the plant.

Leaf initiation in the apex was measured every 48 to 72h from emergence after apex dissection under stereoscopic microscope of three plants *per* plot. Results from the progress in time of the number of leaves were adjusted to a linear regression model, from which the initiation date at the apex of target leaves was estimated.

Dry matter of target leaves was measured every 15 days in three plants per plot. Samples were cut from the plant and oven-dried (with air circulating at 60 ◦C) to constant weight and weighed.

Soluble carbohydrates were measured every 15 days according to Dubois et al. (1956), from a 50mg sub sample of leaves dry matter after milled. Absorbance at 490nm from both the sample and the glucose standard was measured with an spectrophotometer (Bausch & Lomb, Spectronic 20, USA). 19th International Sunflower Conference, Edirne, Turkey, 2016 Quantum yield of PSII was measured every 10 days with a handheld fluorometer (FluorPen FP100 Z990, Photon Systems Instruments, Drasov, República Checa).

Chlorophyll was measured every 5 to 16 days after extraction with 2,3 N, N dimetilformamida from 6 discs of 0.5 cm diameter taken from each leaf. Absorbance at 664.5nm and 647nm was measured with a spectrophotometer (Bausch & Lomb, Spectronic 20, USA). Chlorophyll content was estimated according Inskeep and Bloom (1985).

The timing of the evolution of each of the variables in which its value fell to 80% of its maximum value was considered as an indicator (event) of the onset of senescence evaluated with that variable in each target leaf. In the case of chlorophyll are also considered the 20% ("yellowing") and 0% ("dead"). The occurrence of these related to senescence events, was ordered on a unique thermal time scale. The sequence of events was considered modified when the occurrence of at least two of them was reversed compared to the sequence set in the control (for evaluation of SSR effect) or compared to any of the remaining two leaves (for evaluation of the effect of leaf age).

Data of dry matter, carbohydrates, chlorophyll and quantum yield in each sample date were processed by analysis of variance procedures. Sequences of senescence related events were assessed by the method of analysis of variance using an unique model including treatments and leaves. Differences between the mean values were evaluated from the test of least significant difference (LSD, p <0.05). All analyzes were performed with the statistical analysis program Infostat Professional v.1.1 (Di Rienzo and Robledo, 2002).

RESULTS

Meteorological conditions during the experiment*:*

Daily mean temperature during the experiment was 1ºC higher than the historical average, being lower only in March. Contrarily, daily mean radiation did not differ significantly from the historical (18.3 vs. 18.5 MJ.m-2.d-1). In December and January, radiation in 2012-13 was lower than the historical, while in February it was higher (Table 1). Rainfall was higher than the historical, especially in December and January (134% and 40%, respectively, Table 1).

Table 1: Daily mean temperature and solar radiation and rainfall during the months of the experiment (2012/13), and for an historical series (H) of 41 years (1971-2011).

	Temperature (ºC)		Solar radiation ($MJ.m^{-2}.d^{-1}$)		Rainfall (mm)	
	2012/13	H	2012/13	H	2012/13	H
Nov-12	17.7	16.0	21.7	21.1	64	89
Dec-12	20.2	18.8	21.1	22.0	239	102
Jan-13	21.1	20.2	20.6	22.0	152	108
Feb-13	21.2	19.8	21.1	19.5	33	85
Mar-13	16.4	18.1	15.3	15.2	114	92
Apr-13	17.2	14.4	9.8	10.9	74	80

Characterization of the effect of treatments on the target leaves.

Dry matter:

In leaf 15, after the application of R6 and R7 treatments the loss of dry weight was delayed, while in S it was not affected (Fig. 1.A). In leaves 20 and 25, head removal produced an increase in dry matter (R6) and/or the delay in weight loss in advanced stages (R6, R7 y R8).

Shading accelerated weight loss (Fig. 1 B y C). The weight of the leaves towards the end of the cycle was higher as the demand was interrupted before (R6>R7>R8>C).

Chlorophyll:

After head removal in R6 and R7 a delay in chlorophyll degradation was observed in leaf 15 (75% between R6 and C at 1068°Cd, $p \leq 0.05$, Fig. 1.D). Maximal chlorophyll content in leaf 20 was near 0.04 mg/cm2. Head removal also delayed chlorophyll degradation in this leaf, while in S it was advanced (Fig. 1.E). The same was observed in leaf 25, although degradation in S was faster than in leaf 20 (Fig. 1.F).

Quantum yield of PSII (Qy):

Head removal in R6 and R7 delayed decrease in Qy in leaf 15 (Fig. 1.G). In leaf 20, head removal in R6 maintained maximal Qy 400°Cd more than C (Fig. 1.H). Treatment S advanced Qy fall, which attained 0% near 200°Cd before the control (Fig. 1.H). Treatments R6, R7 and R8 in leaf 25 delayed 100°Cd the decrease in Qy (Fig. 1.I). Conversely, S treatment advanced more than 200°Cd the falling of this variable, presenting at 1200°Cd a Qy 65% lesser than the control ($p \leq 0,05$, Fig. 1.I).

Soluble carbohydrates (HCS):

Head removal in R6 allowed maintaining maximal HCS concentration more than 100°Cd in leaf 15. When treatment was applied in R7, HCS of the C was already reduced to the half of the maximal value; anyway it delayed the falling more than 100°Cd comparing to C (Fig. 1.J).

In leaf 20, head removal allowed in the three treatments (R6, R7 y R8) maintained higher concentration of HCS for a longer period than C, and in the cases of R6 and R7 also delayed the start of the decrease. Conversely, in S advanced more than 300°Cd both the start and the end of the drop in HCS (Fig. 1.K). In leaf 25 the response was rather similar to that of leaf 20 (Fig. 1.L).

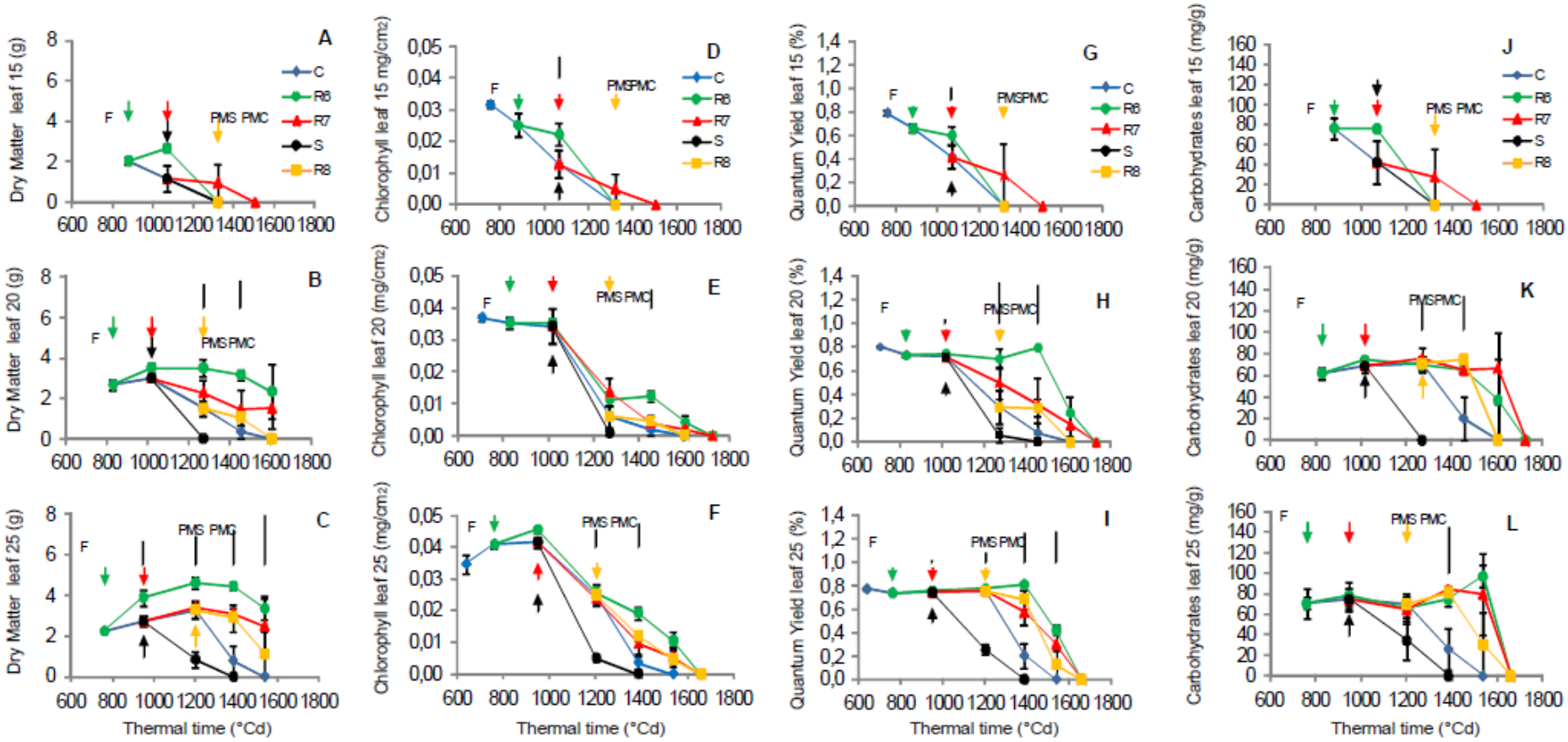

Fig. 1 Dry Matter (A-C), Chlorophyll content (D-F), Quantum yield of PSII (G-I) and Carbohydrates (J-L), in Leaf 15 (A, D, G, J), 20 (B, E, H, K) and 25 (C, F, I, L) as a function of the thermal time after their initiation in the apex. Treatments: control: C, head removal in the developmental stages: R6, R7 and R8, and shading reducing 50% of incident radiation: S, in R7 stage. Vertical lines on the symbols are the standard error of the mean value (n=9). Vertical lines above indicate the least significant difference for each sample date (α=0.05, LSD). Arrows indicate treatment application. F means flowering, and PMC and PMS, physiological maturity in C and S treatments.

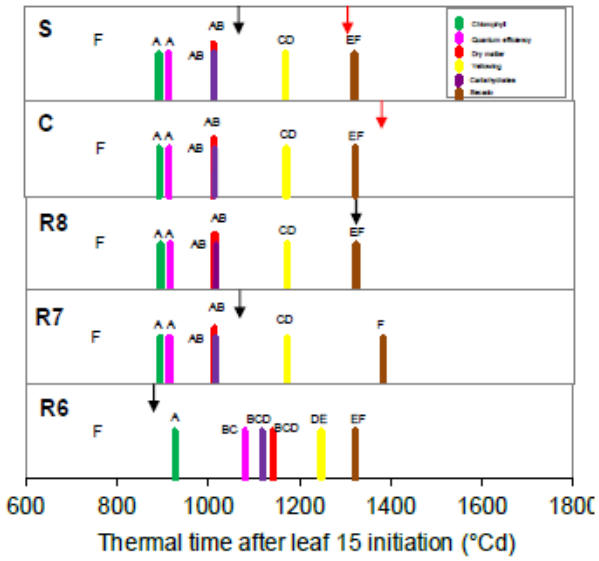

Fig. 2 Events related to leaf senescence sorted in a thermal time scale from the initiation of leaf 15 in the apex. Different letters indicate that 2 events from the same or a different treatment are significantly different (α=0.05). Arrows indicate treatment application (black) or physiological maturity (red). F indicates flowering date.

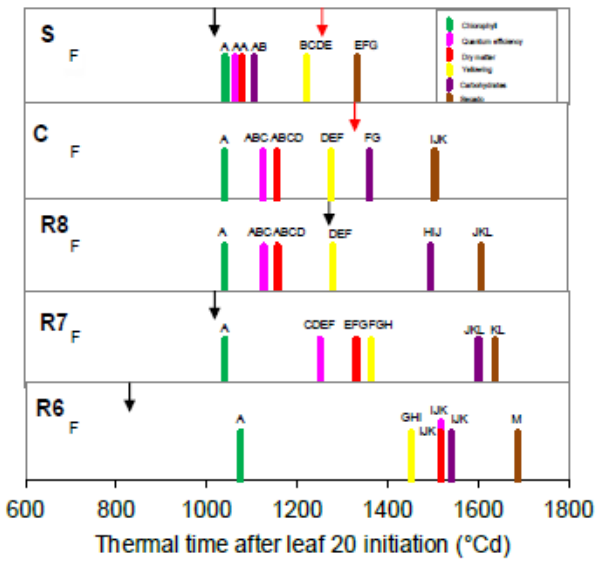

Fig. 3 Events related to leaf senescence sorted in a thermal time scale from the initiation of leaf 20 in the apex. Different letters indicate that 2 events from the same or a different treatment are significantly different (α=0.05). Arrows indicate treatment application (black) or physiological maturity (red). F indicates flowering date.

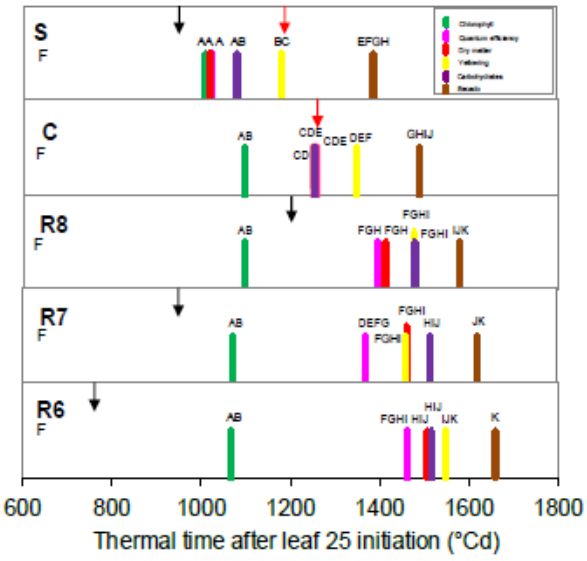

Fig. 4 Events related to leaf senescence sorted in a thermal time scale from the initiation of leaf 25 in the apex. Different letters indicate that 2 events from the same or a different treatment are significantly different (α=0.05). Arrows indicate treatment application (black) or physiological maturity (red). F indicates flowering date.

Effect of SSR on the sequence and the moment of occurrence of the events:

In leaf 15, chlorophyll, Qyield, dry matter and carbohydrates were the first events in the control. More than 150°Cd later happened yellowing ($p<0,05$), and then leaf definitively dead ($p<0,05$, Fig. 2, C). SSR treatments did not affect neither the sequence nor the moment of occurrence of the events, in comparison with the control ($p>0,05$, Fig.2 R7, R8 y S), excepted R6 where Qyield was delayed ($p<0,05$, Fig. 2, R6).

In leaf 20, chlorophyll, Qyield and dry matter were the first events occurred in C treatment ($p<0.05$). Then, 100°Cd and 200°Cd later were observed yellowing and carbohydrates ($p<0.05$), respectively, finally dying at 1500°Cd after its initiation ($p<0.05$, Fig. 3 C). In R6, a delay in all events was observed with exception of chlorophyll (Fig. 3 R6). Qyield and dry matter occurred almost 400°Cd later than C, and yellowing, carbohydrates and death about 180°Cd later ($p<0.05$, Fig. 3 R6). In R7, dry matter and carbohydrates were delayed near 200°Cd from C ($p<0.05$, Fig. 3 R7). The carbohydrates was the only event affected by R8 treatment (delayed 130°Cd in comparison with the C, $p<0.05$, Fig. 3 R8). Shading advanced 250°Cd the occurrence of carbohydrates and dead ($p<0.05$, Fig. 3 S). SSR treatments did not affect the sequence of events in leaf 20, according to our evaluation parameters (see materials and methods).

In leaf 25, chlorophyll was the first event in the control treatment. 160°Cd later ($p<0.05$), occurred Qyield, dry matter and carbohydrates. Near 100°Cd yellowing did not differ from the previous events ($p>0.05$), and finally leaf died 140°Cd later ($p<0.05$, Fig. 4 C). Treatment R6 delayed all the events, excepted chlorophyll, between 170°C and 250°C ($p<0.05$, Fig. 4 R6). When treatment was applied in R7 either dry matter or carbohydrates were delayed from the control ($p<0.05$, Fig. 4 R7). In R8, also Qyield was delayed ($p<0.05$, Fig. 4 R8). SSR decrease by shading, advanced all the studied events ($p<0.05$) save chlorophyll and yellowing, being dry matter and Qyield the events the plus affected (more than 230°Cd in comparison with C, $p<0.05$, Fig. 4 S). SSR treatments did not affect the sequence of events in leaf 25.

Effect of the leaf age on the sequence and the moment of occurrence of the events:

Life duration of leaf 15 was 184°Cd and 166°Cd shorter than those of leaf 20 and 25 (comparing the last event, $p<0.05$, Fig. 5). Sequence of events in leaf 15 and leaf 25 was similar. In leaf 20 carbohydrates occurred between yellowing and dead, in contrast with leaves 15 and 25 in which it occurred before yellowing ($p<0.05$, Fig. 5). In leaf 15, all the events occurred before than in leaves 20 and 25 ($p<0.05$). In this last, Qyield occurred 132°Cd later than in leaf 20 ($p<0.05$), while carbohydrates occurred 107°Cd before, even if difference was not significant ($p>0.05$, Fig. 5).

SSR effect on the events related to the senescence according to the time of the filling period:

The increase in SSR by removing the head at different times of the grain filling period lead to a delay in the occurrence of all events studied in this work except the fall of chlorophyll to 80% of its maximum value (Fig. 6.A, B, C, D, E and F). Depending on the event, the time at which the increase in SSR occurred, and the leaf, this delay reached 400°Cd (Fig. 6.B). Overall, the event lasted more when the time between the application of the treatment and the occurrence of the event was higher. It can be observed in certain events as the fall of the dry matter, yellowing and leaf dead, an approximately linear relationship between both variables (Fig. 6.C, D and F). In other events, such as the fall of carbohydrates, the delay was approximately constant at changes over 450°Cd in the period between the application of the treatment and the occurrence of the event (Fig. 6.E). Conversely to an increase in SSR, its reduction by applying a shade, advanced all the events studied in this work (Fig. 6.A, B, C, D, E and F). Depending on the event and the leaf, this advance ranged between 88°Cd (chlorophyll, Fig. 6.A) and 256°Cd (carbohydrates, Fig. 6.E).

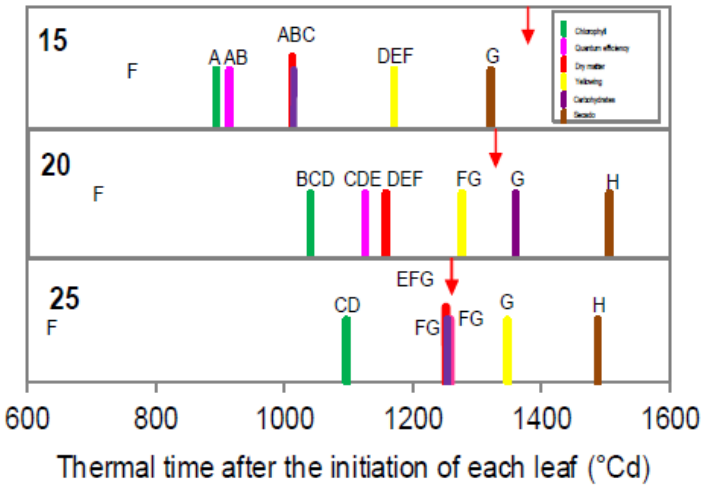

Fig. 5 Events related to leaf senescence in leaf 15, 20 and 25 of the control treatment sorted in a thermal time scale from the initiation of each leaf in the apex. Different letters indicate that 2 events from the same or a different leaf are significantly different (α=0.05). Red arrows indicate physiological maturity, and F the flowering date.

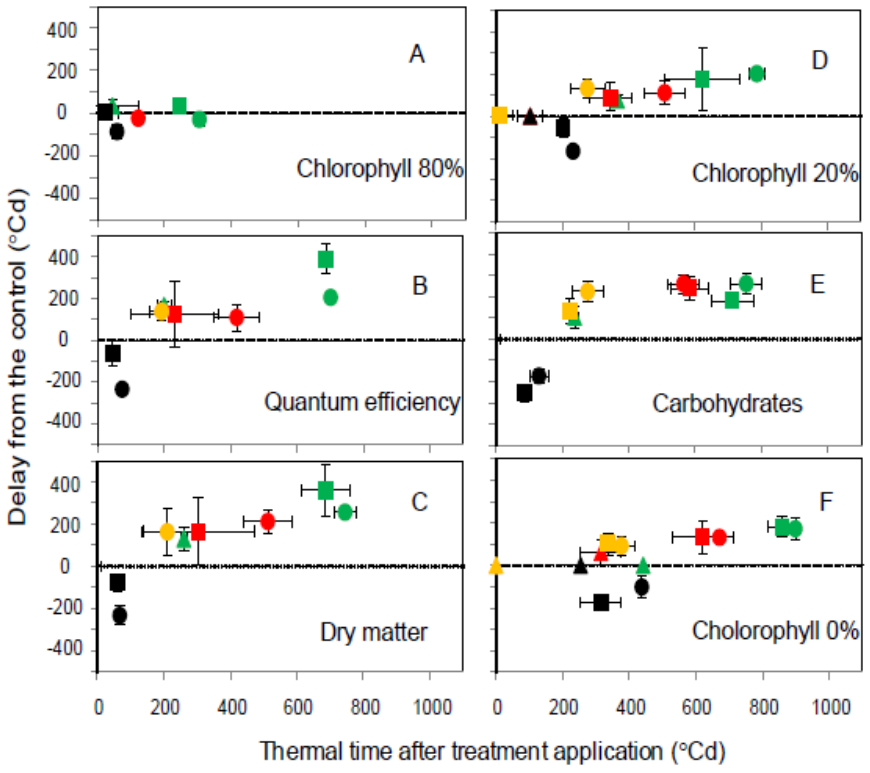

Fig. 6 Delay from the control of the events time of occurrence as a function of the time after the treatment application. Treatments: head removal at the developmental stages R6 (green), R7 (red) and R8 (orange), and shading (S, black). Leaves: 15 (triangle), 20 (square) and 25 (circle). Vertical and horizontal lines on the symbol represent the standard error of the mean value (n=9). Data from events which naturally occurred before treatment application was not included.

The period between treatment application and the occurrence of the events in leaves 20 and 25 was longer than in leaf 15 (Fig. 6.A, B, C, D, E y F). Even, in this last leaf some events occurred previously to the application of certain treatments, and therefore did not appear in the corresponding figures (Fig. 6.A, B, C, D y E). The delay or advance in the occurrence of events by effect of SSR increasing or decreasing, respectively, in the leaves 20 and 25, was generally similar excepted in Qyield and dry matter, where leaf 20 was affected more than 25, in treatment R6, and conversely, leaf 25 was more affected than leaf 20 in shading treatment (Fig. 6.B and C).

DISCUSSION

The increase in SSR by head removal delayed leaf senescence measured through all the variables used as possible senescence indicators in this study. This occurred in a greater or lesser extent in the 3 leaves studied and in spite of the time of the grain filling period in which occurred the increase in SSR (Fig. 1). Ho and Below (1989) and Purohit (1982) also observed a delay in leaf senescence after head removal by measuring chlorophyll content. Sadras et al. (2000) reported comparable results working also with leaf nitrogen and dry matter concentration. The absence of the reproductive sink in annuals prevents grain demand and consequently nutrient remobilization from leaves. This will allow keep longer cell structure and functionality. Otherwise, the lack of the head in sunflower was related to a prolongation of roots functionality, which was associated to leaf duration (Lisanti et al., 2012). Conversely, a decrease in SSR by shading produced an advancement of leaf senescence measured through all studied variables. The decrease of incident radiation causes a decrease in photosynthesis which could be the cause of the onset of senescence.

When we classed our events in a unique thermal time scale we established a sequence. Even if each event, as observed for the complete evolution of the respective variable, mostly delayed or advanced with an increase or a decrease in SSR, respectively, the sequence established remained irreversible not only to changes in SSR, but also to the moment of these changes (Fig 2, 3 and 4) and to the age of the three studied leaves (Fig 5). From these results we could assume that once triggered, leaf senescence would follow the same metabolic signaling pathway.

In other attempt to classify senescence events in sunflower, Moschen et al. (2012) observed that N content in leaf 25 occurred much earlier than our first event, the drop of chlorophyll to 80% of its maximal value. In our work, the first event was the only one remaining stable after changes in SSR and in leaf age, although in several cases (especially in leaf 15) this event occurred before the application of the treatment. From these results we could guess that changes in SSR did not prevent senescence triggering, but they shortened or lengthened senescence period.

As change in SSR was before, the effect on the occurrence of an event was mostly higher. The exceptions were chlorophyll drop to 80% (above mentioned) and carbohydrates drop (Fig. 6). This was probably related to integrating carbohydrates functions in senescence regulation (Wingler and Purdy, 2006).

CONCLUSION

this work demonstrates that SSR during the grain filling period can advance or delay leaf senescence in sunflower. This effect did not involve the onset but the subsequent evolution of senescence. The sequence of studied events related to senescence was not modified by changes in SSR. Senescence in lower leaves occurred earlier than in upper leaves but preserving the same sequence of events.

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TWO SIMPLE MODELS INCLUDING THE SOURCE/SINK RATIO TO EXPLAIN BLACK STEM BY *PHOMA MACDONALDII* IN SUNFLOWER

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ABSTRACT

Black stem (BS) by *Phoma macdonaldii* Boer of sunflower (*Helianthus annuus* L.) is the most prevalent foliar disease in the Buenos Aires province, main sunflower area of Argentina. The source- sink ratio (SSR) of sunflower crop affects the plant susceptibility to BS, although this effect may be influenced by several factors. The aim of this work was to establish simple models to take into account the SSR of sunflower to estimate BS incidence and severity, in different environmental conditions, hybrid cultivars and leaf stratums. Three field experiments, including two hybrids, were performed at Balcarce, Argentina. The SSR was modified by grain excision or shading, during the grain filling period. BS incidence and severity (in nodes 8, 12 and 20) were evaluated weekly from flowering. SSR took account of a significant fraction of the yearly incidence ($R2 \geq 0.619$) and severity variation ($R2 \geq 0.458$), both hybrids comprised. To include the annual variability, regression analyses were performed between meteorological and physiological variables (PAR, % interception, number of rainy days, mm of rainfall) and BS incidence and severity. In the case of severity, the age of the leaf was also included. Multiple linear and non-linear models were selected by the analysis of stepwise and residual methods. About 80% of the total variation in incidence and severity of BS due to hybrids, experiments and treatments, was explained by simple models including SSR and number of rainy days ($p \leq 0.0001$), or SSR, number of rainy days and leaf age ($p \leq 0.0001$), respectively. Simple models like these are potentially useful in the assistance to crop management, and could also be included to improve simulation models of diseases, growth and development in sunflower.

Key words: source- sink ratio, *Helianthus annuus* L., *Phoma macdonaldii* Boer., rainy days, age of the leaf

INTRODUCTION

The "black stem" (BS) caused by the necrotrophic fungus *Phoma macdonaldii* Boer (teleomorph *Leptosphaeria lindquistii*) is the most prevalent leaf disease in sunflower (*Helianthus annuus* L.) in the Buenos Aires province (Lazzaro *et al*., 2013), the main sunflower production area of Argentina (1.16 to 2.40 thousand ton of grains in the last ten years, SIIA-MAGPyA, 2015). Symptoms appear on the stem near flowering stage, progress from bottom to upper leaves, and are usually associated to previous necrosis in veins, petiole and/or leaf lamina (Bordat *et al*., 2011). *P. macdonaldii* also attacks roots and the collar of the plant, producing a stem girdling lesion at the soil level at the beginning of premature ripening (Donald *et al*., 1987). As yet, there are no reports of sunflower genotypes with high resistance to BS or premature ripening. Yield losses between 10 and 30 % were reported to be associated to BS (Debaeke and Pérès, 2003; Velásquez and Formento, 2003) or premature ripening (Carson, 1991) via a

decrease in intercepted radiation, related to premature leaf senescence and/or in radiation use efficiency (Quiroz *et al*., 2014).

Crop models have many current and potential uses for answering questions in research and crop management. Models can assist in synthesis of research understanding about the interactions of genetics, physiology, and the environment, integration across disciplines, and organization of data Boote *et al*., 1996. Often, mathematicians and statisticians models are used to study and describe plant growth, the effect of management practices and development of diseases Campbell y Madden, 1990 Hernandez *et al*., 2009.

BS estimate models have been developed by Debaeke and Peres (2003) and Desanlis (2013). These models consider epidemiological aspects as canopy microclimate (relative humidity and temperature), plant growth (leaf area index) and fungicide treatment, to account for climatic and agronomic limitations to fungus development. Further, in a previous work by our group were determined that BS incidence and severity were inversely related to the source -sink ratio (Nuñez Bordoy *et al*., 2012). These relations suggests the existence of a stem carbohydrates threshold under of this the BS incidence or severity start to increase. The carbohydrate content of the plant could closely relate to the existence of one or more substances that inhibit the growth of fungi, such as phenolics and terpenes (Silva Acuña *et al*., 2000) associated to plant response against pathogens. Because the relation between SSR and BS symptom could be affected by plant intrinsic factors or by meteorological and agronomic conditions we formulate the objective to establish simple models to take into account the SSR of sunflower to estimate BS incidence and severity, in different environmental conditions, hybrid cultivars and leaf stratums.

MATERIALS AND METHODS

Three field experiments (Exp. 1, Exp. 2 and Exp. 3) were the INTA Balcarce Experimental Station, Argentina (37º45' S, 58º18' W). Hybrids VDH 487 (Advanta Seeds SAIC, Argentina), 81 days from emergence to flowering, and Baqueano (KWS Argentina SA), 88 days from emergence to flowering, were sown on the Typic Argiudol soil (USDA taxonomy, organic matter 7.4%, P-Bray 25.8 ppm). Plant density was adjusted manually to 5.6 plants m-2 in the three experiments and rows were 0.7 m apart. The crops were grown under good water and nutrients conditions. Weeds and insects were controlled adequately through cultural and chemical techniques.

The experiments were conducted under conditions of natural inoculation of *P. macdonaldii* in plots infected with the pathogen (verified in previous experiments). Additionally, pieces of infected milled sunflower plants from previous experiments were homogeneously distributed in the plots during V6 stage (Schneiter and Miller, 1981) to assure *P. macdonaldii* presence.

In order to modify the source-sink ratio (SSR) with a different approach (modifying the sink or the source), two sorts of treatments were applied after the end of flowering (R6, Schneiter and Miller, 1981):

1. Grains excision: grains from about two (G↓) or three (G↓↓) quarters of the head were carefully removed (Echarte *et al*., 2012).

2. Reduction of solar radiation: a 38 % uniform shading with black, synthetic and neutral mesh cloth (S) was applied (Dosio *et al*; 2000).

3. An untreated plot was kept as control (C).

Source sink treatments and hybrids were combined in a randomized complete block design with three replicates. Each plot consisted of four rows of 6 m long, spaced at 0.7 m.

Daily global incident radiation and rainfall were measured in a weather station located 400 m from the experiments. Daily mean air temperature in treatments control and shaded (S) was also measured at leaves 8, 12, 20 and 28 level (from the bottom of the plant) with copper/constantan thermocouples. The

average of the temperature at these three levels was used for thermal time estimates. Data were averaged every 3600 s, and recorded by a data logger (Cavadevices.com, Buenos Aires, Argentina). Thermal time was calculated by daily integration of air temperature and a base of 6 °C (Kiniry *et al*. 1992), and cumulated from flowering.

Daily incident photosynthetically active radiation (PAR) was calculated as 0.48 × global daily incident radiation. The proportion of PAR intercepted by the crop at noon was determined according to Gallo and Daughtry (1986) as (1 − Rb/Ro), where Rb is PAR measured below the last green leaf and Ro is PAR measured above the canopy. Rb and Ro were measured weekly at solar noon (±1 h) with a line quantum sensor (LI-191SB, LI-COR, Lincoln, NE, USA). The daily proportion of PAR intercepted between two measurements was calculated by linear interpolation. The daily intercepted PAR (iPAR) was calculated as the product of the daily incident PAR and the daily proportion of PAR intercepted. The iPAR was cumulated from flowering to physiological maturity (PM).

Incidence of BS by *P. macdonaldii* (I %) was evaluated every 7-10 d in 3 plants per plot (n=9), as the ratio between the affected to the total number of nodes per plant. Severity of BS by *P. macdonaldii* (S %) was evaluated every 7-10 days in leaves 8, 12, 20 and 28 (from the bottom of the plant), selected to obtain a suitable plant profile of BS severity, in 3 plants per plot (n=9), following the methodology proposed in *Quiroz et al*. (2014). BS incidence and BS severity were estimated at 300, 350, 400 °Cd and PM by interpolation between two successive measurements.

The source–sink ratio (SSR) was periodically calculated during the grain filling period as the quotient of accumulated iPAR and the grain number per plant affected by the grain weight of treatment G↓↓, considered the closest to the potential weight. SSR was estimated at 300, 350, 400 °Cd and PM by interpolation between two successive measurements.

Data of SSR, incidence and severity of BS were processed by analysis of variance procedures (INFOSTAT Professional v.1.1, Di Rienzo *et al*., 2010). Differences among treatments means were evaluated with the LSD test ($P \leq 0.05$). Data of incidence and severity of BS as a function of SSR were adjusted to exponential models (Nuñez Bordoy *et al*., 2012) at 300, 350 and 400 °Cd from flowering and at PM (Sigma Plot v. 11.0 Systat Software Inc., 2010). For each variable the model with the greatest signification (α) and coefficient of determination (R2) was selected. Models did not include outliers.

To include the annual variability, regression analyses were performed between meteorological variables (PAR, % interception, number of rainy days, mm of rainfall) and BS incidence and severity. In the case of severity, the age of the leaf was also included. Multiple linear and non-linear models were done by the analysis of stepwise and residual methods (INFOSTAT Professional v.1.1, Di Rienzo *et al*., 2010).

RESULTS AND DISCUSSION

Source-sink ratio after flowering slightly increased or remained steady in plants from control and shading treatments, while grain excision (G↓ and G↓↓) mainly increased it in all tested situations (Fig. 1). SSR increase by grain removal is attributable to a higher retention of green leaves which still intercept solar radiation (data not shown), inclusive in some cases, SSR still increases yet after physiological maturity. Although productive sense of SSR after the end of grain filling is questionable, if photosynthesis is yet detectable, assimilates could be stocked in alternative sinks like stem.

An interaction between the effect of the experiment and that of the treatment ($p<0.0044$) was observed for SSR at 400ºCd after flowering (Fig. 1.A, B, C and D). No differences between S and control treatments were observed in Exp.1 and Exp.2 ($p>0.05$), probably because of a mild drop in the grain number (data not shown). Since grain number in sunflower is finally set near 20 days after flowering (Connor and Hall, 1997), in some cases we probably applied shading treatment just before grain number was set. Grain excision treatments showed a higher SSR in Exp.1 (216%, G↓↓) and Exp.2 (47% and 200%, G↓ and G↓↓, respectively) than that of the control (Fig. 1.A, B, C and D). Furthermore, hybrid Baqueano had SSR

more than 30% higher than hybrid VDH 487 at this thermal time after flowering (p<0.0001, Fig. 1.A, B, C and D), due to a lesser demand from grains with a small potential weight than hybrid VDH 487.

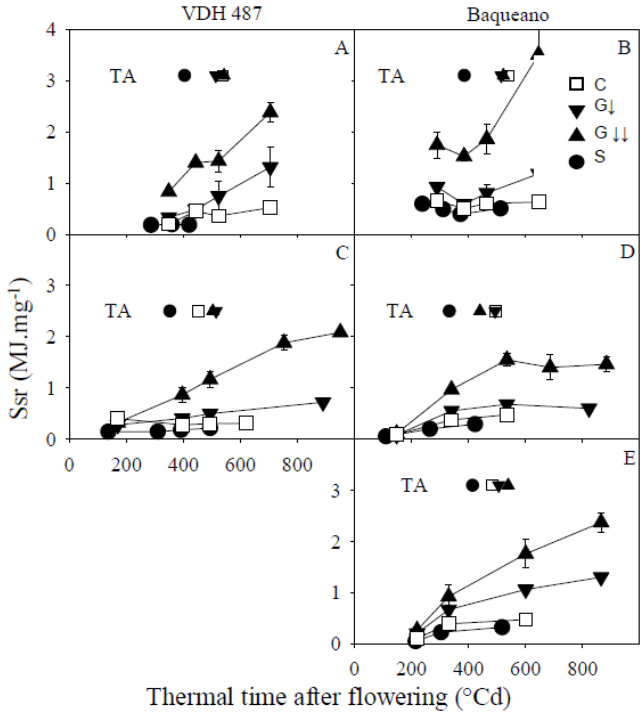

Fig. 1. Source-sink ratio (SSR) as a function of the thermal time after flowering for hybrids VDH 487 (A and C) and Baqueano (B, D and E), in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

Incidence of BS by *P. macdonaldii* increased during the grain filling period in all treatments, hybrids and experiments (Fig. 2). Shading treatment almost always accelerated the incidence increase (Fig. 2.A, B, C and D), while grain excision usually reduced it (Fig. 2.A, C, D and E). BS incidence at 400ºCd after flowering was highly affected by applied treatments (p<0.0001, Fig. 2 A, B, C and D). Plants from shading treatment increased 42% BS incidence (p<0.05), while those from the higher grain excision treatment (G↓↓) decreased it 16%, in comparison with control plants (p<0.05, Fig. 2.A, B, C and D). No hybrid effect was observed for this variable (p=0.4732). The lower values of BS incidence were observed in Exp. 3, while the highest in Exp. 1 (14% and 59%, respectively, mean of all applied treatments, Fig. 2.B, D and E). An interaction between the treatment and the experiment was observed for this variable (p<0.0001).

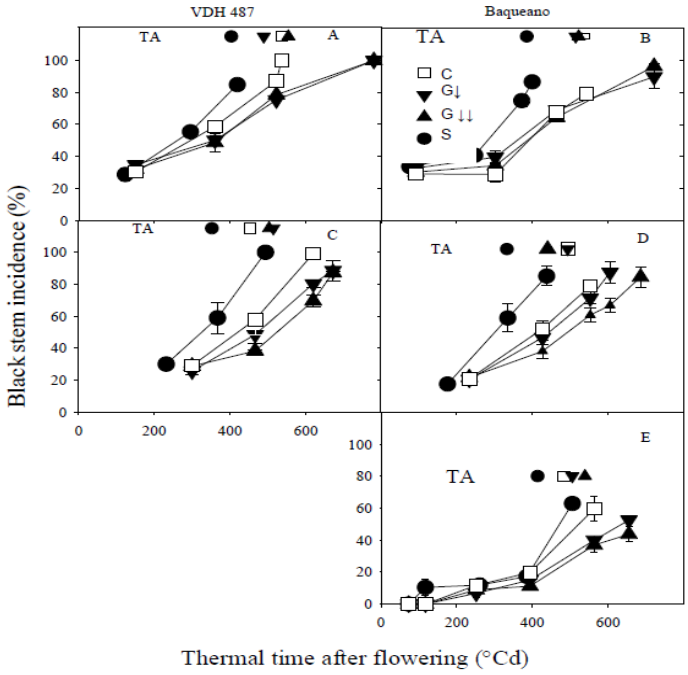

Fig. 2. Black stem incidence (%) as a function of the thermal time after flowering for hybrids VDH 487 (A and C), Baqueano (B, D and E) in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

Symptoms of BS by *P. macdonaldii* appeared first in leaves from the bottom of the plant and progressed upwards to upper leaves in all hybrids, treatments and experiments (p<0.0001). This result corroborates the rise acropetal nature of this disease (Quiroz, *et al.* 2014). In turn, leaf functionality is associated to incident light and therefore to its position on the stem. Bottom leaves receive an intensity and aquality of light (red/far red ratio) poorer than upper ones which make them senesce before (Rousseaux *et al*., 1996), may be more susceptible to *P. macdonaldii* infections for photosynthetic stress-translocation balance.

The onset and the progress on the node of BS severity symptoms were advanced in shading treatment (S) and delayed in grain excision treatments (G↓, G↓↓) in most hybrids and experiments (Fig. 3, leaf 20 as example).

A significant effect of the treatments (p<0.0001), the hybrid (p=0.0074) and the leaf (p<0.0001) on BS severity was observed at 400ºCd after flowering. Shading increased 50% BS severity while grain excision reduced it 18% and 33% (treatments G↓ and G↓↓, respectively, p<0.05) in comparison with the control (Fig. 3.A, B, C and D, leaf 20 as example). Hybrid VDH 487 showed severity symptoms more than 10% higher than hybrid Baqueano (p<0.05). Leaf 8 was the most affected by BS severity, followed by leaf 12 and leaf 20 which was the less affected (difference of 80% between leaves 8 and 20, p<0.05).

The effect of the experiment interacted with those of the treatment and the leaf (p=0.0033 and p=0.0025, respectively) Treatment S during Exp. 1 and Exp. 2 presented higher BS severity values than the rest of the treatments at 400ºCd after flowering (p<0.05). In Exp. 3, we observed the lesser BS severity in all treatments (Fig. 3. B, D and E, leaf 20 as example). Leaves 8, 12 and 20 presented lower BS severity values in Exp. 3 in comparison with Exp. 1 and Exp. 2 (p<0.05, Fig. 3. B, D and E, leaf 20 as example). There were not observed symptoms of BS severity in leaf 28 at 400ºCd after flowering (data not shown).

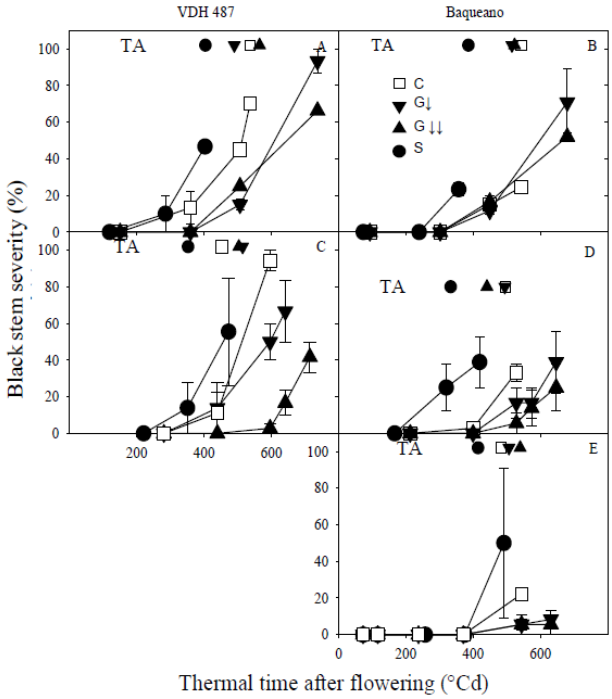

Fig. 3. Black stem severity (%) in leaf 20 as a function of the thermal time after flowering for hybrids VDH 487 (A and C), Baqueano (B, D and E), in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

As stated in MM, results of incidence and severity of BS as a function of SSR were adjusted to exponential models at 300°Cd, 350°Cd and 400°Cd from flowering and at physiological maturity, however we decided to carry out modeling with results from 400°Cd from flowering for three reasons: (i) before this date, disease symptoms were not important yet (Fig. 2 and Fig. 3), (ii) this was the very last date in which we kept all the treatments for the analysis, since in shading treatment, the stem of the plants became black, impeding measurements, and (iii) adjustments at 300°Cd, 350°Cd and 400°Cd from flowering and at physiological maturity presented lower significance and/or determination coefficient (data not shown). Both incidence and severity of BS by *P. macdonaldii* decreased with increasing SSR. As a consequence of the highly significant effect of the experiment and the leaf presented above, results from treatments at 400°Cd from flowering adjusted to negative exponential models for each experiment in the case of BS incidence ($R2 \geq 0.619$, Fig. 4.A), and for each measured leaf in the case of BS severity ($R2 \geq 0.458$, Fig. 4.B).

Similar results were reported by Eslava *et al.* (2007) observed root and presence of mycelia from *Fusarium spp*. on the stem base. This function suggests the existence of a stem carbohydrates threshold under of this the BS severity start to increase, as Davet and Serieys (1987) had shown for *Macrophomina phaseolina* infection at base stem in sunflower.

The higher BS incidence was observed in Exp. 1 (Fig. 4.A), while the higher BS severity was almost always observed on leaf 8 and the lower on leaf 20 (Fig. 4.B). Increasing SSR from 0.1 to 0.6 MJ.mg-1 was associated to a decrease in BS incidence up to near 40% of the maximum incidence observed (Exp. 2, Fig. 4.A), and 70% of the maximum severity observed (leaf 20, Fig. 4.B). Values of SSR higher than 0.6 MJ.mg-1 did not affect significantly, neither incidence, nor severity of BS (Fig. 4.A and B).

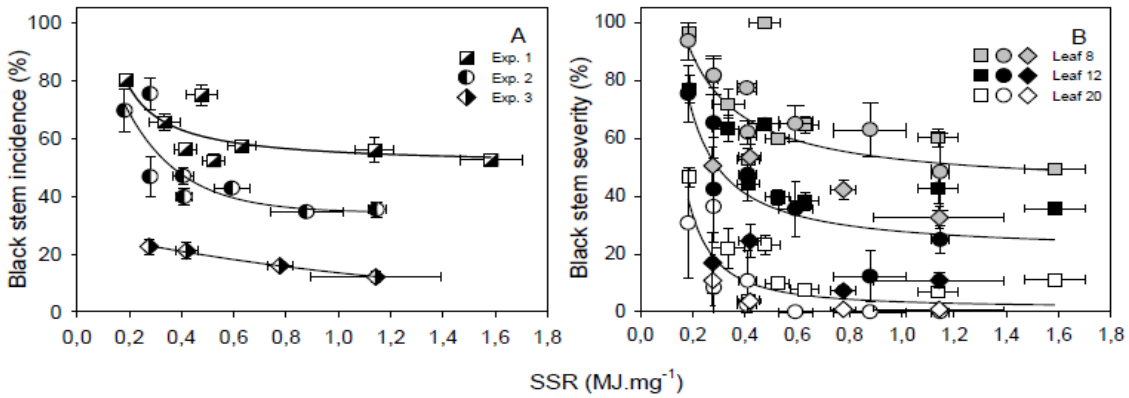

Fig. 4. Incidence (A) and severity (B) of black stem by *P. macdonaldii* as a function of the source-sink ratio (SSR, MJ.mg-1) at 400°Cd after flowering in Exp. 1 (squares), Exp. 2 (circles) and Exp. 3 (diamonds). Symbols filled in combined black/white indicate BS incidence (A). BS severity is indicate with grey (leaf 8), black (leaf 12) or white (leaf 20) symbols (B). Shading value from Exp. 1 in hybrid Baqueano corresponded to 372Cd after flowering. Curvilinear lines illustrate the adjustment of the results to the model: *BSincidence(%)=50.524*exp(0.089/(SSR+0.007)), p=0.0895, R2=0.619, n=8, for Exp.1; BSincidence(%)=34.216+89.563*exp(-4.774*SSR), p=0.05, R2=0.698, n=8, for Exp.2, and BSincidence(%)=27.991*exp(-0.730*SSR), p=0.0025, R2=0.995, n=4, for Exp.3. BSseverity(%)=48.051+70.593*exp(-3.066*SSR), p=0.0053, R2=0.46, for leaf 8; BSseverity(%)=24.721+97.108*exp(-4.186*SSR), p=0.0055, R2=0.458, for leaf 12, and BSseverity(%)=3.962+146.129*exp(-7.9*SSR), p=0.0002, R2=0.63,* for leaf 20.Vertical and horizontal bars on the symbols indicate the standard error of the mean value of BS incidence or severity and SSR, respectively (n=9).

After a multiple regression analysis including, SSR, the number of rainy days, the photosynthetically active radiation (PAR), the % of interception of radiation, the mm of rainfall and the age of the leaf, the models: BS incidence = -26.78+7.56*number of rainy days+56.52*exp(-4.138*SSR), s.e.=11.97%, and BS severity = -374+0.26*age of the leaf+10.96*number of rainy days+95.067*exp(-5.033*SSR), s.e.=12.15%, explained about 80% of the variability in BS incidence and BS severity, in an estimated/observed plot (Fig. 5.A and B).

While in the case of BS incidence a linear model setting between estimated and observed values did not differ from the 1:1 bisector (p=0.17), in BS severity, both severity intercept (p=0.0006) and the slope (p=0.0004) differed from "0" and "1", respectively. The observed values of BS severity were slightly underestimated or overestimated by the model in low and high ranges of the scale (0-20% and 80-100%, respectively, Fig. 5.A and B). Nevertheless, the magnitude of these differences was lower than the s.e. of the model.

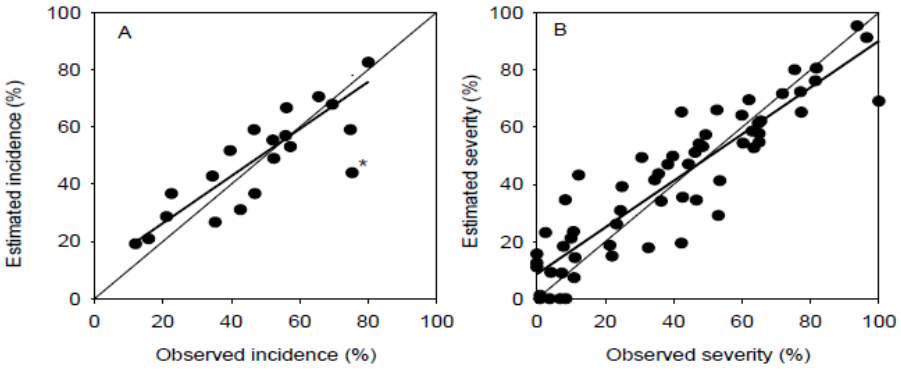

Fig. 5. Observed and estimated values of BS incidence (A) and BS severity (B) obtained from the field experiments and from the models BS incidence=-26.78+7.56*number of rainy days+56.52*exp(-4.138*SSR), s.e.=11.97%, and BS severity=-374+0.26*age of the leaf+10.96*number of rainy days+95.067*exp(-5.033*SSR), s.e.=12.15%, respectively. The thick lines result from the adjustment of the results to linear models (estimated incidence=9.909+0.822*observed incidence; R2=0.798; p<0.0001; n=19, and estimated severity=8.625+0.815*observed severity; R2=0.825; p <0.0001; n=60). The asterisk near a symbol indicates that this result was an outlier (estimated - observed > 2.5 standard deviation), and was not consider in the adjustment. The thin line represents the 1:1 values.

The function between SSR and BS symptom was strongly affected by disease variable (incidence or severity), the experimental years and by number of nude. In concordance with Quiroz (2015), the number of rainy days from flowering to 400ºCd after flowering was the meteorological variable which better explained the effect of the experiment on BS incidence.

Rainfall events were the principal epidemiological indicator affecting diseases expression in the relationship SSR vs BS. In agreement, Délos *et al.* (1997) demonstrated that rainfall correlated with ascospores release from the primary source of inoculum (stubble, seeds, etc.), and not with inoculum amount (Descops *et al*., 2012).

The age of the leaf (in ºCd from its appearance on the apex) was the variable which better explained differences observed among leaves. Time passed between *P. macdonaldii* inoculation in petiole and symptons appearance in stem, depend on phenological stage (related to leaf age) and the cultivar (Larfeil *et al*., 2010), exhibiting later stages the shortest period. Leaf age affects photosynthesis, being older leaves less efficient than younger ones (English *et al*., 1979) resulting in early senescence and probably with a higher susceptible to *P. macdonaldii* infections.

Our models were constructed without water and nutrients limitation during three years and with two hybrids. We considered ecophysiological and epimediological interactions from

the crop and disease during the grain filling period. Other empiric predictive models, developed including a great range of crop management and environments (Debaeke and Péres, 2003), established a positive relationship between LAI or iPAR at flowering and BS and postulate that the more favourable microclimate of dense stands canopies could explain the higher level of infection of *P. macdonaldii*. Later, Desanlis (2013) deepened this epimediological approach proposing a conceptual model where the potential infection rate could be reduced by several reduction factors (RF): microclimate (HR, T), plant growth (LAI) and fungicide treatment, to account for climatic and agronomic limitations to fungus development.

CONCLUSION

The estimate BS models proposed in this paper include a new concept relating SSR, as a crop ecophysiological condition, to the plant susceptibility to *P. macdonaldii* infection. In turn, these models can contemplate different environmental conditions, hybrid cultivars and leaf stratums. This approach could be combined with the other mentioned models. For this it would be necessary to broaden the crop managements (according to Debaeke y Peres 2013) and stressed environments (e.g., crop damage, water or nutrient deficits, etc.).

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CALLUS FORMATION AND PLANT REGENERATION IN SUNFLOWER (*HELIANTHUS* L., ASTERACEAE) IN VITRO TISSUE CULTURE

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ABSTRACT

For the sake of introduction of sunflower to *in vitro* culture the seeds and flowers of different wild species were used: *Helianthus annuus* (three samples), *H. decapetalus*, *H. giganteus*, *H. macrophyllus*, *H. nutalli*, *H. occidentalis* ssp. occidentalis, *H. tuberosus* (four samples), *H. tuberosus* velmoren, and also cultivated variety "Master", lines VIR 114B (fertile) and VIR 114Apet (CMS). Fifteen combinations of plant growth regulators have been used. We were successful in introduction into the culture in vitro and obtain stably growing callus of *H. annuus* wild type (1 sample), *H. giganteus*, *H. occidentalis* ssp. occidentalis, *H. tuberosus* (callus from seeds and from flowers) and sunflower cv. "Master". The optimal medium for callus formation was MS with BAP and NAA (ratio 2:1) or TDZ. We could obtain successful regeneration from callus only for *H. giganteus* on MS media with BAP and NAA (ratio 2:1 or 5:1 in low concentration) when cultivated in the dark.

Key words: Sunflower, *In vitro* culture, Plant regeneration, *Helianthus giganteus*

Abbreviations: BAP - benzylaminopurine, NAA - naphthylacetic acid, TDZ - thidiazuron, AS - adenine sulfate

INTRODUCTION

Different methods for callus induction, adventitious bud formation, shoot multiplication and rooting of *in vitro* formed shoots of sunflower are described (Lupi et al., 1987; Weber et al., 2000; Rath and Pearson, 2004; Ozyigit et al, 2007; Neskorodov, 2011; Sujatha et al, 2012; Khalil et al., 2015 and others). According to the literature it is known that sunflower *in vitro* culture is quite successful, but stable plant regeneration from callus is difficult. The greatest success is achieved using direct regeneration from seed or immature embryos. But the perennial species and interspecific hybrids have very low seed production, or complete sterility (Gavrilova, Anisimova, 2003), so it is difficult to obtain enough amounts of seeds from them. In addition, regeneration by direct somatic embryogenesis (embryoidogenesis) is not suitable for use in genetic transformation protocol (Weber et al., 2000). The second difficulty for finding the optimal protocol of regeneration is a high variability in the response to the conditions of cultivation of species and varieties of sunflower (Ozyigit et al, 2007; Sujatha et al, 2012).

In sunflower investigation the obtaining of a new ways of plants regeneration from callus cultures *in vitro* is of current importance. It is necessary, using different kinds and types of sunflower explants to achieve the production of a stable callus culture, and then to choose the optimal conditions for successful regeneration of plants from callus.

MATERIALS AND METHODS

Helianthus annuus wild type (three different samples), *H. decapetalus*, *H. giganteus*, *H. macrophyllus*, *H. nutalli*, *H. occidentalis* ssp. occidentalis, *H. tuberosus*, *H. tuberosus* velmoren, and also cultivated sunflower *H. annuus* variety "Master", lines VIR 114 B (fertile) and VIR 114 A pet (CMS) were chosen for the study. Seeds were collected from plants grown in the Kuban Experimental Station of VIR.

In most of the experiments we used seedlings obtained from the seeds as primary explants. In addition we used as explants the Jerusalem artichoke (*H. tuberosus*) flowers. Jerusalem artichoke has been chosen as a model, as the most accessible in our region (St. Petersburg and the Leningrad region) perennial sunflower species. Jerusalem artichoke inflorescences at the different flowering stages were collected from the three different samples.

In the first series of experiments, the seeds were superficially sterilized under complete aseptic conditions by soaking in 96% ethanol for 20 min., and then treated over the burner flame for 10-15 seconds. Seeds were germinated for 24 hr on filter paper moistened with distilled water. If at first day of germination the appearance of the hyphae of fungi in the testa was observed, the embryos of these seeds were extracted and subjected to additional treatment of 10% hydrogen peroxide solution in the course of 20 min., then washed twice with sterile distilled water.

Plantlets were transferred to Petri dishes on a ½MS medium (Murashige and Skoog (1962) medium with a half dose of nutrients). Two week later, plantlets were cut into pieces. As primary explants we used all parts of the plantlets: pieces of root, stem, cotyledon, and bud with the leaves. Pieces of plantlets were placed on the MS medium. Three modifications were used, depending on the added growth regulators (MS-1, MS-2, MS-3 - see table). The plates were cultivated at t + 22°C in the dark or light. Then callus was transplanted to five variants of culture medium (MS-4, MS-5, MS-6, MS-7, MS-8). The plates were cultivated at t + 26°C in the dark or under 16 hour photoperiod.

Table. The composition of growth factors added to the culture medium

Growth factors (mg/l)	MS-0	MS-1	MS-2	MS-3	MS-4	MS-5	MS-6	MS-7	MS-8	MS-9	MS-10	MS-11	MS-12	MS-13	MS-14	MS-15
BAP		2		0,1		2	4	4	4	0,2	0,5	1	1	0,5	1	
TDZ			2		2	1	1	1	1							
NAA		1		0,1		0,5	1	1,5	2	0,1	0,1	0,5	0,02		1	0,5
AS				40	40											

In the second series of experiments the tubular flowers were used from the inflorescence of Jerusalem artichoke as explants. The inflorescences were sterilized during 20 min. in 10% solution of hydrogen peroxide, and then transferred to distilled water, disassembled into individual flowers, which were placed for 15 min. in peroxide solution, then washed twice with sterile distilled water. We planted them on five variants of MS medium (MS-4, MS-5, MS-6, MS-7, MS-8).

For plant regeneration, the calluses derived both from seedlings and from flowers were transplanted to a new variant of culture medium (MS-9, MS-10, MS-11 and MS-12). The plates were cultivated at t + 26°C in the dark condition or under 16 hour photoperiod.

Callus with regeneration zones was transferred into a new version of the medium (MS-13, MS-14 and MS-15) to support the growth and rooting. Then, plants both having roots and without them were transplanted into larger flasks on medium without hormones (MS-0).

Subsequently, the plants were transferred from the sterile culture into non-sterile conditions. They were transplanted to pots filled with sand and placed in a climatic chamber at t= + 26°C under 16 hour photoperiod.

RESULTS AND DISCUSSIONS

We had introduced into the culture *in vitro* and obtained stably growing calluses of *H. annuus* wild type (1 sample), *H. giganteus*, *H. occidentalis* ssp. occidentalis, *H. tuberosus* (callus from seeds and from flowers) and *H. annuus* cv. "Master" (Fig. 1a,b,c: 2a,b).

We are faced with the fact that it is difficult to obtain sterile seedlings using freshly collected seeds. These seeds germinated worse and were poorly sterilized. Frequently the re-treatment of emerged embryos was necessary and so we released them from the seed coat, which was struck by mildew, and sterilized again.

To obtain a stable callus cultures from the seeds the following protocol was the most productive: 1) sterilization of seeds, 2) germination on filter paper moistened with distilled water, 3) re-sterilization and transfer of seedlings on a medium ½MS, 4) using all parts of the seedling (root, leaf, stem, cotyledon) as explants, 5) culturing in the dark condition or under 16 hour photoperiod (the second is preferable) and 6) regular transplanting every 6-8 weeks (Fig. 1c,d).

We observed intensive formation of a new callus on MS-1, MS-2, MS-3 or MS-11 media with equal success. So, callus was formed well on media containing NAA and BAR in the ratio 2:1 or in equal low concentration (0,1 mg/l) with AS, as well as on media with TDZ.

The appearance of callus formation centers was noted 7-10 days after transfering. Intensive callus formation continues from 2 to 2,5 months.

Callus formation was observed in different parts of seedling (from pieces, cotyledon, stem, leaves, roots, buds) (Fig. 1d,e).Cytology showed that in callus histogenesis, the formation of centers of meristematic activity and elements of the vascular system occur (Fig. 2h).

In the case of using the tubular flowers as a primary explants the optimal procedure for obtaining a callus is shorter: 1) surface sterilization of inflorescences, then isolation and sterilization of flowers, 2) culturing either in the darkness or under light and 3) regular transfers every 6-8 weeks.

We used for callus induction the five variants of culture medium (MS-4, MS-5, MS-6, MS-7, MS-8). Of these, only a MS-4 medium was bad for callus formation.

Within 7-14 days after the transfer of flowers on a medium, we observed changes similar to flowering in the wild - the disclosure of the tops in anthers (but pollen did not fall out, in contrast to the natural condition). 4-6 weeks later, the beginning of callus formation is noted which occurs intensively within 2,5-3 months, then the callus growth stops (Fig. 2c,d,e).

Callus initiation was observed at the bottom of the disc nectar, and along the upper edge of the tube sepals from anther and stigma tissues. Cytological study showed that in these parts the sections of tissue with small meristem-like cells with dense cytoplasm and nucleus are still persist, while the surrounding tissue consists of large vacuolated cells with badly viewed nucleus (Fig. 2f,g).

We can get the successful regeneration from callus only for *H. giganteus* on MS media with BAP and NAA (ratio 2:1 or 5:1 in low concentration) when cultivated in the darkness. More than one year passed since the seeds have been planted in a Petri dishes and callus was transplanted several times into new versions of the nutrient medium. In the medium MS-9 there were observed gemmo- and rhyzogenesis (shoot and root formation) and rhyzogenesis (root formation), on media MS-10 and MS-11 only gemmogenesis (shoot formation) (Fig. 1f,g,h).

In general, media supplemented with BAP and NAA in ratio 2:1 had a higher callus formation (MS-1, MS-11) as well as media with TDZ (MS-2) and with AS plus low concentration of BAP and NAA (MS-3).

Interestingly, the same range of concentration of 1,0mg/l BAP combined with 0,5mg/l NAA was reported by other researchers to give the highest shoot regeneration (Knittel et al., 1991; Baker et al., 1999; Rath and Pearson, 2004; Ozyigit et al., 2007; Khalil et al., 2015). Some of them noted the

importance of auxins/cytokinin balance for sunflower regeneration, but in our experiments for most samples of sunflower on media with BAP/NAA in such concentration we could get only callusogenesis.

In general it is worth paying attention to presence in cultural medium BAP and NAA in 2:1 ratio, as the most interesting in respect of stimulation of processes of a morphogenesis (callusogenesis, histogenesis, gemmogenesis, rhyzogenesis and a gemmorhyzogenesis) in sunflower culture *in vitro*.

Thus, in literature the different successful protocols which marked for this or that genotype of sunflower are noted, but there is no uniform scheme guaranteeing the success of micropropagation of any investigated sunflower varieties. The response to influence of growth factors depends strongly on the genotype chosen for experiment and is not predictable in advance. Also, the result depends on the choice of the explants and culture conditions (see review in Khalil et al., 2015). Here you can pay attention to more studied culture – corn for which the genes responsible for regeneration *in vitro* are already allocated (Tomes and Smith, 1985; Armstrong et al., 1992; Checheneva and Trukhanov, 1994 and others). Probably, sunflower has similar genes responsible for ability of cells to differentiate on the way of gemmo- or embryoidogenesis *in vitro*. There are works where the positive influence of alleles of several genes to *in vitro* morphogenesis of sunflower is found (Kostina et al., 2013, 2015).

Figure 1. Callus formation and regeneration of plant from seeds. ***a*** – *H. giganteus* plants and flower, ***b*** – *H. annuus* cv. Master, ***c*** – seedling in vitro, ***d*** – different parts of seedling in vitro, ***e*** – callus formation and rhyzogenesis in primary culture after 4 months, ***f*** – gemmogenesis after transferring on new medium, ***g*** – callus with shoots and roots after one year, ***h*** – new plant of *H. giganteus* in the pot. Scale bar – 5 mm

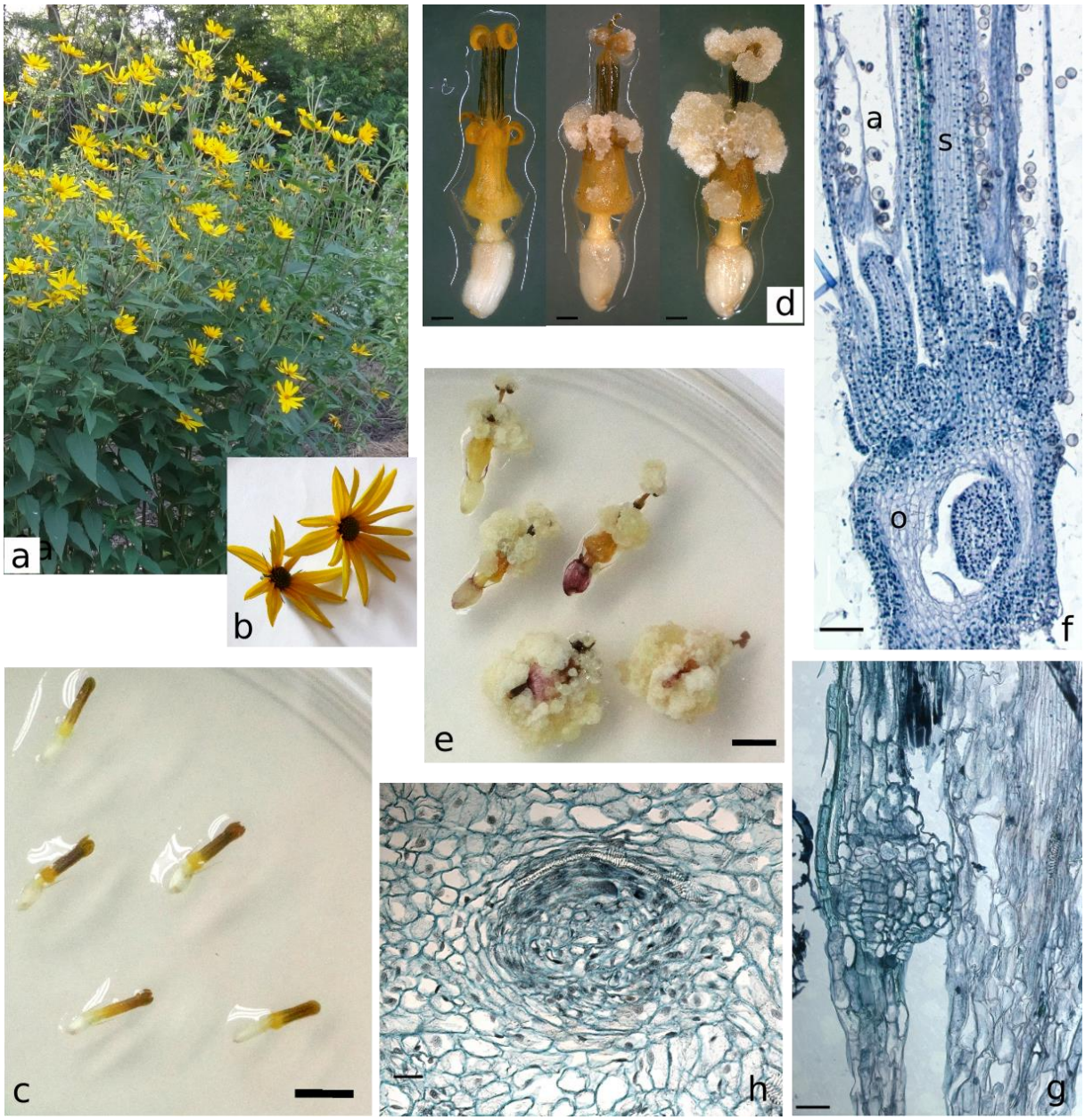

Figure 2. Callus formation from tubular flowers. ***a, b*** – *H. tuberosus* plants and flower, ***c*** – tubular flowers placed on Petri dishes in vitro, ***d*** – three different stage of callusogenesis, ***e*** – callus on tubular flowers after 2 months, ***f*** – longitudinal section of tubular flower (*a* – anther with pollen grain, *o* – ovary, *s* - stigma), ***g*** – zone of callus formation on anther stalk, ***h*** – formation of elements of vascular system in callus.
Scale bar: c, d, e – 5 mm, f – 100 mμ, g, h – 50 mμ.

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OBSERVATIONS ON IMI GROUP HERBICIDES STRESS ON SUNFLOWER LEAVES (*HELIANTHUS ANNUUS* L.) BY SCANNING ELECTRON MICROSCOPY

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ABSTRACT

In this study, IMI group herbicides that used in sunflower cultivation and some sunflower cultivars that have resistance to these herbicides in different levels were used. Effects of herbicide application in different doses on surface of sunflower leaves were observed with light microscope and SEM. Seeds taken from Trakya Agricultural Research Institute was used as a material. Four different sunflower cultivars named SN:8 which is unresisting to IMI and SN:9, SN:10, SN:14 which are resistance to IMI were used in this study. Seed were germinated under controlled conditions in climate chamber and then seedlings were transferred to experimental parcels. Three different doses (125, 250, 375 ml/da) of herbicide were applied to seedlings that have 4-6 leaves. Leaf samples were collected on seventh day of herbicide treatment and observed under the light microscope. Fresh leaves obtained from the plant were used for SEM observations at Trakya University TUTAGEM laboratories. Anatomical investigations showed that there are differences on leaves surface between unresisting and resistance cultivars depending on different herbicide doses. In SEM analysis of the leaves, differences were seen in the number, size and structure of hairs and stoma cells.

Key Words : *Helianthus annuss* L., IMI, Leaves, Anatomy, SEM

A STUDY ON THE STANDARD GERMINATION AND SEEDLING GROWTH OF SOME CONFECTIONARY AND OIL SEED SUNFLOWER(*HELIANTHUS ANNUUS* L.) CULTIVARS

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ABSTRACT

It is very important to know sowing performance for production of sunflower that has wide economic importance. It is very common to observe variation in performance of same plant, the same cultivar seeds, even if the they have same chronological age class during their field propagation and performance. Performance difference among seed lots is due to variation in seed vigor. This study was carried out in the laboratory of the "Variety Registration and Seed Certification Center" between 15 January – 15 April 2014 to determine germination and seedling growth of some confectionary (cv. Palancı 1, Çiğdem 1) and oilseed sunflower cultivars namely (İnegöl Alası and Tunca, Sanbro and 08 TR 003) cultivars. In the study, standard germination tests measuring germination speed and germination vigor, seedling length, root length, above-ground wet and dry weight, below-ground wet and dry weight values were measured. According to the germination speed and germination vigor values, the oilseed cv. Tunca of sunflower that had the highest values of 96,50% and 98%, and confectionary type cv. Çiğdem1 had the lowest value of 77 % for germination speed and lowest value of 86% in germination vigor in terms of the general average for all cultivars.

Key Words: Sunflower (*Helianthus annuus* L.), Germination Speed, Germination Vigor, Seedling Length, Root Length.

INTRODUCTION

The oilseed vegetative groups cultivated in Turkey include sunflower, soybean, sesame, peanut, poppy, canola, safflower, cottonseed. 46 % from our vegetable oil production are meeting from sunflower (Anonym, 2007).Sunflower oil is one of vegetable oils with the highest nutritional value due to the containing high rate of unsaturated fatty acids (69%). Furthermore sunflower is also consumed as confectionary and 2,6 % of the production is for confectionary use. (Arıoğlu, 2000). Sunflower is also an important plant in terms of healthy nutrition. Sunflower seed which is also rich in terms of potassium and vitamin E is an important source of linoleic acid(Anonyms, 2004).

First stage at plant production is sowing of seed and germination. Good germination and ground output constitutes one of the most important stages of the plant productivity. During germination stage, the plant seeds having a difficult and uneven germination result in a heterogeneous output in cultivation environments and become source of significant losses both in terms of production and seed yield. Weeds, diseases and pests appearing together with irregular and late germination slow the growth of plants and have negative effects to productivity and the quality (Muhyaddin and Wiebe 1989). Germination; is the period providing the transition from the resting phase of seed to forming the plant and continuing till output of the radical-rootlets from the seed (Eser et al 2005). Conditions such as low and high soil temperature, thick seed coat, upper crust of the soil, heavy textured soils, soil salinity, aridity causing stress to seeds tending for irregular germination are causes of no seed germination (Heydecker and Coolbear 1977). In this study, standard germination and output performance with early seedling growth of some oil and confectionery sunflower cultivars was investigated.

MATERIALS -METHODS

This study was conducted at the laboratories of Variety Registration and Seed Certification Center, Ankara. For the confectionary cultivar of sunflower Palancı 1, Çiğdem 1 and İnegöl Alası and for the oil sunflower cultivar Tunca, Sanbro and 08 TR 003 were used as materials. During laboratory analyses; germination containers containing silt sand (0,8 mm diameter), calibrated growth rooms, sterilisation equipment, electronic moisture meter, assay balance (with a precision of 0,001) seed storage room, oven and desiccator were used.

Laboratory studies were conducted between 15th of January and 15th of April 2014, standard germination tests, sprouting speed, sprouting vigor, germination speed, germination vigor, length of the seedling, length of the root, aboveground wet and dry weight, underground wet and dry weight were measured and weighed. Field output tests were performed at the pilot fields at Yenikent station of the Variety Registration and Seed Certification Center between the 16th of April and 07th of May 2014.

In accordance with the methods and applications of International Seed Testing Association (ISTA) for each cultivar 100 seeds 4 replications are counted. As germination medium containers with a 25 cm length, w5 cm wide, 15 cm deep were used. With closing the germination containers they are let into the germination cabinets working with an accuracy of ± 1 ° C to a pre-set day temperature of 30 ° C and night temperature of 20 °C. Evaluation of the germination were done in accordance with the criteria established in the seed evaluation manual of ISTA. In the evaluation the % ratios of normal germinations, abnormal germination and dead seeds were determined.

RESULTS

The values of analysis of variance results of determined standard germination for the oil and confectionary sunflower cultivars with germination speed, germination vigor, sprouting speed, sprouting vigor are given in table 1.

Table 1: Analysis of variance for the germination speed, germination vigor, sprouting seed and sprouting vigor values determinate as a result of standard germination result of sunflower cultivars.

Source of variation	Degrees of freedom	Mean Square			
		Germination speed	Germination vigor	Sprouting speed	Sprouting vigor
Overall	23	-	-	-	-
Replication	3	6,44	13,56	49,06	9,94
Cultivar	5	239,47**	80,27**	391,37**	145,77**
Error	15	10,04	7,29	16,26	17,68

** $p<0.01$

Values for germination speed and vigor

As shown in Table 1 germination speed, germination vigor, sprouting speed and sprouting vigor values of different confectionary and oilseed sunflower cultivars were statistically significant at the 1% level in term of cultivars.

When analysing the values of germination speed it is determined that the highest value belongs to the oil sunflower cultivar Tunca with 96,50 % and this is followed with 90 % by oil sunflower cultivar 08 TR 003 and the lowest germination speed value is obtained with 77 % from the confectionary sunflower cultivar Çiğdem 1.

In terms of germination vigor the highest value is determined with 98 % by the cultivar Tunca followed by a value of 96 % by oil sunflower cultivar 08 TR 003 and lowest value of 86 % from the confectionary sunflower cultivar Çiğdem 1. (Table 2)

Table 2: Germination speed and vigor values (%) of sunflower cultivars determined as a result of standard germination

Cultivar	**Germination speed**	**Germination vigor**	**Cultivar**	**Germination speed**	**Germination vigor**
Palancı 1	79,00 c	90,00 cd	**Tunca**	96,50 a	98,00 a
Çiğdem 1	77,00 c	86,00 d	**Sanbro**	87,50 b	95,50 ab
İnegölAlası	79,00 c	91,50 bc	**08 TR 003**	90,00 b	96,00 ab
Germination speed LSD $_{0.01:}$ 4.78			Germination vigor LSD $_{0.01:}$ 3.48		
Germination speed VK : 3.74			Germination vigor VK : 2.91		

Values for sprouting speed and vigor

When analysing the mean values of cultivars at the table 3 it is seen that the highest value belongs in terms of sprouting speed with 95,50 % and the sprouting vigor with 95,60 % from the oil type sunflower cultivar Tunca.

Table 3. Sprouting speed and vigor values (%) of sunflower cultivars determined as a result of standard germination

Cultivar	**Sprouting speed**	**Sprouting vigor**	**Cultivar**	**Sprouting speed**	**Sprouting vigor**
Palancı 1	72,50 c	86,50 b	**Tunca**	95,50 a	96,50 a
Çiğdem 1	71.50 c	78,50 c	**Sanbro**	82,50 b	84,50 bc
İnegölAlası	76,00 c	86,00 b	**08 TR 003**	90,50 a	90,50 ab
Sprouting speed LSD $_{0.01:}$ 6.08			Sprouting vigor LSD $_{0.01:}$ 6.34		
Sprouting speed VK : 4.95			Sprouting vigor VK : 4.83		

Seedling length

When analysing the values for seedling length, it is determined that the highest value of 19,47 cm seedling length of oil type cultivar Sanbro followed with a value of 18,64 cm by the oil sunflower cultivar Tunca and the lowest seedling length with 16,48 cm of confectionary sunflower cultivar Palancı 1.

Root length

When analysing the mean root length values the highest root length was obtained with 11,55 cm from the oil sunflower cultivar Tunca.

Above – ground wet weight

In terms of mean above ground wet weight the highest value was determined with 7,70 g. by oil sunflower cultivar 08 TR 003

Above – ground dry weight

In terms of mean above ground dry weight it is determined, that the highest value belongs with 0,60 g to the confectionary sunflower cultivar İnegölAlası followed with 0,56 g by confectionary sunflower cultivar Palancı 1 and the lowest value obtained with 0,33 g from the oil sunflower cultivar Tunca.

Below – ground wet weight

In terms of mean below ground wet weight it is determined, that the highest value belongs with 1,80 g to the confectionary sunflower cultivar İnegöl Alası followed with 1,73 g by confectionary sunflower cultivar Palancı 1 and the lowest value for below ground wet weight is obtained with 1,12 g from the oil sunflower cultivar Sanbro.

Below – ground dry weight

The highest below ground dry weight is obtained with 0,25 g by confectionary sunflower cultivar and the lowest below ground dry weight with 0,07 g from oil sunflower cultivar 08 TR 003.

Field output vigor

In terms of field output vigor values for the cultivars the highest field output belongs with 91,50 % to the oil sunflower cultivar Tunca followed with 86 % by oil sunflower cultivar 08 TR 003 and the lowest germination vigor value obtained with 75 % from the confectionary sunflower cultivar Çiğdem 1.

CONCLUSION

Evaluating the data obtained from the study in optimal and controlled manner, it was possible to distinguish genetic structure after measurements of seedling length, root length, above ground wet and dry weights, below ground wet and dry weights. Besides laboratory based data the data was also taken out of field conditions that also affirmed similar trends despite some minor differences similar. In addition to the genetic structure environmental factors were also effective under field conditions.

When comparing the germination speed and vigor values of oil and confectionary cultivars it is observed that germination vigor of oil cultivars are higher than others.

In terms of field output vigor it is determined that there are no important differences between oil and confectionary cultivars. It is to be pointed out, that the oil sunflower cultivar Tunca is the best among cultivars tested under field conditions.

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DETERMINATION OF ACCELARATED AGING AND FIELD GERMINATION TEST VALUES OF SOME CONFECTIONARY AND OILSEED SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVARS

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ABSTRACT

It is very important to know sowing performance for production of sunflower that has wide economic importance. It is very common to observe variation in performance of same plant, the same cultivar seeds, even if the they have same chronological age class during their field propagation and performance. Performance difference among seed lots is due to variation in seed vigor. This study was carried out in the laboratory of the "Variety Registration and Seed Certification Center" between 15 January – 15 April 2014 to determine the germination and seedling growth of some confectionary and oilseed sunflower cultivars namely Palancı 1, Çiğdem 1 and İnegöl Alası and Tunca, Sanbro and 08 TR 003 respectively. In the study, standard germination tests measuring germination speed and germination vigor, seedling length, root length, above-ground fresh and dry weight, below-ground wet and dry weight values. According to the germination speed and germination vigor values, the oilseed cv. Tunca of sunflower that had the highest values of 96,50% and 98 %, and confectionary type cv. Çiğdem1 had the lowest value of 77 % for germination speed and lowest value of 86% in germination vigor in terms of the general average for all cultivars.

Key Words: Sunflower (*Helianthus annuus* L.), Accelerated Aging, Field Germination Vigor

INTRODUCTION

Oilseed plants within vegetative production are defined as vital basic necessities for human nutrition (Yurdagül and Ersoy, 1997). With regard to cultivation area and production of oilseed plants sunflower has first place containing high proportion of oil (22-50 %) it is an important vegetable oil plant for raw oil production. (Kızıloğlu, 1992., Kara,1996). We meet 46 % of our oil demands from sunflower(Anonymous, 2007). Furthermore 2,6 % of sunflower is consumed for confectionery purposes (Arıoğlu, 2000). Sunflower seed which is also rich in terms of potassium and vitamin E is an important source of linoleic acid. Foods which are rich in terms of linoleic acid are helping to decrease the cholesterol level in blood (Anonymous, 2004).

It is very important to know the planting values at the production phase of sunflower that has a wide area of usage and economic importance. As the seeds growing on sunflower table mature at different times, therefore, sunflower seeds performance varies even we take same kinds of seeds from the same plants under field conditions and in their germination performances. These performance differences observed between seed samples arise from seed vigor (ISTA 1995).

In Turkey, there is no legal obligation for performing vigor test on other cultivars as well as on sunflower seeds parties. Therefore standard germination test performed by seed producer companies are considered as adequate and performing of an additional vigor test is considered unnecessary. A vigor test allows us to obtain reliable opinion on estimating the quality of seed samples, classification, output and their storage capabilities.

Vigor test is a test performed to measure the performance and vigor out of the vitality value of seed sample. To determine the seed vigor, various tests such as electrical conductivity test (EC), controlled deterioration test, cold test (CT), cool germination test (CG) and accelerated aging tests (HY) (Anonymous, 2013).

The aim of seed vigor test is; to be aware of sowing values for a wide range of environments and estimating the outputs in unfavourable planting conditions besides their classification of seed samples and also to obtain data for storing potentials of seeds. Seed vigor in a broad sense represents the sum of the properties confirming the activity and performance of seeds.

At the discussions of seed qualities a quality variable on agenda in recent years is a feature of seed vigor. The seed vigor feature as a basic quality feature of seed can be defined as a measure of germination and formation of plantlets potential get through all the hindering stress factors under uncontrolled field conditions.

ISTA describes the seed vigor as a sum of features that performing germination activities and performances under various environmental conditions of seeds. And also with the feature to give an opinion for formatting a plant under field condition of seed, given a chance for seed inventory management demonstrates the importance of the possibility of seed vigor measurement (Başak, 2006).

MATERIALS and METHODS

This study was conducted at the laboratories of Variety Registration and Seed Certification Center, Ankara as accelerated aging studies on confectionary and oilseed sunflower cultivars between the dates of 15th of January and 15th of April 2014. All materials used in the study were obtained from the harvest of the year 2013. As cultivars of confectionary sunflower Palancı 1, Çiğdem 1 and İnegöl Alası and as oil sunflower the cultivars Tunca, Sanbro and 08 TR 003 were used as materials.

For laboratory analysis; at the accelerated aging tests aging containers consisting of three parts were used. Aging container consisted of an outer box with a size of 11×11×4 cm and inside thereof from sieve mesh made inner chamber with a size of 10×10×3 cm and a cover on the container (Figure 1). With the aim to maintain a high relative humidity 40 ml pure water was put in the outer chamber. Seeds are spread on sieve mesh in a single layer and the sieve mesh was placed into the outer container. At this stage, it is important not to splash water on sieve mesh and seeds. After seeds are put on the mesh the cover was closed. At all seed samples from each 100 seeds 4 replications were applied. In addition to this also necessary laboratory equipment was used. Thousand grain weight and grain moistures of cultivars was determined before the start of the test.

Accelerated aging containers were left in the aging chamber operated at 41 ºC. Aging was performed for duration of 24-48-72 and 96 hours. Temperature control of aging chamber was made on a continuous and regular basis. 1 hour after getting the seeds out of the aging chamber a standard germination test in accordance to the rules of ISTA was applied with seeds in each of 4 replications.

Statistical analysis and evaluation**:** Intra-period comparison values obtained in the study were subjected to analysis of variance according to the factorial randomised randomized blocks design",

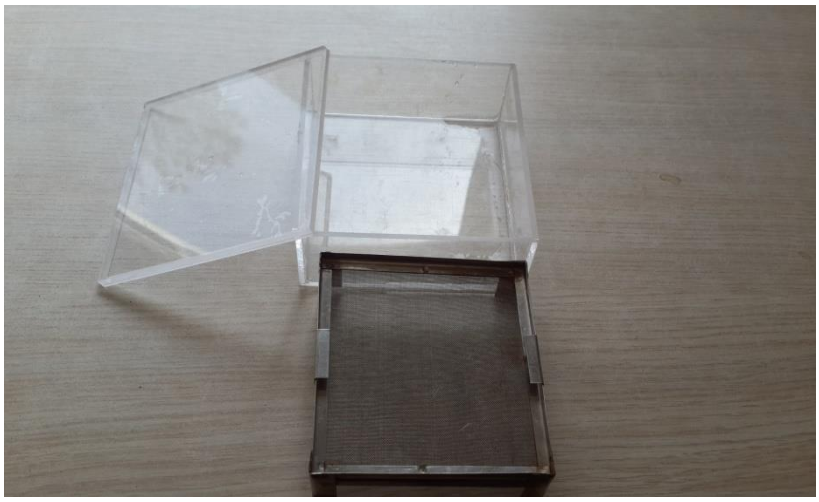

Figure 1. Accelarated aging Equipments

RESULTS AND DISCUSSION

Germination vigor values after 24 hours of accelerated aging

Analysis of variance results for the germination vigor test results of accelerated aging for a duration of 24,48,72 and 96 hours for oil and confectionary type sunflower cultivars are given in table 1.

Table 1: Analysis of variance for the germination vigor values determined after an accelerated aging test of 24, 48, 72 and 96 hours applied to sunflower cultivars.

Source of variation	Degrees of freedom	Mean Square			
		24 hours	48 hours	72 hours	96 hours
Total	23	------	-----	-----	-----
Replication	3	5.83	40.50	63.83	1.26
Cultivar	5	174.07**	596.97**	852.58**	821.14**
Error	15	5.33	10.50	3.88	15.70

** $P<0.01$

As shown in Table 1 germination vigor values after 24, 48, 72 and 96 hours of accelerated aging of different confectionary and oilseed sunflower cultivars were statistically significant at the 1% level on the basis of cultivars.

When analysing average germination vigor values of accelerated aging for 24 hours of sunflower cultivars it is seen that the highest value with 94,50 % belongs to the oil sunflower cultivar Tunca and this is flowed with 91,75 % by oil sunflower cultivar Sanbro and the lowest germination vigor value is getting by confectionary sunflower cultivar Çiğdem 1 with a value of 75.50 % (Figure 2).

When analysing average germination vigor values from the table 3 of accelerated aging for 48 hours of cultivars it is seen that the highest value with 88,50 % belongs to the oil sunflower cultivar Tunca and this is followed with 73,25 % by oil sunflower cultivar 08 TR 003 and the lowest germination vigor value of 50,25 % was noted on confectionary sunflower cultivar İnegöl Alası.

When analysing average germination vigor values from the table 4 of accelerated aging for 72 hours of cultivars it is seen that the highest value with 82,50 % belongs to the oil sunflower cultivar Tunca and

this is followed by 55,75 % by confectionary sunflower cultivar Palancı 1 and the lowest germination vigor value was noted as with 37,45 % by confectionary sunflower cultivar İnegöl Alası.

Table2: Germination vigor (%)values determined after 24 hours of accelerated aging test by cultivars

Cultivar	**Accelerated Aging (HY) -24 hours**	**Cultivar**	**HY-24 hours**
Palancı 1	87,75 c	**Tunca**	94,50 a
Çiğdem 1	75,50 d	**Sanbro**	91,75 ab
İnegöl Alası	90,00 bc	**08 TR 003**	89,00 bc
LSD $_{0.01:}$ 3.48 VK : 2.62			

Table 3: Germination vigor (%)values determined after 48 hours of accelerated aging test by cultivars

Cultivar	**HY-48 hours**	**Cultivar**	**HY-48 hours**
Palancı 1	71,25 b	**Tunca**	88,50 a
Çiğdem 1	68,75 b	**Sanbro**	71,50 b
İnegöl Alası	50,25 c	**08 TR 003**	73,25 b
LSD $_{0.01:}$4.88 VK : 4.60			

Table 4: Germination vigor (%)values determined after 72 hours of accelerated aging test by cultivars

Cultivar	**HY-72hours**	**Cultivar**	**HY-72hours**
Palancı 1	55,75 b	**Tunca**	82,50 a
Çiğdem 1	47,00 d	**Sanbro**	51,00 c
İnegöl Alası	37,45 e	**08 TR 003**	52,00 c
LSD $_{0.01:}$2.99			
VK : 3.60			

When analysing average germination vigor values from the table 4.27 of accelerated aging for 96 hours of cultivars it is seen that the highest value with 60,25 % belongs to the oil sunflower cultivar Tunca and this is followed with 37,75 % by oil sunflower cultivar Sanbro and the lowest germination vigor value of 21.75 % was obtained on confectionary type sunflower cultivar İnegöl Alası.

Table 4. Germination vigor (%)values determined after 96 hours of accelerated aging test by cultivars

Cultivar	**HY-96Hours**	**Cultivar**	**HY-96hours**
Palancı 1	24,00 c	**Tunca**	60,25 a
Çiğdem 1	25,50 c	**Sanbro**	37,75 b
İnegöl Alası	21,75 c	**08 TR 003**	35,00 b
LSD $_{0.01:}$5.97			
VK : 11.64			

CONCLUSION

As a result of variance analyses performed in terms of accelerated aging test values between six different cultivars a statistic difference of 1 % is determined. When analysing aging values of cultivars with a duration of 24, 48, 72 and 96 hours it is determined that the highest value with 94,50 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value 75,50 % belonged to the confectionary sunflower cultivar Çiğdem 1. As the result of accelerated aging for 48 hours it is determined that the highest value with 88,50 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value belonged to 50,25 % for confectionary sunflower cultivar İnegöl Alası. As the result of accelerated aging for 72 hours it is determined that the highest value with 82,50 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value of 37,45 % belonged to confectionary type sunflower cultivar İnegöl Alası. As a result of accelerated aging for 96 hours it was determined that the highest value with 60,25 % belonged to the oil sunflower cultivar Tunca and the lowest germination vigor value of 21,75 % belonged to the confectionary sunflower cultivar İnegöl Alası.

When comparing the accelerated aging values for 24, 48, 72 and 96 hours for oil and confectionary cultivars it was observed that the accelerated aging values of oil cultivars are higher compared to confectionary type cultivars.

A steady decline in the germination rate of 24-48-72 and 96 hours aging process was observed for sunflower cultivars. Critical point for the sunflower cultivars used in this study is the accelerated aging test performed for 96 hours. It is to mention, that each aging performed over 96 hours can reduce the germination vigor and perhaps be a dead end for some cultivars (İnegöl Alası and Çiğdem 1). Accordingly if an accelerated aging test has to be performed for sunflower, it is recommended to carry out for 96 hours.

As a result of the study; when the result get from the accelerated aging increase an increase at the germination vigor, sprouting vigor and field output vigor were observed. We can indicate for the cultivars having a great acceleration aging result, that this cultivar is resistant for storage, transport and also has a higher field output rate. To determine the storage, transport and field output values is not a determining factor for germination vigor and sprouting vigor. Since germination and sprouting vigor tests are conducted under controlled conditions and without forcing the seeds. But at the accelerated aging test the seed is aging with fatiguing through moisture and temperature and germination value is determined after this aging.

As a result observed by the cultivars of sunflowers used the accelerated aging values are higher by oil cultivars compared to confectionary cultivars. It is to point out, cultivar Tunca is a superior cultivar among all cultivars in terms of transport, storing and field output.

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GENETICS AND BREEDING

GENETIC ANALYSIS OF SEED YIELD RELATED TRAITS UNDER OPTIMUM AND LIMITED IRRIGATION IN SUNFLOWER

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ABSTRACT

In order to estimation of genetic components of variance for agronomic traits of sunflower, 16 single cross hybrids obtained by crossing between four restorer line and four CMS line as tester were evaluated as Randomized Block Design with three replications in two separate optimum and water limited conditions. The resulted data were analyzed as Line × Tester mating design fashion. According to the results under optimum irrigation condition days to maturity, seed weight and oil content were under control of additive effects while plant height was under control of none-additive effects. Flowering time, head diameter and seed and oil yield were under control of both additive and none-additive effects. Under water limited condition days to maturity was under control of additive while plant height, and seed and oil yield were under control of none-additive effects. Flowering time was under control of both additive and none-additive effects. There was a high (82%) general heritability for flowering time and an intermediate for plant height and oil yield (61-62%) under optimum irrigation. Under water limited condition the highest general heritability was obtained for flowering time, seed weight and oil yield. The values for heritability were lower under limited irrigation compared to the optimum condition which it could be resulted because of more environment effect under drought stress. The results of this study implies that selection under stressed condition is complicated and may bring illusory results, so controlling of the environmental condition is very critical in proper estimating of genetic components of variance and it is dependent to homogeneity of genetic materials and environmental condition. With environmental control, selection based methods may be efficient for production of early mature sunflower hybrids under drought condition while there is a necessity of hybridization for improvement of plant height and seed and oil yield.

Key words: Additive effect, Dominance, Heritability, Line × tester

INTRODUCTION

Sunflower whit an annual production of about 41M tones (fao.org) is the third major supplier of edible oil in the world following soybean and rapeseed. Development of hybrids with high genetic potential for seed yield and optimum plant architecture capable of adapting to the specific area of cultivation is the main objective of sunflower breeding programs (Hladni *et a*l., 2011). Breeding for desirable plant characteristics requires information about the nature of gene action and the mode of inheritance of quantitative traits as well as general and specific combining abilities of parental inbred lines. Generally different traits of any plant are under control of additive or none-additive gene action. The relative importance of these components has been reported by many authors in sunflower.

El-Hity (1992) indicated the importance of both additive and non-additive effects in controlling of 1000 seed weight and oil content. Putt (1996) reported that non-additive component was more important than the additive component in governing seed yield in sunflower. Bajaj *et al.* (1997) reported the significance of additive genetic effects in the inheritance of days to maturity, plant height, and 100 achene weight and oil contents. Kandalkar (1997) reported that seed yield was governed by both additive and non-additive genetic effects. Over dominance gene action is reported for plant height, head diameter, oil content, 100 seed weight and seed and oil yield (Gangappa *et al*, 1997). Singh *et al.* (1999) reported the predominance of non-additive genetic effects for achene yield, oil content. Ashok *et al.* (2000) found

additive gene effects for seed yield. Sharma *et al.* (2003) reported the importance of additive genetic effects in the inheritance of head diameter, achene yield per plant and oil contents. Farrokhi (2003) reported that plant height, growth duration, head diameter, 1000 achene weight, achene yield and oil contents were under control of both additive and non additive effects. Parameshwari *et al.* (2004) reported the dominance of non-additve genetic effects for days flowering, plant height, head diameter, 100 achene weight and oil contents. Devi *et al*. (2005) reported that achene yield and its components were predominantly governed by non additive genetic effects. Jan *et al*. (2005) showed dominance of non additive genetic effects for achene yield. Mijic *et al*. (2006) reported that both additive and dominant variances were involved in inheritance of 1000 achene weight.

Skoric *et al.* (2007) stressed the non-additive gene effects on oil percentage. Karasu *et al.* (2010) reported significant general combining ability for plant height, 1000 seed weight and seed number per head. They found that the non-additive effects were the most effective than other type of polygenetic effects. The gene action was changed across the years, for example additive gene action was significant for number of achenes per head and 1000-achene weight in one year but not in the second. Ghaffari *et al.* (2011) reported that days to maturity, 100 achene weight, number of achenes per head and achene yield were under the control of both additive and dominant effects, however plant height and oil contents were controlled predominantly by additive effects and life cycle duration and achene yield were controlled by dominant effects. Nooryazdan *et al*. (2011) reported additive genetic effects for days to 50 percent flowering, branching and plant height. Machikowa *et al*. (2011) reported that the additive genetic effect for these traits was more important than non additive effect for 1000 achene weight and plant height, achene yield, head diameter and oil content. According to the results of Tabrizi et al. (2012) plant height, head diameter, empty seeds per head, days to beginning of flowering, days to maturity, stem diameter and 1000 seed weight were found to be controlled mainly by additive gene effects and over-dominance effect was important for days to end of flowering. Oil yield, oil percent, head dry weight, seed weight per head, seed yield and hulled seed yield were under the control of both additive and non-additive effects.

MATERIALS AND METHODS

The experiment was carried out in Khoy Agricultural and Natural Resources Research Station in Iran. The station located in 38° 32' north latitude and 44° 58' east altitudes. The 16 single cross hybrids obtained by crossing between four restorer lines by four CMS lines as tester were evaluated as Randomized Block Design with three replications under optimum and water limited conditions. Each experimental plot consisted of 3 rows of 4 m length with 60 x 25 cm spacing between and within rows. Fertilizers were applied at the rate of 100:70:90 kg/ha for N: P: K. Drought stress was imposed by water withholding in R4-R6 growth stage. During the growth season agronomic traits as days to flowering and maturity, plant height, head diameter and seed and oil yield and the related components were measured. The resulted data subjected to line x tester analysis (Kemptorne, 1957) to estimate respective genetic variance components.

RESULTS AND DISCUSSIONS

According to the results the restorer lines explained the most part of genetic variance for flowering time, plant height and seed weight, while testers (CMS lines) had the main role in explaining of the genetic variance of days to maturity, head diameter, oil content and oil yield under optimum irrigation condition. Line × tester interaction effects were important for seed number per head and seed yield (Fig. 1A). The lines had also explained the most of variability for days to flowering, plant height and 1000 seed weight under water limited condition, however except days to maturity the variability of other traits were explained by line × tester interaction effect (Fig. 1B). This indicate that different line tester combinations can provide more variability under drought condition which itself could be aroused from activation of drought responsive genes (Oncel et al. 2000; Shao et al. 2008; Skoric, 2009). These changes may act as a protective and adaptability factor against drought condition; however the response of genotypes could be different under drought condition which affects the line × tester interaction component.

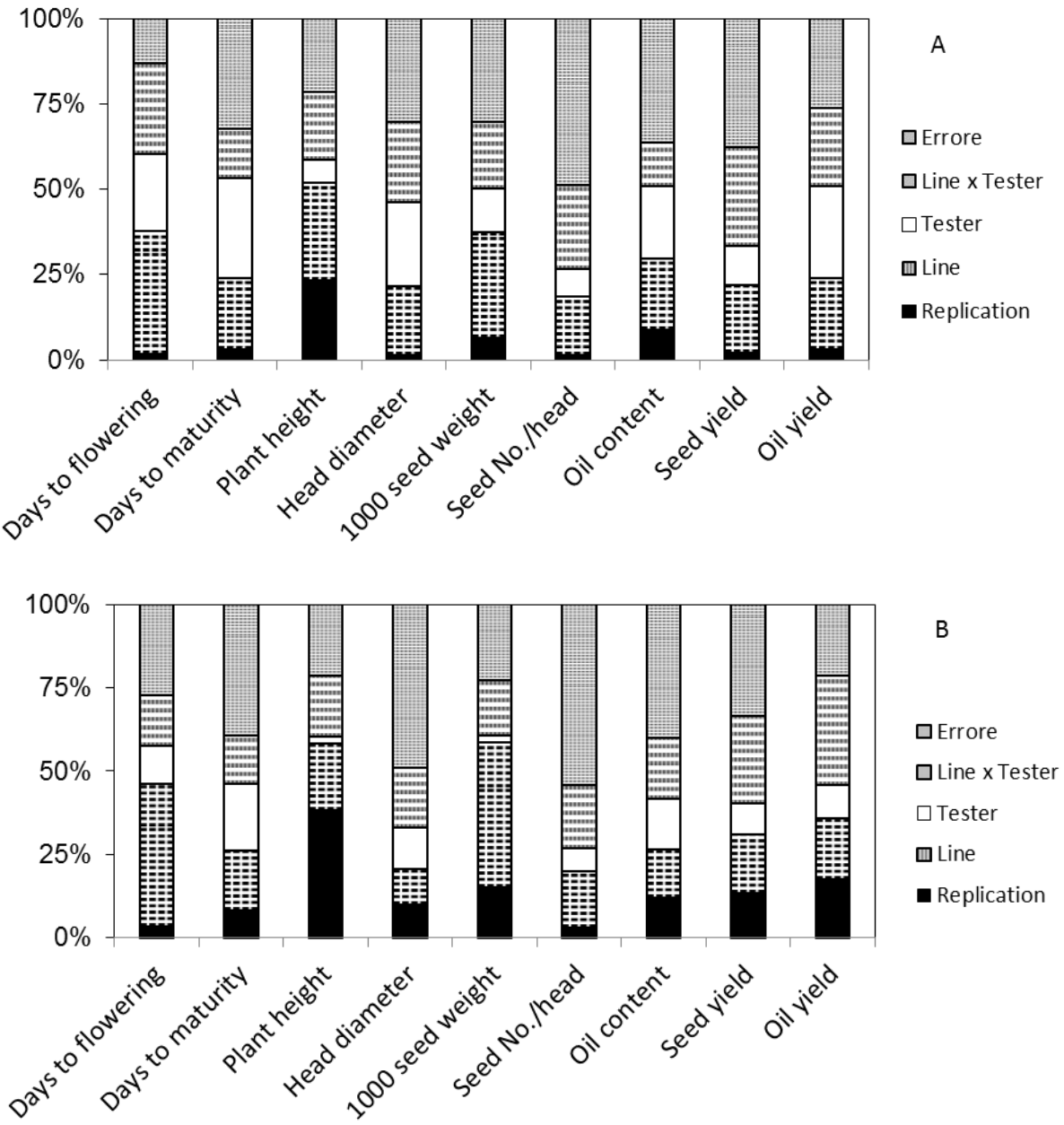

Fig.1. Relative contribution of different components on genetic variance components of agronomic traits under A) Optimum irrigation and B) water limited conditions.

The results indicated that under optimum irrigation condition growth period, seed weight and oil content were under control of additive effects, while plant height was under control of none-additive effects (Fig.2). Flowering time, head diameter and seed and oil yield were under control of both additive and none-additive gene effect. It is concluded from literature that generally the qualitative traits are predominantly under additive gene action (Singh *et al*. 1999; Sharma *et al.* 2003; Ghaffari *et al.* 2011; Nooryazdan *et al*. 2011), while quantitative characteristics as seed/oil yield are under control of both additive and non-additive gene actions (Putt, 1996; Gangappa *et al*, 1997; Devi *et al*. 2005; Jan *et al*. 2005a; Ghaffari *et al.* 2011; Tabrizi et al. 2012). Because of controversy reports it is impossible to determine absolute effect of gene action for a given trait.

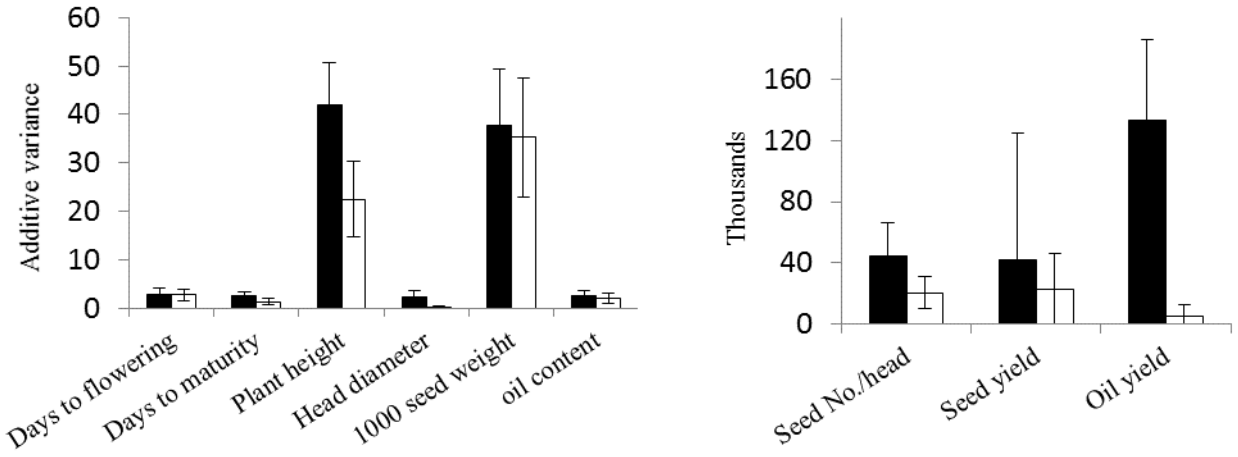

Fig. 2. Additive variance for agronomic traits under well watered (dark bars) and drought stressed condition (white bars)

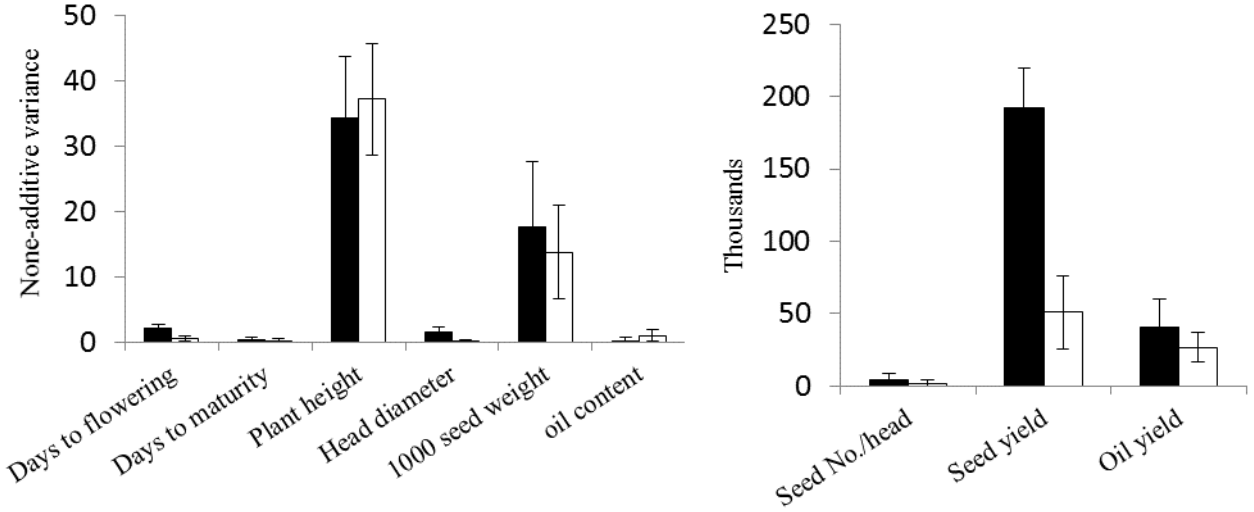

Fig.3. None- additive variance for agronomic traits under well watered (dark bars) and drought stressed condition (white bars)

Under water limited condition days to maturity was under control of additive while plant height, and seed and oil yield were under control of none-additive effects (Fig.3). Flowering time was under control of both additive and none-additive effects. These results is in accordance with other reports under non-stressed condition (putt, 1996; Bajaj et al. 1997; Gangappa et al. 1997; Devi et al. 2005; Parameshwari *et al.* 2004 and Nooryazdan *et al*. (2011). Comparison of genetic components showed that additive effects for plant height, head diameter and oil yield are significantly higher in optimum condition compared with stressed condition. There were also significant differences for days to flowering, head diameter and seed and oil yield. To the best of author's knowledge there is no information about genetic control of sunflower traits under drought condition. The results of this study indicated that additive gene action is restricted under drought condition which it could be resulted because of higher environmental effects on estimated variances. Karasu *et al*. (2010) indicated that the gene action was changed across the years, for example additive gene action was significant for number of achenes per head and 1000-achene weight in one year but not in the second.

There was high (82%) broad sense heritability for flowering time and intermediate for plant height and oil yield (61-62%) under optimum irrigation (Table 1). There was an intermediate heritability for days to flowering and maturity and oil content. Narrow sense heritability Estimates were low for all traits except seed number per head. Under water limited condition the highest broad sense heritability was obtained for

flowering time, seed weight and oil yield (Table 2). The values for heritability were lower under limited irrigation compared to the optimum condition which it could be resulted because of more environment effect under drought stress. These results indicate that the effect of environment is higher than genotype under stressed condition which could reduce the efficiency of selection under drought condition. Alza and Fernandez (1997) reported higher narrow sense heritability estimates for various sunflower traits as seed yield, number of seeds per head, seed weight, head diameter; oil content and days to bloom. On the contrary, Sayed *et al*. (2013) reported low narrow sense heritability estimates for seed and oil yields. The lower narrow sense heritability estimates in this study indicated the importance of non-additive gene effects for the agronomic traits in the sunflower genetic materials which were used in this experiment.

Table 2. Heritability estimates for agronomic traits under well watered condition

	Days to flowering	Days to maturity	Plant height	Head diameter	Seed weight
h^2_B	0.82	0.55	0.61	0.57	0.51
h^2_N	0.47	0.47	0.33	0.34	0.32

	Seed number	Oil content	Seed yield	Oil yield
h^2_B	0.70	0.45	0.39	0.73
h^2_N	0.64	0.42	0.07	0.56

Table 2. Heritability estimates for agronomic traits under drought stressed condition

	Days to flowering	Days to maturity	Plant height	Head diameter	Seed weight
h^2_B	0.62	0.40	0.50	0.21	0.64
h^2_N	0.51	0.35	0.19	0.15	0.46

	Seed number	Oil content	Seed yield	Oil yield
h^2_B	0.49	0.35	0.12	0.31
h^2_N	0.46	0.24	0.09	0.07

CONCLUSIONS

The results of this study implies that selection under stressed condition is complicated and may bring illusory results, so controlling of the environmental condition is very critical in proper estimating of genetic components of variance and it is dependent to homogeneity of genetic materials and environmental condition. With environmental control, selection based methods may be efficient for production of early mature sunflower hybrids under drought condition while there is a necessity of hybridization for improvement of plant height and seed and oil yield.

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A UNIQUE CYTOPLASMIC-NUCLEAR INTERACTION CAUSING SUNFLOWER PLANTS WITH REDUCED VIGOR AND THE GENETICS OF VIGOR RESTORATION

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ABSTRACT

Plants with pale yellow leaves and reduced vigor were observed in backcross progenies of inbred line HA 89 or HA 410 in the cytoplasms of 12 perennial *Helianthus* species, but not in the cytoplasms of annual *H. niveus*, *H. praecox*, *H. anomalus***,** and *H. neglectus*. Segregation ratios of normal (N) to reduced-vigor (RV) plants in testcrosses and self-pollination of heterozygous normal plants, respectively, suggested that single dominant gene (*V*) controls vigor restoration. A high frequency of vigor restoration genes was found in 11 cultivated sunflower lines, with the exception of HA 89, HA 410, RHA 801, and Seneca. Testcross progenies of the half-diallel crossed F1s among HA 271, HA 234, VNIIMK, Armavir, Issanka, and HA 821 onto the RV cmsRIG1 were all normal, suggesting that all these lines possess the same *V* gene. Extensive use of *H. tuberosus* in early sunflower breeding programs might explain the presence of *H. tuberosus V* gene in many cultivated sunflowers, and the possible selective advantage of the *V* gene. A new *V* gene derived from *H. giganteus* was identified, which differed from the *V* gene commonly existing in cultivated lines. Other *V* genes derived from *H. hirsutus* and *H. salicifolius* will be compared among all the *V* genes. The *V* gene commonly existing in cultivated lines has been mapped to the linkage group 7 of the sunflower genome using SSR markers. The tightly linked markers will help select for normal vigor progenies when using perennial *Helianthus* cytoplasms in a breeding program

Key Words : sunflower, cytoplasmic-nuclear interaction, reduced vigor, wild perennial *Helianthus*

CORRELATION STUDIES OF SSR MARKER BASED GENETIC DISTANCE AND HETEROSIS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) one of the most important oilseed crops in India known for its quality oil. However, in recent years the area under cultivation is decreasing owing to crop being affected by biotic and abiotic stress. This situation necessiated development of higher heterotic hybrids involving diverse germplasm to break the yield plateau. Anexperiment was conducted at Main Agricultural Research Station, UAS - Raichur to evaluate 49 sunflower hybrids along with parents to determine the correlation between SSR based genetic distance (GD) and heterosis for nine quantitative traits. The 49 hybrids were derived by crossing seven CMS lines and seven restorers in line x tester design. . Significant heterosis was recorded in hybrids for all nine traits studied. Genetics distance between pairs of tested CMS lines and testers ranged from 0.18 to 0.68. The correlation between genetic distance and heterosis was not significant for the most of characters studied. A highly significant positive correlation was observed between genetic distance and head diameter both at mid-parent ($r=0.48$; $p<0.01$) and better parent ($r=0.475$; $p<0.01$) heterosis level. However, significant negative heterosis was recorded between genetic distance and mid-parent heterosis for number of seeds per head ($r=-0.348$; $p<0.05$) and oil content ($r=-0.391$; $p<0.01$). The SSR markers included in the study are solely for their high PIC values. The poor correlation of GD with hetersis accept for head diameter indicates the need to include the markers linked to yield contributing traits to help in to rely on marker based GD to predict hybrid performance. .

Key words: Sunflower, heterosis, genetic distance, correlation

INTRODUCTION

The cultivation of sunflower at commercial scale as an oilseed crop is worldwide. The largest traditional producer is Russia and other sunflower producing countries include Argentina, the Eruopean Union, USA, China, India, Turkey and South Africa. The world sunflower production is around 30 million tones and is being cultivated over an area of 20 million hectares. . In India, sunflower is being grown over an area of 0.69 million hectares with a production of 0.54 million tones with the productivity of 791kg per ha (Anon., 2015).

Sunflower being a highly cross pollinated is an ideal crop for exploitation of heterosis. The discovery of Cytoplasmic Male Sterility by Leclercq (1969) followed by fertility restoration system by Kinman (1970) provided the required breakthrough in the development of hybrids. Hybrids are also highly self fertile and resistant to diseases, thus resulting in enhanced seed set and seed filling (Seetharam, 1981). After sunflower being introduced to India as oil seed crop in early 1970's, the first sunflower hybrid BSH-1 was released during 1980 and thereafter several hybrids have been released. The exploitation of heterosis through hybrid breeding is one of the landmark achievements in plant breeding (Duvick, 2001) and particularly in sunflower (Seetharam, 1984). In last decade (2001-2010), 6 varieties and 11 hybrids have been released for commercial cultivation (Anon., 2014) in India.

In heterosis breeding programmes, a large number of experimental hybrids need to be and are routinely produced and tested to identify hybrid vigour. This requires huge resources and manpower. In

general, heterosis is considered as an expression of the genetic divergence among inbreds/parents used for crossing. Reliable prediction of single-cross performance is crucial in hybrid breeding as the evaluation of large number inbred lines in numerous cross combinations is difficult. . Several prediction approaches have been suggested using phenotypic data with co-ancestry coefficients calculated from pedigree records or marker data (Schrag et al., 2009). The information on the genetic diversity and distance among the breeding lines and correlation between genetic distance and hybrid performance are important in determining breeding strategies, classifying the heterotic groups and predicting the hybrid performance.

Studies of genetic diversity in relation to hybrid performance have been undertaken in several crops. Investigations in corn, *Zea mays* L., have shown that the genetic diversity of parents was significantly correlated with hybrid performance and that yield heterosis could be predicted using molecular markers (Schrag et al.,2006). Genetic diversity of different sunflower gene pools has been studied with enzymes (Tersac et al., 1993), RFLP markers (Hongtrakul, 1997) and SSR markers (Solodenko et al., 2005). However, the literature data on the predication of sunflower heterosis and hybrid performance by marker based genetic distance of the parental lines is scarce (Tersac *et al*.1994, Cheres et al. 2000). The objective of the study wasto identify the reliability of SSR markers to determine the genetic diversity and association between SSR based genetic diversity and heterosis for yield component traits in sunflower.

MATERIAL AND METHODS

Seven CMS lines (CMS-2A, CMS-821A, CMS-850A, R-10-46-2A, CMS-4A, CMS-6A, CMS-10A) and seven R-lines (R-GM-39, R-GM-41, R-GM-49, R-GM-69, 83-Br, R-393 , R2F01120B) were crossed in L x T fashion during kharif-2013-14. The resultant 49 hybrids along with parents were evaluated for nine yield and yield contributing characters in RCBD design with three replications. Heterosis, expressed as per cent increase or decrease of derived F_1 over mid parent (average heterosis) and better parent (heterobeltiosis) was calculated for each character as per the method of Turner (1953) and Hayes *et al.* (1956).

Table 1: list of sunflower SSR primers used for the study

1. ORS-287	16. ORS-324	31. ORS-677
2. ORS -290	17. ORS-332	32. ORS-769
3. ORS-296	18. ORS-333	33. ORS-780
4. ORS-300	19. ORS-339	34. ORS- 807
5. ORS-301	20. ORS-337	35. ORS- 811
6. ORS-309	21. ORS-358	36. ORS- 852
7. ORS-310	22. ORS-378	37. ORS- 930
8. ORS-311	23. ORS-388	38. ORS- 938
9. ORS-315	24. ORS-407	39. ORS- 959
10 ORS-316	25. ORS- 484	40.ORS- 1068
11.ORS-318	26. ORS-546	41.ORS- 1088
12.ORS-319	27. ORS-552	42. ORS- 1159
13 ORS-321	28. ORS-578	43. ORS- 1220
14.ORS-322	29. ORS- 628	44. ORS- 1245
15.ORS-323	30. ORS- 671	

The genomic DNA of 14 parental lines was extracted by following modified CTAB method. Fourty four sunflower SSR primers were used for PCR amplification using gradient thermocycler. The amplified products were separated using 3.5% agarose gel electrophoresis. DNA polymorphism between two inbreds was estimated by comparison of amplified fragments. Jaccard similarity coeffiecient (j) was calculated according to Staub et al., (2000). Genetic distance (GD) among all parental lines was estimated as per formula GD=1-j given by Spooner et al., (1996).

The values of genetic distances as measured by SSR markers were correlated with mid-parent heterosis and better parent heterosis to estimate their relationship using Pearson's coeefficient of correlation. Correlations were done for hybrid combinations from each tester and lines separetely. Significance of correlation was determined using the table os Snedecor (1959).

RESULTS AND DISCUSSION

The 49 sunflower hybrids derived by crossing seven CMS and seven restorers in LxT fashion were evaluated for yield and yield component traits along with parents. High degree of variation was observed for all characters studied in both parents and hybrids. The mean values of the hybrids were significantly higher than the parental lines for plant height, head diameter, 100 seed weight and seed yield per plant (Table 2).

Table 2: Mean values and coefficient of variation (V) for the sunflower parental lines and their hybrids.

Character	Female parent		F1 Hybrid		Restorer lines	
	Mean	V (%)	Mean	V (%)	Mean	V (%)
Plant height (cm)	102.38	29.9	144.90	9.98	107.5	15.07
Days to 50% flowering	64.00	2.77	65.50	1.94	63.00	5.59
Head Dia (cm)	14.76	1.85	17.50	1.49	11.04	1.20
No. of leaves	20.46	3.78	27.32	2.59	21.34	2.23
100 seed wt (g)	3.27	0.71	3.94	0.48	2.39	0.33
No. of seeds/head	1206.6	127.7	1179	117.0	1349	148.9
Volume wt (g/100ml)	36.36	2.06	40.06	1.74	37.66	2.83
Seed yield/pl (g)	30.13	2.59	35.42	2.98	27.30	4.06
Oil content (%)	34.65	3.98	37.81	1.38	34.70	2.95

The heterosis level for most of the traits studied was signicantly superior viz., plant height, head diameter, 100 seed weight, seed yield per plant (Table 3). The highest level of mid-parent heterosis observed for 100 seed weight(39.28) followed by plant height (38.70) and head diameter (35.78). Whereas the highest level of better parent heterosis observed forplant height (34.65) followed by 100 seed weight(28.04) and head diameter (24.60).

Table 3: Mean and range of heterosis for nine quantitative traits in 49 sunflower hybrids

Trait	Mid-parent Heterosis		Better Parent Heterosis	
	Mean	Range	Mean	Range
Plant height (cm)	38.70**	6.8 – 88	34.65**	-5 – 61
Days to 50% flowering	3.25	-10.41 – 15.40	2.45	- 11.90 – 7.90
Head Dia (cm)	35.78**	8.35 – 79.03	24.60**	-2.73 – 46.51
No. of leaves	31.00	-2.75 – 37.43	28.20	-14.51 – 30.51
100 seed wt (g)	39.28*	11 – 45.40	28.04*	6.01 – 36.38
No. of seeds/head	-3.13	-11.77 – 11.37	-10.60	-23.20 – 11.54
Volume wt (g/100ml)	8.80	6.56 – 20.53	6.44	4.41 – 12.51
Seed yield/pl (g)	24.40*	6.96 – 58.16	19.50*	1.42 – 55.78
Oil content (%)	6.70	-4.51 – 18.80	5.81	-11.39 – 12.36

Forty four sunflower SSR primers were used to study genetic diversity among fourteen parental lines. Out of 44 primers used three primers failed to amplify and ten primers showed monomorphic amplification bands. The remaining 31 primers showed polymorphism with an avarage polymorphism of 39.65 % (PIC=39.65%). The number of amplified products ranged from 1 to 3 with an average of 1.21 bands per primer and 1.13 bands per primer were polymorphic.

The frequency of SSR polymorphism was calculated based on presence (taken as 1) or absence (taken as 0) of common bands. The binary data was used to compute pair wise similarity coefficient (Jaccard, 1908). The genetic similarity computed considering data of SSR markers showed a wide range from 0.32 to 0.82 indicating the presence of high variability among 14 sunflower genotypes.

The highest similarity was observed between the parental genotype CMS 821A and CMS 2A (0.82) while the lowest similarity was observed between the parental genotypes *viz*,. R-GM-41 and R-10-46-2A (0.32), R-GM-49 and CMS A6 (0.32).

Genetic diversity is the extent to which the heritable material differs within a group of plants, which is a result of evolution, including domestication and plant breeding. Assessing genetic diversity of cultivated crop plants is important to select proper genotype for hybridization programme. The sunflower genetic diversity and co-ancestry analysis have been carried out using RAPD (Arias *et al*., 1995). The placement of individual cultivars into different accessions based on morphological attributes do not necessarily reflect the real genetic relationship.

The recent advances in molecular biology have provided the descriptors based on protein and DNA as an aid to plant breeding programme. Genetic diversity caused by sexual reproduction i.e. hybridization, selection and mutation results in genome changes from one base pair to entire chromosome. The molecular markers that are not influenced by environmental changes provide an opportunity to examine the genetic relationship between accessions more precisely. This can help in the rationalization of existing germplasm collections and allow future collection strategies towards specific objective. Molecular markers can be used as a valuable tool for identification of parental lines and varieties for protection of plant breeder's right. DNA markers also help in studying the evolutionary/phylogenetic relationship between inbreds and varieties.

The relation between SSR based genetic diversity among inbred lines and their hybrids performance dependent on the trait of interest examined. Correlation coefficient between genetic distance,

parental means and hybrid performance in terms of heterosis were not significant for most of the characters studied. However, significant correlation was observed for GD with both mid-parent (r=0.48) and better parent (r=0.475) heterosis. For both number of seeds per head and oil content, correlation between GD and mid-parent heterosis was significantly negtive (r=-0.348 & r=-0.391).

The correlation between genetic distance and heterosis levels expressed was not significant for most of the traits studied except for head diameter, number of seeds per head and oil content. The SSR markers included in the study are solely for their high PIC values. The poor correlation of GD with hetersis accept for head diameter indicates the need to include the markers linked to yield contributing traits to help in to rely on marker based GD to predict hybrid performance. (Charcosset *et al*., 1991 and Bernardo *et al*., 1992).

Tersac *et al*. (1994) described relationships between heterosis and enzymatic polymorphism of 39 sunflower populations. The correlation coefficients for all enzyme systems were too low to be used as predictors of the general combining ability, but when enzymatic systems were analyzed separately, four of them turned out to be useful markers for breeding purposes. Zeid et al. (2004) pointed out that the lack of association between heterosis and genetic dissimilarities for inter group hybrids may be explained by the absence of crosses between related parents i.e. by the absence of variation for parental relatedness: all crosses have unrelated parents.

In the present study the GD showed poor correlation with both mid-parent and better parent heterosis except for head diameter. Similar reports were done in previous studies on pepper, alfalfa, wheat and rapeseed (Diers *etal*. 1996, Geleta *et al*. 2004, Zeid *et al*. 2004, Riday *et al*. 2003).

CONCLUSION

The conclusion on the use and reliability of SSR based genetic distance to predict hybrid performance in terms of heterosis depends on use of large number of specific markers linked to yield contributing traits. A higher accuracy would also be possible by identification of molecular markers linked to combining ability.

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STABILITY OF THE LEVEL OF PARTIAL RESISTANCE TO WHITE ROT IN SUNFLOWER

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ABSTRACT

In the southeast of Buenos Aires Province, Argentina, sunflower cultivars must be moderately resistant to *Sclerotinia sclerotiorum* infections on capitula. The stability of 32 sunflower hybrids for the relative incubation period of white rot during six years in Balcarce was assessed. Two stability methods were proposed: the linear regression model (univariate method) and the additive main effects and multiplicative interaction model (AMMI) (multivariate method). Combined analysis of variance detected highly significant effects of hybrids, years and hybrid-year interaction (GxE) effect. The linear regression model explained only 24% of the GxE sum of squares, while the two first principal components of the AMMI model retained the 66.8% of that sum of squares. Consequently, the AMMI model seemed to be more effective for characterizing the GxE effect. Two statistics based on AMMI models were considered afterwards: the AMMI Stability Value (ASV) and the Genotype Selection Index (GSI). The hybrids Paraiso 20, Paraiso 22, ACA 863, MG 60, Tehuelche CL, CF31 and Pampero DM had stability since the ASV index. According to GSI, Paraiso 22, Paraiso 20, Tehuelche CL and MG 60 showed high level of resistance to white rot besides of general adaptability across six years in Balcarce.

Key words: hybrids, partial resistance, *Sclerotinia sclerotiorum*, genotype-environment interaction, AMMI, linear regression.

INTRODUCTION

Sunflower *(Helianthus annuus*) has traditionally been an important crop in Argentina given the Premium quality of its seed-oil. In the period 2013/14, our country contributed about 5% of worldwide grain production (USDA, 2014). The Southeast of the Buenos Aires Province (SEB) produces around the third of the Argentinean production and, consequently it became the main sunflower grown area (BCBA, 2014).

Sclerotinia sclerotiorum on sunflower capitula produces white rot, a disease that may cause direct and/or indirect damages in all regions where sunflower is grown (Pereyra and Escande, 1994; Gulya *et al.*, 1997). An efficient control of white rot involves the use of sunflower hybrids with a moderate level of resistance that allows reducing the epiphyte risk and the variation of seed-productivity given the pathogen infection.

In sunflower, the resistance to white rot is of horizontal type and its inheritance mainly depends on additive gene effects (Castaño *et al.*, 2001; Godoy *et al.*, 2012). Studies conducted by the Sunflower Breeding Group in the "Unidad Integrada Balcarce" suggested that white rot can be considered as a series of phases starting in the flowering period, when the *S. sclerotiorum* infection and the mycelium invasion into capitulum are made, and ending at maturity (Castaño, 2007). These phases are assessed through components of partial resistance and among them we found the relative incubation period (RIP), which measures the relative period of time of inoculated capitulum to show white rot symptoms (Castaño and Giussani, 2009).

In conventional breeding programs, sunflower selection is carried out in the field. However, the level of hybrids resistance to white rot is affected by the genotype-environment (GxE) interaction effect (Godoy *et al.*, 2005). Recently, Delgado *et al.* (2013) found significantly GxE interaction effect when white rot partial resistance was evaluated in hybrids sown in the SEB. Therefore, in sunflower breeding for white rot resistance it will be important to determine the GxE interaction effect in order to make easier the selection of hybrids and do not overestimate the expected genetic progress (Kang and Gorman, 1989).

The genotype stability is the ability of certain genotypes to have consistently relative performance across environments. Becker and León (1988) distinguished two concepts of stability: 1) biological or static, where the stable genotype has minimum variance across environments, and 2) dynamic or agronomic, where stable cultivars are those that show a constant gain in performance with increasing potential environment. Last definition is that one preferred by agronomists and plant breeders (Alwala *et al.*, 2010).

Methods to analyze the GxE interaction effect and to detect the most stable genotypes are numerous. Among univariate methods, the join linear regression analysis (Finlay and Wilkinson, 1963) has been mostly used because its mathematical simplicity and its easy biological inferences that could be made. However, it has some disadvantages when the linearity fails and/or when there are not several genotypes and environments involved in the analysis. Another weakness is related to that the regression coefficient tends to simplify the genotype response in a single plane, when it may be described in a multidimensional space (Crossa, 1990; Flores *et al.*, 1998; Cubero and Flores, 2003).

Regarding to multivariate methods, the analysis of additive main and multiplicative interaction effects (AMMI) (Gollob, 1968; Mandel, 1971) has been used to evaluate genotype stability. Given that the AMMI method considers the sources of variation genotype and environment as additive effects and the GxE interaction effect has multiplicative effect, analyses of variance and of principal components must be done (Cubero and Flores, 2003; Farshadfar and Sutka, 2003; Alwala *et al.*, 2010; Williams Alanís *et al.*, 2010). Based on this method, the nonparametric statistics AMMI Stability Value (ASV) (Purchase *et al.*, 2000) and the Genotype Selection Index (GSI) (Farshadfar, 2008) were also proposed.

In sunflower, genotype stability has been determined for seed-yield (Lorenzo and Lorenzo, 1987; Aguirrezábal *et al.*, 2002), but there are not many studies made for other quantitative traits like horizontal disease resistances. Therefore, the objective of this study was to evaluate the stability of sunflower cultivars released in the SEB, when the white rot resistance is measured, by uni- and multivariate methods.

MATERIALS AND METHODS

Plant material and experimental design

Field experiments were at the 'Unidad Integrada Balcarce', Argentina (37 ° 45'S, 58° 18'W, 130m ASL), during six consecutive years (e.g. 2010-15). Thirty two sunflower cultivars were grown following a randomized complete block design, with three replications (Table 1). The hybrids ACA 884 and Paraíso 20 were also sown next to the experiments to be used as flowering checks.

Inoculum, inoculation protocol and measured variable

Assisted infections were carried out following the protocol suggested by Vear and Tourvieille (1984). The capitula of 12 plants/plot in the R5.3 stage (Schneiter and Miller, 1981), or its homologous F3.2 (Cetiom, 1992) were inoculated with 5ml of an aqueous suspension containing about 5000 ascospores/ml of *Sclerotinia sclerotiorum*. Given the heterogeneity of flowering period (i.e. within and among cultivars) there were four to six inoculation dates in the field experiments. Inoculated capitula were immediately covered with Kraft paper bags and trials received irrigations by micro-sprinklers once or twice a week until white rot data were scored. White rot partial resistance was evaluated by capitulum

through one of its components: the relative incubation period (RIP). The RIP is the ratio of the number of days between inoculation and first detected symptoms for each inoculated capitulum and that for the checks inoculated on the same day. A plot mean RIP value was calculated by hybrid. Hybrids with RIP higher than unity had a favorable level of resistance because their symptoms appeared after that the checks (Castaño *et al.*, 1993; Godoy *et al.*, 2005).

Statistical analyses

Analysis of variance

There was a set of six consecutive years of RIP responses for each evaluated hybrid. The assumptions required for the combined analysis of variance were evaluated by checking the residuals and using Hartley's F test of homogeneity of variances. The following mathematical model was proposed, considering both hybrids and years as fixed effects:

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \gamma_{k(j)} + e_{ijk}$$

where: i=1,…,32; j=1,…,6; k=1,…,3;

y_{ijk}: RIP of *i*th hybrid, at *k*th block within *j*th year;
μ: RIP general mean;
τ_i: main effect of hybrid *i*;
β_j: main effect of year *j*;
$(\tau\beta)_{ij}$: *i* hybrid-by-*j* year interaction effect;
$\gamma_{k(j)}$: effect of block *k* within year *j*;
e_{ijk}: residual associated with *ijk*th observation of RIP.

The joint linear regression (Finlay and Wilkinson, 1963) as well as the AMMI (Gollob, 1968; Mandel, 1971) methods were used to characterize the GxE interaction. Both methods are based on the partition of the GxE sum of squares of the analysis of variance (ANOVA).

Joint-regression analysis

The slopes of regressions of individual hybrid's RIP against annual mean RIPs, based on all hybrids in the trial (i.e. environmental index) were determined. The GxE interaction effect was partitioned into the heterogeneity of regression coefficients and the deviations from the regression model.

Additive Main Effects and Multiplicative Interaction (AMMI) model

In the AMMI model, the sum of squares of the GxE interaction was decomposed by a Principal Component Analysis (PCA). Also, two analytical indices derived from AMMI model were calculated: the AMMI Stability Value (ASV) (Purchase et al., 2000), and the Genotype Selection Index (GSI) (Farshadfar, 2008).

The ASV, that weights the contributions of each hybrid to the GxE sum of squares through the two main components selected from the PCA, was calculated by hybrid using the following formula:

$$ASV_i = \sqrt{\left[\frac{SS_{PC1}}{SS_{PC2}}(PCA1score)\right]^2 + (PCA2score)^2}$$

where:

$\frac{SS_{PC1}}{SS_{PC2}}$: is the weight given to the PCA1-score by dividing the PCA1 sum of squares by the PCA2 sum of squares;

PCA1score: is the value obtained by the 1st main component for each hybrid;

PCA2score: is the value obtained by the 2nd main component for each hybrid.

Hybrids with lower ASV values showed high stability level because of their low contributions to the GxE interaction. The GSI is an index that allows simultaneous selection of those hybrids with high level of partial resistance as well as stability. For each hybrid, it was calculated as:

$$GSI_i = rASV_i + rRIP_i$$

Where: $rASV_i$: is the ranking of hybrids according to the ASV index (value 1 corresponds to the highest stability level); $rRIP_i$: is the ranking of hybrids according to the average of RIP across evaluated environments (value 1 corresponds to the highest RIP values). According to this index, the hybrid with the least GSI is considered as the most stable with high RIP. All statistical analyses were performed by the R program (R Core Team, 2014). Also, the software 'agricolae' (De Mendiburu, 2014) was used to adjust some specific functions in the AMMI model.

RESULTS AND DISCUSSION

The RIP means of hybrids across years ranged from 0.8196 (ACA 885) to 1.2674 (SPS 3109) and the environmental indices (i.e. mean of all hybrids in a year) ranged from 0.9111 (2012) to 1.0868 (2011) (Table 1).

Table 1. Means of RIP obtained from the 32 sunflower hybrids evaluated, differentiated by year, and the average over the six years. Values obtained by the ASV (AMMI stability value) and GSI (Genotype selection index) for each hybrid. RIP averages for each year (Environmental Index) are also indicated.

HYBRIDS	YEARS						AVERAGE 6 YEARS	STABILITY INDEX	
	2010	2011	2012	2013	2014	2015		ASV	GSI
64A89	0.9063	1.2707	0.9431	0.9396	0.9902	1.0155	1.0109	0.3202	45
65A25	0.9141	0.8555	0.8593	1.0093	0.8995	0.9068	0.9074	0.2122	45
ACA 863	0.7343	0.9242	0.7565	0.8789	0.8914	0.8266	0.8353	0.0491	34
ACA 884	1.0831	1.0671	0.9958	0.9709	1.0125	1.0635	1.0322	0.129	25
ACA 885	0.8027	0.7095	0.6904	0.9951	0.8277	0.8920	0.8196	0.3502	63
ACA 886 DM	0.9356	1.0055	0.6407	0.8989	0.9029	0.9561	0.8899	0.2372	51
Agrobel 963	0.9827	1.2467	0.8277	1.0317	0.9460	0.9303	0.9942	0.2275	38
Albisol 2	1.2039	1.1105	0.9973	1.0178	1.0715	1.0630	1.0773	0.1575	22
Albisol 20	0.8240	1.0447	0.8482	0.9896	0.8843	0.9599	0.9251	0.1012	33
BuckSurcoflor	0.9161	1.1607	1.1032	0.9933	1.0021	1.0053	1.0301	0.2857	41
Cauquen	1.0453	1.0856	1.0843	0.9803	1.0363	1.0280	1.0433	0.2172	28
CF 31	1.0099	1.0162	0.8751	0.9554	0.9338	0.8790	0.9449	0.0938	29
Dekasol 3820	1.0704	1.1145	1.0003	1.0096	1.0185	1.0028	1.0360	0.114	23
Dksol OP3845	0.9731	1.0867	0.8552	1.0248	0.9205	1.0965	0.9928	0.1139	29
DM 230	0.8851	1.1183	0.9052	0.9299	0.9255	1.1432	0.9845	0.1463	33
GS 3190 RDM	1.2201	1.0384	0.9991	1.1316	1.1853	1.0960	1.1118	0.3083	32
HS-03	0.7780	1.1300	0.8228	0.8935	0.9206	0.8662	0.9019	0.2642	54
KWSBaqueano	1.0723	0.9770	0.9881	1.0016	1.0142	1.0077	1.0102	0.2217	36
Macon	1.0229	1.2322	1.0366	1.0221	0.9816	0.9700	1.0442	0.2395	32
MG 2	0.9652	1.0300	0.9138	0.9544	0.9267	0.8975	0.9479	0.1058	31
MG 60	1.0631	1.1768	0.8658	1.0121	0.9918	0.9779	1.0146	0.0758	18
NK 70	0.9645	0.9588	0.7853	0.9940	0.9510	1.0011	0.9425	0.1919	41
Pampero DM	0.8956	1.0083	0.7762	0.8960	0.9170	1.0030	0.9160	0.0973	33
Pan 7031	0.9868	1.1478	0.8725	0.9120	1.0074	0.9455	0.9787	0.1127	30

Paraíso 20	1.0374	1.1421	0.9644	1.0579	0.9966	1.0572	1.0426	0.0245	11
Paraíso 22	1.1230	1.1857	0.9816	1.2048	0.9850	1.0209	1.0835	0.0263	7
Paraíso 27	1.0747	1.2638	1.1036	1.1350	0.9824	0.9796	1.0898	0.2383	27
Paraíso 75	1.2030	1.2083	1.2238	1.1463	1.0910	1.0910	1.1606	0.2684	29
SPS 3109	1.3043	1.3165	0.9399	1.4329	1.3344	1.2763	1.2674	0.4273	33
Tehuelche CL	0.9675	1.1810	1.0380	1.1734	0.9887	1.1061	1.0758	0.0869	12
Tobsol 3004	0.8047	0.9507	0.7107	0.9471	0.8808	0.9133	0.8679	0.1546	45
VDH 487	0.9894	1.0133	0.7502	1.0089	0.9464	1.0702	0.9631	0.2434	46
ENVIROMENTAL INDEX	0.9925	1.0868	0.9111	1.0171	0.9801	1.0015			

Combined analyses of variance

Although Hartley's F test detected heterogeneity of variances among years, experimental residuals across environments did not show strong anomalies. According to Annicchiarico (2002), combined analysis of variance was made with the original data (e.g. RIP values) without transforming them.

Results of the analysis of variance are in Table 2. All evaluated effects were significant ($p<0.01$). Total sum of squares was decomposed into constituents. This showed that 13% of that total could be attributed to environmental effects (i.e. years), 40% to genotype effects (i.e. hybrids) and 21.8% to the GxE interaction effect.

Table 2. Combined analysis of variance for RIP responses obtained from 32 sunflower hybrids evaluated at Balcarce during six years.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	p-value
Year	5	1.5997	0.3199	12.69	$1.91\ 10^{-4}$
Blocks within years	12	0.3026	0.0252	3.36	
Hybrid	31	4.9123	0.1585	21.03	$1.25\ 10^{-63}$
Hybrid xYear (GxE)	155	2.6718	0.0172	2.29	$6.90\ 10^{-11}$
Error	371	2.7957	0.0075		

Joint-regression analysis

The two components of the GxE interaction effect, that is the linear regression and the deviation from regression, were both significant ($p <0.01$) and contributing with 24% and 76% to the GxE sum of squares, respectively (Table 3).

Table 3. Partition of the sum of squares of the GxE interaction according to the joint-regression method.

Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean squares**	**F ratio**	**p-value**
Hybrid x Year (GxE)	155	2.6718	0.0172	2.29	$6.90\ 10^{-11}$
Linear regression	31	0.6411	0.0207	2.76	$3.39\ 10^{-6}$
Residual	124	2.0307	0.0164	2.19	$7.16\ 10^{-9}$

According to Romagosa and Fox (1993), the contribution of the linear regression effect to the GxE sum of squares (24%) could be considered relatively low value. In addition, if we compare the linear regression component with its residual ($F= 1.26$, $p= 0.1878$) it would indicate that the variability explained by linear regression does not differ from the unexplained variability. These results suggest that

the joint linear regression would not be a suitable model to evaluate, in our case, the stability of sunflower hybrids by their level of resistance to white rot.

Additive Main Effects and Multiplicative Interaction (AMMI) model

The use of the multivariate method allowed decomposing the GxE sum of squares in five main axes (i.e. principal components, PC) (Table 4). Whereas the first three PC's showed significant effects ($p < 0.01$), the analysis stopped at PC2 given that with these two components more than two-thirds (i.e. 66.8%) of the sum of squares of GxE interaction effect were considered.

Table 4. Decomposition of the sum of squares of the GxE interaction by the main components obtained, absolute and cumulative percentages of the GxE sum of squares explained by each, and p-values associated.

Principal component (PC)	Absolute percentage	Cumulative percentage	Degrees of freedom	Sum of squares	Mean squares	p-value
PC1	39.7	39.7	35	1.0710	0.0306	$3.35\ 10^{-12}$
PC2	27.1	66.8	33	0.7323	0.0222	$3.23\ 10^{-7}$
PC3	15.2	82	31	0.4105	0.0132	$8.52\ 10^{-3}$
PC4	12.4	94.3	29	0.3347	0.0115	0.04
PC5	5.7	100	27	0.1528	0.0057	0.80

AMMI Stability Value (ASV) and Genotype Selection Index (GSI)

Estimated ASV index values ranged from 0.0245 (Paraíso 20) to 0.4273 (SPS 3109) (Table 1). In their paper, Purchase *et al.* (2000) did not suggest the most extreme ASV value from which genotypes with or without stability were determined. Therefore, in our work we considered stability in sunflower hybrids to those ones who have weighted contribution to the GxE interaction effect at a rate lower than 0.1. Consequently, there were seven hybrids accomplishing this requirement: Paraiso 20, Paraiso 22, ACA 863, MG 60, Tehuelche CL, CF 31 and Pampero DM.

The calculated GSI index values varied from 7 (Paraiso 22) to 63 (ACA 885) (Table 1). To determine the sunflower genotypes with low ASV value as well as moderate level of resistance we considered those ones whose GSI values were lower or equal than the first decile (18.4). So, the hybrids Paraiso 22, Paraiso 20, Tehuelche CL and MG 60, with RIP means across years higher than one, were selected.

CONCLUSIONS

The cumulative contribution of principal components 1 and 2 to the sum of squares of GxE interaction effect (66.8%) was 2.78 times that one explained by the linear regression (24%). Then, the AMMI model seemed to be more effective than the joint-regression analysis to evaluate the stability of sunflower hybrids by the RIP to white rot.

Further trials would allow describing better the sunflower hybrid capacity to do not change their relative RIP across different years in Balcarce. Under our experimental conditions, the selection index (GSI), derived from AMMI analysis, detected the hybrids Paraiso 22, Paraiso 20, Tehuelche CL and MG 60 having stability and moderately resistance to white rot.

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COLLECTION OF WILD *HELIANTHUS ANOMALUS* AND *DESERTICOLA* SUNFLOWER FROM THE DESERT SOUTHWEST USA

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ABSTRACT

Genetic resources are the biological basis of global food security. Collection and preservation of wild relatives of important crop species such as sunflower provide the basic foundation to promote and sustain the crop. Acquisition through exploration is the initial step in the germplasm conservation process. There are 53 species of wild *Helianthus* (39 perennial and 14 annual) native to North America. An exploration covering 3700 km to the desert southwest US in June of 2015 led to the collection of five populations of *H. deserticola* (desert sunflower) and eight *H. anomalus* (sand sunflower) accessions. All populations were collected throughout the broad distributional range of the species. Based on sand sunflower's occurrence in desert sand dune habitats of Utah and Arizona, it frequently has been recognized as drought tolerant, with the largest achenes of any wild species and high oil concentration potential, and thus is a candidate for improving cultivated sunflower. Desert sunflower is a xerophytic annual species found in sandy soils on the floor of the Great Basin Desert in small populations in western Nevada, west central Utah, and along the border of Utah and Arizona. Population size, habitat, soil type, seed set, the presence of diseases and insects, and other wild sunflower species located near the collection sites were recorded for each population. This germplasm will be important now and in the future as a genetic resource to combat emerging pests and environmental challenges, helping maintain sunflower as a viable global crop and to preserve it for future generations.

Key words: Sunflower, Crop wild relatives, Wild species, Germplasm resources, Exploration

INTRODUCTION

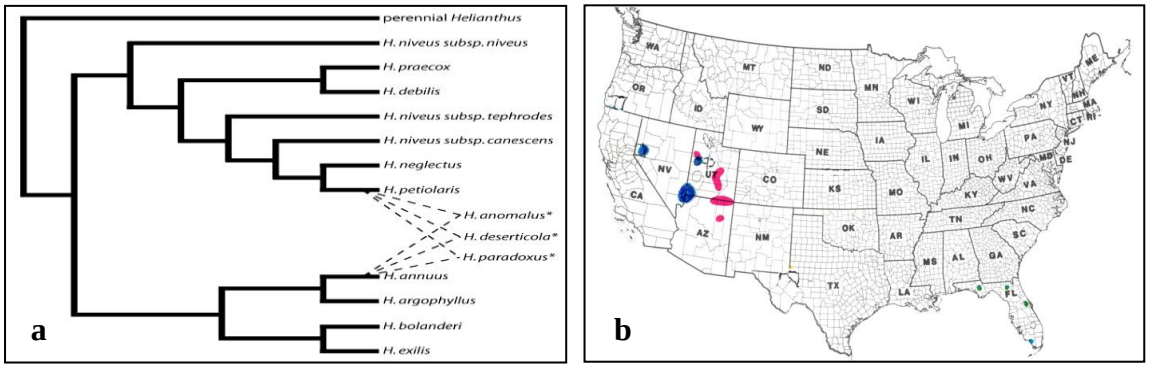
Collection and preservation of wild relatives of important crop species such as sunflower provide the basic foundation to promote and sustain the crop. Genetic resources are the biological basis of global food security, and acquisition through exploration is the initial step in the germplasm conservation process. There are 53 species of wild *Helianthus* (39 perennial and 14 annual) native to North America (Heiser et al., 1969; Schilling, 2006). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continuous source of agronomic and economic traits for cultivated sunflower (Seiler and Rieseberg, 1997; Seiler and Marek, 2011; Kane et al., 2013; Seiler and Jan, 2014). In a survey of the use of wild relatives in crop improvement over a 20 year period, among 13 crops of international importance, sunflower ranked fifth with seven traits incorporated (Hajjar and Hodgkin, 2007).

Helianthus anomalus (sand sunflower) is a rare endemic species adapted to sand dune and swale habitats in Utah and northern Arizona (Heiser, 1958, Heiser et al., 1969, Thompson et al., 1981; Nabhan and Reichhardt, 1983). It is a confirmed homoploid diploid hybrid species based on comparison of isozyme, nuclear ribosomal DNA, and cpDNA with its parental species, *H. annuus* and *H. petiolaris* that occupies an extreme environment relative to its parental species (Rieseberg, 1991; Gross et al., 2004; Ludwig et al., 2004)(Fig. 1a). *Helianthus annuus* is distributed throughout the central and western United States and typically inhabits heavy, clay-based soils. *Helianthus petiolaris*, the smaller of the two parental species, is distributed mainly through the central United States and inhabits sandier soils than *H. annuus*. The two parental species co-occur and often hybridize throughout their range. The species are all annual,

outcrossing, and have a haploid chromosome number of 17 (Heiser, 1947; Heiser et al., 1969; Rogers et al., 1982). *Helianthus anomalus* has been frequently recognized as drought tolerant, with the largest achenes of any wild species and high oil concentration potential (Seiler, 2007), and thus is a candidate for improving cultivated sunflower (Nabhan and Reichhardt, 1983; Seiler and Marek, 2006). It also appears to be more tolerant of nutrient stress than its ancestral parents based on a lower relative growth rate and higher nutrient-use efficiency (Brouillette and Donovan, 2011).

Helianthus deserticola (desert sunflower) is a xerophytic species found in sandy soils on the floor of the Great Basin Desert and distributed in small populations located in western Nevada, west central Utah, and along the border of Utah and Arizona, USA (Heiser et al., 1969) (Fig. 1b). It is also a homoploid diploid annual hybrid between two annual parental diploid species, *H. annuus* and *H. petiolaris* (Rieseberg, 1991, Gross et al., 2004). This species inhabits the desert floor, an extreme environment relative to its parental species (Gross et al., 2004) (Fig. 1a). Based on desert sunflower's occurrence in sand dune desert habitats, it frequently has been recognized as drought tolerant with high oil concentration potential, and thus a candidate for improving cultivated sunflower germplasm (Seiler, 1992; Seiler, 2007). Both species are excellent candidates for diversifying the genetic base of cultivated sunflower by enhancing oil concentration and quality improvement, as well as drought tolerance.

Figure 1a. Evolutionary relationships among annual *Helianthus* species. Homoploid hybrid species *H. anomalus* and *H. deserticola* are indicated with asterisks. Figure is redrawn from Gross et al., 2005, and based on combined nuclear ribosomal and chloroplast DNA data reported by Rieseberg, 1991; and, **1b.** Distribution of *Helianthus anomalus* (sand sunflower) in Utah and Arizona, and *H. deserticola* (desert sunflower) in Nevada, Utah and Arizona in the desert southwest US.



Unfortunately, very few populations of *H. anomalus* and *H. deserticola* have been collected and only a few are available for research purposes from the USDA-Agricultural Research Service, National Plant Germplasm System (NPGS) wild sunflower germplasm collection. Also, it is very difficult to regenerate the limited number of original seed from some of the earlier collected accessions. The objective of the study was to undertake an exploration to the desert southwest USA in Utah and Arizona in June to collect the winter-spring populations instead of the summer-fall populations previously collected in September-October of the two desert species, *H. anomalus* and *H. deserticola*, and preserve them for future generations to combat emerging pests and environmental challenges, helping to maintain sunflower as a viable and competitive global crop. The exploration was supported with funding from the Plant Exchange Office, National Germplasm Resources Laboratory, USDA-ARS, Beltsville, MD.

MATERIALS AND METHODS

The sunflower exploration for *H. anomalus* and *H. deserticola* took place from June 14 to June 22, 2015. Some populations were revisited in late July-early August 2015 to collect additional seed, and two additional populations without mature seed in June were collected. The exploration covered 3700 km in two states, Utah and Arizona. Seed heads were collected from 20 to 250 plants within each population

and bulked into a single sample. Herbarium specimens were deposited in the USDA-ARS wild *Helianthus* herbarium at Fargo, ND. The achene samples were deposited at the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, where they are maintained and distributed.

All populations were collected from the restricted distributional range of the species, Utah and Arizona for *H. anomalus*, and Utah, Arizona and Nevada for *H. deserticola* (Fig. 1b). Prior locations, generalized distribution maps, and herbaria voucher records were used to locate populations in cooperation with local natural resource officials and botanists who provided valuable information about the current year population distributions and status of the two species. Landownership was determined and all necessary permits were obtained for seed collection and inclusion of the seed in the NPGS genebank. Population size (number and extent), habitat, soil type, seed set per head, and the presence of diseases, insects, and other wild sunflower species were recorded for each population.

RESULTS AND DISCUSSION

The exploration was successful in collecting 10 representative populations of *H. anomalus* from its distributional range in Utah and Arizona (Table 1). A single population of *H. anomalus* was located in Arizona, but the plants were just flowering and no seeds were collected. It had been 15 years since this species was last collected for the NPGS (Seiler and Brothers, 2003). Attempts to recollect this endemic species over the last quarter century have met with mixed results. In September of 2000, none of 12 populations collected in the October of 1980 could be relocated in the fragile sandy habitats (Seiler and Brothers, 2003). The species appears to be very sensitive to the prevailing fall-winter and spring-summer moisture conditions. The current exploration during June located numerous populations of sand sunflower probably due to the excessive spring rains in several parts of the species' distributional range.

Figure 2 shows the typical habitat of the only population of *H. anomalus* we located in Arizona. Unfortunately, only a few plants were observed with no mature seeds to collect. Figure 3 shows one of the diverse habitats in Utah where a typical *H. anomalus* plant with multiple branches and heads, light shiny green leaves, and whitish stems grows on top of sandy hummocks with the wind causing the sand to shift and appear as waves in the sand. Figure 4 shows the unique tap root that develops to help plants survive the constant shifting sand on the dunes. Figure 5 shows a unique habitat for *H. anomalus* in a draw on the steep slope of a shifting sand dune. Figure 6 shows dried white plant stalks from the previous season(s) confirming a persistent and thriving population.

The exploration was also successful in collecting five representative populations of *H. deserticola* from its distributional range in Utah and Arizona (Table 1). *Helianthus deserticola* was not collected in Nevada because of restricted access to the areas where the species occurs. In September of 2000, an attempt to recollect several populations previously collected in October of 1980 was not successful in the fragile sandy sagebrush habitat probably due to the extremely dry 2000 growing season, although one new population was discovered (Seiler and Brothers, 2003). As with *H. anomalus*, the species appears to be very sensitive to the prevailing fall-winter and spring-summer moisture conditions. The current exploration during June 2015 located several populations of desert sunflower mainly due to the excessive spring rains in several parts of the species' distributional range.

Table 1. *Helianthus anomalus* and *H. deserticola* identification number, elevation, location, habitat, and population size collected in June 2015.

Identification Number	Elevation (m)	Location	Habitat	Population Size
ANO-2810	1310	**Utah;** San Juan Co., SE of Cal Black Memorial Airport	Shifting sand dunes, roadside	200
ANO-2811	1450	**Utah;** San Juan Co., Nokai Dome Rd, SE of Halls Crossing	Shifting sand dunes, roadside	750
ANO-2813	1147	**Utah;** Garfield Co., Notum-Bullfrog Rd	Shifting sand dunes, roadside	1,000
ANO-2815	1769	**Utah;** Kane Co., Hole-in-the-Rock Rd, Grand Staircase Escalante National Monument	Shifting sand dunes, roadside	200
ANO-2817	1394	**Utah;** Garfield Co., unnamed draw into North Wash, west side of Hwy 95	Shifting sand dunes, steep slope	250
ANO-2818	1425	**Utah;** Wayne Co., Near Hanksville	Shifting sand dunes, roadside	200
ANO-2819	1661	**Utah;** Wayne Co., Lower San Rafael Rd	Shifting sand dunes	100
ANO-2820	1565	**Utah;** Emery Co., Hans Flat Rd	Shifting sand dunes	1,000
ANO-2821	1231	**Utah;** Grand Co, White Wash Dunes	Shifting sand dunes	1,000
ANO-2822	1532	**Utah;** Emery Co, west side of Hwy 24	Shifting sand dunes	1,000s
DES-2802	1214	**Utah;** Kane Co., Beside High Desert Lodge, Big Water	Sandy desert shrub pasture	750
DES-2803	1290	**Utah;** Kane Co., End of Church Wells Rd, Grand Staircase Escalante Natl. Monument	Sand dunes, near pasture	500
DES-2805	1261	**Utah;** Kane Co., Jacobs Tanks Rd, west of Grand Staircase Escalante National Monument visitor center	Sandy swale	500
DES-2806	1294	**Arizona;** Coconino Co., Vermilion Cliffs Natl. Monument; Ferry Swale Wash	Undulating swale wash, sandy soil	1,000
DES-2807	1295	**Arizona;** Coconino Co., Southeast of Page	Sandy roadside ditch	1,000

Figure 7 shows the typical habitat of a population of *H. deserticola* located in southern Utah. While this species shares some of the habitat types of *H. anomalus*, the main difference is that it is found on the floor of the Great Basin desert in sandy soils interspersed mainly with sagebrush and other desert shrubs. Figure 8 shows one of the diverse habitats where *H. deserticola* grows in sandy soils and hummock type of topography near desert shrubs. Figure 9 shows typical plants with multiple branches and heads, dull green leaves and darker greenish-red pubescent lower stems. Figure 10 shows the unique habitat in an undulating swale wash in sandy soil among the desert shrubs. Figure 11 shows a unique habitat of *H. deserticola* scattered in a sandy pocket on an undulating swale wash underlain by shale rock.

Figure 2. Gerald Seiler standing next to the largest *Helianthus anomalus* plant in a very small population found near Dennehosto, AZ in a shifting sand dune. Only found a few plants were found with no mature seed to collect.

Figure 3. *Helianthus anomalus* (ANO-2810) on hummock sand dunes in San Juan County, UT, SE of Cal Black Memorial Airport. Notice the wave pattern in the sand from the wind shifting the sand in the dunes. Typical plants with multiple branches, light shiny green leaves, and whitish stems.

Figure 4. Population ANO-2813 in Garfield County, UT along Notum-Bullfrog Rd in shifting sand dunes near roadside. Notice the distorted exposed roots that developed to anchor the plant in the actively shifting sand dunes.

Figure 5. Population ANO-2817 in Garfield County, UT, North Wash, west side of Hwy 95, with sunflowers growing in a draw of a steep slope of a shifting sand dune.

Figure 6. Laura Marek collecting seed in population ANO-2813 in Garfield County, UT. Along Notum-Bullfrog Rd, in shifting sand dunes. Note the dead white plant stalks from previous season(s).

Figure 7. *Helianthus deserticola* (DES 2802) in Kane Co., UT near Big Water UT in a typical desert shrub habitat interspersed among the shrubs in open sandy areas.

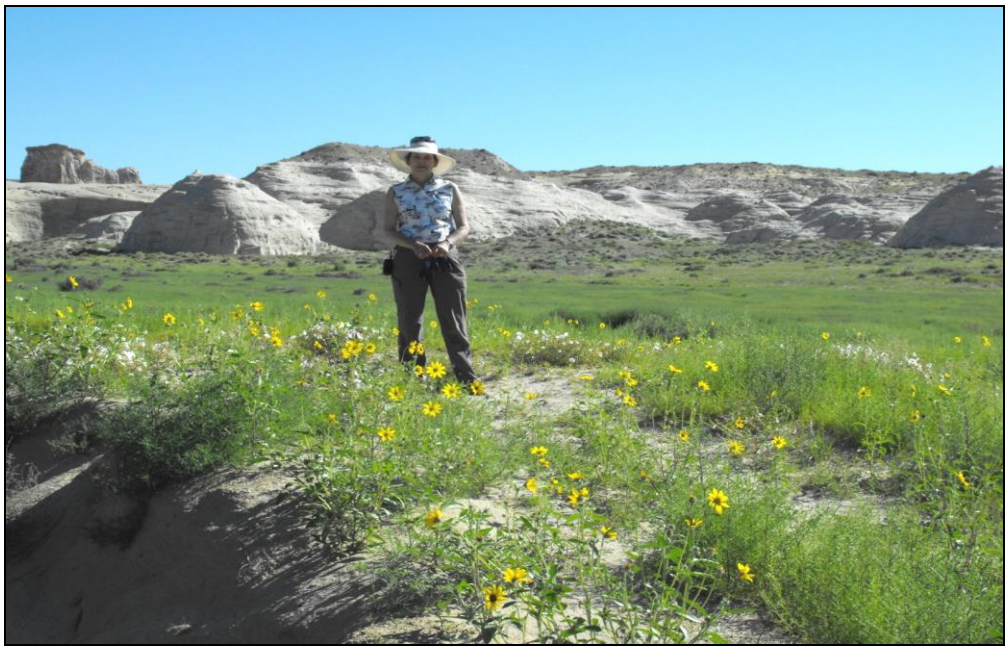


Figure 8. Laura Marek collecting seed of population DES 2803 in Kane Co., at the end of Church Wells road, west of Big Water, UT. Note the different habitat with more grayish sandy soils and hummock type topography in the background near desert shrubs.

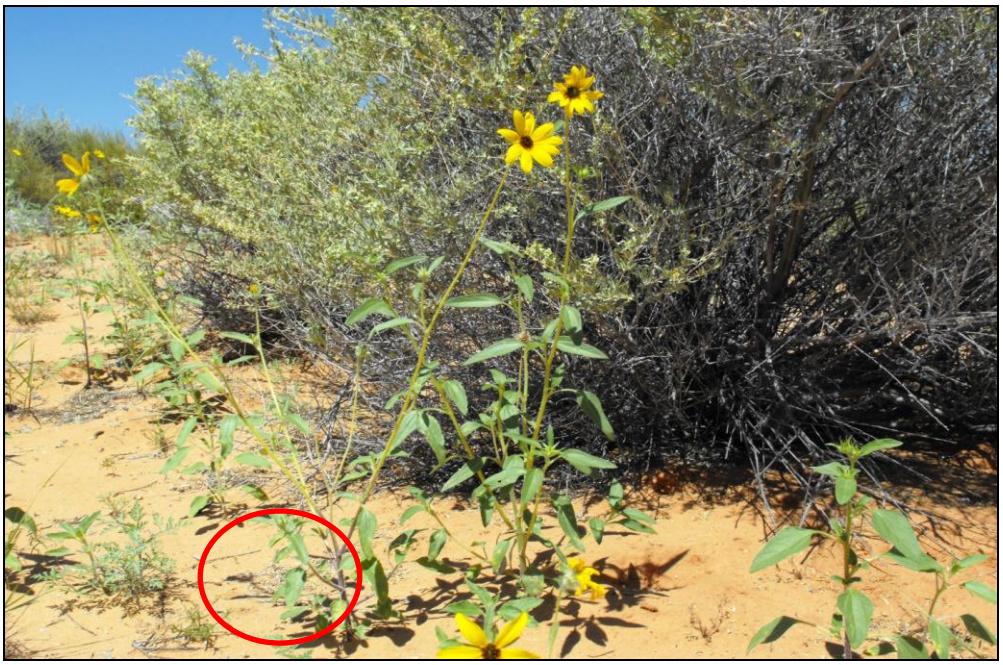


Figure 9. Population DES-2805 in Kane Co., UT, along Jacobs Tank Rd, west of the Grand Staircase Escalante National Monument (BLM), sandy pocket among the desert shrubs. Typical plant with multiple branches, dull green leaves, and darker greenish-red pubescent lower stems.

Figure 10. Population DES-2806 in Coconino Co., AZ, Ferry Swale Canyon, Vermillion Cliffs National Monument (BLM) in an undulating swale wash in sandy soil among the desert shrubs.

Figure 11. Gerald Seiler collecting seed of DES-2806 in Coconino Co., AZ, Vermillion Cliffs National Monument (BLM) in sandy soil in the undulating swale wash among the desert shrubs. Note the white shale outcropping in the background that underlies this area.

CONCLUSION

The addition of 10 *Helianthus anomalus* and five *H. deserticola* populations to the NPGS wild sunflower germplasm collection represents the first germplasm of these species collected Utah and Arizona in almost 15 years. The added populations are important as a genetic resource to combat emerging pests and environmental challenges, helping to maintain sunflower as a viable and competitive global crop and to preserve it for the future generations.

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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF 400 NEW SUNFLOWER PRE-BRED LINES

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ABSTRACT

Crop wild relatives are key genetic resource for the continued improvement and diversification of sunflower. Here we describe the development of new pre-bred lines for sunflower, as well as the phenotypic and genotypic characterization of these lines. We created circa 400 pre-bred lines, each of which contain introgressions from a wild *Helianthus* genotype. The wild samples were selected to encompass as much genetic diversity as possible, and the rounds of backcrossing and selfing did not include any intentional selection. This approach was taken to maximize the wild diversity introduced into the crop gene pool for evaluation. All lines are freely available to the sunflower community under the standard material transfer agreement. Collaborators at the Uganda National Agriculture Research Organization (NARO) and SOLTIS (France) evaluated these lines, and analyses show that they contain a great deal of promising variation for valuable traits such as drought tolerance, disease resistance and flowering time. Additionally, 55 previously developed pre-bred lines were evaluated at UBC. The multi-locus genotypes of each line were assessed with genotyping by sequencing, and the number, size and locations of the wild introgressions were identified. Patterns of introgression across the genome are discussed.

Key Words : wild sunflowers, introgression, germplasm, genotyping by sequencing, phenotypic characterization

THE EVALUATION OF ANNUAL WILD *HELIANTHUS* SPECIES FOR THEIR MORPHOLOGICAL, PHENOLOGICAL AND SEED CHEMICAL CHARACTERISTICS IN FIELD CONDITIONS

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ABSTRACT

Twenty-seven wild annual *Helianthus* species and subspecies, from the USDA-ARS North Central Plant Introduction Station sunflower collection were evaluated in field trials for several morphological and phenological characteristics, concentrations of seed protein and oil, and fatty acid profile during 2012 and 2013. These were *H. agrestis*, *H. annuus* Ames 4114, *H. annuus* Ames 7111, *H. annuus* Ames 29273, *H. annuus* Ames 29348, *H. anomalus*, *H. argophyllus*, *H. bolanderi*, *H. debilis* ssp. *cucumerifolius*, *H. debilis* ssp. *debilis*, *H. debilis* ssp. *silvestris*, *H. debilis* ssp. *tardiflorus*, *H. debilis* ssp. *vestitus*, *H. deserticola*, *H. exilis*, *H. neglectus*, *H. niveus*, *H. niveus* ssp. *canescens*, *H. niveus* ssp. *tephrodes*, *H. petiolaris*, *H. petiolaris* ssp. *fallax*, *H. petiolaris* ssp. *petiolaris*, *H. porteri*, *H. praecox*, *H. praecox* ssp. *hirtus*, *H. praecox* ssp. *praecox*, *H. praecox* ssp. *runyanii*. The flowering dates of *H. argophyllus* in 2012 and 2013 were the latest with 202.00 and 202.33 days, respectively. The earliest flowering dates were observed in *H. debilis ssp. cucumerifolius* (91 days), *H. praecox* (91.00 days) and *H. praecox* ssp. *hirtus* (91.67 days) in 2012, and *H. annuus* Ames 4114 (105.33 days) in 2013. *H. argophyllus* had also the highest plant heights in both years with 325.67 and 303.0 cm. Oil concentrations of annual wild sunflower species changed between 8.02 and 31.16%. The highest linoleic acids were observed in *H. debilis* ssp. *tardiflorus* (66.77%) in 2012 and *H. praecox* ssp. *praecox* (69.13 %) in 2013. Seed protein contents changed from 6.41 to 37.05 % depend on genotypes and year.

Key words: *Helianthus* ssp., oil fatty acids, seed protein, yield components, flowering date

INTRODUCTION

Sunflower is one of the world's main sources of plant-based oil. It is also remarkably widely grown crop. Sunflowers were grown on 25 million hectares of worldwide in 2013 (FAO, 2014). Its oil ranks among the best quality edible vegetable oils and its essential fatty acids are an important part of the human diet (Skoric et. al. 2008). Common sunflower is also an important crop grown globally for production of cut flowers, fuel, commercial fiber, and seeds for snacks and bird food. Originally it was domesticated from the self incompatible common sunflower approximately 4,000 years ago. Sunflowers have historically been used by humans as a nutritious food source, a medicinal treatment for many ailments, and as a dye for body paint and coloring basketry. (Mandel et al., 2011; Anonymous, 2014).

Major goals in *sunflower breeding* remain high seed and oil yield, improved oil quality, as well as resistance to different stresses (Kaya et al., 2012). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continued source of agronomic traits for crop improvement. The main reason for establishing a collection of wild sunflower species is the reduced genetic variability of the domesticated sunflower for a number of agronomic characteristics. The transfer of useful characters from *Helianthus* species to the cultivated sunflower started at the beginning of the 20th century and continues nowadays purposefully (Christov, 2008). In recent years, interspecific hybridization has been extensively applied in sunflower breeding. Wild sunflower species have been used as sources of desirable genes for a number of characteristics. Many

traits dealing with morphology, architecture, and disease resistances have been transferred from *Helianthus* species to sunflower. The genetic researches on the developing of new CMS - restorers of fertility have contribute to enrich diversity and to increase heterosis in sunflower (Atlagic et al., 2006; Seiler, 2007; Nooryazdan et al., 2010; Whitney, et al., 2010). Wild species are a potentially important source of abiotic tolerance; therefore, it may be desirable to introgress drought and salinity tolerant genes from wild relatives. They also contains considerable variability for biotic stress such as disease, insect pest resistance. Similarly, fatty acid composition and protein quality can also be modified by introgression from wild species. The increase in sunflower production and seed quality has been largely connected to the inclusion of wild *Helianthus* species into the improvement work on sunflower (Fernandez-Martinez, 1991; Perez et al., 2007; Christov, 2008; Nooryazdan et al., 2010)

Although, interest in using wild species in breeding programs has increased, the limited genetic variability in cultivated sunflower has slowed the future improvement of the crop, and has placed the crop in a vulnerable position should any major shifts of disease races or pests occur. In order to be competitive with other premium oils in the market, the reduction of saturated fatty acids in sunflower oil will play a key role in maintaining our continuing success. Evaluations of wild species have provided information about useful genes for future sunflower improvement. However, there are still numerous genes in wild sunflower species yet to be identified and introgressed into cultivated sunflower. The understanding of wild *Helianthus* species will increase the number of useful genes available from wild *Helianthus* species, making it possible to transfer cultivated sunflower (Jan et al., 2008, Baute et al., 2015).

In the present study, we focus on the evaluation of annual wild *Helianthus* species for their morphological, phenological and seed chemical characteristics in field conditions to provide useful features for future sunflower improvement.

MATERIALS AND METHODS

In this research, twenty-seven wild annual *Helianthus* species and subspecies getting from the USDA-ARS North Central Regional Plant Introduction Station-Iowa State University were used as materials. Their names and origins are given in Table 1. In this research, annual wild *Helianthus* species for were evaluated their morphological, phenological and seed chemical characteristics in field conditions of 2012 and 2013.

In the first year, seeds of wild sunflower were sown into multiple pots in the glasshouse on March 13, 2012, and their seedling were planted into fields on April 25, 2012. The second year, sowing time of seeds into multiple pots and planting time of seedlings on field were March 12, 2013 and May 17, 2013, respectively. The distance of plants on the rows and between rows were one meter. Irrigation was not applied into fields in both years except planting time of seedlings, and weeds were cleaned by hoeing.

The experiments were carried out on the field of the Faculty of Agriculture at Namık Kemal University in Tekirdağ, Turkey (40º59´N, 27º33´E, elevation 3 m), on soil with clay loam and low organic matter content in 2012 and 2013.

Table 1. *Helianthus* species and subspecies in this research, and their origin's

	Helianthus species and subspecies	Origin's
1	*H. agrestis*	Florida, USA
2	*H. annuus* Ames 4114	North Dakota, USA
3	*H. annuus* Ames 7111	California, USA
4	*H. annuus* Ames 29273	Texas, USA
5	*H. annuus* Ames 29348	South Australia, Australia
6	*H. anomalus*	Utah, USA
7	*H. argophyllus*	Texas, USA
8	*H. bolanderi*	California, USA
9	*H. debilis* ssp. *cucumerifolius*	Texas, USA

10	*H. debilis* ssp. *debilis*	Florida, USA
11	*H. debilis* ssp. *silvestris*	Texas, USA
12	*H. debilis* ssp. *tardiflorus*	Florida, USA
13	*H. debilis* ssp. *vestitus*	Florida, USA
14	*H. deserticola*	Nevada, USA
15	*H. exilis*	California, USA
16	*H. neglectus*	New Mexico, USA
17	*H. niveus*	Arizona, USA
18	*H. niveus* ssp. *canescens*	Utah, USA
19	*H. niveus* ssp. *tephrodes*	Mexico
20	*H. petiolaris*	South Dakota, USA
21	*H. petiolaris* ssp. *fallax*	New Mexico, USA
22	*H. petiolaris* ssp. *petiolaris*	Oklohama, USA
23	*H. porteri*	Georgia, USA
24	*H. praecox*	Texas, USA
25	*H. praecox* ssp. *hirtus*	Texas, USA
26	*H. praecox* ssp. *praecox*	Texas, USA
27	*H. praecox* ssp. *runyanii*.	Texas, USA

The main chemical characteristics of soils are shown in Table 2. Organic matter in the research area was very low, and changed between 0.92 and 1.37 % according to different depths. Phosphorus (P), potassium (K) and pH of soils ranged from 55.9 to 108.3 kg/ha, 124.5 to 209.6 ppm and 7.78 to 7.85, respectively.

Table 2. Some chemical properties of the experimental field soil

Depth (cm)	SOM (%)	WS (%)	EC µS cm^{-1}	PH	Lime (%)	P_2O_5 kg/ha	K ppm	Ca ppm	Cu ppm	Fe ppm	Mn ppm	Mg ppm	Zn ppm
0-30	1.37	42	866	7.78	1.82	108.3	209.6	6076	0.75	3.81	8.83	240.6	0.15
30-60	1.18	43	720	7.82	3.71	72.6	151.3	6055	0.67	3.62	6.60	246.9	0.10
60-90	0.92	43	631	7.85	8.06	55.9	124.5	5911	0.62	3.62	7.08	263.2	0.09

SOM = soil organic matter, WS = soil water saturation

Climatic data during growing periods of *Helianthus* ssp. in 2012 and 2013 are given in Table 3. Generally, the values of rainfall, relative humidity and temperature in the vegetative growth period and flowering duration of wild sunflower genotypes in the first year field conditions were higher than in 2013 except June rainfall and May temperature.

Table 3. Climatic data during growing periods of *Helianthus* ssp. in 2012 and 2013

Month	Rainfall (mm)		Relative humidity (%)		Temperature (°C)		The highest temperature (°C)		The lowest temperature (°C)		Sunshine duration (hour/day)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
March	18.0	52.8	81.8	98.5	7.9	9.6	12.3	13.5	3.6	5.9	6.3	4.5
April	61.4	16.0	82.4	84.8	14.1	13.5	19.3	17.7	9.6	9.4	7.4	6.7
May	62.4	8.0	91.2	69.7	18.1	19.5	22.5	23.8	14.2	15.1	7.1	9.4
June	0.2	35.0	78.2	68.7	24.1	22.4	28.4	26.7	18.9	18.1	10.9	8.4
July	6.0	0	68.7	61.4	27.0	24.7	31.5	28.8	22.1	20.0	10.6	10.5
August	7.8	0.2	62.7	62.3	26.0	25.9	31.1	30.1	20.9	21.7	10.3	9.6
September	8.4	10.2	73.6	61.4	22.2	21.6	26.6	25.6	18.1	16.9	8.1	8.4
October	54.0	96.4	87.3	76.2	19.2	14.3	23.5	17.9	15.1	10.4	6.5	6.5
November	24.8	36.6	97.0	79.0	13.7	12.9	16.9	15.9	10.7	9.6	3.4	3.6
December	184.6	2.4	97.3	74.1	6.4	6.2	9.7	9.7	3.1	3.0	2.6	2.7

Statistical analysis was conducted according to Standard procedures for a randomized complete block design with three replications. The SAS System was used to generate the analysis of variance (ANOVA) for determining treatment effects on the dependent variables (SAS Institute, 1997). Treatment mean comparisons were based on F-Protected Least Significance Differences (LSD) comparisons at $P \leq 0.05$.

RESULTS AND DISCUSSION

In this research, twenty-seven wild annual *Helianthus* species and subspecies were grown under field conditions in 2012 and 2013. But some of them such as *H. deserticola* dried during vegetative growth period under field conditions depend on ecological conditions of 2012 or 2013. Table 4 shows the results of variance analyses on fifteen morphological, physiological, yield and quality components of wild sunflower species.

Analysis of data identified that genotype and genotype x year interactions were highly significant ($p < 0.01$) for all characters. Year effects on the characters except plant height. were also significant. Therefore, data of all character were analyzed separately for each year in the study.

Statistical significant groups by the LSD test at $P < 0.05$ for plant height, canopy cover diameter of single plant and head number per plant are given in Table 5. Although genotypes displayed variable plant height between 2012 and 2013, *H. argophyllus* had the highest plant height with 325,7 cm in 2012 and 303 cm in 2013. The first year; *H. debilis* ssp. *vestitus*, *H. debilis* ssp. debilis, H. *praecox* ssp. *praecox* and *H. debilis* ssp. *tardiflorus* were the same latest group for plant height. For canopy cover diameter of single plant, the highest value was measured in *H. annuus Ames* 29273 although *H. debilis* ssp. *cucumerifolius*, *H.* neglectus, H. *bolanderi*, *H. annuus* Ames 29273, *H. praecox* ssp. *runyanii*, *H. debilis*

ssp. *tardiflorus* and *H. debilis* ssp. *vestitus* had the highest values in 2013. Head number per plant in 2013 was changed from 5 to 800. The highest head number per plant was counted on *H. debilis* ssp. *cucumerifolius. H. neglectus*, *H. praecox* ssp. *praecox*, *H. praecox, H. praecox* ssp. *runyanii* and *H. debilis* ssp. *tardiflorus* were also the same LSD groups.

Table 4. Analyses of variance of some morphological, physiological, yield and quality components of wild sunflower species under the field conditions in 2012 and 2013

	Genotype	Year	GenotypexYear	CV
Plant height (cm)	26543.64**	978.98ns	1920.42**	16.53
Canopy cover diameter of single plant (cm)	5910.17**	17368.25**	3166.62**	17.81
Head number per plant+	127995.46**			
Head diameter (cm)	45.43**	33.67**	13.57**	37.13
1000 seed weight (g.)	2409.99**	116.48**	92.71**	36.52
The first flowering day number	3275.72**	10360.63**	125.56**	4.77
50 % flowering day number	4879.93**	40480.63**	645.04**	4.71
Flowering period day	5612.44**	1400.82**	1060.68**	9.22
Seed yield per plant (g.) +	9305.93**			
Seed protein content (%)+	40.04**			10.25
Seed oil content (%)	96.16**	446.57**	70.72**	0.94
Oleic acid content of seed oil (%)	350.66**	481.78**	57.90**	0.87
Linoleic acid content of seed oil (%)	235.73**	446.55**	44.58**	0.54
Palmitic acid content of seed oil (%)	6.45**	0.07**	1.07**	0.71
Stearic acid content of seed oil (%)	2.87**	0.12**	0.54**	0.78

**: Significant differences are shown at $P < 0.01$ based on ANOVA. Statistical analysis was made only in 2013

Head diameter of wild sunflower species was changed from 1.1 to 18.3 cm in 2012 and 1.1 to 6.4 cm in 2013 (Table 6). *H. annuus* Ames 4114 had the biggest heads in both years. Table 6 also shows 1000 seed weights of genotypes. 1000 seed weight had the highest variable among wild annual sunflower species changing from 1.10 to 101.20 g. Generally *H. debilis* subspecies gave the lowest seed weights while *H. annuus* genotypes had the highest weights. Previous researchers for morphological characters, in annual wild sunflower species had similar results in this research (Seiler, 1997; Seiler and Rieseberg, 1997; Skoric, 2009, Onemli and Gucer, 2010).

Table 5. Plant height, canopy cover diameter of single plant and head number per plant of wild sunflower species

Helianthus species and subspecies	Plant height (cm)		Canopy cover diameter of single plant (cm)		Head number/plant
	2012	2013	2012	2013	2013
H. annuus Ames 4114	138.3ef	79.3j	100.7gh	37.7g	5.0e
H. annuus Ames 7111	187.7cd	165.3efg	210.0bc	143.7bcde	117.0cde
H. annuus Ames 29273	251.0b	234.7b	274.0a	157.3abc	203.7bcde
H. annuus Ames 29348	166.3cde	228.0bc	123.3efgh	129.0cdef	223.70bcde
H. argophyllus	325.7a	303.0a	156.0de	125.7cdef	228.0bcde
H. bolanderi	194.3c	198.7cd	175.0cd	159.7abc	479.0abcd
H. debilis ssp. *cucumerifolius*	118.7fgh	192.7de	152.7def	192.3a	800.7a
H. debilis ssp. *debilis*	80.3hi	61.3j	147.0def	95.7f	83.7de
H. debilis ssp. *silvestris*	145.0def	89.7ij	155.0de	112.3def	221.3bcde
H. debilis ssp. *tardiflorus*	108.0fghi	136.7gh	150.0def	156.0abcd	417.0abcde
H. debilis ssp. *vestitus*	71.0i	78.3j	143.3defg	151.7abcde	217.70bcde
H. exilis	105.7fghi	[redacted]	93.7h	[redacted]	
H. neglectus	188.0cd	171.7def	150.0def	183.0ab	593.3ab
H. petiolaris	197.7c	155.7fg	220.0b	112.0ef	245.0bcde
H. praecox	102.3fghi	91.0ij	176.0bcd	126.3cdef	501.7abc
H. praecox ssp. *hirtus*	84.0ghi	89.0ij	110.0fgh	135.3cdef	297.7bcde
H. praecox ssp. *praecox*	81.7hi	62.7j	139.0defgh	133.3cdef	536.7ab
H. praecox ssp. *runyanii*	130.7efg	121.0hi	170.0cd	157.3abc	418.3abcde
LSD	46.88	32.18	44.54	43.69	413.35

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$

Table 6. Head diameter and seed weight of wild sunflower species

Helianthus species and subspecies	Head diameter (cm)		1000 seed weight (g.)	
	2012	2013	2012	2013
H. annuus Ames 4114	18.3a	6.4a	101.20a	69.20a
H. annuus Ames 7111	6.5b	3.2c	12.50b	10.13b
H. annuus Ames 29273	5.8b	4.1b	4.40d	6.50bcd
H. annuus Ames 29348	7.6b	4.4b	12.80b	9.20bc
H. argophyllus	2.3c	3.2b	9.00c	6.53bcd
H. bolanderi	2.2c	2.3d	3.20e	5.60bcd
H. debilis ssp. *cucumerifolius*	1.9c	2.2e	1.30ij	2.86bcd
H. debilis ssp. *debilis*	1.1c	1.1h	1.40hij	0.73d
H. debilis ssp. *silvestris*	1.7c	1.8efg	1.60ghi	1.03d
H. debilis ssp. *tardiflorus*	1.4c	1.6fgh	1.10j	1.33d
H. debilis ssp. *vestitus*	1.4c	1.3gh	1.20ij	0.90d
H. exilis	1.8c	[redacted]	1.90fg	[redacted]
H. neglectus	2.2c	2.1def	1.40hij	2.93bcd
H. petiolaris	2.5c	2.3de	4.60d	3.77bcd
H. praecox	2.3c	1.8defg	1.80gh	1.90cd
H. praecox ssp. *hirtus*	1.6c	1.9def	1.40hij	1.37d
H. praecox ssp. *praecox*	1.9c	1.6fgh	1.80gh	1.43d
H. praecox ssp. *runyanii*	2.2c	1.9def	2.30f	1.23d
LSD	2.56	0.54	0.47	7.32

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$

H. argophyllus showed the latest flowering in both years (Table 7). This genotype flowered 202.0 and 202.3 day later from emerging in 2012 and 2013, respectively. Generally *H. praecox* subspecies had earlier flowering than the others. The longest flowering period was determined on *H. debilis* ssp. *vestitus* in 2012 while all *H. praecox* subspecies and *H. debilis* ssp. *vestitus* had the longest flowering in 2013. There were a few study on flowering with annual species. Generally they were on *H. annuus* and not similar detailed.

Seed yield per plant, seed protein content and seed oil content of wild sunflower species are given in Table 8. Generally *H. annuus* genotypes had higher seed yields per plant in 2013. Seed protein content of wild annual sunflower species was changed from 6.41 to 22.09 % in 2012, and from 14.01 to 44.13%

in 2013. In the first year, *H. praecox* ssp. *hirtus* gave the highest seed protein content while *H. exilis, H. petiolaris* ssp. *fallax, H. neglectus, H. debilis* ssp. *debilis and H. argophyllus* had the highest protein content in 2013.

Table 7. The first flowering day number, 50 % flowering day number and flowering period day of wild sunflower species

Helianthus species and subspecies	The first flowering day number		50 % flowering day number		Flowering period day	
	2012	2013	2012	2013	2012	2013
H. annuus Ames 4114	95.0h	105.3g	107.0j	122.0f	78.3m	40.7g
H. annuus Ames 7111	109.3cd	129.7cd	113.3gh	160.7cde	121.7i	120.3ef
H. annuus Ames 29273	108.0d	138.0bc	112.3h	161.7cde	128.3gh	126.0ef
H. annuus Ames 29348	103.0e	126.0cd	114.0g	164.7cd	127.7h	111.7f
H. argophyllus	202.0a	202.3a	221.0a	223.0a	50.0o	51.7g
H. bolanderi	102.0ef	122.7de	107.0j	162.7cde	130.3g	133.3def
H. debilis ssp. *cucumerifolius*	91.0i	125.7cd	97.0l	158.0cde	150.7c	132.7def
H. debilis ssp. *debilis*	108.3d	144.0b	158.0c	216.3a	156.7b	134.3def
H. debilis ssp. *silvestris*	111.0c	128.0cd	151.0d	169.7bc	91.0l	137.7cdef
H. debilis ssp. *tardiflorus*	101.7ef	130.0cd	141.7f	150.3de	101.3k	130.7def
H. debilis ssp. *vestitus*	103.0e	121.0def	144.0e	216.3a	162.7a	157.7abcd
H. exilis	129.0b	[redacted]	159.3c	[redacted]	73.0n	[redacted]
H. neglectus	98.0g	122.7de	143.3e	165.0cd	104.0j	142.7bcde
H. petiolaris	108.0d	126.3cd	164.7b	185.7b	134.7f	113.0f
H. praecox	91.0i	112.0efg	97.0l	149.3de	142.7d	164.3abc
H. praecox ssp. *hirtus*	91.7i	110.0efg	97.3l	146.7e	138.0e	168.3ab
H. praecox ssp. *praecox*	94.0h	108.0fg	101.7k	151.7de	141.3d	171.0a
H. praecox ssp. *runyanii*	101.0f	109.3fg	110.0i	154.0cde	120.0i	169.3ab
LSD	1.93	13.09	1.52	16.06	2.63	27.09

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.

Seed oil content of wild sunflower species in 2012 and 2013 were changed from 16.74 to 27.45 % and 8.02 to 31.16%, respectively. *H. argophyllus* in 2012 and *H. annuus* Ames 4114 in 2013 had the highest seed oil contents.

Table 8. Seed yield per plant, seed protein content and seed oil content of wild sunflower species

Helianthus species and subspecies	Seed yield per plant (g.)		Seed protein content (%)		Seed oil content (%)
	2013	2012	2013	2012	2013
H. agrestis			27.79+		
H. annuus Ames 4114	16.07c	10.69+	18.53def	24.86c	31.16a
H. annuus Ames 7111	51.67bc	19.24	17.34def	24.74c	21.53c
H. annuus Ames 29273	104.84b	13.54	14.01g	16.74h	16.68e
H. annuus Ames 29348	233.20a	14.25	22.33bc	25.76b	23.54b
H. anomalus			23.51		
H. argophyllus	15.50c	17.10	24.70ab	27.45a	8.02k
H. bolanderi	56.99bc	6.41	18.77de	21.37f	16.70e
H. debilis ssp. *cucumerifolius*	102.38b	17.10	17.34def	23.21d	14.52f
H. debilis ssp. *debilis*	1.57c	18.53	26.13a	23.14d	
H. debilis ssp. *silvestris*	15.41c	14.25	17.72def		13.82g
H. debilis ssp. *tardiflorus*	12.85c	7.84	18.53def	20.09g	9.29j
H. debilis ssp. *vestitus*	16.25c	13.57	17.10defg		10.68i
H. exilis		7.84	44.13	20.24g	
H. neglectus	56.30bc	6.41	26.84a		10.44i
H. niveus			19.95		
H. petiolaris	32.84c	14.96	18.29def	22.18e	18.02d
H. petiolaris ssp. *fallax*			37.05		
H. petiolaris ssp. *petiolaris*			17.81		
H. porteri			29.93		
H. praecox	23.75c	17.10	17.34def	22.26e	14.64f
H. praecox ssp. *hirtus*	28.93c	22.09	16.62efg		13.91g
H. praecox ssp. *praecox*	32.00c	17.10	19.95cd		16.92e
H. praecox ssp. *runyanii*	41.18bc	6.41	15.44fg		12.53h
LSD	64.97	3.28	3.29	0.26	0.33

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.. + Values were not analyzed statistically.

Table 9 shows fatty acid compositions of *Helianthus* ssp. Oleic acid content of annual wild sunflower seed oil in 2012 and 2013 varied from 17.35 to 48.69% and 16.89 to 37.02%, respectively. Both of years, *H. annuus* Ames 7111 and *H. annuus* Ames 4114 had the highest oleic acid contents. Generally, oleic acid content of wild annual *H. annuus* species were higher than the other species. For high oleic acid content, this species was followed by *H. bolanderi*, *H. argophyllus* and *H. petiolaris*.

Table 9. Important oil fatty acids of the *Helianthus* ssp

Helianthus species and subspecies	Oleic acid (C18:1) content of seed oil (%)		Linoleic acid (C18:2) content of seed oil (%)		Palmitic acid (C16:0) content of seed oil (%)		Stearic acid (C18:0) content of seed oil (%)	
	2012	2013	2012	2013	2012	2013	2012	2013
H. annuus Ames 4114	42.46b	37.02a	46.38i	53.02l	6.01f	5.57j	3.94e	2.76o
H. annuus Ames 7111	48.69a	37.00a	41.39j	53.27l	5.17i	5.36k	2.67k	2.94n
H. annuus Ames 29273	38.65c	23.95f	51.39h	65.57e	5.11j	5.26l	3.33g	3.63i
H. annuus Ames 29348	36.31f	36.92a	53.99f	54.21k	4.82k	4.87m	2.79i	2.43p
H. argophyllus	37.12e	23.94f	52.92g	61.68i	5.66g	7.22e	2.72j	3.68h
H. bolanderi	38.13d	31.25b	51.38h	57.56j	5.23i	5.58j	3.13h	3.59j
H.debilis ssp. *cucumerifolius*	23.38i	27.10c	63.62c	62.55h	7.03d	5.81i	4.16d	3.94g
H. debilis ssp. *debilis*	17.35k		66.09b		9.88a		5.90a	
H. debilis ssp. *silvestris*		20.92h		66.93d		6.70g		4.33e
H. debilis ssp. *tardiflorus*	18.62j	17.91j	66.77a	68.63b	8.71b	7.67c	3.75f	3.53k
H. debilis ssp. *vestitus*		16.89l		68.12c		8.63a		5.12b
H. exilis	25.92h		59.41d		7.89c		4.92b	
H. neglectus		24.89e		65.07f		6.06h		3.09l
H. petiolaris	33.40g	26.45d	55.66e	63.20g	5.51h	5.58j	2.64k	3.05m
H. praecox	23.50i	22.06g	63.66c	62.05i	6.65e	7.69c	4.74c	5.22a
H. praecox ssp. *hirtus*		20.28i		66.59d		7.39d		4.56c
H. praecox ssp. *praecox*		17.51k		69.13a		7.92b		4.50d
H. praecox ssp. *runyanii*		20.98h		65.74e		7.13f		4.26f
LSD	0.48	0.34	0.44	0.49	0.07	0.07	0.04	0.04

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P< 0.05$.

Linoleic acid changed between 41.39 and 66.77% in 2012. *H. debilis* ssp. *tardiflorus* gave the highest linoleic acid content while *H. annuus* Ames 7111 had the lowest value. In the second year *H. praecox* ssp. *praecox* with 69.13% had the highest linoleic acid content while *H. debilis* ssp. *tardiflorus* was second wild sunflower genotypes. In contrast to oleic acid *H. annuus, H. bolanderi, H. argophyllus* and *H. petiolaris* gave the lower oil linoleic acid contents than the other species..

H. debilis ssp. *debilis* in 2012 and *H. debilis* ssp. *vestitus* in 2013 had the highest palmitic acid contents with 9.88% and 8.63%, respectively. *H. annuus* Ames 29348 in both of years gave the lowest palmitic acid contents.

Stearic acid content changed from 2.64 to 5.90% in 2012 and from 2.43 to 5.22% in 2013. *H. debilis* ssp. *debilis* in 2012 and *H. praecox* in 2013 had the highest stearic acid contents. Wild *H. annuus* genotypes except *H. annuus* Ames 29348 gave lower stearic contents than the other annual wild sunflower species in this research.

Although there were differences between 2012 and 2013 for observed characters in this research, really significant differences were found among annual species. Seiler (1998), Turhan et. al., (2010) and Onemli (2012a and 2012b) found effects of climate change on oil content and especially fatty acid composition of sunflower. Seiler (1998) also determined the lowest palmitic acid contents in *H. annuus* as in this study. In order to be competitive with other premium oils in the market, the reduction of saturated fatty acids in sunflower oil will play a key role in maintaining success

In previous studies, *Helianthus argophyllus* for drought tolerance breeding has been extensively used by the sunflower breeders (Rauf, 2008). Perez et al.(2007), studied to characterize and examine the use of wild sunflower species (*Helianthus petiolaris* Nutt), some physical properties and morphological characteristics of seeds from different locations in Argentina were determined. A collection of 168 accessions belonging to 62 species and subspecies was evaluated for fatty acid composition of the seed oil by De Haro and Fernandez-Martinez (1991) in Spain. Seventy-seven wild sunflower accessions in France for 13 quantitative characters were evaluated to assess the patterns of morphological and climatic variation (Nooryazdan et al., 2010). The wild *Helianthus* species were determined rich sources for genes determining resistance to different diseases, parasites, pests, drought and other important traits. (Christov, 2008)

In spite of these past successes, there are still numerous genes in wild sunflower species yet to be identified for cultivated sunflower breeding The understanding of wild *Helianthus* species will increase the number of useful genes available from wild *Helianthus* species, making it possible to transfer cultivated sunflower (Jan et al., 2008, Baute et al., 2015).

Generally previous studies in wild sunflower included limited number species with limited characters. In this study, almost all annual wild sunflower species were determined for their so many morphological, physiological, yield and quality components. Although the results were similar previously studies, there were a lot of new findings with especially flowering and seed quality characters in this research on a large wild annual sunflower species.

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PRINCIPAL COMPONENT ANALYSIS FOR CARBON ISOTOPE DISCRIMINATION-RELATED TRAITS IN RECOMBINANT INBRED LINES OF SUNFLOWER

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ABSTRACT

We used principal component analysis (PCA) as statistical method to analyze and grouping genetic diversity in sunflower. To utilize PCA, carbon isotope discrimination (CID) and its related traits were measured on a population of 148 F8 recombinant inbred lines (RILs) of sunflower. The RILs were treated in water-stressed as a randomized block design with two replicates. The result of Bartlett's sphericity test showed the significant value was less than alpha level. Correlations among CID-related traits were determined. The CID was negatively correlated with water use efficiency (WUE), biomass (BM) and cumulative water transpired (CWT) expressed in the biplot diagram. The first two components showed 69.28% of the cumulative variability. Based on the biplot diagram, three distinct groups could be differentiated including high WUE genotypes, high BM genotypes and high WUE-BM genotypes. The correlation between CID and the related traits and distribution of the RILs groups among the traits allow in understanding the genetic diversity of the RILs which could be used as a basic consideration before applying selection program in plant breeding.

Key Words : PCA, CID, RILs, Sunflower

NEW VIRULENCES OF *OROBANCHE CUMANA* APPEAR IN ROMANIA

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ABSTRACT

Broomrape (*Orobanche cumana*) is one of the must dangerous pathogen of sunflower in Romania. Breeding for resistance has been crucial for protecting the crops against this pathogen. In many countries area with sunflower was so much increaset beeing more and more difficult to respect a well crop technology, that beeing the must important reason which caused appearence of new and more virulent races. Since all sources up to now are attacked, new investigations should be done to discover new genes which can protect sunflower against parasite attack.

Key Words : broomrape, virulence, pathogen

THE CULTIVATED SUNFLOWER PAN GENOME PROVIDES INSIGHTS ON THE WILD SOURCES OF INTROGRESSIONS AND THEIR ROLE IN BREEDING.

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ABSTRACT

Since domestication, cultivated sunflower has accumulated gene introgreessions from its direct ancestor and closely related species. Wild relatives of sunflower are known for their enhanced tolerance for both biotic and abiotic stress and therefore are a promising genetic resource for breeding robust verieties. As the wild source used for crossing in breeding programs varies, different introgressed segments are obtained in different lines. The outcome of these introgressions is variation among lines in the genomic composition and presence/absence of specific genes. Therefore, some genes found in the cultivated gene pool are absent from the sunflower reference genome corresponding to a specific line, HA412. Identifying the overall repertoire of genes across lines, referred as the species pan genome, is of great interest for both evolutionary biologists and breeders. Here we present the cultivated sunflower pan genome based on the public association mapping population, which is comprised of 288 lines that capture most of the diversity in the cultivated gene pool. Sequences from each accession were aligned to the reference genome and badly mapped reads were extracted and used for *de-novo* assembly of sequences not found in the reference genome. Assembled sequences were further annotated to reach a unified non-redundant set of 21,081 sequences corresponding to the dispensable fraction of the cultivated sunflower pan genome. To identify the potential introgressions, low coverage whole genome sequences from 192 accessions of different wild *Helianthus* taxa were aligned to the dispensable portion of the pan genome, and 831 genes of wild parentage were identified. Thus, wild introgressions have contributed both new alleles and new genes to the cultivated sunflower gene pool. We further explore the wild origin of each introgressed gene and identify their underlying contribution to phenotypic variation in the cultivated sunflower genepool.

Key Words : Pan genome, Introgressions

STABILITY PERFORMANCE OF NEW INTRODUCED SUNFLOWER HYBRIDS FOR SEED YIELD AND ITS COMPONENTS UNDER SUDAN CONDITIONS

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ABSTRACT

Four introduced sunflower hybrids (SY-4200, SY-4045, NK Kondi and Neoma, from Syngenta Seed Company) were testing under Sudan conditions against three checks during the period of 2011-2013. The hybrids were tested in five locations (irrigated and rainfed) for their uniformity, adaptability and yield potential. The hybrids were arranged in a RCBD with three replications overall environments. Results of combined analysis of variance for seed yield showed significant effects of hybrids, environments and hybrids x environments interaction. Mean seed yield ranged from 1646 kg ha^{-1} to 2041 kg ha^{-1}. The hybrid SY-4045 out-yielded Hysun-33 by 19 % and Bohooth-1 by 23 %. While, the hybrid Kondi out-yielded the two checks by 14 % and 17 %, respectively. The results of yield stability showed that, the two hybrids (SY-4045 and Kondi) were leading, according to their means seed yield across environments. The hybrid SY-4045 had a slope of (1.16) and Kondi of (1.09) and considered as more stable hybrids. Mean oil content of Kondi (47 %) was leading; while of SY-4045 (43 %) was similar to Hysun-33 (42 %) and Bohooth-1 (44 %). Mean oil yield of SY-4045 (884 kg ha^{-1}) and Kondi (917 kg ha^{-1}) was higher than that for Hysun-33 (714 kg ha^{-1}) and Bohooth-1 (731 kg ha^{-1}). Also, both hybrids (SY-4045 and Kondi) are a single cross hybrid, in addition to their credits of earliness, medium plant height and high self-fertility for good seedset compared to checks. The introduced hybrids SY-4045 and Kondi were released in May 2015 for commercialization under Sudan conditions.

Keywords: Sunflower, Helianthus annuus, G x E interaction, Stability performance, Seed Yield,

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a member of the family Asteraceae. In Sudan, sunflower recently become an important cash crop, to strengthen the economy and fill the gap in vegetable oil production and provides a high value animal feed. The crop is grown both as a summer and winter crop under irrigated system and as a summer crop under rainfed system. Sunflower as a non-traditional crop provides an excellent alternative to cover large areas in the production of oil crops beside the major oil crops. The sunflower cultivated area in Sudan had shown an increasing trend in the last ten years. This is because sunflower is one of the crops which attracted the interest of both farmers and private companies. In addition to its wide adaptability, suitability to mechanization, low labor needs, short duration, high yield potential and good quality as a major reasons for increasing sunflower areas.

Sunflower producers in the country depended almost exclusively on imported seeds. Therefore, virtually 98% of oilseed sunflower production is with hybrid cultivars, which necessitate the need for the development and release of more hybrids that can meet farmers' standards (Mohamed *et al,* 2014). Also, this situation necessitates considerable research efforts by the Agricultural Research Corporation (ARC) to cope with increasing demand for seeds of high yielding well adapted hybrids. Hence, attempts have been made to improve hybrid seed supply through testing and releasing more sunflower hybrids as collaboration between private and public institutes. The collaboration program between ARC with some

international sunflower seed companies (Pannar, Advanta, Syngenta, May, Nuseed …etc) was started during 2006-2007 as main step for improving stability/sustainability of seed supply and probably lowering seed prices through creating free competition among more seed companies and ensures the seed supply at the optimum time. For the last five years, this program resulted in the releasing of new introduced sunflower hybrids such as Pan-7049, Pan-7033 and Aguara-4 (Mohamed *et al*, 2011), Opera and Sirena (Mohamed *et al*, 2012) Nugold Dowana and Nugold Darya (Mohamed *et al*, 2013).

Moreover, in a plant breeding program, potential genotypes are usually evaluated in different environments before selecting desirable ones that show stability across environments. For stabilizing yield it is necessary to identify the stable genotypes suitable for wide range of environments. The most widely used way to biometrically assess stability is the regression method, which is based on regression of the mean value of each genotype on the environmental index. The technique to measure stability was previously proposed by Finlay and Wilkinson (1963) and was later improved by Eberhart and Russell (1966). Therefore, the objectives of the present study were to determine the yield performance and stability of four Syngenta sunflower hybrids a cross five locations; and release of the most promising hybrids adapted to the sunflower growing areas of the Sudan.

MATERIALS AND METHODS

The performance of four Syngenta sunflower hybrids (SY-4200, SY-4045, NK Kondi, and Neoma) along with three checks; Hysun-33, Bohooth-1, and Sirena were evaluated during the period of 2011-2013 at five locations (Table 1). The seven sunflower hybrids were tested over 21 environments (seventeen environments at irrigated sites and four environments at rainfed sites). At each environment, sunflower hybrids were arranged in a randomized complete block design with three replications. The plot size was 4 rows and 8 m long ridges spaced 0.80 m apart. The effective sowing dates were during second week of July for Kharif plantings and second to third week of November for winter plantings. Seeds were sown in hills spaced 0.30 m apart within ridges and thinned to one plant per hill two weeks after planting. Irrigation was at intervals of 12-14 days depending on weather conditions. Plots were kept weed-free through frequent hand weeding. Nitrogen was applied only at irrigated sites at 80 kg urea (46% N) per hectare. All recommended agronomic practices were followed throughout the season. Data were collected from the middle two rows on days to 50% flowering, plant height (cm), number of seeds per head, percentage of empty seeds, 1000-seed weight (g), seed yield (kg ha^{-1}), seed oil content and oil yield (kg ha^{-1}). For easy reference the location/year/season combination was considered as an environment and given a number (Table 1). Analysis of variance was performed on individual trials (each environment) and the F-test was made. Combined analysis was performed separately on irrigated and rainfed environments and pooled over 21 environments, assuming random environment and fixed hybrid (Gomez and Gomez, 1984). Stability performance of each hybrid over twenty one environments was determined following the model of Eberhart and Russell (1966). IRRISTAT statistical analysis package for windows (2006) was used for the data analysis.

RESULTS AND DISCUSSION

The pooled analysis of variance showed highly significant differences ($p>0.01$) among the hybrids (G) and environments (E) for seed yield (Table 2), indicating the presence of variability among the hybrids (G) as well as environments under study. The hybrid x environment (G x E) interaction was also highly significant for seed yield, this shows that hybrids react differently at different environments for seed yield. The genotype x environment (G x E) interaction was further partitioned into linear and non-linear components (Table 2). Both hybrid-environment (linear) and pooled deviation (non-linear) were highly significant, indicating involvement of linear as well as non-linear components of variation shared G x E interaction. The significant hybrid x environment (linear) indicated that linear response of genotypic stability to change in environment was not the same for all hybrids evaluated. However the significant deviation from regression revealed the importance of linear regression component in

determining the interaction between hybrids with environments. Different models have been proposed to evaluate the yield stability of the genotypes. Finlay and Wilkinson (1963) proposed linearity of regression as a measure of stability, however, Eberhart and Russell (1966) emphasized that both linear (b_i) and non-linear components of G x E interaction should be considered in judging the phenotypic stability of a particular genotype. Further, Samuel *et al*. (1970) suggested that the linear regression could simply be regarded as a measure of response of a particular genotype that depend largely upon a number of environments whereas the deviation from regression line was considered as measure of stability, genotypes with the lowest or non-significant standard deviation being the most stable and vice versa.

Therefore, a hybrid must not only yield well in the area of its initial development or evaluation, but preferably maintains a high yielding and quality capacities in a wide range of environments intended for commercial production. Stability parameters were calculated according to procedure described by Eberhart and Russell (1966). The stability analysis results are presented in Table 3. The results showed clear differences in values of regression coefficients (b_i) greater than or around unity and relative minimal deviation from regression. This means these introduced sunflower hybrids are more responsive to environmental changes, which give the breeder an advantage to select hybrids for both adverse and favorable environments. Therefore, the resultant regression coefficient (b_i) and deviation from regression (S^2_{di}) and mean yield for each hybrid are parameters for estimating the stability of yield over the environments. The two Syngenta hybrids SY-4045 (2041 kg ha^{-1}) and Kondi (1947 kg ha^{-1}) gave the highest seed yield over the grand mean with the regression coefficients of 1.16 and 1.09, respectively; these results were very close to unity indicating their adaptability to a range of environments. They also had lower deviation from regression (S^2_{di}), indicating that these hybrids had stable seed yield over a wide range of environments.

Hybrid vigor has been the main driving force for acceptance of this oilseed crop in both the world and Sudan. The overall means seed yield for hybrids across the 21 environments ranged between 1646 kg ha^{-1} (Sirena) to 2041 kg ha^{-1} (SY-4045) with grand mean of 1772 kg ha^{-1}(Table 4). The highest means seed yield were recorded by the Syngenta hybrid SY-4045 followed by Kondi. Under irrigated sites (environments) seed yield ranged between 1183 kg ha^{-1} (E9) to 2951 kg ha^{-1} (E7) and for rainfed sites varied from 361 kg ha^{-1} (E18) to 2272 kg ha^{-1} (E20). The maximum seed yield of 2951 kg ha^{-1} was recorded by the hybrid Kondi followed by Sirena (2769 kg ha^{-1}) and SY-4045 (2749 kg ha^{-1}) at irrigated environment E7. The minimum means of seed yield were recorded by all hybrids under rainfed environments (E18 and E21). These results indicated wide variability for seed yield among Syngenta sunflower hybrids over different environments (i.e., irrigated and rainfed environments). The hybrid SY-4045 out-yielded the three checks (Hysun-33, Bohooth-1 and Sirena) across the twenty one environments. Also, Kondi (across the 21 environments) out-yielded both Hysun-33 and Sirena in 18 environments, and out-yielded the local Sudanese hybrid (Bohooth-1) in across the 21 environments. The mean seed yield across the twenty one environments for SY-4045 was 19% more than that of Hysun-33 (1709 kg ha^{-1}) and 23% more than Bohooth-1 (1664 kg ha^{-1}). Also, Kondi showed about 14 % and 17% more seed yield than Hysun-33 and Bohooth-1 respectively (Table 4). Therefore, the mean seed yield rank of the Syngenta hybrids (SY-4045 and Kondi) across the 21 environments was first for SY-4045 and second for Kondi.

Results for days to 50% flowering are shown in Table (5). There was significant difference among the hybrids for flowering time that ranged from 64 to 70 days with a mean of 68 days. Kondi was earlier than Hysun-33 by four days and earlier than Bohooth-1 by two days. Hence, this hybrid can be planted late under rainfed areas and/or under irrigation to make use of available water and to reduce irrigation cost. Across all environments the overall mean of hybrids plant height was 139 cm (Table 5). Plant heights for the selected hybrids were 141 cm for SY-4045, and 138 cm for Kondi compared to 149 cm for Hysun-33 and 141 cm for Bohooth-1. However, development of dwarf or semi-dwarf plant height is the recent trend in breeding work to avoid lodging of sunflower hybrids during storm or heavy rains. Hence, these hybrids had reasonable height, suitable for mechanical harvest and had good resistance to lodging. The mean numbers of seeds per head of individual hybrid are presented in Table (5) only for irrigated sites. Overall the irrigated environments, the hybrids; SY-4045, Kondi and Neoma recorded a higher number of seeds as compared to the three checks. This indicated that irrigated environments were the

most favorable conditions and the selected hybrids (SY-4045 and Kondi) had the capacity to exploit such environments by attaining the highest number of disc flowers formed seedset and hence high number of seeds per head. On the other hand, the lower percentage of empty is an indicator of higher seed setting and consequently high self-fertility and increased seed yield. Also, the results in Table (5) showed that the Kondi had the lowest mean percentage of empty seeds per head (6.2 %) compared to 7.7 % for Hysun-33 and 7.8 % for Bohooth-1. These results confirmed that the higher number of seeds and lower number of empty seeds per head were greatly influence by the genetic and the level of self-compatibility of hybrid and the environmental stress during the reproductive phase. Seed mass or weight is one of the most important yield components. The overall mean of thousand seed weight was 55 g (Table 5). SY-4045 (61 g) showed a mean of thousand seed weight different from that of Hysun-33 and Bohooth-1 (57 g for both), while Kondi (55 g) had a mean 1000-seed weight to some extended not different from that of checks across the 21 environments. The oil content was determined by Soxhelt method only for Medani irrigated site during kharif and winter season of 2012-2013 (Table 5). Oil content is an important component of oil yield per unit area. Results showed that the mean of oil content of SY-4045 (43%), Hysun-33 (41%), Bohooth-1 (39%) and Sirena (44.6%) were very similar in oil content percentage. While, Kondi (47%) showed higher mean oil content compared to the three checks. Regarding the mean of oil yield in Table (5), SY-4045 out-yielded Hysun-33, Bohooth-1 and Sirena by 24%, 21% and 20%, respectively. Also, Kondi out-yielded Hysun-33 by 28%, Bohooth-1 by 26% and Sirena by 25% under the environments of the study.

CONCLUSION

Consistent high mean seed and oil yields demonstrated by two Syngenta sunflower hybrids (SY-4045 and NK Kondi) and their adaptability and stability make them suitable hybrids for cultivation over a wide range of environments. In addition to credits of both hybrids for earliness, medium plant height and high self-fertility for good seedset compared to checks. Hence, the both hybrids were released last May 2015 for commercial production under both irrigated and rainfed (500-800 mm) systems in the central clay plains of the Sudan. Therefore, introduction and release of a large number of hybrids with high yielding from different countries is expected to ensure timely delivery of seeds and will probably result in lower seed prices due to free competition between suppliers.

Table 1: Locations and environments under which Syngenta sunflower hybrids were evaluated during 2011-2013

Location	**Medani**	**Suki**	**Rahad**	**Damazin**	**Gedarif**
Latitude	14° 23ˉ N	13° 25ˉ N	13° 28ˉ N	11° 49ˉ N	14° 20ˉ N
Longitude	33° 29ˉ E	33° 51ˉ E	33° 31ˉ E	34° 24ˉ E	35° 21ˉ E
Elevation (m.a.s.l.)	405 m	430 m	421 m	470 m	592 m
Environment (Season) code	E1,E2 (2011) E3,E4(2012) E5,E6(2013)	E7 (2011) E8,E9(2012) E10,E11(2013)	E12,E13(2011) E14,E15(2012) E16,E17(2013)	E18(2012) E19(2013)	E20(2012) E21(2013)
Soil type (%)	Clay 54 %	Clay 68 %	Clay 60 %	Clay 63%	Clay 75 %
pH	8.0	7.8	8.1	7.2	
Available P	3.0 mg kg^{-1}	3.0 mg kg^{-1}	1.8 mg kg^{-1}	1.8 mg kg^{-1}	3.3 mg kg^{-1}
O.C. %	0.36	0.60	0.60	0.63	0.60
N %	0.03	0.03	0.05	0.03	0.03

Source: Soil Survey Department, ARC-Sudan

Table 2: Pooled analysis of variance of seed and yields (kg ha^{-1}) in Syngenta sunflower hybrids evaluated in twenty one environments

Source of variation	d.f	Mean square
Hybrid (G)	6	603661.95**
Environment (E)	20	1592153.12**
Hybrid (G) x Environment (E)	120	61649.74**
Environment + (Hybrid x Environment)	140	280293.08**
Environment (Linear)	1	7035229.11**
Hybrid x Environment (Linear)	6	60456.53**
Pooled deviation	133	52896.46**
Pooled error	294	14092.19

** Denote significant at 0.01 probability level

Table 3: Estimates of stability parameters for seed yield of Syngenta sunflower hybrids evaluated at 21 environments.

Hybrid	Seed yield (kg ha^{-1})	Slope (b_i)	SE ±	S^2_{di}
SY-4200	1708	0.94	0.104	35138
SY-4045	2041	1.16	0.089	21870
NK Kondi	1947	1.09	0.119	49272
Neoma	1692	1.08	0.065	5086
Hysun-33	1709	0.87	0.066	5512
Bohooth-1	1664	0.87	0.070	7871
Pan-7351/Sirena	1642	0.99	0.099	30090
Mean	1772			

Where; b_i = regression coefficient, S^2_{di} = deviation from regression

Table 4: Means of seed yield (kg ha^{-1}) of sunflower hybrids evaluated over 21 environments.

Location	**Medani**						**Suki**				
Hybrid/Envi.	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
SY-4200	1775	1917	2049	1435	1533	1765	2026	2024	1205	1795	2012
SY-4045	2356	2405	2243	1740	1731	1896	2749	2263	1611	2373	2571
NK Kondi	2129	2178	2209	1633	1629	1807	2951	2107	1547	2753	1538
Neoma	1781	1864	1715	1201	1407	1579	2311	1739	1183	2167	2004
Hysun-33	1564	1746	1828	1457	1468	1737	2302	1828	1401	2051	2346
Bohooth-1	1311	1782	1996	1495	1536	1761	1903	1816	1536	2251	1758
Sirena	1430	1479	1802	1610	1597	1742	2769	1816	1240	2104	1538
Mean	*1764*	*1910*	*1977*	*1510*	*1557*	*1755*	*2430*	*1942*	*1389*	*2213*	*1967*
SE ±	*196*	*154*	*73.5*	*45.2*	*18.4*	*32.6*	*156*	*77.9*	*99.5*	*184*	*139*
Level of sig.	**	*	**	**	**	**	**	**	*	*	**
C.V.%	*19.2*	*14.0*	*6.4*	*5.2*	*2.0*	*3.2*	*11.1*	*6.9*	*12.4*	*14.4*	*12.2*
Mean loca.	*1746*						*1988*				
Location	**Rahad**						**Damazin**		**Gedarif**		**Overall**
Hybrid/Envi.	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	**mean**
SY-4200	2362	2397	2154	1459	1216	2000	465	1340	2111	823	**1708**

SY-4045	2674	2553	2153	1945	2570	2250	382	1635	1816	949	**2041**
NK Kondi	2362	2188	2188	1598	2049	2333	677	2133	2272	603	**1947**
Neoma	2431	2397	1945	1494	2188	2000	361	1451	1578	735	**1692**
Hysun-33	2119	2345	1945	1598	1563	1958	616	1590	1571	856	**1709**
Bohooth-1	2084	2292	1841	1424	1841	1917	469	1601	1603	722	**1664**
Sirena	1911	2501	1910	1355	1841	1792	528	1132	-	702	**1646**
Mean	*2277*	*2382*	*2019*	*1553*	*1896*	*2036*	*500*	*1555*	*1825*	*770*	***1772***
SE +	*130*	*80.0*	*96.8*	*88.2*	*124*	*79.8*	*44.3*	*18.0*	*227*	*43.3*	***26.04***
Level of sig.	*	*	*ns*	*	**	**	**	**	*ns*	*Ns*	**
C.V.%	*9.9*	*5.8*	*8.3*	*9.8*	*11.4*	*6.8*	*15.4*	*2.0*	*21.5*	*21.0*	***11.7***
Mean loca.	*2027*						*1028*		*1298*		***1617***

Table 5: Means of some traits of SYNGENTA sunflower hybrids evaluated over 21 environments.

Hybrid/trait	***DF**	**PH**	**NSH**	**ES**	**SW**	**OC**	**OY**
SY-4200	70	136	1092	10.4	54	41.53	709.33
SY-4045	68	141	1115	8.2	61	43.32	884.16
NK Kondi	66	138	1152	6.2	55	47.10	917.04
Neoma	64	133	1178	7.3	48	45.53	770.37
Hysun-33	70	149	1094	7.7	57	41.76	713.68
Bohooth-1	68	141	1047	7.8	57	43.90	730.50
Sirena	68	139	1045	9.0	56	44.69	733.81
Mean	***68***	***139***	***1103***	***8.1***	***55***	***43.97***	***779.15***
SE +	*0.18*	*0.85*	*17.1*	*0.47*	*0.58*		
Sign. Level	**	**	**	**	**		
C.V.%	*2.1*	*4.8*	*12.3*	*46.6*	*8.3*		

**DF= days to 50% flowering, PH = plant height (cm), NSH = number of seeds per head, ES = percentage of empty seeds (%), SW = 1000- seed weight (g), OC = oil content (%) & OY = oil yield (kg ha^{-1}).*

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ADVANCEMENTS IN CLEARFIELD® PLUS SUNFLOWER HYBRID VARIETY DEVELOPMENT

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ABSTRACT

Clearfield® Plus sunflowers, the next generation herbicide tolerance trait based on a single mutation in the acetohydroxyacid synthase gene, *Ahas1-3,* or *CLHA-Plus,* was first launched in Argentina in 2010. Since then, Clearfield Plus hybrids have been introduced to the market in the USA, Romania, Bulgaria, and South Africa with additional countries anticipated in 2016. To speed the introduction of Clearfield Plus hybrids to the market, first generation hybrids combine the *CLHA-Plus* mutation, homozygous, on one parent with the *Ahas1-2* (*ImiSun*) mutation, homozygous, on the second parent (hetero combo). In this manner breeding companies optimize resources and inbreds from existing Clearfield breeding programs in combination with converted or new *CLHA-Plus* inbreds. Clearfield breeding programs, though, have been hampered by the necessity to spray select tolerant individuals containing both the *ImiSun* mutation plus enhancing (E)-factor(s), required for full commercial tolerance. To date, no reliable molecular markers have been developed to detect the presence and zygosity of the E-factor(s), making marker assisted selection unfeasible. This paper investigates the learnings from the past 5 years comparing Clearfield and Clearfield Plus hybrid systems both from the breeding perspective and from the grower perspective. The next generation Clearfield Plus hybrids coming to the market include CLHA-Plus (*Ahas1-3/Ahas1-3*) homozygous hybrids which benefit from more efficient breeding improvements and, like their hetero combo counterparts, demonstrate improved herbicide tolerance leading to more reliable tolerance in diverse environments. All Clearfield Plus hybrids benefit from improved herbicide products and increased weed control spectrum in South America as well as in Europe and Eastern Europe.

Key Words : Clearfield, Clearfield Plus, breeding, E factor, CLHA-Plus

GRAIN, KERNEL AND HULL CHARACTERIZATION OF OILSEED AND OILSEED X CONFECTIONARY GENOTYPES

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ABSTRACT

Sunflower breeding programs developed, through the years, an increase in grain oil concentration by increasing the kernel proportion in the grain or the oil concentration in the kernel without affecting potential grain size. Bigger grain sizes (globular form indeed) were reported as less affected by dove consumption. Although quantitative data is not available, birds appear to prefer oilseed hybrids of sunflower over confectionary types. Agronomics characteristics, hull and kernel proportion and oil content were investigated in several commercial hybrids (oilseed genotypes), two novel oilseed x confectionary (O*C) cultivar and three inbreed lines (progenitors of O*C genotypes). Fields experiments were conducted in Reconquista (Argentina) during 2014-2015 in two sowing dates (optimal and late). The measured traits were phenology, grain weight, hull and kernel percentage, whole, hull and kernel grain oil percentage, length and width grain. Days after flowering (DAF) of commercial and O*C hybrids ranged among 71 and 80, while days after maturity (DAM) varied from 99 to 123. No differences were found between oilseed and O*C genotypes in phenology. Oil percentages of commercial hybrids were 49.5±2.6 (59.7±1.4 and 19.9±1.3 kernel and hull oil percentage, respectively) while O*C genotypes were 41.7±1.9 (55.8±1.5 and 18.4±1.4 kernel and hull oil percentage, respectively). Seeds of O*C genotypes were 41 and 42% wider and longer, respectively, as well as 82% heavier than commercial hybrids. In ABSTRACT, we have assessed the reduction in oil percentage on O*C genotypes in comparison with commercial hybrids. Sunflower breeding programs may incorporate these genotypes for dove tolerance improvement.

Keywords: kernel oil, hull oil, oilseed, confectionary hybrids

INTRODUCTION

Sunflower (*Helianthus annuus* L.) seed is considered to be an important oilseed crop because it contains highly nutritious oil in large quantity (Shukla *et al.*, 1992). Sunflower kernel has between 57 to 67% oil content (Aguirrezabal & Pereyra; 1998). The hull comprises 20–30% of the seed, depends on the variety, and contains mostly crude fiber and non-significant quantity of fat (Tranchino *et al*., 1984).
Changes in environmental conditions during the grain filling period could potentially affect oil content of kernel, hull and whole seeds. For sunflower crop management, hybrids selection and his interaction with environment could also affect oil content of kernel, hull and whole seeds.
In Argentina, some species of dove (Columbidae) and parakeet (Psitticidae) can cause significant damage to sunflower (Bucher, 1990). The eared doves (*Zenaida auriculata* Des Murs) is the most numerous granivorous bird in Argentina and Uruguay and cause serious sunflower damage (Bucher, 1992; Canavelli, 2010).
In most bird-resistance studies, two main strategies were recognized: chemical repellents (Crase y Dehaven, 1976; Rodriguez *et al*, 1995) and morphological characteristics such as head angle and down-facing head (Zuil & Colombo, 2012). More uniform crops are apparently less susceptible to damage by

cockatoos (Allen, 1986). Canavelli (2010) recommended using hybrids of sunflower with bigger head angle and/or down facing head as part of crop management practices. Larger grain sizes (globular form indeed) were reported as less affected by dove consumption (Linz & Hanzel, 1997). Oilseed cultivars appear to be more attractive to doves than confectionary ones (Bernardos & Farrell, 2012). Novel hybrids were obtained from ACA breeding programs to increase grain size using an oilseed by confectionary lines.

The aim of this work was to evaluate the agronomic characteristics, hull and kernel proportion and oil content in fifteen commercial hybrids (oilseed genotypes), two novel oilseed x confectionary (O*C) cultivar and three inbreed lines (progenitors of O*C genotypes)

MATERIALS & METHODS

This research was carried out during the 2014-2015 growing season in Reconquista (29° 11' S; 59° 52' W), Santa Fe, Argentina in optimum and late sowing dates, RQTA1: 14/08 and RQTA2: 15/10, respectively. The experiment was conducted with fifteen commercial hybrids (oilseed genotypes), two novel oilseed x confectionary (Oil*Con) cultivar and three inbreed lines (progenitors of O*C genotypes). Genotypes were arranged in a randomized complete block design with four replications in each environment. Each plot was 4 rows and 5 m long, consisting of four rows of a single genotype. Mean plant densities were 4.5 pl m^{-2}. The inter-rows spacing was 52 cm and inter-plant spacing was 30 cm. The soil analysis showed the following result: 1.83 % of organic matter, pH 6.2, 14.3 $mg.kg^{-1}$ of available phosphorus and 19.9 $mg.kg^{-1}$ of $N\text{-}NO_3$. Pest and diseases were effectively controlled.

The meteorological conditions (daily temperature, precipitation and solar radiation) were measured in a weather station located 500 m approximately from the experiment. Daily incident radiation corresponding to the photosynthetically active range of the spectrum was calculated as 0.48 × global daily incident radiation (PAR incident).

Phenology (Schneiter & Miller, 1981) and plant height were recorded. The length, width and hull thickness of seeds samples were measured with calipers. Seed coats were removed with tweezers and their percentage to total seed weight was calculated. The oil percentage of whole seed, kernel and hull were measured with NMR equipment (Nuclear Magnetic Resonance Spinlok, Córdoba, Argentina).

The broad-sense heritability was calculated as the ratio of total genetic variance to total phenotypic variance (Visscher *et al.*, 2008). Results are presented as mean ± standard error. Analysis of variance was performed with the statistics program Infostat (Di Rienzo *et al.*, 2015). Data were subjected to Tukey's multiple-range tests to compare mean values at 5% level of significance. Sigmaplot software (Sigmaplot 8.0, SPSS Inc., Chicago, IL) was used to establish the relationships.

RESULTS AND DISCUSSIONS

In RQTA 1, maximum, minimum and mean air temperature averages were 27,6, 15,7 and 21,6 ºC respectively, whereas in RQTA 2 were much higher (30,2, 19,1 and 24,6 ºC). Cumulative photosynthetically active radiation incident was 1363 and 1218 $Mj.m^{-2}$ in RQTA 1 and RQTA 2, respectively. Furthermore, cumulative rainfall was 738 and 1042 mm in RQTA 1 and RQTA 2, respectively. Daily mean temperature, PAR incident and rainfall are illustrated in Figure 1.

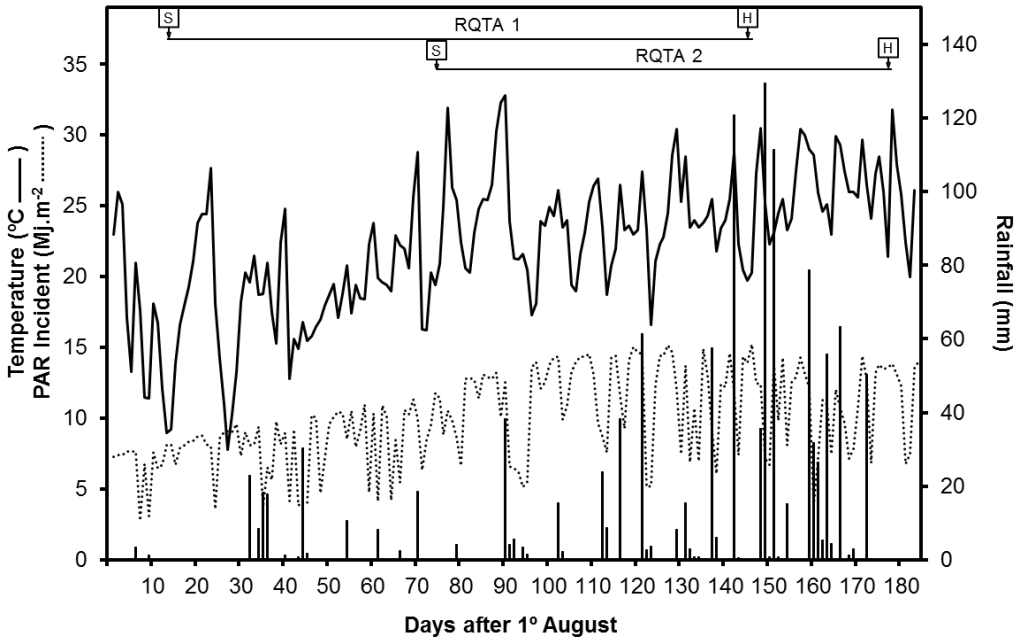

Figure 1. Daily mean temperature (ºC), photosynthetically active radiation incident (PAR incident $Mj.m^{-2}$) and rainfall (mm) since August 1º. Horizontal lines at the top of the figure represent the date of sowing (S) and harvest (H) for RQTA 1 and RQTA 2.

Days after flowering (DAF) of commercial and O*C hybrids ranged among 71 and 80 DAF, while days after maturity (DAM) varied from 99 to 123. In RQTA 1, the average days from sowing to flowering among commercial hybrids were 80 (ranged among 75 and 86), while the mean days from sowing to physiological maturity was 123 (varied among 114 and 129 days). In RQTA 2, the average days from sowing to flowering between hybrids was 71 (ranged among 65 and 79), while the mean days from sowing to physiological maturity was 99 (varied among 90 and 109 days). Furthermore, the height of commercial and Oil*Con hybrids were 137 ± 18 and 148 ± 8 cm, respectively. The Oil*Con hybrids showed a similar behavior than commercial ones in phenology and height.

Sunflower genotypes differed from each other in length ($p<0.0001$). Regarding width and hull thickness, statistically significant differences were found between genotypes and environment ($p\leq0.0009$, Table 1). Significant interaction G*E was found in width seed. The confectionary parental line "Male 2" had the highest length and width seed, compared to the commercial hybrids. Seeds of Oil*Con genotypes were 41 and 42% longer and wider, respectively, as well as 82% heavier than commercial hybrids. However, the hull was 48% thicker than commercial hybrids and similar to the confectionary males lines.

Table 1. Length, Width and Hull Thickness (in mm) of seeds for different environments (RQTA 1 and 2) of 15 commercial genotypes of sunflower, 3 parental lines and two confectionary*oilseed hybrids.

Hybrids	Type	Length		Width		Hull Thickness	
		RQTA 1	RQTA 2	RQTA 1	RQTA 2	RQTA 1	RQTA 2
		mm					
ACA 861	Oilseed	10,5 ± 0.1*	10,6 ± 0,6	4,8 ± 0,2	5,9 ± 0,1	0,5 ± 0,048	0,5 ± 0,025
ACA 885	Oilseed	10,9 ± 0,1	10,6 ± 0,2	5,3 ± 0,3	5 ± 0,2	0,4 ± 0,001	0,3 ± 0,021
ACA 887	Oilseed	11,0 ± 0,0	11,4 ± 0,3	4,7 ± 0,4	5,4 ± 0,2	0,4 ± 0,048	0,4 ± 0,036
AD66 CL	Oilseed	9,6 ± 0,2	9,8 ± 0,1	4,9 ± 0,1	5,4 ± 0,2	0,3 ± 0,046	0,5 ± 0,01
Cacique CL	Oilseed	10,4 ± 0,2	10,3 ± 0,3	4,6 ± 0,3	5,4 ± 0,2	0,3 ± 0,031	0,4 ± 0,044
Diagora	Oilseed	10,4 ± 0,2	10,4 ± 0,4	4,8 ± 0,3	4,8 ± 0,1	0,4 ± 0,043	0,5 ± 0,01
DK 3970 CL	Oilseed	10,3 ± 0,3	9,8 ± 0,3	4,7 ± 0,1	5,3 ± 0,3	0,3 ± 0,038	0,4 ± 0,041
DK 4045	Oilseed	10,4 ± 0,1	10,8 ± 0,3	4,8 ± 0,3	5,3 ± 0,1	0,5 ± 0,001	0,5 ± 0.001
DK 4065	Oilseed	8,8 ± 0,5	9,1 ± 0,3	4,8 ± 0,1	5,0 ± 0,0	0,3 ± 0,027	0,3 ± 0,0087
NEON	Oilseed	10,8 ± 0,1	10 ± 0,4	5,3 ± 0,1	5,3 ± 0,1	0,5 ± 0,025	0,5 ± 0,025
P 1100 CLP	Oilseed	10,1 ± 0,3	9,5 ± 0,5	4,6 ± 0,2	4,9 ± 0,2	0,3 ± 0,001	0,4 ± 0,044
P102CL	Oilseed	8,9 ± 0,1	9,1 ± 0,1	4,4 ± 0,1	5,3 ± 0,3	0,5 ± 0,043	0,4 ± 0,041
PAN 7031	Oilseed	10,8 ± 0,3	10,4 ± 0,3	4,8 ± 0,3	5,2 ± 0,1	0,4 ± 0,04	0,4 ± 0,027
PAN 7076	Oilseed	10,9 ± 0,3	10,1 ± 0,5	4,5 ± 0,3	5,3 ± 0,3	0,4 ± 0,001	0,4 ± 0,01
PROTON 290	Oilseed	10,1 ± 0,1	10,6 ± 0,2	4,8 ± 0,3	5,9 ± 0,1	0,3 ± 0,047	0,4 ± 0,042
Female (F)	Oilseed	12,0 ± 0,0	11,5 ± 0,3	5,5 ± 0,0	5,3 ± 0,1	0,2 ± 0.01	0,3 ± 0,029
Male 1 (M1)	Con	15,5 ± 0,3	14,5 ± 0,3	6,0 ± 0,0	6,5 ± 0,3	0,6 ± 0,029	0,6 ± 0,029
Male 2 (M2)	Con	17,0 ± 0,0	16,5 ± 0,3	7,5 ± 0,0	8,0 ± 0,6	0,5 ± 0,029	0,6 ± 0,029
Hybrid 1 (F*M1)	Oil*Con	14,5 ± 0,0	15,0 ± 0,0	7,5 ± 0,0	7,0 ± 0,0	0,6 ± 0,029	0,6 ± 0,029
Hybrid 2 (F*M2)	Oil*Con	13,8 ± 0,1	14,3 ± 0,4	6,8 ± 0,1	7,3 ± 0,4	0,5 ± 0,029	0,7 ± 0,029

	Significance		
Genotype	<0,0001	<0,0001	<0,0001
Environment	0,2341	<0,0001	0.0009
G*E	0,0915	0,0211	0,0539
DMS (Tukey p<0,05)	1,6	1,3	0,23

*mean ± standard error

Sunflower genotypes were statistically different in term of hull/kernel ratio and hull proportion among genotypes, environment and the interaction G*E ($p \leq 0.0311$, Table 2). Although the difference found in hull thickness was 48% between commercial and oil*con genotypes, the proportion of hull between them was 6%. The hull/kernel ratio were higher in RQTA 2 (47.6 and 50.5 for commercial and oil*con genotypes, respectively) than RQTA 1 (41.2 and 45.5 for commercial and oil*con genotypes, respectively).

Table 2. Hull/Kernel ratio and Hull proportion of seeds for different environments (RQTA 1 and 2) of 15 commercial genotypes of sunflower, 3 parental lines and two confectionary*oilseed hybrids.

Hybrids	Type of hybrid	Hull/Kernel Ratio		Hull Proportion	
		RQTA 1	RQTA 2	RQTA 1	RQTA 2
		……%.....			
ACA 861	Oilseed	42 ± 1,8	48 ± 4	30 ± 0,9	32 ± 1,8
ACA 885	Oilseed	44 ± 1,7	49 ± 1,1	31 ± 0,8	33 ± 0,5
ACA 887	Oilseed	46 ± 2,7	62 ± 4,9	31 ± 1,3	38 ± 1,8
AD66 CL	Oilseed	32 ± 0,9	33 ± 3,6	24 ± 0,5	24 ± 1,8
Cacique CL	Oilseed	36 ± 1,8	37 ± 2,1	26 ± 1,0	27 ± 1,1
Diagora	Oilseed	31 ± 2,5	41 ± 2,2	24 ± 1,4	29 ± 1,1
DK 3970 CL	Oilseed	29 ± 1,1	39 ± 1,7	22 ± 0,6	28 ± 0,9
DK 4045	Oilseed	55 ± 4,6	56 ± 1,5	35 ± 1,9	36 ± 0,6
DK 4065	Oilseed	36 ± 2,8	46 ± 7,3	26 ± 1,6	31 ± 3,4
NEON	Oilseed	60 ± 6,7	57 ± 5,0	37 ± 2,4	36 ± 2,1
P 1100 CLP	Oilseed	34 ± 1,0	40 ± 0,6	25 ± 0,6	28 ± 0,3
P102CL	Oilseed	41 ± 1,1	48 ± 3,7	29 ± 0,6	32 ± 1,6
PAN 7031	Oilseed	44 ± 1,7	51 ± 3,3	30 ± 0,8	34 ± 1,4
PAN 7076	Oilseed	45 ± 1,4	57 ± 5,2	31 ± 0,7	36 ± 2,1
PROTON 290	Oilseed	44 ± 2,7	51 ± 5,9	30 ± 1,3	34 ± 2,4
Female (F)	Oilseed	24 ± 1,4	28 ± 0,4	19 ± 0,9	22 ± 0,3
Male 1 (M1)	Con	73 ± 2,1	63 ± 1,2	42 ± 0,7	39 ± 0,5
Male 2 (M2)	Con	65 ± 0,3	69 ± 1,6	39 ± 0,1	41 ± 0,5
Hybrid (F*M1)	Oil*Con	41 ± 2,2	45 ± 0,1	29 ± 1,1	31 ± 0,0
Hybrid 2 (F*M2)	Oil*Con	50 ± 0,5	56 ± 0,4	33 ± 0,2	36 ± 0,2
		Significance			
Genotype		<0,0001		<0,0001	
Environment		<0,0001		<0,0001	
G*E		0,0311		0,0305	
DMS (Tukey p<0,05)		17,1		7,5	

*mean ± standard error

Whole seed oil, kernel oil and hull oil percentage showed statistically differences among genotypes, environment and the interaction G*E ($p \leq 0.0001$, Table 2). Oil percentages of commercial hybrids were 49.5±2.6 (59.7±1.4 and 19.9±1.3 kernel and hull oil percentage, respectively) while O*C genotypes were 41.7±1.9 (55.8±1.5 and 18.4±1.4 kernel and hull oil percentage, respectively). The whole seed oil percentage was higher in RQTA 1 (50.2 and 45.5 for commercial and oil*con genotypes, respectively) than RQTA 2 (48.8 and 39 for commercial and oil*con genotypes, respectively). The kernel oil percentage was higher in RQTA 1 (63 and 56.5 for commercial and oil*con genotypes, respectively) than RQTA 2 (56.6 and 55 for commercial and oil*con genotypes, respectively), while hull oil percentage was 18.7 and 19.5 for commercial and oil*con genotypes, respectively in RQTA 1 and 21.2 ad 17.5 for commercial and oil*con genotypes respectively in RQTA 2. The confectionary males lines resulted in less oil percentage in kernel and hull.

Table 2. Percentage of Whole Seed Oil, Kernel Oil and Hull Oil for different environments (RQTA 1 and 2) of 15 commercial genotypes of sunflower, 3 parental lines and two confectionary*oilseed hybrids.

Hybrids	Type	Whole Seed Oil		Kernel Oil		Hull Oil	
		RQTA 1	RQTA 2	RQTA 1	RQTA 2	RQTA 1	RQTA 2
		……%……					
ACA 861	Oilseed	47 ± 1,0*	49 ± 0,4	62 ± 1,3	55 ± 1,1	18 ± 0,6	22 ± 0,7
ACA 885	Oilseed	49 ± 1,4	48 ± 0,7	64 ± 1,4	55 ± 0,9	18 ± 1,0	21 ± 0,3
ACA 887	Oilseed	49 ± 0,5	47 ± 1,7	66 ± 0,7	58 ± 1,8	17 ± 0,5	20 ± 0,4
AD66 CL	Oilseed	51 ± 1,6	52 ± 1,1	61 ± 1,2	55 ± 0,7	20 ± 0,6	24 ± 0,5
Cacique CL	Oilseed	51 ± 1,4	50 ± 1,1	62 ± 1,3	54 ± 0,5	20 ± 0,3	23 ± 0,4
Diagora	Oilseed	55 ± 0,8	49 ± 1,3	64 ± 0,7	54 ± 1,1	20 ± 0,2	23 ± 0,3
DK 3970 CL	Oilseed	54 ± 0,8	57 ± 0,4	60 ± 2,1	60 ± 0,5	21 ± 1,3	23 ± 0,3
DK 4045	Oilseed	46 ± 0,9	49 ± 3,0	63 ± 1,4	57 ± 1,3	17 ± 0,6	21 ± 1,5
DK 4065	Oilseed	57 ± 0,9	47 ± 4,4	64 ± 1,8	55 ± 2,3	21 ± 1,1	21 ± 1,0
NEON	Oilseed	43 ± 0,7	46 ± 0,7	62 ± 0,9	57 ± 1,7	16 ± 0,4	20 ± 0,6
P 1100 CLP	Oilseed	51 ± 1,2	49 ± 1,6	63 ± 1,6	57 ± 1,7	19 ± 0,7	21 ± 0,3
P102CL	Oilseed	48 ± 1,1	45 ± 2,5	63 ± 0,4	56 ± 0,8	18 ± 0,3	20 ± 1,4
PAN 7031	Oilseed	50 ± 1,9	48 ± 0,8	64 ± 1,2	58 ± 1,7	18 ± 0,5	20 ± 0,8
PAN 7076	Oilseed	51 ± 0,4	46 ± 1,2	65 ± 1,8	60 ± 3,2	18 ± 0,9	18 ± 1,6
PROTON 290	Oilseed	51 ± 2,2	50 ± 1,5	63 ± 1,3	58 ± 1,5	19 ± 0,7	21 ± 0,7
Female (F)	Oilseed	55 ± 0,1	47 ± 0,1	54 ± 0,6	50 ± 0,7	25 ± 0,3	23 ± 0,3
Male 1 (M1)	Con	35 ± 0,1	27 ± 1,8	50 ± 1,3	51 ± 0,5	17 ± 0,4	13 ± 0,8
Male 2 (M2)	Con	26 ± 0,6	23 ± 0,6	45 ± 0,5	48 ± 0,2	14 ± 0,2	12 ± 0,3
Hybrid 1 (F*M1)	Oil*Con	45 ± 0,2	41 ± 0,0	55 ± 0,1	54 ± 0,0	20 ± 0,0	19 ± 0,0
Hybrid 2 (F*M2)	Oil*Con	44 ± 0,6	37 ± 0,2	58 ± 0.0	56 ± 0,8	19 ± 0,2	16 ± 0,4
				Significance			
Genotype			<0,0001	<0,0001		<0,0001	
Environment			<0,0001	<0,0001		<0,0001	
G*E			<0,0001	<0,0001		<0,0001	
DMS (Tukey p<0,05)			7,8	3,9		7,4	

*mean ± standard error

Hull/kernel ratio was curvilinear and negatively in relation to whole seed oil percentage (Fig 2). Hull/kernel ratio accounted for the variation in whole seed oil percentage of all genotypes ($p < 0.0001$, $r^2=0.74$, Fig. 2 a) where lower hull/kernel ratios showed the highest seeds oil percentages (commercial hybrids and female line). The oil*con genotypes had mean values of hull/kernel ratio and seed oil percentages. Whole seed oil percentage was curvilinear and positively related to kernel oil ($p<0.0001$, $r^2=0.58$ Fig 2 b) and hull oil ($p<0.0001$, $r^2=0.67$, Fig. 2 C). Dedio (1982) reported that each of the two components of the seed (kernel and hull oil) have a significant effect on the oil content of the whole seed. Regarding both variables, oil*con genotypes has shown an intermediate performance between confectionary and oilseed genotypes.

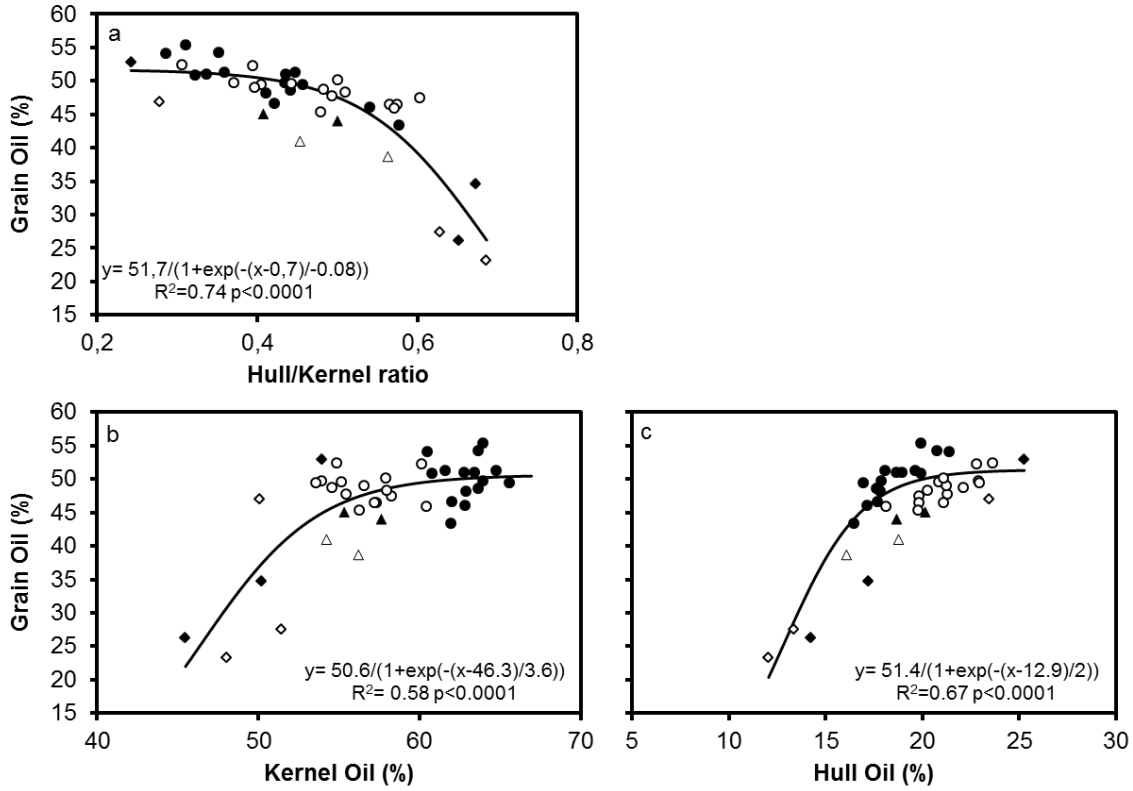

Figure 2. Relationship between grain oil and Hull/Kernel ratio (a), Kernel Oil (b) and Hull oil (c) of sunflower hybrids. Data correspond circle, diamond and triangle to commercial hybrids, parental lines and oil*con genotypes, respectively. Full and empty icons represent RQTA1 and RQTA 2, respectively.

For whole seed oil, hull and kernel weight and seed length, the higher component of variance was genetic and the heritability (broad sense) estimated ranged between 61 and 98 % (Table 4). High heritability estimates for oil content has been reported by Shrinivasa (1982) and Khair *et al.* (1992). Dedio (1982) reported that due to the lack of genetic linkage between kernel and hull oil, a breeder could screen for both component while striving to improve oil content. Further investigations should be developed to understand the effect of the environmental conditions over the oil parameters measured in this work. Furthermore, it would be interesting to develop studies involving a wider range of genetics background of confectionary, oilseed and their crossing genotypes.

In ABSTRACT, oil*con genotypes referred in this work has a good performance in oil percentage compared to confectionary and commercial oilseed. Thus, the grain size (length and width) of Oil*Con genotypes was higher than commercial hybrids and could be used in bioassays against eared doves to confirm the reduction of birds consumption. We have assessed the reduction in oil percentage on O*C genotypes in comparison with commercial hybrids.

Table 4. Genotypic variance, environment variance, interaction G*E variance and heritability in wide sense for Seed Oil percentage, hull weight, kernel weight, seed length, seed width, kernel oil (%), hull oil (%), Hull/Kernel Ratio and Hull.

	σ^2_g	σ^2_{ga}	σ^2_a	H^2
Whole Seed Oil	432	32,2	242,4	0,61
Hull Weight	599	30,8	37,1	0,90
Kernel Weight	966	81,8	170,2	0,79
Seed Lenght	36	0,5	0,4	0,98
Seed Width	6	0,4	6,8	0,47
Kernel Oil percentage	127	23,4	945,9	0,12
Hull Oil percentage	47	12,7	71,3	0,36
Hull/Kernel Ratio	0,1	0,01	0,11	0,45
Hull percentage	0,02	0,0013	0,02	0,48

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DEVELOPING WELL ADAPTED HYBRIDS IN EUROPE BY USING A G*E APPROACH

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ABSTRACT

The expression of sunflower yield is determined by biotic and abiotic factors. Breeding work allows, most of the time, to find efficient answers for pests or diseases damages, but the interaction between varieties and environmental criteria is more difficult to analyze and therefore to select. First studies carried out in France by INRA and TERRES INOVIA demonstrated the possibility to structure the Genotype *Environment interaction using pedo-climatic parameters, at different stages of the sunflower cycle, and highlighted differences of behavior between hybrids. On that basis, we developed our own research program using data collected for the last 10 years on our European sunflower testing network, in order to find original agro-climatic indicators that explain the Genotype *Environment interaction. Using strong statistical methods and computer tools, we were able to identify a limited number of different "climates" that summarize most frequent agro-climatic conditions present in the European sunflower area (10 different countries), and to find specific combinations of indicators for each "climate". Moreover, a statistical model established with these indicators significantly explains part of the Genotype * Environment interaction and highlights the capacity of some varieties to obtain stable and high enough yield in all the identified "climates". The model is a powerful tool for the breeding or the marketing teams to characterize *a posteriori* testing locations and better analyze how relevant their network is. The use of that method in the breeding process will also be helpful in creating new hybrids broadly adapted to abiotic stresses.

Key Words : abiotic stress, Europe, G*E interactions

OPTIMIZATION OF AGROBACTERIUM-MEDIATED GENE TRANSFER SYSTEMS IN TURKISH SUNFLOWER (HELIANTHUS ANNUUS L.) VARIETIES

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ABSTRACT

This study aimed to establish the plant tissue culture and gene transfer systems in some elite Turkish sunflower (*Helianthus annuus* L.) varieties. Plant tissue culture systems were established on Murashige and Skoog (MS) media supplemented with various plant-growth regulators using cotyledonary nodes and meristematic shoots as explants. After surface sterilization, seeds were germinated in MS media for 12 days in growth chamber under 16/8 photoperiods, %60 humidity and 24oC temperature. Following germination, explants were isolated and cultured in MS media containing 0.25 mg/l NAA (1-Naphthaleneacetic acid) and 1 mg/l BAP (6-Benzylaminopurine). Isolated shoots were inoculated with an upper-virulent strain of *Agrobacterium tumefaciens*, which included a kanamycin resistance gene as selective marker, a cauliflower-mosaic-virus-derived 35S promoter, a GFP coding sequence and an antibiotic resistance gene (BAR) for selection of transformed plants. All regenerated shoots were rooted on MS media supplemented with 1 mg/l IBA. GFP protein expression was detected on gel as well as visualized using Fluorescent microscope. *Agrobacterium*-mediated gene delivery system in the meristematic tissues is regarded as an efficient method in production of transgenic sunflowers as well as it forms a baseline for the effective delivery of agronomically valuable gene/s in some Turkish elite sunflower varieties. Many commercial sunflower varieties are seriously affected by various biotic and abiotic stress factors, and also require the chemical control while maintaining the product quality. Traditional breeding strategies do not have ability to address all these limitations but biotechnology does. Thus, present study emphasized the application of molecular and biotechnological methods to improve the some elite sunflower varieties in Turkey.

Key Words : Sunflower, tissue culture, gene transfer, regeneration, organogenesis

INCLUSION OF DOMINANCE EFFECT IN GENOMIC SELECTION MODEL TO IMPROVE PREDICTIVE ABILITY FOR SUNFLOWER HYBRID PERFORMANCE

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ABSTRACT

Hybrids dominate the world sunflower production, mainly because of the important heterosis in this crop. Therefore, predicting hybrid performance is of high importance to improve the efficiency of sunflower breeding programmes. Rather than seeking to identify individual loci significantly associated with a trait, genomic selection (GS) uses all marker data as predictors and consequently delivers more statistically accurate predictions. Dominance, a major factor of heterosis together with epistasis, is often neglected in GS model, adding to the residual error. So the inclusion of dominance effect in the GS model may improve the predictive ability. An incomplete factorial design of 36 males and 36 females was used in this study. We used genotypic data on 635,155 SNPs and phenotypic data (flowering time and leaf senescence) on 452 hybrids in six environments. We used a mixed model to correct spatial variation in each environment and we performed genomic prediction of the genetic hybrid value with a genomic best linear unbiased prediction (GBLUP) method, based on relationship matrices. Two models were compared: an additive model with the male and female relationship matrices and a dominance model taking into account the male, the female and the dominance, the latter defined as the interaction between male and female. Prediction accuracies of these models were estimated as the correlation between genetic hybrid values and observed phenotypes. Comparison of these models and prediction gain of the inclusion of the dominance in GS will be discussed for the two traits chosen for their contrasting heritability

Key Words : genomic selection, parents effect, dominance

ASSESSMENT OF SUNFLOWER GERMPLASM SELECTED UNDER AUTUMN PLANTING CONDITIONS

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ABSTRACT

Agronomic potential of traditional sunflower spring varieties is low because its flowering and grain filling are often exposed to mid and end-season drought. To overcome this, new breeding strategy consisted of selecting varieties tolerant to winter cold in order to shift to autumn or early winter planting. Nowadays, 'Ichraq' is the only one registered autumn variety. The objective of this research is to evaluate various genotypes having been selected in different environments under autumn planting conditions. This germplasm was planted early at winter during two years (2013 and 2014) at 'Annoceur', a mountain site known for its pronounced winter cold. Morphological, physiological, agronomic and technological parameters were considered for the germplasm assessment. Analysis of variance showed significant differences between genotypes for most of these parameters. Plantlet initial vigor average was 3.5 varying from 1 for genotype M32 to 5 for AN8. Leaf area average was 162 cm² varying from 25 to 375 cm² for genotypes M17 and AN34, respectively. Total chlorophyll content average was 43 mg/g, varying from 28 to 79 mg/g for genotypes K7 and M29, respectively. Number of days from sowing to flowering varied from 162 for genotype AN21 to 180 for genotypes M27 and M29. Mean seed yield per plant was 49 g, with a large variation from 8 to 110 g for M18 and K8, respectively. Mean seed oil content was 36%, ranging from 22% for M8 to 47% for K4. Genotypes having exhibited more performance than 'Ichraq' were selected to develop new sunflower germplasm suitable for autumn or early winter sowing.

INTRODUCTION

Agricultural sector continues to dominate Morocco's economic activity. The rural population accounts for 40% of the total population. The Agriculture thus proved an effective engine of economic growth and guaranteed food security. To upgrade and boost domestic agriculture, different strategies have been implemented during the Moroccan contemporary history. The latter being the Green Morocco Plan implemented since 2008. Owing to its importance in the cropping system and the food security challenge in vegetable oils, the oilseed is considered among the priority sectors. Since 2001, the year of oilseed sector reform, and until 2013, the year of signature of a sector program contract, sunflower was the major annual oilseed crop grown in Morocco, with an average area about 50000 ha and an average seed yield below 1 t/ha. Indeed, national seed oil production covers barely 2-3% of the overall needs of the edible oil in the country estimated at over 410.000 t. The gap is covered by importation which has negative repercussion on the economy and food security of our country (Nabloussi et al., 2015).

In Morocco, sunflower traditionally sown in spring has limited productivity as it does not benefit the fall and/or winter precipitation, and it is often exposed to drought and high temperatures of mid and late cropping cycle. Such constraints coincide with periods of flowering and seed filling that are critical for determining seed productivity and seed oil content (Ouattar et al., 1992). Its cultivation is often secondary and is considered as catch crop, following early droughts or floods that affect growing of autumn crops, mainly cereals. However, several studies have shown the benefits of early planting (autumn or early winter) in improving the seed yield and oil content in Morocco (Boujghagh, 1993 ; Gosset and Vear, 1995 ; Aboudrare et al., 2000), Spain (Gimeno et al., 1989) and France (Allinne et al., 2009). Early

sowing, two to three months earlier than conventional sowing, induced a significant drop in temperature at planting and during early stages of vegetative growth (Allinne et al., 2009). The characterization and evaluation of sunflower genotypes adapted to low temperature conditions, during early vegetative growth stages, requires analyzing the impact of such conditions on the physiological processes associated with initial seedling vigor and plant cold tolerance. Agronomic, morphological, physiological, technological and biochemical attributes could be taken as valuable criteria to identify and select genotypes adapted to winter cold conditions. Nowadays, "Ichraq" is the only autumn variety registered in the Moroccan Official Catalogue (Nabloussi et al. 2008). It is a late maturing, winter cold tolerant and combines good seed yield and high seed oil content. Current research continues to develop new sunflower populations, resistant (or tolerant) to winter cold and agronomically performant, which would be the basis for selection of new improved varieties better than the variety "Ichraq". Thus, the present work aimed to evaluate new sunflower genetic materials for agro-morphological, physiological and technological traits under early winter planting conditions.

MATERIALS AND METHODS

Plant materials

The plant material used in this study consisted of 46 sunflower genotypes including 'Ichraq', the first and only one autumn variety, considered as check, and 45 individual selected plants derived from 'Ichraq'. As this latter is a population variety (Nabloussi et al., 2008), there was opportunity to select individual plants (PS) in order to release new germplasm that will be more performant than 'Ichraq'. The 45 PS were selected in various environments for their vigor, habit and agro-morphological performances.

Methods

The 46 genotypes were planted on 2 January 2014 at the INRA experimental station located at 'Annoceur', mountainous area known for its rough winter cold. It is located 50 km from Fez city, in the north of Morocco, at an altitude of 1350 meters. During the cropping cycle, the minimum temperature was -5°C, registered in January and February whilst the maximum temperature was 37°C, recorded in May.

Trial was conducted under rainfed conditions following a randomized complete blocks with two replications. Each genotype was sown in two 5 m rows spaced by 60 cm. In each row, plants were spaced by 30 cm. Initial N-P-K fertilization was 80-80-30 units, respectively, followed by cover N fertilization with two inputs of 40 units, one at stem elongation stage and the other at flowering stage. No phytosanitary treatment was applied.

Morphological, phenological, physiological, agronomic and technological parameters were studied. During plant vegetative growth, plant height (cm), growth rate (cm/d), collar diameter (mm), initial vigor of young seedlings (following a grading scale of 1 to 5), number of leaves per plant, leaf area (cm^2) and number of branches per plant were measured. Flowering time of each genotype was determined by counting the number of days between planting date and the date when 50% of plants of this genotype have flowered. Chlorophyll content (mg/g) was calculated according to the method of Billore and Mall (1975). The optical density (OD) of all the supernatant obtained was measured in a spectrophotometer at 645 nm and 663 nm. The concentrations of chlorophyll pigments are given by the following formulas:

CHL A = 12.7 (OD 663) – 2.69 (DO645)

CHL B = 22.9 (OD 645) – 4.56 (DO663)

At maturity, head diameter and head aborted diameter were measured (cm). After harvest, total seed yield (q/ha), seed yield per plant (g) and its components (number of seeds per propeller and 1000 seeds weight) are determined. Also, seed oil content was determined using RMN method (Oxford 4000).

Descriptive analysis of gathered data, analysis of variance and analysis of correlation were performed using different procedures of SAS program. Duncan's new multiple range test was applied to compare genotypes means.

RESULTS AND DISCUSSION

Morphological parameters

Analysis of variance showed there were significant differences ($P < 0.001$) between the 46 genotypes for all studied parameters (Table 1). Initial vigor of young seedlings varied from 1 for genotype M32 to 5 for genotype AN8, with an average of about 3.5, higher than the check vigor (3). In many studies, seedling and plantlet initial vigor was found as a good selection criterion correlated with the adaptation and the performance of genotypes under environmental abiotic stresses (Foolad and Lin, 2001). In the present work, all genotypes having an initial vigor of 4 or 5 will be selected for further evaluation and germplasm improvement. For growth rate, the overall mean was 2.31 cm/d, with a minimum of 0.53 cm/d, registered for genotype AN21 and a maximum of 3.92 cm/d for genotype M34, slightly higher than that of the check, which was 3.15 cm/d (Table 1). Genotypes having growth rate higher than that of the check will be selected. The average plant height was 147 cm, with a variation from 75 to 200 cm for M18 and K20, respectively. Plant height of the check was about 167 cm. Higher is a plant more it is susceptible to lodging and late drought (Sposaro et al., 2008). Plants with a height less than the observed average (< 145 cm) could be interesting for selection. Number of leaves per plant varied from 17 for M4 to 38 for K3, with an average of 27.5 leaves per plant. The check had 25 leaves per plant. The average leaf area was 162 cm², which is equal to the check value. The genotypes M17 and AN34 exhibited the extreme values: 24.5 and 374.85 cm², respectively. Elevated number of leaves per plant and high leaf area are correlated with high plant transpiration (Romero-Aranda et al., 2001). Thus we aimed to select those plants having less than 25 leaves and a leaf area less than 162 cm². Regarding collar diameter, genotype M7 exhibited the strongest value which was about 31 mm, whilst genotype M18 showed the lowest value which was 11 mm. The overall mean value was 20 mm and the check value was 23 mm. Like initial vigor, collar diameter in an indicator of good adaptation under stressed environments (Liua et al., 2012). Therefore, all the genotypes exhibiting a collar diameter more than the observed average (20 mm) could be selected for further evaluation. Among the 46 studied genotypes, 27 ones, including 'Ichraq' the check variety, had no branching, whilst 19 ones were branched, with a number of branches per plant varying from one to six. Genotype M30 was the most branched, having six branches per plant. The overall average was 0.93. Sunflower branching is an indicator of plants susceptibility to cold conditions (Alba et al., 2010). The plants selected for further evaluation and new germplasm constitution should have no branching.

Physiological parameters

Analysis of variance revealed significant effect of genotype on flowering earliness, chlorophyll a content and chlorophyll b content ($P < 0.001$), and non-significant effect on total chlorophyll content (Table 1). However, a large variation was observed, ranging from 28 mg/g for genotype K7 to 79 mg/g for genotype M29. The average total chlorophyll content was 43.21 mg/g, while the content concerning the check variety was 54.6 mg/g (Table 1). Genotypes maintaining high chlorophyll content under abiotic stresses, like as drought or cold, exhibit tolerance to such stresses (Yang et al 2015). All genotypes having total chlorophyll content higher than that of the check will be selected. Regarding chlorophyll a and chlorophyll b content, the genotype K4 exhibited the highest values for both types, 11.3 and 19.8 mg/g, respectively. The lowest contents were 0.86 mg/g, registered in genotype K9, and 1.74 mg/g, registered in genotype K8, for chlorophyll a and chlorophyll b, respectively. The check variety had 1.86 and 2.77 mg/g for these parameters, respectively. Vegetative period before flowering was too long, with an average duration exceeding 170 days from sowing date to flowering date. It ranged from 162 days for genotype AN21 to 180 days for genotypes M27 and M29. The check variety has bloomed in 170 days after sowing.

Flowering earliness is a desired character in environments under terminal drought stress (Ribot et al., 2012). Thus, genotypes having a sowing-flowering period shorter than that of the check will be selected.

Table 1. Analysis of variance (Mean square and significance level of differences) for agromorphological, physiological and technological traits of 45 sunflower accessions evaluated under early winter planting conditions in Annoceur 2014.

Parameter	Genotype	Average	Minimum		Maximum		value from Control Ichraq	Threshold for selection
			Value	Genotype	Value	Genotype		
IV(1)	*** (2)	3.49	1	M32	5	AN8	3	4-5
GR	***	2.31	0.53	AN21	3.92	M34	3.15	>3.15
PH	***	146.77	75	M18	200	K20	166.66	<145
NLP	***	27.46	17	M4	38	K3	25.33	<25
LA	**	162.23	24.5	M17	374.85	AN34	161.7	<162
CD	***	20.08	11.11	M18	30.82	M7	23.11	>20
CHLT	ns	43.21	28.2	K7	79.1	M29	54.6	>54
CHLA	***	4.04	0.86	K9	11.31	K4	1.86	>1.86
CHLB	***	6.77	1.74	K8	19.89	K4	2.77	>2.77
DSF	***	170.48	162	AN21	180	M29 M27	170	<170
NBP	***	0.93	0		6	M30	0	0
THD	***	12.79	6	M22 M18	23	K9	16.33	>16
AHD	ns	2.48	0		7	K3 K9	2	<2
PAD	ns	21.2	0		62.5	M32	13.96	<13.96
NSP	***	18.39	8	M22	27	K20 K8	20	>20
HSY	***	49.36	8.184	M18	110.3	K8	62.14	>62
TSW	***	47.52	12.4	M18	83.6	K8	56.86	>56
SOC	***	36.43	21.83	M8	46.85	K4	38.52	>38
TSY	***	27.42	4.54	M18	61.3	K8	34.52	>34

(1) IV: initial vigor, GR: growth rate, PH: plant height, NLP: number of leaves per plant, LA: leaf area, CD: collar diameter, CHLT: total chlorophyll content, CHLA: chlorophyll a content, CHLB: chlorophyll b content, DSF: days from sowing to flowering, NBP: Number of branches per plant, THD: total head diameter, AHD: aborted head diameter, PAD: percentage of aborted diameter, NSP: number of seeds per propeller, HSY: head seed yield, TSW: 1000 seeds weight, SOC: seed oil content, TSY: total seed yield (per hectare).

(2) *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively. ns not significant.

Agronomic and technological parameters

Analysis of variance showed there were significant differences ($p < 0.001$) between the studied genotypes for all agronomic and technological parameters, excepted aborted head diameter (AHD) and percentage of aborted diameter (PAD) (Table 1). However, one could observe some variation between genotypes for AHD and PAD (Table 1). Most of the evaluated genotypes had no AHD, and among the few ones having

AHD, genotypes K3 and K9 exhibited the largest AHD, 7 cm. The overall average AHD was about 2.5 cm. The overall average PAD was about 21%, ranging from 0%, for most of the genotypes, to more than 62%, for genotype M32. All genotypes exhibiting some AHD should be discarded from the selected population as aborted sunflower head is an indicator of plant susceptibility to cold (Hladni et al., 2010). Average total head dimeter (THD) was 12.8 cm, ranging from 6 cm, for genotypes M22 and M18, to 23 cm, for genotype K9. The check variety 'Ichraq' had a THD of 16 cm, an AHD of 2 cm and a PAD of 14%. A large range was observed for number of seeds per propeller, from 8 in genotype M22 to 27 in genotypes K8 and K20. The check variety had a number of 20 seeds per propeller. Regarding seed yield per head, the overall mean was slightly higher than 49 g, and a large range was found, from 8 g in genotype M18 to 110 g in genotype K8, which is much higher than head seed yield of the check (62 g). Thousand seed weight (TSW) ranged from 12.4 g in genotype M18 to 83.6 g in genotype K8, and the average was 47.52 g. TSW of the check was about 57 g. The average total seed yield (TSY) was around 27 q/ha and there was a large variation from 4.54 q/ha in genotype M18 to 61.30 q/ha in K8. TSY of the check was slightly higher than 34 q/ha. Total head diameter, number of seeds per propeller, single head seed yield and TSW are components of TSY which are correlated with this latter, and thus could be considered as selection criteria for seed yield breeding (Yasin and Singh, 2010). In our study, we will select all those genotypes showing values higher than those of the check. Finally, seed oil content (SOC) fluctuated from 21.80% in genotype M8 to 46.85% in genotype K4, and had a mean value of 36.43%. The check 'Ichraq' had a SOC of 38.52%, which was slightly higher than the overall average. Genotypes with SOC exceeding that of the check will be selected. Table 2 shows the pools of genotypes selected, according to described threshold, for each of the studied parameters.

Pearce 1999 subdivided the plants into three categories according to their tolerance to cold and ability to adapt to low temperatures. Susceptible plants to low temperatures that suffer damage as early as 12 °C, tolerant plants to low positive temperatures and plants capable to acclimate to survive under temperatures below zero degree. Xin and Browse 2000 showed that they are a large number of physiological mechanisms that allow plants to better withstand severe stress (temperatures below zero) after a long time at low temperature (acclimatization). Many studies have shown low temperature direct effects on cells (Pearce, 1999), on seed germination (Durr et al., 2001), on photochemical reactions of photosynthesis and carbon fixation (Liua et al., 2012). Likewise, cold causes reduction of cell water content (Kacperska, 2004).

Our findings have shown there was a genetic diversity among the sunflower genotypes evaluated for most of the studied parameters. In all cases, these genotypes were compared with the check variety 'Ichraq'. This study allowed us to identify and select genotypes more interesting than the check for morphological, physiological, agronomic and technological parameters under winter early planting conditions. Globally, taking into account all these parameters, the genotypes AN8, AN3, AN34, AN33, AN27, AN23, AN21, AN24, K30, K20, K10, K3, K8, K7, K4 seemed to be performant and promising. After confirming their performance in further seasons, they could be useful for intercrossing to develop a new variety more performant and more tolerant to winter cold than 'Ichraq', the only one autumn variety registered to day in Morocco.

Table 2. Pools of sunflower genotypes selected for their performance on basis of morphological, physiological, agronomic and technological parameters under early winter planting conditions.

Parameters	Sunflower genotype pools
IV	AN8;AN6;AN3;M45;M43;M30;M26;M17;K8;K7;K6;K5;K4; A35;A34;A33; A32;A27;A23;A21;A13;A11
RG	M34;M32;M30;K30;K20;K10;K9;K8;K5;
PH	AN8;AN3;M43;M41;M37;M34;M32;M29;M26;M27;M22;M19;M18;M17;M13;M8;M7;M6; M5;M4;AN21
NLP	AN8;AN3;M41;M37;M34;M32;M29;M27;M22;M19;M18;M17;M8;M6;M4;K8;AN33;AN27;AN21;
LSA	AN6;AN3;M45;M37;M34;M32;M29;M26;M22;M19;M18;M17;M8;M7;M6;M4;K30;K20;K9; K8;K4;K3;AN32;AN31;AN27;AN24;AN23;AN21;AN13;AN11;AN9;
CD	AN8;AN3;M45;M41;M37;M34;M30;M26;M22;M13;M7;M5;K30;K20;K10;K9;K8;K7;K6; K5; K4;K3;AN35;AN34;AN32;AN31;AN27;AN24;AN23;AN21;AN13;AN11;
CHLT	M29;M27;M22;M8;M6;K30;K3;AN31;
CHLA	All genotypes except: M45;M34;M30;K9;K8;AN35;AN31;AN23;AN9
CHLB	All genotypes except: M30;K8;AN35;AN34;AN31;AN23;
DSF	AN8;AN6;M22;M18;M7;M4;K30;K7;K4;AN35;AN34;AN33;AN27;AN31;AN24;AN23; AN21;AN13;AN9
NGB	AN8;AN6;AN3;AN45;M43;M41;M37;M13;M7;K30;K20;K10;K9;K8; K7;K3;K4;K5;K6;AN35;AN32;AN27;AN24;AN9;AN11;AN13
DTC	AN8;M45;M37;K20;K10;K9;K8;K7;K5;K4;K3;AN31;AN24;AN27;
DFA	AN3;M43;M34;M32;M27;M29;M30;M18;M7;K20;K10;K9;K8;K7;K5 ;K3;AN34;AN32;AN27;AN24;AN23;AN13;AN9
PDA	AN3;M43;M37;M34;M32;M27;M29;M30;M18;M7;K20;K10;K9;K8;K7; K5;K3;AN34;AN32;AN31;AN27;AN24;AN23;AN13;AN9
NGP	AN8;AN3;M45;M43;M41;M37;M34;M32;M30;M13;M8;M7;M6;M5;K30;K20; K10;K9;K8;K7;K6;K5;K4;K3;AN34;AN31;AN27;AN24; AN23;AN11;AN9
SYC	AN8;AN6;AN3;M45;M41;M37;M32;M30;M8;M7;K30;K20;K10;K8;K7;K4;K3; AN35;AN34;AN27;AN24;AN23;AN21;AN11;
TSW	AN8;AN6;AN3;M45;M43;M41;M37;M34;M32;M30;M18;M7;K30;K20;K10;K8;K7;K5; K4;K3;AN35;AN34;AN33;AN27;AN24;AN23;AN21;AN13;
SOC	AN8;AN6;AN3;M41;M22;M19;M17;M6;M5;M4;K30;K20;K10;K9;K8;K7; K6;K5;K4;K3; AN34;AN33;AN32;AN31;AN27;AN24;AN23;AN21;AN13; AN11;AN9
SYP	AN8;AN6;AN3;M45;M41;M37;M32;M30;M8;M7;K30;K20;K10;K8;K7;K4;K3 ;AN35;AN34;AN33;AN27;AN24;AN23;AN21;AN11;

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TESTING ANNUAL WILD SUNFLOWER SPECIES FOR RESISTANCE TO *OROBANCHE CUMANA* WALLR.

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) is a holoparasitic weed that attacks the roots of sunflower (*Helianthus annuus* L.) causing yield losses in excess of 30%. It affects mostly warm and dry regions. Development of resistant cultivars and optimization of agricultural practices are the most important tasks for broomrape control in affected countries. In Serbia, first severe infestations were recorded in the early 90s. IFVCNS breeding program for transfer of *O. cumana* resistance from wild *Helianthus* species first pointed to *H. petiolaris* ssp. *petiolaris* as an excellent donor of *Or* genes. Resistance of annual wild species to *O. cumana* has been evaluated in a long term characterization program. Starting from 1996, multiple tests were performed in the greenhouse and in the field with broomrape presence. Total of 7 annual *Helianthus* species and 182 accessions were screened for resistance. The highest percentage of accessions with no segregation for resistance was found in *H. petiolaris* (81%) followed by *H. niveus* and *H. argophyllus*. If resistance is expressed per plant, *H. petiolaris* and *H. niveus* had more than 90% of plants with no infection. *H. argophyllus, H. debilis, H. praecox* and *H. neglectus* were in the range of 77-86%, while *H. annuus* had only 37% of resistant plants and proved to be the most susceptible of the tested annual species. The obtained results pinpoint the most useful species and accessions for further work on keeping cultivated sunflower resistant to broomrape.

Key Words : wild annual Helianthus, resistance, Orobanche cumana

STUDY OF THE CHARACTERISTICS OF CULTIVATED VARIETIES OF SUNFLOWER, REGARDING THE PRODUCTION OF HIGH QUALITY SUNFLOWER MEAL WITH DEHULLING PROCESS

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ABSTRACT

Dehulling sunflower seeds, before crushing, increases the protein content in the meal up to 36%, whereas a cake obtained without dehulling contains 27-29% protein. The quality of sunflower seeds directly impacts the possibility of obtaining a high protein meal. The purpose of this study was to assess the varietal effect on the protein content and the hullability of sunflower seeds. Genetic effect was studied with seed samples from a network of variety evaluation trials in France during the two years. The protein content in seeds was expressed as a percentage of Defatted Dry Matter. Hullability was obtained by measuring the initial weight of the seeds and the weight of extracted hulls, removed by a laboratory dehulling equipment. Other measured characteristics were oil content, seed size, crude fibre content. Significant differences between varieties for protein content were observed within the medium early/medium late group in 2013 (from 33.2% to 41.3%), as for hullability (from 3.7% to 14.7%). As a consequence, the potential protein content of their dehulled meals also ranged widely (34-44%). Crude fibre content was closely correlated to hullability. An equation was established to estimate the protein content of dehulled sunflower meal as a function of protein content and crude fibre content in seeds. The protein content of sunflower seeds proved to be the key characteristic determining the quality of sunflower meal. Genetic selection, which allowed great improvements in the oil content and fatty acid composition, should therefore also help to improve the quality of sunflower meal.

Key words: Sunflower, Hullability, Protein, Variety

INTRODUCTION

Sunflower seed processing produces 2 principal co-products: oil, mainly for human consumption, and meal, for animal feed. Two main variants exist in the crushing industry: oil extraction from whole seeds and oil extraction from partially dehulled seeds. In the first process, the resulting meal is of low protein content (27-29%). In the second process, the resulting meal has a higher protein content (36% protein content is a standard quality for this type of meal) and reduced fibre. The second process is highly developed in Eastern Europe and Argentina. In France, until recently, dehulling was carried out in only one oil mill which has a limited dehulling capacity offering only a modest improvement in meal protein content. Capacity has begun to develop since 2013, with a larger factory now partially dehulling prior to crushing.

Dehulling offers 2 advantages:

- The higher protein and lower fibre content meal has an increased economic value on the animal feed market. Peyronnet *et al.* (2012) demonstrated that the interest price of a 36% protein content

meal (i.e., that maximum price at which it remained competitive) was 70% of the soybean meal price, whereas for a 29% protein meal it was only 43%.

- The hulls removed can be used as an energy source for steam production in a high-performance biomass boiler. Rising energy costs and environmental concerns have led to a growing interest within the crushing industry for using hulls as energy source instead of fossil fuels (Tostain *et al*., 2012).

In the 1980s, energy prices were very high; towards the end of the decade and the early 1990s, research was undertaken in France to prepare the crushing industry for greater use of the dehulling process. Genetic studies were carried out concerning the ease with which hulls could be removed from sunflower seeds (hullability). These studies showed that this characteristic could be introduced through breeding programmes. Cultivars producing seeds with a smaller hull mass, higher oil content but a good hullability offered the most promise for improving the quality of sunflower meal. Such genotypes were rare, but a recurrent selection programme could be used to increase the frequency of favourable genes (Denis and Vear, 1996). Although all this work constituted a favourable basis for the growth of sunflower dehulling in the French crushing industry, the technology was not developed. In France, the oil mill that only recently reintroduced dehulling actually abandoned the technique in the early 1990s, the context being one where the oil content of the new cultivars was improving but hullability was decreasing, resulting in considerable losses of oil from the hull fraction. At that time moreover, boiler technology for burning hulls had not achieved an adequate level of efficiency. So, the economics were against dehulling. As a consequence, sunflower breeding has ignored the characteristic of hullability, and likewise protein content.

The supply of vegetable protein to livestock is now a matter of political concern. Oilseed meals are an attractive source of proteins. Moreover, sunflowers have the advantage of containing no anti-nutrients or toxic components. Increasing the protein content in sunflower meal would therefore be advantageous. The quality of seeds, notably the protein content expressed as a percentage of Defatted Dry Matter (DDM) and their hullability, determines the potential protein content of the resulting meal. It has been shown that the most profitable way to reach a set requirement of protein content in the meal is to produce seeds with high protein content as a percentage of DDM, in order to require extraction of only a minimum amount of hulls (Dauguet *et al.*, 2015).

The economic focus remains on sunflower oil (about 700-800€/t in 2015, as compared with approximately 180-200€/t for non-dehulled meal and 250-280€/t for 36% protein meal). Hence, breeding has always been centred on obtaining varieties combining high yield and high oil content. These are the 2 criteria that are currently taken into account in the registration of new sunflower varieties; protein content is not a criterion in the registration of new sunflower varieties and nor is it measured in the official trials. Studies have shown that soil and climatic conditions exert a greater influence on protein content than genetics (Nel, 2001; Oraki *et al.*, 2011; Dauguet *et al.*, 2015). This can be explained by the fact that breeding programmes have not searched for variability in protein content. No relationship has been observed between oil content and protein content as a percentage of DDM (Dauguet *et al.*, 2015). So, the independence of these 2 features would suggest that there is considerable scope for improving the protein content of the defatted fraction without penalizing oil content.

Hullability increases with the size of seed and decreases with their oil content; these are varietal characteristics and so genetic improvements of hullability might be considered (Baldini *et al.*, 1994; Denis *et al.*, 1994; Evrard *et al.*, 1996; Nel, 2001; Sharma *et al.*, 2009; Dauguet *et al.*, 2015).

In a previous study (Dauguet *et al.*, 2015), we examined seed samples taken from a wide network of farmers' fields in South West France, looking at 3 varieties, over 2 years (2 varieties each year). Both protein content and hullability were found to be influenced by the environment, with water stress having a substantial effect. Some differences between cultivars could be identified, affecting protein content and

hullability. In contrast, the influence of agricultural practices such as nitrogen fertilization could not be established. In order to improve meal quality, and the competitiveness of sunflowers in the food chain, boosting the protein content of sunflower seeds through breeding would be very beneficial, so long as there was no negative effect on oil content and hullability remained adequate.

The objective of the present study, designed in close collaboration with Terres Univia, the French oil and protein crops inter-branch organization, was to improve knowledge of the sunflower cultivars traded on the French market, in particular with regard to their characteristics that impact the possibility of producing good quality meal: seed protein content as a percentage of DDM and hullability. We studied genetic and climatic effects on these characteristics, with samples from a network of varietal evaluation trials, during 2 consecutive years to evaluate the variability in of marketed sunflower varieties for these or other characteristics not taken into account in breeding programmes. We also measured crude fibre content in order to study its correlation with hullability, and the possibility of evaluating hullability using this more simple analytical result rather than employing laboratory dehulling equipment.

MATERIALS AND METHODS

Samples: Seed samples were collected from the Terres Inovia experimental network. This is constructed each year to evaluate the performance of varieties marketed in France (agronomic performance such as yield and diseases resistance; quality traits of the seeds such as oil content and Thousand Seed Weight). For the purposes of this study, additional analyses were performed on the seed samples to measure their protein content, hullability and crude fibre content. The varieties studied were oleic and linoleic types in 2 maturity groups (early or medium early/medium late). Each year, the Terres Inovia experimental network includes about 30 variety trials for each maturity group.
The seed samples collected for this study came from 2012 and 2013. Each year, we collected samples from several experimental locations, from regions where sunflowers are commonly cultivated: South-West, West and Central France (see Figure 1).

Each year, we studied the protein content of 5 to 7 different varieties in each maturity group, in the 8 different trial locations. For hullability and crude fibre analyses, the number of varieties studied was reduced to 2 per maturity group, as the measurements were time-consuming and costly.

In order to investigate the variability of profiles for protein content and hullability, we also studied each year a larger number of varieties (12 and 16) in only 2 or 3 trials (see Table 1).

Overall, the data set included 275 samples of sunflower seeds, with 40 different sunflower varieties, at 23 different locations (see Table 1).

Table 1: Cultivar distribution according to year and locations

Year	Maturity group	Trial locations (postal code of French department)	Cultivars
2012	Early	Antoigné (79), Frozes (86), Levroux (36), Saint Branchs (37), Rhodon (41), Maslacq (64)	ES Biba, Vellox, Extrasol, ES Balistic, ES Ethic
		Saint-Martial (16), Vibrac (17)	ES Biba, Vellox, Extrasol, ES Balistic, ES Ethic, Ullys, Fydgi, Volltage, ES Violetta, P64LL41, ES Atletic, SY Valeo
	ME/ML	Virson (17), Loudun (86), Vicq / Nahon (36), Lévignac (31), Tané (32), Le Saumont (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO
		Vibrac (17), Duras (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Breha, Sherllok, Dougllas, Mobill, SY Edenis, ES Akustic, NK Adagio, LG5625, ES Tektonic, ES Unic
2013	Early	Vibrac (17), Levroux (36), Meung sur Loire (45), Ivoy le pré (18), Maslacq (64)	ES Biba, Vellox, Extrasol, SY Valeo, ES Violetta, Fydgi
		Antoigné (79), Triaize (85), Trouy (18)	ES Biba, Vellox, Extrasol, SY Valeo, ES Violetta, Fydgi, SY Sanbala, P63LL78, LG5377, Bering, MAS83R, ES Lumina, ES Columbella, SY Revelio, ES Atletic, ES Balistic
	ME/ML	Antoigné (79), Benet (85), Lévignac (31), L'Isle Jourdain (32), Montagnac Auvignon (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Dougllas, ES Tektonic
		Pompertuzat (31), Tané (32), Duras (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Dougllas, ES Tektonic, Clloser, Meddia CS, LG5687HO, SY Explorer, LG5528, SY Edenis, ES Akustic, LG5625

ME/ML=Medium early/medium late

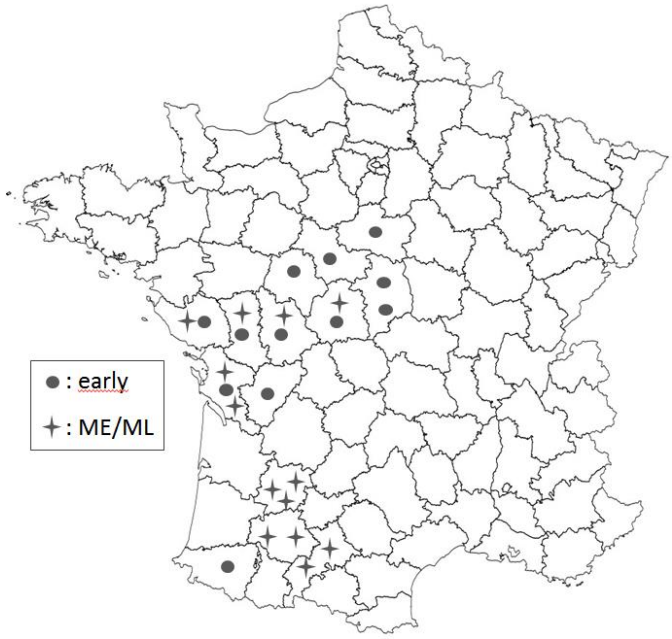


Figure 1. Location in France of the selected sunflower variety trials in 2012 and 2013, by maturity group.

Chemical analyses: For each seed sample collected, the oil content was assessed by Nuclear Magnetic Resonance (NF EN ISO 10565) and expressed as a percentage at marketing standard (9% moisture content and 2% impurities level), as commonly used in varietal trials. Protein content was assessed by the Dumas method (NF EN ISO 16634-1) and expressed as a percentage of Defatted Dry Matter (DDM) or Dry Matter (DM). The crude fibre content was measured by the Weende method (NF V03-040 with previous oil extraction by hexane), and was expressed as a percentage of Defatted Dry Matter (DDM) or Dry Matter (DM). The expression of results as a percentage of DDM, for protein or crude fibre content, has an obvious interest from an end-user perspective, as it gives information on the content that would be obtained in the meal, after oil extraction.
An indicator of the seed size, the Thousand Seeds Weight (TSW), was measured on clean dry grain (0% moisture).
While each sample was analysed for it oil and protein content and TSW, the crude fibre content was assessed in only 2 varieties in each maturity group each year.
All of these analyses were carried out at Terres Inovia's Analysis Laboratory in Ardon.
Hullability determination: What we refer to as "hullability" was obtained by measuring the initial weight of the seeds and the weight of extracted hulls, removed by a standard procedure: Hullability (%) = (mass of extracted hulls (g))/ (mass of initial seeds (g)).
Seed hullability is affected by water content (Sharma et al, 2009). Since the seeds had been stored at various levels of humidity, they were taken out of cold storage and placed in Petri dishes that were then left open for 48 hours, to facilitate equilibration of water content prior to dehulling. The water content of the seeds was low, as they had previously been dried slightly to favour long-term storage: about 5.5-6.0% (mean moisture 5.7%) and sufficiently uniform (standard deviation 0.7%) to permit a comparison of hullability.

A conical divider was used to produce 4 identical subsamples of approximately 15g from the primary sample. Three replicates were used in the dehulling test; the 4th was used to measure water content. Employing a method determined by a previous study (see Dauguet et al., 2015), the weighed samples were passed 3 times through the laboratory dehulling equipment, a Techmachine, at 2 000 revolutions per minute (rpm). This is equivalent to limited or moderate dehulling in an industrial dehulling process which would result in 10% hull extraction, whereas 15% hull removal is current practice in industry.

After sorting using laboratory sorting equipment, the various fractions (kernels, whole seeds, fines and hulls) were weighed (to the nearest 0.01 g). The percentage of extracted hulls was taken from the average of 3 replicates. Water content was assessed from the difference in seed weight before and after 15 hours in an oven at 103°C (NF V03-909).

Hullability also was measured only for 2 varieties in each trial, except for the trials with a wider range of varieties studied (2 or 3 trials each year in each maturity group) where hullability was measured for each cultivar.

Statistical analyses: Data were analysed using analysis of variance (ANOVA). F-test and differences were evaluated via the Student-Newman-Keuls Test (software SAS 9.4). The coefficients of determination, and associated probability (Student) were also established using SAS software. Shapiro-Wilk tests were performed to check the normality of the residuals; homoscedasticity was verified visually. *Calculation of the protein content in sunflower meal post-dehulling:* Given the defined quality characteristics of sunflower seeds (protein content, hullability), we developed a formula to estimate for each sample the protein content of the dehulled meal. In this way, we were able to assess the potential of a particular variety to produce meal of the required quality. This formula is based on measured values: initial seed protein content and the degree of hullability (percentage of extracted hulls), as well as assumptions regarding oil and moisture content of the meal, and the protein, oil and moisture content of the sunflower hulls. These assumptions were based on a yearly study of meal quality in the French crushing industry (Terres Inovia's unpublished results from a particular factory) and from an online database on feedstuffs (Feedipedia) for parameters on hulls.
Assumptions:

A = Oil content in meal in raw matter (RM) = 1.2%

B = Moisture content in meal = 11.5%

C = Protein content in hulls in RM = 6%

Moisture content in hulls = 10%

Oil content in hulls in RM = 2%

X = mass of removed hulls (g/100g seeds)

Formulae:

D = Defatted Dry Matter (DDM) of seeds = 1 – (moisture content of seeds) – (oil content of seeds on NMR)

E = Protein content of seeds (%DDM) = protein content of seeds (%DM)/ (1 - oil content of seeds (%DM))

F = Defatted Dry matter of the hulls = 1 – (moisture content of hulls) – (oil content of hulls) = 88%

G = Protein content of non-dehulled meal (% RM) = $E * (1 - A - B) * \left(\frac{D}{1-A-B}\right)$

H = Protein content of extracted hulls (% RM) = X * C

I = Protein content of dehulled meal (%RM) = $\frac{G-H}{D-X*F} * (1 - A - B)$

RESULTS AND DISCUSSION

Analysis of variance

The results were aggregated by maturity group and by year, see Tables 2 and 3. Here, we assessed the influence of location and cultivar on the seed characteristics in 8 trials.

Table 2: Results for the Early group cultivars grown in eight trial locations (mean by cultivar, t comparison tests at 5% and levels of significance of ANOVA of seed components).

Year	Factor		Oil content (% at marketing standards)	Thousand Seeds Weight (g DM)	Protein content (% DDM)	Protein content (% DM)	Crude fibre (% DDM)	Crude fibre (% DM)	Hullability (% extracted hulls)	Calculated protein content in dehulled meal (% RM)
2012	Cultivar	ES Biba	46.1 (B)	42.4 (BC)	34.5 (A)	16.7 (B)				
		Vellox	47.9 (A)	39.6 (C)	36.2 (A)	16.8 (B)	28.3 (B)	13.5 (B)	11.0 (B)	38.9 (A)
		Extrasol	45.4 (B)	45.4 (AB)	35.5 (A)	17.4 (B)	30.3 (A)	15.0 (A)	15.5 (A)	41.5 (A)
		ES Balistic	42.9 (C)	46.3 (A)	35.9 (A)	18.6 (A)				
		ES Ethic	46.0 (B)	42.4 (BC)	34.5 (A)	16.7 (B)				
	Level of significance	Location	***	***	***	***	**	**	NS	NS
		Cultivar	***	***	NS	***	***	***	**	NS
2013	Cultivar	ES Biba	48.1 (C)	49.7 (C)	32.4 (AB)	14.9 (AB)				
		Vellox	52.3 (A)	50.2 (C)	33.3 (A)	13.8 (B)				
		Extrasol	47.7 (C)	57.9 (A)	33.5 (A)	15.6 (A)				
		SY Valeo	48.2 (C)	50.4 (C)	31.3 (AB)	14.4 (B)	35.5 (A)	16.6 (A)	10.2 (A)	33.1 (A)
		ES Violetta	47.7 (C)	55.6 (AB)	30.9 (B)	14.3 (B)				
		Fydgi	50.7 (B)	52.1 (BC)	32.9 (AB)	14.2 (B)	35.2 (A)	15.3 (B)	9.6 (A)	34.7 (A)
	Level of significance	Location	***	***	**	**	NS	NS	*	NS
		Cultivar	***	***	**	**	NS	*	NS	NS

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per year within a column followed by the same letter are not significantly different ($P < 0.05$)

Table 3: Results for the ME/ML group cultivars grown in eight trial locations (means by cultivar and levels of significance of ANOVA of seed components)

Year	Factor		Oil content (% at marketing standards)	Thousand Seeds Weight (g DM)	Protein content (% DDM)	Protein content (% DM)	Crude fibre (% DDM)	Crude fibre (% DM)	Hullability (% extracted hulls)	Calculated protein content of dehulled meal (% RM)
2012	Cultivar	NK Kondi	44.9 (A)	41.1 (B)	35.3 (A)	17.5 (C)				
		Kapllan	44.9 (A)	42.7 (AB)	36.1 (A)	17.9 (BC)				
		Extrasol	43.6 (AB)	46.0 (A)	36.7 (A)	18.7 (AB)	29.1 (A)	14.8 (B)	16.8 (A)	43.7 (A)
		DKF3333	43.2 (B)	45.8 (A)	36.1 (A)	18.6 (AB)	30.0 (A)	15.4 (A)	15.3 (A)	41.4 (A)
		LG5656HO	42.3 (B)	44.1 (AB)	36.3 (A)	19.0 (A)				
	Level of significance	Location	***	***	***	***	**	**	**	NS
		Cultivar	***	*	NS	**	NS	*	NS	NS
2013	Cultivar	NK Kondi	47.9 (A)	49.1 (D)	35.5 (C)	16.4 (C)	36.8 (A)	17.4 (A)	9.7 (B)	37.3 (B)
		Kapllan	48.2 (A)	52.4 (CD)	38.0 (AB)	17.5 (B)				
		Extrasol	46.6 (B)	56.3 (B)	37.2 (ABC)	17.8 (AB)				
		DKF3333	46.9 (B)	51.0 (CD)	37.7 (ABC)	17.9 (AB)				
		LG5656HO	44.5 (C)	49.8 (CD)	37.4 (ABC)	18.7 (A)				
		DOUGLLAS	47.6 (A)	60.1 (A)	39.0 (A)	18.0 (AB)				
		ES TEKTONIC CL	46.0 (B)	53.7 (BC)	36.2 (BC)	17.6 (B)	36.1 (A)	17.9 (A)	14.4 (A)	41.8 (A)
	Level of significance	Location	***	***	***	***	*	**	*	*
		Cultivar	***	***	**	***	NS	NS	***	**

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per year within a column followed by the same letter are not significantly different ($P < 0.05$)

ANOVA were performed on the parameters studied using location and cultivar as explicative factors (see Tables 2 and 3). The impact of location was significant, except on hullability and crude fibre content in the early group and for the calculated protein content of the dehulled meal. This impact might be attributable to different meteorological and soil conditions affecting plant growth.

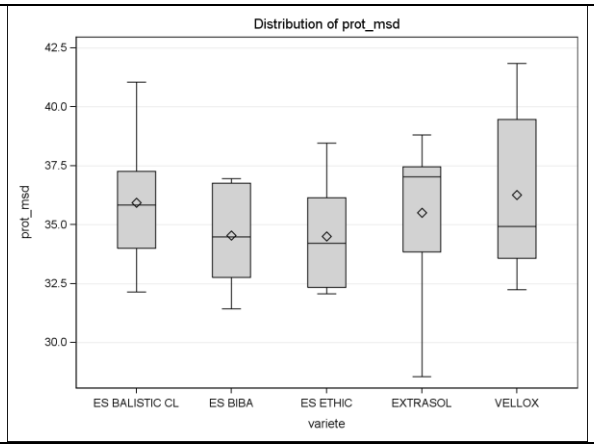
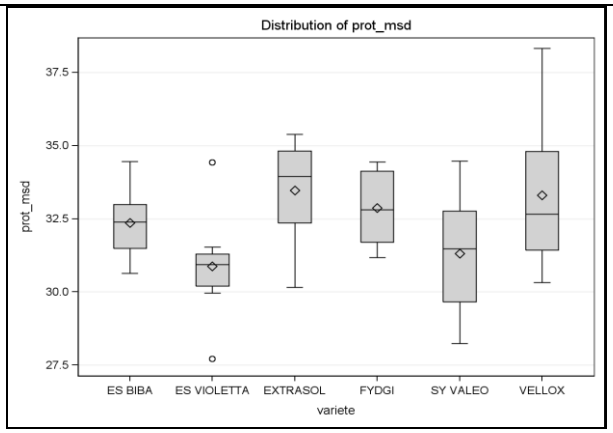
Cultivar was the principal factor affecting oil content, seed size (TSW) and percentage protein of DM. It did not however systematically affect the protein content of DDM: the variability for each variety was high (see Figure 2). Significant differences between varieties for protein content as a percentage of DDM were observed only in 2013: ES Violetta was significantly lower than Extrasol and Vellox (30.9%

versus 33.5 and 33.3%) within the early group. Within the ME/ML group, NK Kondi and ES Tektonic had significantly lower protein contents than Dougllas (35.5% and 36.2 % versus 39%). For this parameter, in 2012 the differences between locations were greater than the differences between cultivars.

For hullability, Vellox was significantly more difficult to dehull than Extrasol in 2012 (11% extracted hulls versus 15.5), and NK Kondi had also a lower hullability than Es Tektonic in 2013 (9.7% extracted hulls versus 14.4%). However, there was no significant difference between SY Valeo and Fydgi in 2013, or between Extrasol and DKF3333 in 2012, as the differences between locations were high (see Figure 3).

It is difficult to conclude from the results for crude fibre content. Crude fibre associated with higher hull content in seeds could be a favourable factor for hullability. For example, Extrasol had significantly higher crude fibre content (on DDM and on DM) than Vellox, which could be related to a better hullability, but, ES Tektonic showed better hullability than NK Kondi, although these 2 varieties had comparable crude fibre contents. This led to a conclusion that crude fibre content was not the unique factor affecting hullability. Seed size, hull structure and the phenomenon of adherence were probably important also.

The final aspect, the right hand column in Tables 2 and 3, a calculation of the potential protein content in meal that would be obtained after dehulling, based on protein content of the seeds and hullability, did not show significant genetic differences, except between NK Kondi and ES Tektonic, as the second had a better hullability and gave a richer meal. This parameter suggests that the protein content of meal could be quite high, above the standard level in high-protein meal (36%), since for some cultivars in some years it exceeded 40%. It was only in the early group in 2013 that the protein content was low for all the varieties, and hullability was moderate; so the calculated protein richness in the meal was less than the standard 36%. This highlighted the importance of initial protein content in seeds in the production of good quality meal.

Group	2012	2013
Early		

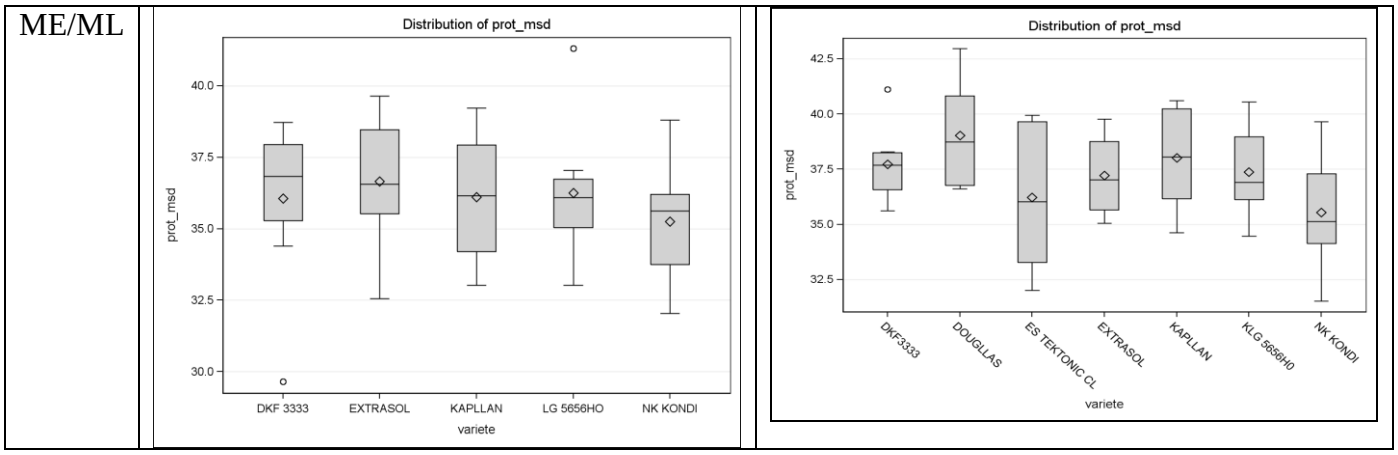


Figure 2: Boxplots of cultivar effect on protein content (%DDM) showing the median (line in the middle), mean (diamond), interquartile range (box) and total range (whiskers) not including atypical values (circle symbols, where they exist)

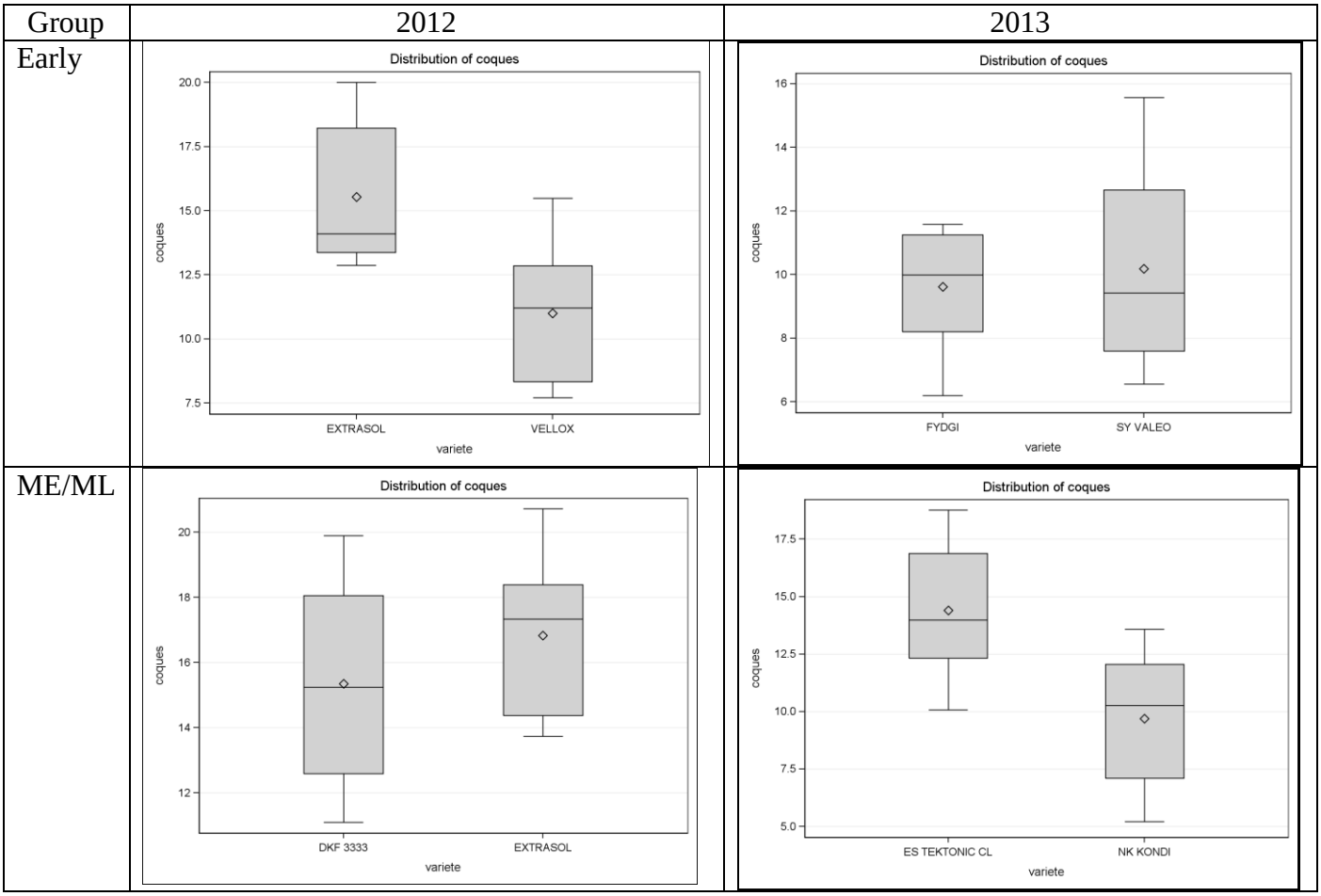


Figure 3: Boxplots of cultivar effect on hullability (% of extracted hulls) showing the median (line in the middle), mean (diamond), interquartile range (box) and total range (whiskers)

3.2. Year effect

Some trial locations and cultivars were constant in both years, which enabled the evaluation of the effect of year (including the climatic effect) (see Table 4):

- for the early group, 4 locations (Antoigné, Levroux, Vibrac and Maslacq) and 3 varieties (ES Biba, Vellox, Extrasol),

- for the ME/ML group, 3 locations (Duras, Tané, Lévignac) and 5 varieties (NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO).

Table 4: Analysis of variance for year, location and cultivar effects on seed characteristics (means, t comparison tests at 5% and levels of significance)

Factors		N	Oil content (% at marketing standards)	TSW (g DM)	Yield (t/ha at marketing standards)	Protein content (% DDM)	Protein content (% DM)
Early	2012	12	47.5 (B)	45.4 (B)	3.80 (A)	35.8 (A)	16.7 (A)
	2013	12	49.3 (A)	55.3 (A)	3.57 (A)	33.3 (B)	14.9 (B)
	Year		*	***	NS	*	**
	Location		NS	NS	NS	**	**
	Cultivar		**	*	NS	NS	NS
ME/ML	2012	15	44.3 (B)	41.6 (B)	3.42 (B)	37.4 (A)	18.8 (A)
	2013	15	48.0 (A)	47.5 (A)	3.66 (A)	37.2 (A)	17.2 (B)
	Year		***	**	*	NS	***
	Location		**	NS	*	NS	NS
	Cultivar		**	NS	***	NS	**

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means within a column followed by the same letter are not significantly different ($P < 0.05$) with t test

It was rainier and less sunny in 2012 than in 2013. During 2013, water stress occurred after flowering, which led to lower yields in the South and West of France and in the country as a whole (respectively 2.38 t/ha in 2012, and 2.14 t/ha in 2013, according to public statistics). In the studied, this trend was observed for the early cultivar group, but it was not substantial (yield 2012 3.8 t/ha and yield 2013 3.57 t/ha); while the situation was the opposite for the ME/ML cultivar group with better yields in 2012 (3.66 t/ha) than in 2013 (3.42 t/ha). This is due to the fact that the varietal evaluation trials were grown in more optimal conditions than normal farmers' fields, and therefore were not representative of national sunflower production. The climatic effect influenced the oil content and seed size (TSW), higher in 2013 than in 2012, and percentage protein content of DM, higher in 2012, which was a consequence of lower oil content in 2012. For the percentage protein content of DDM, a significant difference was observed only in the early cultivar group, with higher levels observed in 2012 than in 2013.

The wider range of varieties

Each year, 15 cultivars were sampled and analysed in 2 (2012) or 3 (2013) locations for each maturity group, to obtain some idea of the diversity of cultivar profiles for protein content as a percentage of DDM and their hullability, and for their potential to produce good quality meal.

This larger panel of cultivars made it possible to assess the potential variability of the protein content in dehulled meals. The range for the calculated protein content of dehulled meal (see Table 5) is large, with 10 percentage points between the poorest and the best cultivars within ME/ML group in 2013 (for other maturity groups and years, the range was 7 to 9 points; the results are not presented here).

Table 5: Analysis of variance for seed components from 15 ME/ML group cultivars, grown in 3 locations in 2013 (means and level of significance of ANOVA on seed components)

Cultivar	Oil content (% at marketing standards)	TSW (g DM)	Protein content (% DDM)	Hullability (% extracted hulls)	Calculated protein content of dehulled meal (% RM)
CLLOSER	49.7 (A)	53.2 (ABC)	39.0 (AB)	7.3 (CD)	39.1 (ABCD)
MEDDIA CS	49.0 (AB)	47.1 (C)	41.3 (A)	3.7 (E)	38.6 (BCD)
KAPLLAN	48.5 (BC)	53.1 (ABC)	38.9 (AB)	10.3 (BC)	41.5 (ABC)
DOUGLLAS	47.6 (CD)	61.3 (A)	39.4 (AB)	11.0 (B)	42.5 (AB)
NK KONDI	47.4 (CD)	51.3 (BC)	36.1 (BC)	9.5 (BCD)	37.4 (BCD)
LG5687HO	47.2 (DE)	48.0 (C)	35.6 (BC)	8.6 (BCD)	36.2 (CD)
SY EXPLORER	46.9 (DEF)	51.4 (BC)	36.9 (ABC)	9.8 (BCD)	38.5 (BCD)
DKF3333	46.8 (DEF)	51.8 (BC)	38.3 (AB)	7.0 (D)	37.8 (BCD)
LG5528	46.8 (DEF)	51.5 (BC)	38.4 (AB)	8.7 (BCD)	39.3 (ABCD)
SY EDENIS	46.6 (DEF)	50.8 (BC)	33.2 (C)	10.0 (BCD)	34.7 (D)
EXTRASOL	46.5 (DEF)	57.9 (AB)	38.5 (AB)	9.7 (BCD)	40.2 (ABC)
ES AKUSTIC	46.2 (DEF)	57.0 (AB)	39.2 (AB)	10.0 (BCD)	41.1 (ABC)
LG5625	45.7 (EF)	55.4 (ABC)	35.0 (BC)	14.3 (A)	39.9 (ABCD)
ES TEKTONIC CL	45.6 (F)	54.6 (ABC)	37.0 (ABC)	13.5 (A)	41.5 (ABC)
LG5656HO	44.4 (G)	50.8 (BC)	38.8 (AB)	14.7 (A)	44.4 (A)
Cultivar effect	***	***	***	***	***
Location effect	***	***	***	***	***

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per cultivar within a column followed by the same letter are not significantly different ($P < 0.05$) with Student-Newman-Keuls comparison test

Location and cultivar effects were significant for all parameters in Table 5: oil content, seed size (TSW), percentage protein content of DDM, hullability and the calculated protein content of the dehulled meal. We were able, therefore, to distinguish varieties with contrasting characteristics, not only for oil content, but also concerning parameters that affect the possibility of obtaining meal with high protein content.

Cultivars LG5656HO and Dougllas had significantly higher calculated protein content in their dehulled meal (44.4% and 42.5%) than cultivars LG5687HA and SY EDENIS (34.8% and 34.9%). However, for all other varieties, we could not draw a firm conclusion, as the differences concerning this parameter were not significant. Thus, with this wider number of varieties, various combinations of seed characteristics were identified:

- Varieties with low or medium oil content, but high protein content (as a percentage of DDM) and good or medium hullability, giving a high protein meal (LG5656HO, ES TEKTONIC, Extrasol, ES Akustic)
- Some varieties with high oil content, high protein content (as a percentage of DDM) and medium hullability, giving a high protein meal (Dougllas, Kapllan)
- Some varieties with medium oil content, poor protein content (as a percentage of DDM) and medium hullability, giving a lower protein meal compared to other varieties (SY Edenis, LG5687HO)
- Some varieties with very high oil content, high protein content (as a percentage of DDM) but low or very low hullability, giving a medium protein meal (Clloser, Meddia CS).

Turning to the economic aspect, some varieties would be more profitable than others. The outlines of an economic approach can be suggested, but would require further development if sunflower ideotypes

are to be determined. Using 2015 market data (oil price of 750€/t, 36% protein meal at 260€/t and hulls at 80€/t), and by calculating the rate of hull removal necessary to produce a 36% protein-content meal (based on the formula presented in section 2.5), we calculated the likely achievable income of some cultivars. Oil content was the main factor affecting income, with percentage protein content of DDM as the second factor (using high protein content seeds, a lower percentage of hulls can be removed to produce 36% protein meal, the quantity of which is therefore greater). The cultivars that would produce the highest expected incomes belonged to the varietal groups combining high or very high oil contents and high protein contents: Meddia CS, Clloser, Kapplan, Douglllas (494 to 505 €/ton of processed seeds). The lowest incomes were obtained for cultivars displaying low/medium oil contents: LG5625, SY Edenis, LG5656HO and ES Tektonic (472 to 477 €/ton of processed seeds).

Crude fibre content analysis could replace hullability tests?

In 2013, hullability (percentage of extracted hulls) was significantly correlated with: oil content, Thousand Seeds Weight, percentage protein content of DDM, percentage crude fibre content of DDM and percentage crude fibre content of DM (Table 6). Only percentage protein content of DM was not correlated. The closest correlation was with crude fibre content in DM (see Figure 4). The results of the 2012 correlation matrix gave the same conclusions.

Table 6: Pearson correlation matrix concerning 2013 trials (first line Pearson correlation coefficient (R), second line number of samples)

	TSW	**Protein content (%DDM)**	**Protein content (%DM)**	**Crude fibre (%DDM)**	**Crude fibre (%DM)**	**% extracted hulls**	**Calculated protein content of dehulled meal (% RM)**
Oil content	-0.38452*** 157	-0.20423* 157	-0.59629*** 157	-0.17711 *NS* 32	-0.78159*** 32	-0.59706*** 107	-0.51507*** 107
TSW		0.08842 *NS* 157	0.23748** 157	0.33684 *NS* 32	0.31760 *NS* 32	0.40733*** 107	0.39198*** 107
Protein content (%DDM)			0.90667*** 157	0.08418 *NS* 32	0.43585* 32	-0.28675** 107	0.76529*** 107
Protein content (%DM)				0.13256 *NS* 32	0.63598*** 32	-0.00416 *NS* 107	0.85291*** 107
Crude fibre (%DDM)					0.72126*** 32	0.35681* 31	0.25643 *NS* 31
Crude fibre (%DM)						0.70678*** 31	0.64457*** 31
% extracted hulls							0.38716*** 107

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

This could be explained by the fact that the percentage cellulose content of dry matter was both highly correlated with the percentage cellulose content of DDM (the richer is the whole seed in fibre, the

richer also in fibre is the defatted fraction of the seed) and oil content (the richer is the seed in oil, the greater is the reduction of the defatted fraction, which lowers the proportion of cellulose). Previous studies had shown on the one hand that hullability is significantly and negatively correlated with the seed oil content (Denis *et al.*, 1994; Dauguet *et al.*, 2015); and on the other, since the crude fibre is concentrated mainly in the hulls, that hullability is strongly and positively correlated with the seed hull content (not assessed in this present study, but demonstrated by Denis *et al.*, 1994; Baldini *et al.*, 1994; Nel, 2001). Thus, the crude fibre content as a percentage of DM incorporates both the effect of fibre content as a percentage of DDM, and the effect of oil content on hullability.

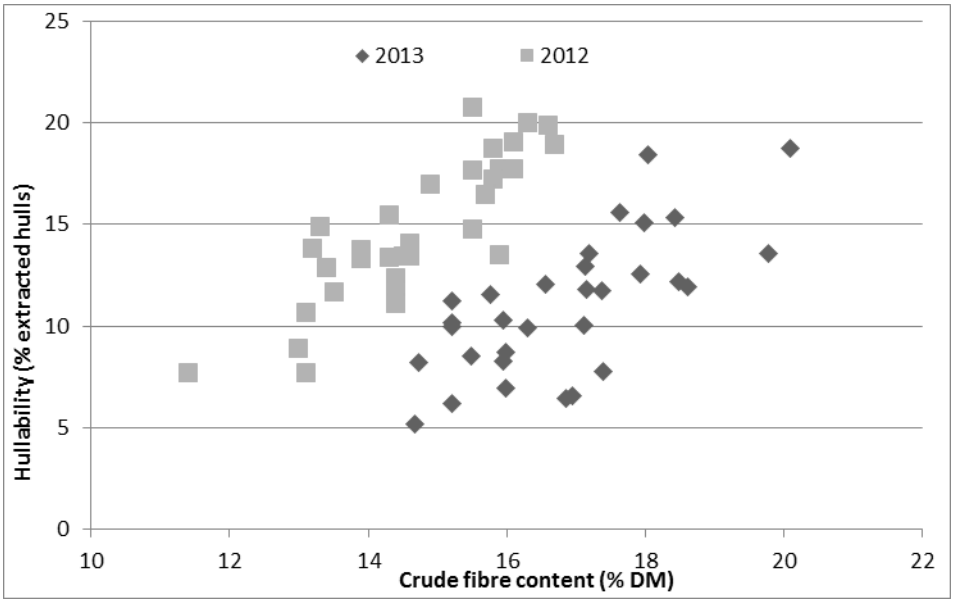


Figure 4. Relationship between on hullability (% of extracted hulls) and crude fibre content of sunflower seeds

An analysis of covariance was conducted, testing the effects of crude fibre content expressed as a percentage of DM, year and the interaction "Crude fibre DM * Year" on the extracted hull rates (Table 7). It showed a year effect, i.e. a different intercept but, no interaction effect (similar slopes).

Table 7: Analysis of covariance results for the percentage of extracted hulls variable (2012 and 2013 data)

Parameter	**Estimated value**	**Standard error**	**Pr > |t|**
Intercept	-22.85	3.57	<0.0001
Crude fibre content	2.01	0.21	<0.0001
Year 2012	8.04	0.72	<0.0001
Year 2013 (reference)	0		
R^2 model = 0.69			

The crude fibre content analyses were performed on: DKF3333, Vellox and Extrasol in 2012 and NK Kondi, ES Tektonic, Fydgi and SY Valeo in 2013. However, the climatic context was probably the most impacting parameter since hullability was lower overall in 2013 compared to 2012 (see Tables 2 and 3), which may be linked to a higher oil content in 2013 compared to 2012 (see Table 4).

Thus, if significant advances are made in the near future in the development of rapid non-destructive analysis methods, determining the crude fibre content as a percentage of DM would be an appropriate way to assess the hullability of varieties in sunflower breeding programmes. An annual calibration does, however, appear necessary.

Predicting the potential of a variety for producing a meal with high protein content?

It appears that protein content of dehulled sunflower meal (calculated data for each sample from the seed protein content and rate of extracted hulls by the method outlined in section 2.5) was most closely correlated with seed protein content as a percentage of DDM ($p < 0.0001$ and $R^2 = 0.59$, see Table 6 for 2013 data) and much less related to the rate of hulls extracted ($p < 0.0001$, $R^2 = 0.15$, see Table 6 for 2013 data). From this, it may be concluded that the initial seed protein content is of paramount importance for obtaining high protein meals.

Taking all the data for 2012 and 2013 together, we obtained Equation 1.

Equation 1. Relationship between protein content of dehulled meal and protein content as a % of DM (2012 and 2013 data, Figure 5)

Prot_dehulled_meal = 18,198 + 0,6391 * (protein content DDM) [$R^2 = 0.43$]

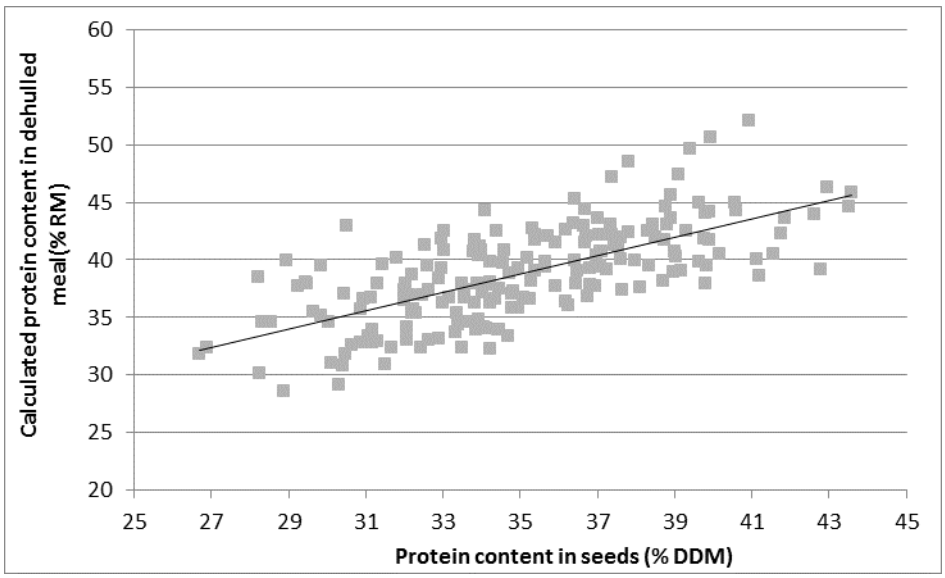


Figure 5. Relationship between calculated protein content in dehulled meal (% RM) and protein content of sunflower seeds (% DDM)

Adding crude fibre content to the model could improve the equation, as this parameter was correlated with hullability. Taking into account the year effect, as shown in section 3.4, could further improve the predictive model.

Equation 2. Protein content of dehulled meal (% RM) as a function of protein content DM and crude fibre content DM, and including year effect (2012 and 2013 data)

2013: Prot_dehulled_meal = -26.22 + 1.12*(protein content DDM) + 1.48*(Crude Fibre content DM)

2012: Prot_dehulled_meal = -20.73 + 1.12*(protein content DDM) + 1.48*(Crude Fibre content DM) Significance: General model $p<0.0001$; Intercept $p<0.0001$; protein content DDM $p<0,0001$; Crude Fibre content DM $p<0.0001$; Year effect $p<0.0001$

R^2 (model)=0.86 ; partial R^2 (protein content DDM)=0.66 ; partial R^2 (Crude Fibre content DM)=0.16 ; partial R^2 (year effect)=0.05

A calibration of this Equation 2 according year results in a more accurate estimate, and enables classification of varieties according to their capacity to produce meal with improved protein content after dehulling.

4. CONCLUSION

At present, sunflower breeding programmes do not take into account the characters of protein content and hullability. So if the crushing industry wishes to produce a high protein meal it would have to review the dehulling process. In this study, we identified sunflower varieties that combine both high oil and high protein content. The protein content of sunflower seeds proved to be the key characteristic determining the quality of sunflower meal; improvement by breeding would help to improve both meal quality and the profitability of the crushing process. Selection of varieties with particularly high oil contents could have a negative impact on hullability; it may be worthwhile checking this in order to avoid difficulties at crushing plants.

Results observed in this study proved that for selection of cultivars producing sunflower meal with more than 40% protein content is perfectly feasible without having to remove more than 13% of seed mass in hulls. This study also highlighted important environmental effects (year and location) on protein content and hullability; this indicates that cultivar selection alone is not sufficient to ensure the production of a precise quality target for the seeds, although it should reduce the risk of failing to deliver meal of a commercial standard and/or losing too much oil in the hulls extracted. The question remains open as to whether the stakeholders in sunflower oil mills would benefit from negotiating specifications with their suppliers to segregate crops that have strong potential for producing high protein meal. A framework for sharing the earnings attributable to seed protein content could be set up for farmers. Further technical and economic assessments are needed to comprehensively address these possibilities. Action on them could lead to the adoption of new breeding strategies and significant improvements in the quality of sunflower meal.

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THE B1 LOCUS THAT CONTROLS APICAL SHOOT BRANCHING IN *HELIANTHUS ANNUUS* EXHIBITS A MOLECULAR DIVERSITY LINKED TO THE BREEDING HISTORY OF HYBRIDS

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ABSTRACT

During domestication, shoot branching has been discarded for selection in sunflower. However, when selection entered the F1 hybrid era approximately 50 years ago, a recessive shoot branching gene was widely deployed in male lines in numerous breeding programs, mainly because multi-head architecture allows a more extended time window for pollen availability, thus securing hybrid seeds production. The vast majority of male lines used in breeding programs for sunflower have the branched phenotype. The *b1* locus controlling apical shoot branching mapped on LG10 in a segregating population (recombinant inbred lines) obtained from the cross between XRQ (an unbranched line) and PSC8 (a branched line). We developed two near isogenic lines (NILs) that only differed one from the other by the genomic region of the chromosome 10 containing the *b1* gene. A large F2 population (approximately 6500 individuals), derived from the two NILs, was used to reduce the genetic window containing the *b1* locus. The entire population was genotyped with two markers surrounding the *b1* locus. All recombinant plants were phenotyped and the locus was mapped in a 0.3cM window. BAC clones located in the b1 region were identified *in silico* and sequenced. The genomic region didn't fully cover the genetic interval but candidate genes were identified. Re-sequencing experiments inside and around the *b1* locus, on a set of 192 lines, allowed us to analyze the molecular diversity. We performed diversity analysis (HKA test, Tajima's D, π) in order to describe the history of the branching in sunflower. Our results suggest that the *b1* locus, including the branching gene, was under selection during domestication and modern breeding. We also developed universal molecular markers to follow this trait in breeding programs.

Key Words : shoot branching, map-based cloning, molecular diversity, breeding

EFFECTS OF OSMOTIC STRESS WITH DIFFERENT HORMON COMBINATIONS ON CALLUS INDUCTION IN SUNFLOWER ANTHERS

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ABSTRACT

Sunflower is one of the most important oil crop in the world. Recently, genetic and breeding programs involve to improve new hybrids which have better properities. Anther culture is an alternative technique to obtain desired properities in a short time. Effective callus induction is critical for successful anther culture. Callus induction can be effected by different treatments. The purpose of this research is to identify the best callus induction treatment using different hormon combinations with the pretreatment of Mannitol for osmotic stress in sunflower anthers. An oil seed variety, 08TR003, of *Helianthus annuus* L. was used for this study. Sunflowers were grown in greenhouse under semi controlled conditions (16 h photoperiod). Buds collected from mid to late uninucleate stage of microspores. Anthers were pretreated 0.5 M Mannitol solution in the dark for 5 days after transferred to B5 media supplemented with 2 mg/L 2-4D, 0.5mg/L Kin; 1mg/L 2-4D, 0.5mg/L Kin; 2mg/L NAA, 0.5mg/L BAP; 1mg/L NAA, 0.5mg/L BAP. The best result was observed using 1mg/L NAA, 0.5mg/L BAP with compared the other applications and control group.

Key Words : sunflower, callus, mannitol, anther culture

CONFECTIONERY SUNFLOWER HYBRID BREEDING IN VNIIMK (RUSSIA)

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ABSTRACT

Experiments were conducted at the Central Station (Krasnodar) of All-Russia Research Institute of Oil Crops (VNIIMK). Released, prospective and experimental sunflower hybrids and inbred lines of VNIIMK breeding were used as a material. Randomized block design was used to test the obtained hybrids. Plant density was 50 000 plants per ha. Plots were 25.2 m^2 in size and had four rows; two central rows were harvested to evaluate seed yield, oil and husk content, 1000-seed weight. Field resistance to all pathogens was registered under the natural conditions. The aim of our breeding efforts was to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid. No such commercial hybrids were available at this time in Russia. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight. Results show that the best hybrid (VK-905 A × VK-944) have significantly higher seed yield in comparison with the check. Seed yield level was rather high in the trial. Check OP variety Oreshek gave 3.23 t/ha. Oil content in the absolutely dry seeds was 454 g/kg for the check variety, and varied from 431 to 480 g/kg in the seeds of studied hybrid combinations. As a result tested hybrids could be used in two different ways (for oil production and for confectionery use) along with released confectionery OP varieties (Oreshek, SPK and Lakomka). Significantly less oil content is typical for the confectionery sunflower produced outside the Russia. To evaluate general combining ability (GCA) of our new confectionery lines we crossed two CMS-lines with four restorer lines. As a conclusion line with the best GCA value for the seed yield was VK-944 (0.40) and VK-905 A was the best line among the testers. Combination of two the most important traits (seed yield and seed size) allowed us to define the most prominent hybrids and lines. It was proved that to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid – is quite possible. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight. Three-year trial allows us to define the most prominent hybrid combination (VK-905 A × VK-944).

Key words: Hybrid – sunflower – confectionery – broomrape resistance

INTRODUCTION

Sustainable market demand for confectionery sunflower seeds made VNIIMK initiate a special breeding program with the aim to develop modern confectionery open-pollinated varieties. Dr. S. Borodin with his colleagues released three OP varieties – SPK, Lakomka and Oreshek (Gontcharov and Beresneva, 2009). Their seeds are close to the oil-type one by structure but larger in size and 1000-seed weight, has bigger husk content and less oil content (450-490 g/kg). Husk is black or black with grey stripes in color. This type of seeds has special Russian name “mezheumok” and means intermediate. People in Russia and Ukraine prefer such types of sunflower seeds for the direct consumption. The seeds also could be easily dehulled by the machinery for confectionery use. Now these four OP varieties covered more than 700 thousands hectares in Russia. Commercial success of confectionery OP varieties encouraged us to start confectionery hybrid breeding program also. This program started in 1999. Russian market demands for sunflower with 1000-seed weight 80 g or more, oil content on the level 450-490 g/kg and seeds should be easily dehulled.

As a new initial breeding material we used non-oil samples of sunflower from Iran and Syria, Russian modern confectionery OP varieties and high-oil inbred lines of our breeding with relatively big seed size.

As a result of crossing this material and self-pollination we developed a number of inbred lines for confectionery hybrid breeding. Lines were crossed with CMS-lines to test their ability to restore pollen fertility. So we found some restorer lines and some maintainer lines. Several of such lines were converted to CMS-lines by back-crossing.

The aim of our breeding efforts was to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight.

MATERIALS AND METHODS

Experiments were conducted at the Central Station (Krasnodar) of All-Russia Research Institute of Oil Crops (VNIIMK). Krasnodar region is situated in the Southern part of Russia near the Black Sea. Climatic conditions are very favorable here for sunflower production. Sunflower usually covers about 0.5 million ha in this region.

Released, prospective and experimental sunflower hybrids of VNIIMK breeding were used as a material. To produce confectionery hybrids we used two CMS-lines of our own breeding (VK-905 A and VK-934 A). Restorer lines were developed from crosses of our elite lines with dolichocarpous sunflower. The most interesting sample was bought in the local Iranian market. It was very specific dolichocarpous sunflower *Helianthus annuus var. armeniacus Wenzl.& Anashcz* (Anaschenko, 1971). This botanical variety of cultivated sunflower considered to be the most genetically distant from usually used sunflower cultivars. Main traits for individual selection were early flowering time (Iranian sample was very late in our conditions), short stem (initial material population was very tall – up to 3 m and more), bigger seed and kernel size, resistance to diseases.

Randomized block design was used to test the obtained hybrids. Plant density was 50 000 plants per ha. Plots were 25.2 m2 in size and had four rows; two central rows were harvested to evaluate seed yield, oil and husk content, 1000-seed weight. Field resistance to all pathogens was registered under the natural conditions. Shneiter and Miller's method (1981) was used for phenological observations. Oil content was evaluated by NMR-analyzer.

RESULTS AND DISCUSSION

New breeding program with the aim to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid – started at VNIIMK in 1999. As a first result the most prominent hybrid combination Katyusha (VK-905 A × VK-944) was put in the State trial (table 1).

Oil content in the absolutely dry seeds was 454 g/kg for the check variety, and 477 g/kg in the seeds of studied hybrid combination. As a result seeds could be used in two different ways (for oil production and for confectionery use) along with released confectionery OP varieties (Oreshek, SPK and Lakomka). Significantly less oil content is typical for the confectionery sunflower produced outside the Russia. But such material had no commercial success here.

1000-seeds weight of all tested hybrid combinations was higher 80 g, thought superiority of check variety was obvious. Comparison of 1000-seeds weight of all tested combinations showed big variation for this trait. 1000-seed weight varied from 79.1 g (VD-354 × K-3) to 109.9 g (VK-905 × K-3).

To evaluate general combining ability (GCA) of our new confectionery lines we crossed two CMS-lines (VD-354 A and VK-905 A) with four restorer lines (K-1, K-3, K-4 and K-5). CMS-lines were used as testers. Obtained hybrids were tested for the seed yield. Analysis of results allowed us to calculate GCA values (Table 2).

Table 1. Trial results of new confectionery sunflower hybrid Katyusha (Krasnodar, 2009-2011)

Hybrid or OP variety	Seed yield		Oil content, %	Oil yield		1000-seed weight, g
	t/ha	± to check		t/ha	± to check	
Oreshek (check)	2.29	-	45.4	0.94	-	117.2
Katyusha	2.66	+0.37	47.7	1.14	+0.20	106.8

After three years of State trial this hybrid was released.

Table 2. General combining ability evaluation of confectionery sunflower lines for seed yield (Gontcharov and Beresneva, 2011)

Line	Type	GCA value
K-1	Paternal (pollinator) line	-0.35
K-3	Paternal (pollinator) line	0.13
K-4 (VK-944)	Paternal (pollinator) line	0.40
K-5	Paternal (pollinator) line	-0.18
VK-905 A	Mother line (tester)	0.17
VD-354 A	Mother line (tester)	-0.17

As a conclusion line with the best GCA value for the seed yield was K-4 (0.40), average value was demonstrated by K-3 line (0.13). Other two lines showed poor results. VK-905 A was the best line among the testers. Next breeding effort allowed us to develop new CMS-line 934 A (confectionery type) and to identify restorer line VK-930 (oil-type) with high combining ability. New hybrids were tested in 2015 (Table 3).

Table 3. Trial results of new confectionery sunflower hybrids (Krasnodar, 2015)

Hybrid or OP variety	Seed yield		Oil content, %	Oil yield		1000-seed weight, g
	t/ha	± to check		t/ha	± to check	
Oreshek (check)	3.49	-	44.5	1.40	-	104.1
VK-934 A×VK-930	4.12	+0.63	45.6	1.69	+0.29	85.2
VK-934 A×VK-944	3.92	+0.43	39.4	1.39	-0.01	106.6
VK-905 A×VK-930	3.61	+0.12	47.2	1.53	+0.13	75.6
LSD_{05}	0.26					

It was proved that to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid – is quite possible. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight. The most prominent hybrid combinations (VK-934 A×VK-930, VK-934 A×VK-944 and VK-905 A×VK-930) are recommended for the future trials. Their parental forms (CMS-lines VK-905 A and VK-934 A and restorer lines VK-944 and VK-930) will be used for the future breeding work.

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POPULATION STRUCTURE, LINKAGE DISEQUILIBRIUM AND ASSOCIATION MAPPING FOR MORPHOLOGICAL TRAITS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower is one of the most important oil crops worldwide. Ago-morphological traits are important for sunflower breeders in selection of genotypes with high performance and other traits. The objectives of this study were to determine the population structure and linkage disequilibrium among 106 dispersed sunflower genotypes and to identify the genomic regions associated with agro-morphological traits using the association mapping approach. High genetic variability was observed among the sunflower genotypes for the studied agro-morphological traits. In molecular experiments, the genetic variability among the genotypes was assessed by using simple sequence repeat (SSR, or microsatellite), inter-retrotransposon-amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP) markers. In this study, 248 loci were detected using 28 IRAP and REMAP primers and also a total number of 67 alleles were detected using 30 SSR loci. The studied sunflower lines were divided into two subpopulations using IRAP+REMAP data and into five subpopulations using SSR data. By using a mixed linear model procedure, 224 loci showed significant association with quantitative trait loci (QTL) controlling the investigated traits. The identified and associated markers are expected to be useful in marker-aided selection in sunflower breeding programs

Key Words : Association Mapping, Linkage Disequilibrium, Microsatellite, Mixed Linear Model, Retrotransposon-Based Molecular Markers, Sunflower

MAPPING QTL CONTROLLING SALT TOLERANCE INDICES IN SUNFLOWER (*HELIANTHUS ANNUS* L.)

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ABSTRACT

Salt stress is an important limiting factor for plant growth. Using selection indices, it is possible to identify cultivars with high yield in normal and stressed conditions. So, the genetic analysis of salt tolerance indices can play important role in plant breeding programs. In order to identify molecular markers associated with salt tolerance indices in sunflower, recombinant inbred lines produced from a cross between RHA266 and PAC2, were studied in a factorial experiment with a completely randomized design with three replications under normal and salinity stress conditions. Stress tolerance indices such as mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), stress tolerance index (STI), yield stability index (YSI) and tolerance index (TOL) was calculated based on yield data under normal and salinity conditions. High correlation was observed between yield under normal and salinity conditions with geometric mean, geometric mean productivity and harmonic mean. So, these indices are introduced as most appropriate measures to identify sunflower lines tolerant to salinity stress. Based on three dimensional plots constructed by yield in normal and salt stress conditions and each one of appropriate indicators (GMP, MP and HM), lines such as C86, C61, C142, C134a, C62, C70a, LR1, C153, C108, C6, C106, C98b and C148 are considered tolerant lines. Using composite interval mapping, a total of 9 QTL were identified for salt tolerance indices. The results indicate co-localization of the identified QTL for GMP, MP and HM in linkage group 14 with QTL identified for grain yield under salt stress conditions.

Key Words : Biplot, Molecular Markers, Stress Tolerance Indices, Sunflower

GENETIC DIVERSITY OF SUNFLOWER (*HELIANTHUS ANNUS* L.) LINES UNDER NORMAL AND SALT STRESS CONDITIONS USING MULTIVARIATE STATISTICAL ANALYSIS

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ABSTRACT

To study genetic diversity of several agronomic and physiologic traits and the effect of salt stress on these characters in 100 inbred lines of sunflower, an experiment was conducted as a split-plot based on randomized complete block design with three replications outside the greenhouse in an open air area under natural environmental conditions with 2 salinity stress levels (0 and 8 dS/m) in Research Station of University of Urmia in 2014. Analysis of variance revealed significant differences among genotypes for all studied traits, indicating the existence of genetic variation among population. The highest coefficient of genetic variation was observed for head dry weight, plant grain yield and the lowest for date of flowering time in both stressed and non-stressed conditions. The results of correlation analysis showed that there is significant and positive correlation between seed yield per plant with most of the studied traits in both stress conditions. Stepwise regression analysis revealed that under salt stress condition 40.3 percent of seed yield per plant variation was determined by head diameter, one hundred seed weight, bottom leaf length, leaf number, bottom petiole length, upper leaf width, chlorophyll concentration and in normal condition 30.3 yield grain per plant variation explained by head diameter, one hundred seed weight and plant height. Cluster analysis grouped lines into 3 clusters in each one of normal and salt stress conditions but the disruption of lines within groups were different depending to stress environment that present the genetic variability for salt tolerance in sunflower lines.

Key Words : Cluster Analysis, Genetic Correlation, Phenotype Correlation, Salt Stress, Split-Plot, Sunflower

FOUR DECADES OF SUNFLOWER GENETIC RESOURCES ACTIVITIES IN INDIA

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ABSTRACT

Sunflower crop is introduced in India during early 1970's with commercial hybrids, parental lines and germplasm accessions received from USA and erstwhile USSR. The work on collection, evaluation and maintenance of sunflower germplasm was carried out at Germplasm Management Unit (GMU) located at the Project Coordinating Unit (PC, Unit Sunflower), Bangaluru from 1983 till 2001. During 2001 PC unit sunflower was transferred to Indian Institute of Oilseeds Research (IIOR). IIOR gene bank maintains 3273 sunflower accessions under the Germplasm Management Unit. The collection includes germplasm **(**GMU 1200**)** exotic collection (350), genetic stocks (97), inbreds (360), populations (5), gene pool (GP) for high oil, yield and autogamy (390), back cross converted lines (15) and wild species (42) including their derivatives (154). Screening of wild *Helianthus species*lead to the identification of both annual and perennial species confirming resistance to major biotic stresses *viz*., powdery mildew, *Alterneria helianthi*, rust and *Spodoptera litura*. Appropriate strategies are being developed for utilization of the resistance sources in introgression breeding progrmamme. Characterization of the germplasm resulted in identification of 37 trait specific germplasm *viz*., high yield (7), high to medium yield coupled with medium to high oil% (8), high oil (6), early maturity (3), dwarfness (4), late maturity (2), powdery mildew tolerance (2), white pollen(1), high 'p' acquisition(1), high oleic acid content (2) and ornamental type (1). Recently a total of 660 germplasm accessions including core germplasm are augmented in the gene bank from European countries. Wide variability among the available accessions exists for key quantitative traits like seed yield/plant (3 to 55 g), oil content (14 to 42 %), 100-seed weight (2 to 16.0 g) and plant height (50 to 360 cm). Utilization of germplasm resulted in identification of two sunflower varieties from Solapur centre i.e. Phule Bhaskar (SS 0808) from germplasm selection (GP-688-1) and Bhanu from gene pool selection (GP-775). Till date 21 varieties; populations and 35 hybrids were released in India. The present focus is an augmentation of trait specific germplasm and utilization of promising cultivar germplasm and wild *Helianthus species* in inbred development, maintainer/restorer gene pool development, parental lines improvement and resistance breeding programme.

Key Words : sunflower , gene bank, genetic resources , utilization, India

QTL MAPPING FOR BROOMRAPE (*OROBANCHE CUMANA* WALLR.) RESISTANCE IN SUNFLOWER

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) is one of the most important biotic factors *causes* reduction in sunflower *yield. Although breeding for* broomrape resistance is most effective method to control the disease, development of cultivars resistant to broomrape is not easy due to quantitative nature of the resistance. Although few QTLs were identified for broomrape race F resistance, none of the QTLs is suitable for marker assisted selection (MAS) due to small effect. In the present study three major QTLs were identified on LG7, LG11 and LG12 for broomrape race F resistance in sunflower by using high density SNP map constructed by *genotyping by sequencing (GBS) approach. Total* phenotypic variation (PVE) explained by the identified QTLs was 82%. This is the first report of *QTL mapping for* broomrape race F resistance *using a high density SNP map.* QTLs identified in this study will be valuable molecular genetics tools for broomrape resistance.

Key Words : *Helianthus annuus*, molecular breeding, genotyping by sequencing (GBS), broomrape race F

PERSPECTIVE AND CHALLENGES TO DEVELOP HIGH YIELDING, DISEASE RESISTANT AND OIL QUALITY SUNFLOWER HYBRIDS IN INDIA

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the third important oilseed crop in the world after soybean and groundnut. It is grown over an area of 25.6 m ha with a production of 44.8 m. tons and average productivity of1749 kg/ha in the world. Due to its wide adaptability, it is grown in all the continents. Important sunflower growing countries are Argentina, CIS countries, France, Spain, USA, China and India. China, France, Turkey are the highest yielding countries with an average yield of >2 tonnes/ha as against the lowest yielding countries like Kazakistan, Myanmar and India with <1 tonne per ha. Russia and Ukraine have largest share of about 50% in total sunflower production in the world. The crop has become an important oilseed crop in India covering an area of 0.55 m ha with a production of 0.41 m tons with the average yield of 752 kg/ha.(Annon.2014). In India the cultivation of sunflower is confined to southern states of Karnataka, Tamil Nadu, Maharashtra and Andhra Pradesh. During the last 20 years, the crop has made a dent in nontraditional areas in the northern states of Punjab, Haryana and Uttar Pradesh, in Spring/zaid season. The productivity of sunflower in these states is highest (2111 Kg/ha) in the country.

HISTORICAL DEVELOPMENT

Breeding in sunflower began around 1912 in the former Soviet Union and the most successful early breeding programme was that of V.S.Pustovoit. The concerted efforts for four decades resulted in increasing oil content from about 30 to 52%. High oil sunflower varieties, such as Peredovik, Armavirskiy 3497, Mayak,VNIIMK 8931, VNIIMK 6540 and Smena developed by V.S.Pustovoit and his associates enabled the spread of sunflower crop not only in Soviet Union but also to other continents. In the 1940s, Putt in Canada developed shorter, early maturing cultivars (Miller,1992). Resistance to rust was incorporated from wild species. Breeding programs were started about the same time in Argentina and several other countries. In the USA, Kinman began breeding programme around 1950, intensive breeding programmes were pursued in several countries around the world, as a result of which sunflower is now grown over a large area in many countries.

Development of hybrids in maize and other crops stimulated sunflower breeders to work towards developing hybrids in sunflower. Kinman in USA, Putt in Canada and several other workers started a hybrid production scheme taking advantage of self-incompatibility system in the crop (Fick, 1978). Although a high per cent of hybrid seed could be obtained on the female line, the % seed set varied with the lines and environmental conditions. Evolving hybrids using genetic male-sterility was developed by Vranceanu (1974) in Romania. Male-fertility (MS ms) was linked with red anthocyanin pigmentation enabling rouging of fertile plants in the female line in the seed production plots. Hybrid seed production cost was high in view of the labour requirement to remove fertile plants.

The landmark in the development of commercial sunflower hybrids was the discovery of cytoplasmic male-sterility by Leclercq (1969) in the progeny of a cross between Helianthus petiolaris Nutt and cultivated sunflower. The system was stable as evidenced by sterility obtained in the progeny of male-sterile plants crossed with fertile cultivated sunflower plants. Genes for genetic restoration of

fertility were found in the wild species by Kinman (1970). Subsequently, Leclercq (1971), Enns et al. (1970) and Vranceanu and Stoenescu (1971) also reported fertility restoring genes. First commercial hybrids based on cytaoplasmic male-sterility were made available in1972 in the USA. Subsequently, the cultivation of sunflower hybrids spread to all parts of the world.

Development of Hybrid Sunflower in India

The value of hybrids and heterosis breeding was recognized with the inception of AICRP on sunflower in 1972-73. Experimental hybrids were developed at Bangalore in 1974-75 using 4 CMS lines(CMS 2, CMS 124, CMS 204 and CMS 234) and 2 restorer lines (RHA 266 and RHA 274) introduced from the USA. All the hybrids were distinctly superior to the check variety EC 68415 both in seed and oil yield .Thus, the first sunflower hybrid BSH-1 (CMS 234A X RHA 274) was released for commercial cultivation in 1980(Seetharam et.al. 1980).Since then the hybrid base has been further widened in the country through extension of heterosis breeding work to other research centres. Many hybrids have been developed by different Public and private Institutes/universities (Table 1).

HETEROSIS STUDIES

Heterosis studies carried out in sunflower have been presented for inter-line, inter-varietal and top cross hybrids involving genetic and cytoplasmic male sterility. Kovacic (1960) in a study of inter varietal crosses observed superior response with an increase in seed yield. But only few lines exceeded the parents in oil content. However, F_1s were more vigorous and flowered earlier than the parents. Popov and Lazarav (1963) developed inbred lines from high yielding varieties and reported that single cross and top cross hybrids surpassed their parents in oil content and seed yield. Some inter-varietal hybrids exceeding their parents in seed yield were also obtained. Schuster (1964) observed heterosis for seed yield to the extent of 70%, heterosis for plant height to an extent of 47% and heterosis for head diameter was upto 60 percent.

Leclercq (1971) observed heterosis to an extent of 12-40% over standard variety Peredovik. Shuravina (1972) found that 16 out of 24 hybrids exhibited heterosis over tester parent to an extent of 39% and 20% for seed weight and seed yield, respectively. In another study, 14 out of 18 hybrids showed heterosis upto 90% for seed weight and 40% for seed yield. However, ten hybrids had reduced hull per cent and three exhibited heterosis upto 4.8% for oil content. Kloczowskii (1972) observed heterosis upto 90 to 160% for seed yield in the F_1, but in F_2 achene yields and oil content were dropped by 20 and 4%, respectively. Fick and Zimmer (1976) reported an increase in yield upto 31% over Peredovik in hybrids. Hybrids were also found to have higher oil content. Kloczowskii (1975) reported heterotic effect upto 43% for achene yield in inerline hybrids while in line x variety hybrids it was18%. In a diallel crosses between short and tall varieties.. The highest yielders were obtained by crossing short lines, families and varieties with variety Cernyanka-66. Skoric (1977) observed that four single cross hybrids yielded 25 to 30% higher seed yield, earlier, shorter and more resistant to diseases than Peredovik and Vniimk-8931. The hybrids had approximately three % higher oil content.

Seetharam *et al*. (1977) while studying the performance of hybrids produced by four cytoplasmic male sterile and two fertility restorer lines observed a significant positive heterosis for days to flower, plant height, head size, test weight, oil content and seed yield. But heterosis was not significant for stem girth and number of leaves. BSH-1 and BSH-2 were considered as best hybrids which surpassed the check variety EC-68415 by 30% in seed yield. In a study of single, double and three way cross hybrids. Shrinivasa (1982) observed a significant heterosis for plant height, stem girth, head diameter and yield per plant in all nine crosses, heterosis for oil content was significant only over the mid parental valve and was negative for 100 achene weight.While evaluating100 F1 involving 20 inbred lines and 5 pollen parents, Choudhary and Anand (1984)observed 62.3% heterosis for 1000-seed weight, 62.8% for seed yield,

64.6% for head diameter, 23.2% for oil content and negative heterosis of 7.7% for days to flowering over better parent.

Singh *et al.* (1984) in a study on performance of variety x inbred crosses observed heterosis for yield to an extent of 47-206%. The studies of Shivaraju (1984) on ten F_1 hybrids indicated an average heterosis to an extent of 175% for seed yield, 129% for number of filled seeds, 39%for head diameter, 22% for stem girth and 7% for oil content. Majority of the hybrids showed negative heterosis for days to 50% flowering and days to maturity.

Among the 49 hybrids studied, Reddy *et al*. (1985) recorded heterobeltiosis for achene yield and oil percentage in 46 and 41 hybrids, respectively. In eight hybrids, heterobeltiosis for achene yield exceeded 100% while in ten, heterobeltiosis for oilyieldwas10%. Giriraj *et al*. (1986) by crossing five CMS lines with two restorers observed average heterosis of -8% for days to flowering and 192% for achene yield per plant. Low heterosis was exhibited for oil content and number of leaves. In a study of 18 hybrids. Wali (1987) observed that heterosis varied considerably for yield and its component characters. The highest heterosis of 259% and 363% over mid-parent was observed for seed yield per plant in summer and kharif seasons, respectively. Heterosis was high and positive for leaf area index,100 seed weight, head diameter and number of filled seeds per plant while it was negative for days to flowering over both mid-parental and better parental value.

Fernandez et al. (1989) crossed lines breeding true for oil with high oleic acid (at least 85%) with standard lines having an oleic acid content of 30%. They analyzed the oil of F_1 seed and showed that high oleic acid content was a dominant trait and had maternal influences. Dedio (1993) observed heterosis for kernel oil content as well as achene oil content. In a comparative study of single cross and three-way cross hybrids, Naresh (1993) indicated that more number of three-way cross hybrids have manifested significant positive average heterosis for all the characters studied except days to 50% flowering and seed filling. The average heterosis registered for seed yield ranged upto 128%.

Disease resistance:

Sunflower (*Helianthus annuus* L.), is prone to attack by several pests and diseases (Mayee, 1997).
Several diseases are known to cause yield loss in sunflower. In India the important diseases are: alternaria leaf spot caused by *Alternaria helianthi*; rust caused by *Puccinia helianthi*; downy mildew caused by *Plasmopora helstedii* and various root and stem rots caused by *Sclerotium sp.* and *Rhizoctonia sp*. But little information is available about genetic control of disease resistance. Wild species of sunflower are known to harbor genes for resistance against diseases.

Sunflower Necrosis Disease:

Sunflower cultivation was seriously affected in India by an unusual necrosis disease caused by Sunflower necrosis virus (SNV). It was first observed in Karnataka state in 1997 and in the subsequent years it spread to other states viz., Tamil Nadu, Andhra Pradesh and Maharashtra with the average disease incidence up to 50%.Necrosis appears on the part of leaf lamina near the mid rib resulting in twisting of leaf and then extends through one side of the lamina to the petiole and stem and finally terminate to the shoot of the plant leading to partial paralytic symptoms . Necrosis at bud formation stage leads to partial twisting of the capitulum .Thrips suspected to act as a vector in transmission of this disease. No resistance source has been reported so far. However, through management this disease can be controlled Sunflower necrosis virus disease (SNVD) became a major threat to the successful cultivation of all the sunflower hybrids and varieties and devastating the crop since 1998. Significant reductions in terms of total crop loss up to 90% were reported due to early infection in the farmers' fields (Bhat et al., 2001; Ramaiah et al., 2001a; Lavanya et al., 2005). This has resulted in the substantial loss of sunflower production to 0.733 M tones in 2000–2001 in comparison to 2.0 M tones in 1998–1999 (Bhat et al., 2002a; Jain et al., 2003). According to Ravi et al. (2001) SNV belongs to the ilarvirus sub group I and is related to tobacco streak virus (TSV) as the former shared 90% amino acid sequence identity with the latter. It has been

reported that the SNV is a single stranded circular RNA virus with isometric virions; the sunflower ilarvirus was related to TSV on the basis of coat protein gene sequence (Prasada Rao et al., 2000; Bhat et al., 2002b). Initially, Jain et al. (2000) reported that the SNV was associated with tospovirus, but later it was confirmed that the ilarvirus, antigenically related to TSV was associated with SNVD (Jain et al., 2003). A disease similar in nature to SNVD has been reported in the Netherlands (Dijkstra, 1983) and Australia (Brunt et al., 1996). Thrips mediated SNV transmission has already been reported in sunflower (Jain et al., 2003; Lokesh et al., 2005). Notably, a groundnut (Arachis hypogaea L.) isolate of TSV was transmitted by a thrip, Frankliniella schultzei Trybom (Reddy et al., 2002). Although ilarvirus is transmitted through seeds (van Regenmortel et al., 2000), there is no report confirming the transmission of SNV through seeds in sunflower. Limited attempts were made for the management of SNVD using border crops and insecticides mainly to control the SNV carrier, thrips (Jain et al., 2000, 2003; Ramaiah et al., 2001b). Apart from vector control, no effective control measures are available for the management of SNVD. Therefore, the majority of the farmers depend only on chemical control of the insect to minimize the virus spread, despite the fact pesticides can be hazardous to the environment and public health. In this scenario, biological control, an eco-friendly disease control. strategy is worth testing as a supplement or an alternative to chemical control. Application of biocontrol agents (BCAs) is an important strategy in crop protection against plant pathogens. The most important control to this disease is to develop sunflower hybrids which show resistance to this virus.

Alternaria leaf spot:

It is a common disease in many countries but causes more damage in India. Only field resistance has been reported in cultivated sunflowers and no information is available on the inheritance of resistance to this disease.

Rust:

Rust is one of the most destructive diseases of sunflower in the world and can appear throughout the plant growth. Racial differentiation exists in this fungus. So far, four races and the corresponding resistant genes have been identified. Resistance to rust found commonly among wild species of *Helianthus* has been successfully incorporated into commercial cultivars. In India, although rust is of a common occurrence, the race pattern is not known.However, resistant source for local races have been identified and incorporated in the hybrids under cultivation.

Downy mildew:

At present, downy mildew is more serious under temperate conditions than in tropics. Its occurrence in India was reported in Maharashtra in 1984 (Mayee and Patil, 1986). The pathogen shows wide variation with many races and corresponding resistant genes in the host. Resistance to each race was found to be controlled by a single dominant gene. Apart from major genes several modifiers are also known to influence resistance. The resistance to the disease appears to be rare in cultivated annuals but more common in wild perennials. One of the popular restorer lines, RHA 27A, has the P12 gene for resistance to races 2and 3.

Wilt: Resistance to wilt is present in both cultivated and wild sunflowers. The
available information indicates that resistance is both simple and complex depending upon the material involved in the study.

This disease was reported first time in India during 1997 in Bagepally area of Kolar distt. in Karnataka and also in Rangareddy distt. of A.P.This disease was also reported in Parts of Maharastra and TamilNadu.

High Oil Quality hybrids:

Oil percentage and fatty acid profile is an important trait to develop high quality hybrids of sunflower. Several environmental factors also influence oil percentage as well as fatty acid composition. Low temperature at seed development stage increases the linoleic component and decreases the other fatty acids whereas it is reverse under high temperature conditions (Sheoran *et. al*, 2014.) For this purpose breeder must go for selection of inbred lines which shows high oil content and fatty acid profile and

emphasis should be laid on the stable lines so that the hybrids with high yield and high oil quality trait could be developed.

Perspective

Sunflower has become a crop of major economic importance worldwide. It is cultivated mainly as an edible oil seed crop. As a source of edible vegetable oil, it is one of the important oil seed crops in the world. Sunflower has made a significant dent in a number of tropical and temperate countries because of the following desirable features including wide adaptability of the crop enabling its cultivation in different agro-climatic regions and soil types. Being day neutral, the crop can be grown in different seasons. Being a short duration crop, it can fit into various multiple cropping systems. Ideal crop for contingency cropping plans. The versatile nature of crop and its increasing contribution to oilseeds production calls for concerted efforts to evolve hybrids with higher productivity. To achieve quantum jumps in the productivity levels among the large areas of Asia, Africa and other countries, production of quality hybrid seed is required. Further to keep pace of the new challenges, broadening the genetic base of male sterile as well as restorer lines, development of superior hybrids and supply of genetically pure hybrid seeds to commercially exploit maximum heterosis assumes greater importance. The gains in productivity of sunflower crops have been achieved primarily through exploitation of available genetic variability Conventional breeding coupled with modern tools such as biotechnology should now be the primary focus in crop improvement programs. Heterosis breeding should be the major focus in this crop. To facilitate better exploitation of the available gene pools and overcome the production constraints, research emphasis needs to be on (i) augmentation/ identification of trait specific germplasm; (ii) prebreeding and genetic enhancement; (iii) allele mining, (iv) functional genomics, proteomics, metabolomics, and interactomics; (v) marker assisted breeding and gene pyramiding; and (vi) trait improvement through genetic engineering.

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MOLECULAR AND GENETIC ASPECTS OF SUNFLOWER DEFENSIVE RESPONSE TO DOWNY MILDEW

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ABSTRACT

Sunflower represents one of the most important oilseed crops worldwide. In Republic of Moldova it is placed third after wheat and maize according to cultivated area. An excessive extension of cultivated areas and high susceptibility of this crop to wide number of diseases determine necessity of obtaining of resistant hybrids. One of the most devastating pathogens of sunflower is downy mildew (DM) *Plasmopara halstedii*, which causes significant yield losses in rainy years. In this context, the aim of this study was to determine resistance potential among sunflower genotypes from RM and some key processes within molecular mechanisms of genetic control of sunflower downy mildew resistance. Performed study included genotyping of sunflower lines using SSR markers, screening of *Pl1* and *Pl6* downy mildew resistance genes and expression studies of 22 genes involved in ROS metabolism and Systemic Acquired Resistance (SAR). The resistance potential of sunflower genotypes cultivated in RM was estimated. *Pl1* gene was identified in 36 genotypes, *Pl6* gene – in 37 and both genes were identified in 24 genotypes. Investigations related to gene expression revealed new insights of sunflower DM resistance mechanism such as differential expression of genes involved in maintenance of oxido-reduction homeostasis in function of infection degree; involvement of transcription factor *Why1* from Whirly family in insurance of sunflower response to *P. halstedii* attack.

Key Words : sunflower, downy mildew, resistance genes, defensive response

COMPARATIVE ASSESSMENT OF ANDROGENIC RESPONSE IN SUNFLOWER (HELIANTHUS ANNUS)

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ABSTRACT

A number of factors include genotype, donor plant growing conditions, developmental stage of pollen, pretreatments and media constituents influence anther culture. The androgenetic ability of four hybrid sunflower genotype using two different growth environments (field and growth chamber), two pretreatment (cold and heat) and also different media compositions were tested and compared. Flower buds (capitulum) containing anthers were collected when the microspores were at the late uninucleate stage. Capitulums from field grown plants were reaching to this stage after 50-60 days after planting but this time is prolonged up to 70 days in the growth chamber. The capitulum diameter of growth chamber grown plants (1-2 cm) was smaller than field grown plants (2-5 cm). The capitulum obtained from growth chamber grown plants includes anthers at optimal developmental stages more than growth chamber grown plants as well. Anthers were excised from capitula of field and growth chamber grown plants and pretreated with cold for 24, 48 or 72 hours at 4°C in the dark. Cold pretreatment for 24 hours produced the highest frequency of embryonic calli induction (34,4 %) on the medium that was supplemented with NAA (0,5 mg/l) and heat pretreatment at 35 0C for 0, 2, 4, 8 or 12 days were applied to anthers and 2 days pretreatment produced the highest frequency of embryogenic calli induction (41%) in growth chamber grown capitulum. The studies on capitulum collected from field have been currently under investigation. This research has been supported by TUBITAK KBAG (Project No: 214O274).

Key Words : Sunflower, anther culture, growth environment.

APPLYING THE TOOLS OF GENOMICS TO SUNFLOWER BREEDING ISSUES

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ABSTRACT

The last four years have been an illuminating time in the history of sunflower genomics. We have seen the release of the first draft of a public sunflower genome. This reference has been wrought with complexity that is still being investigated, but has presented opportunities to understand our favorite species in ways that we have only dreamed of before. From the perspective of the evolutionary biologist, we can obtain greater understanding of how selection has changed this crop, in both expected and unexpected ways. We are beginning to understand how our inbred lines and hybrids are different in terms of genome size and organization, and not simply on their phenotypic trait structure. In this plenary, I will discuss some of the practical tools that arise out of the sequenced genome. At the USDA in Fargo, together with collaborators at University of Colorado, we have used our historical breeding records complete with phenotypic data to develop a Genomic Selection system. Genomic Selection takes sequence information from parents and progeny in the breeding program, trains a model that assigns breeding values to each polymorphic site in the genomic dataset, and uses the model to make informed selection decisions, fully utilizing the historical phenotypic data that is pertinent to the environments of interest. This should improve accuracy and balance in selection in early generations of progeny, resulting in optimized genetic gain compared to previous inefficient, unbalanced methods. A more familiar model is the GWAS or association mapping model. Community resources have been developed around this system, and can result in greater understanding of quantitative traits of large importance (e.g. fatty acid variation in oilseed sunflower). Genomics has also allowed us to better understand the importance of mutation in breeding programs, allowing us to see for the first time the variety in mutations found in mutagenesis experiments. These advances allow the sunflower community to enter a new age of informed breeding, which could lead to acceleration of genetic gain in the future.

Key Words : genomics, breeding, quantitative genetics, genomic selection, GWAS

DETERMINATION OF SUPERIOR HYBRID COMBINATIONS IN SUNFLOWER AND TESTING OF THEIR RESISTANCE TO BROOMRAPE (OROBANCHE CUMANA WALLR.) IN INFESTED AREAS

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ABSTRACT

This research was conducted during 2004-07 in order to study the F_1's hybrid vigor and genetic structure of a hybrid sunflower population in terms of phenological characters, agronomical traits, yield and quality characters and to identify suitable parents and promising hybrid combinations showing superior general and specific combining abilities and resistant to Broomrape. Twenty five experimental hybrids were created using 5 cytoplasmic male sterile (CMS) and 5 pollen tester (restorer) lines having different levels of resistance to Broomrape in sunflower. Field trials of the research were made at three different locations (Center, Ferhadanli and Banarli districts) in Tekirdag province. The experiments were designed in a randomized complete block with three replications. According to the results, the general and specific combining ability (sca) variances were highly significant for all traits investigated except days to 50% flowering. According to the general combining ability effects obtained from the all locations, A_3 (TTAE 4156A) for oil content, seed yield and oil yield were determined as the most suitable parents. The significant SCA effect and high mean values of hybrids combinations showed that the crosses A_4 x R_7 A_3 x R_7, A_4 x R_8, A_5 x R_6, A_3 x R_9 and A_3 x R_8 were promising hybrid combinations for seed and oil yields. It was found that A_3 x R_6, A_3 x R_7, A_3 x R_{10} and A_4 x R_7 hybrids produced 20-25 % more oil yield compared with the mean of checks varieties in some locations. The results of Broomrape test indicated that only R_{10} male line was resistant to broomrape population in all locations. But instead of that male line's hybrids, A_3 x R_6 and A_3 x R_7 experimental hybrids were found highly tolerant to broomrape in all locations. As a result, genotypes A_3 (TTAE 4156A), A_4 (TTAE BAH8 A), R_6 (RHA14) and R_7 (RHA 20) were the parents involved in the best-yielding crosses. Among these parents, A_3 and B_7, which possesses a considerable positive gca effect, might be utilized as a good parent in hybrid sunflower breeding programs. On the other hand, A_3 x R_6, and A_3 x R_7 might be considered as promising hybrid combinations for higher yield based on their sca effects and resistance to Broomrape.

Key Words: Broomrape, Combining ability, *Helianthus annuus* L., heterosis, line x tester, sunflower, yield and quality.

INTRODUCTION

In general, hybrid varieties in open-pollinated crops provide heterosis in terms of yield performance, some agronomic traits such as plant height, earliness and product quality. Therefore, most of the sunflower growers in many countries prefer to use hybrid varieties. Hybrid sunflowers are more stable, highly self fertile and more uniform at maturity (Dedio and Enns, 1976, Seetharam, 1979). Resistance to diseases and broomrape has also increased the importance of hybrid varieties. The heterotic performance of a hybrid combination depends upon to combining abilities of its parents (Kadkol et al.,1984; Allard 1999). Due to high heterosis occurring generally in hybrids between genetically unrelated inbred lines, all plant breeders use as a selection criterion the heterosis to find good combiners. Breeding programs can take advantage from such information on combining abilities to find best selection strategy for developing high yielding lines and hybrids. In that reason, general and specific combining abilities are becoming very important information in plant breeding. (Skoric, 1992). Heterosis and combining ability

studies are frequently used by breeders to improve the superior synthetics and hybrids in sunflower as well as in the other open-pollinated crops (Goksoy et al., 2000).

Regarding combining ability analysis, SCA variance higher than GCA variance means that non-additive genes have higher effects than additive genes in determining the studied characters. Conversely, higher GCA variance indicates that additive gene effects play a more important role in determining these traits. If neither variance is significant, it implies the existence of epistatic gene effects (Marinkovic *et al*., 2000; Joksimovic *et al*., 2000). Various researchers have studied on the general and specific combining ability variances for certain characters in sunflower. Some researchers found that additive gene effects had more important roles in certain yield traits such as plant height (Mruthunjaya *et al*., 1995; Mihaljcevic, 1988; Goksoy *et al*., 1999; Joksimovic *et al*., 2000), 1000-seed weight (Tyagi, 1988; Mruthunjaya *et al*., 1995; Mihaljcevic, 1988; Goksoy *et al*., 1999; Khan, 2001), flowering time (Mihaljcevic, 1988), physiological maturity date (Mihaljcevic, 1988; Tyagi, 1988), etc. Others observed that non-additive genes affected dominantly some yield components such as head diameter (Mruthunjaya *et al*., 1995; Mihaljcevic, 1988) and physiological maturity date (Mihaljcevic, 1988).

The environmental conditions of course influence the evaluation of combining abilities (Petakov, 1996) but, most of the breeders obtained their superior hybrids by crossing inbred (*cms)* female and male (restorer) lines having high GCA and SCA values.

In this study, it was aimed to estimate the genetic structure in 25 hybrids obtained from five CMS and five restorer sunflower lines and to identify parental lines having good combining abilities and superior sunflower hybrids the resistant to broomrape.

MATERIAL AND METHODS

Broomrape observations were evaluated as frequency (F), intensity (I) and attacking rate (AR) according to Pustovoit's method. The plants having 0 – 10 % frequency and 0-1 AR values were considered as resistant (Vranceanu *et al.*, 1980 and Pacureanu-Joita *et al.*, 1998).

Test of the resistance to broomrape was made on natıral infected fields at Karaevli, Tekirdag in 2004. The results of orobanche test showed that except P4223 (Pioneer Seed Co.) all other commercial checks and released inbred lines from UUAF (Uludag University Agricultural Faculty) and TARI (Thrace Agricultural Research Institute) were susceptible to the new broomrape race. Five female and five male were selected from broad-sense sunflower germplasm according to their plant vigor and yield abilities and resistance to broomrape (Table 1).

Five cytoplasmic male sterile lines and five fertility restorer lines illustrated in Table 1 were crossed in all possible combinations in 2004 and 2005. All resultant 25 experimental hybrids, 10 parents and three commercial hybrids (as check) were planted in randomized complete block design with three replications at Center in 2005 and Ferhadanli and Banarli locations of Tekirdag in 2006. In the experiments, parent lines, experimental hybrids and check varieties were planted by hand in mid-April in a well prepared soil. Plot size was 12.6 m^2 (6.0 x 2.10 m) ; row spacing was 0.70 m; plant-plant spacing was 0.30 m. Sixty kilogram of nitrogen per hectare as ammonium nitrate was applied prior to sowing and a further 60 kg N ha^{-1} was added when the plants were 25-30 cm in height. Hand hoeing was done when necessary.

The data were recorded on ten randomly selected plants from middle row of each plot for plant height, stem diameter and head diameter. Additionally, days to 50 % flowering and days to physiological maturity were also observed and recorded to field book. Yield components such as hectoliter, plot yield, 1000 seed weight and number of seeds per head were measured based on plot harvest at the Ministry of Agriculture, Tekirdag Province Control Laboratory. Oil content was measured by NMR at Thrace Oil Seeds Union Laboratory.

All data were subjected to analysis of variance for each character using MINITAB (University of Texas, Austin, version 14) software. Analysis of variance for combining ability was done according to the Line x Tester method in which estimates gca variances and sca variances were obtained as suggested by Singh an Chaudhary (1977). Analysis of combining ability was made using TARPOPGEN (Ege University, Izmir, Turkey) software as outlined by Ozcan and Acikgoz (1999).

Table 1: Selected male and female lines

Parents		**Female/Male**	**Type**	**Source**
Code	**Pedigree**			
A_1	CMS 16 X N 42	Female	CMS	UUAF
A_2	CMS 10 X N 11	Female	CMS	UUAF
A_3	4156 A	Female	CMS	TARI
A_4	BAH 8 A	Female	CMS	TARI
A_5	H1 CMS 88 X N Record (109)	Female	CMS	UUAF
R_6	RHA 14	Male	Restorer	UUAF
R_7	RHA 20	Male	Restorer	UUAF
R_8	RHA 22	Male	Restorer	UUAF
R_9	RHA 03	Male	Restorer	UUAF
R_{10}	RHA 09	Male	Restorer	UUAF

UUAF: Uludag University Agriculture Faculty

TARI : Thrace Agriculture Research Institue

RESULTS AND DISCUSSION

Broomrape screening results are illustrated in Table 6 (at the final page). Commercial varieties used as check in the study, Sanbro (Sygenta Seed Co.) and C70165 (Advanta Seed Co.) are known resistant to 5 races (A to E) of Broomrape. None of them were found even tolerant to broomrape populations in trial areas. Third check variety, hybrid P4223 (Pioneer Seed Co.) known as resistant to new race was found resistant to broomrape in all trial areas.

The results showed that obviously trial areas were infested by new race or races. In Ferhadanli (2006) in spite of high frequency level of the genotypes, all hybrid combinations and parent lines except check variety Sanbro had low attack degree. The results of Broomrape test indicated that only R_{10} (Rha 09) was found resistant in all locations. But instead of hybrid combinations including R_{10} (Rha 09) male line, A_3 x R_6 and A_3 x R_7 experimental hybrids were found highly tolerant to broomrape in all locations. The commercial checks Sanbro and C70165 were susceptible to the new races.

Broomrape races A to E are controlled by the single dominant gene (Sunko et al. 1999). These results confirmed by the earlier researchers revealed that the new race resistant gene actions is mainly determined by dominant - recessive epistatic gene effects. In that reason, depends on its broomrape

sensitivity would be variable one hybrid combination obtained from the cross between broomrape resistant line and non resistant line. (Martinez et al. 2005).

Some of the experimental hybrids had less attacking rate than critical limit level but none of them had less frequency level than 10 %. Therefore none of these experimental hybrids were found resistant to the new races. However, some of experimental hybrids had lower frequency level and attacking rate than commercial checks Sanbro and C70165. Therefore these hybrids can be described as tolerant to the new races. Shindrova and Encheva (1994) reported that orobanche parasite reduced mainly seed yield and some yield components such as 1000 seed weight, plant height, head diameter, oil and protein content but fatty acid and quality composition of kernel were not affected by broomrape. Therefore, using of varieties with resistant to broomrape is very important in infested areas.

In the research, commercial checks reached to the flowering stage at 75 – 78 days and also to the physiological maturity stage at 111 – 112 days while the experimental hybrids required 69 – 86 days to reaching flowering stage and 100 – 120 days for the physiological maturity. Our findings on this subject are in agreement with those of Kaya (1998 and 2001) and Ergen and Saglam (2005) who reported that the days to 50 % flowering and days to physiological maturity ranged between 63 and 81 days and 94 and 110 days, respectively. As seen from general combining ability effects for days to 50% flowering in Table 3, gca effects were negative for A_1 (CMS 16 x N42), A_3 (TTAE 4156 A) and A_4 (TTAE BAH 8 A) whereas that effect was positive for A_5 (H1 CMS 88 X N Record 109) in all locations but gca effects were only statistically significant for A_1 and A_5 parental lines at Banarli location. For the male lines, gca effects were only found positively significant in R_8 (Rha 22) line at Ferhadanli location. Specific combining ability (sca) effects were no significant for days to 50 % flowering (Table 4).

For the days to physiological maturity, only A_1 (CMS 16 x N42) line had significantly negative effect whereas the restorer lines' gca effects were not significant. Sca effects of experimental hybrids were not significant in Tekirdag and Banarli locations. The experimental hybrids A2 x R_{10} and A_3 x R_{10} had significant sca effects in Ferhadanli. In observed p*henological* traits, gca variance was lower than sca variance. Due to σ^2_{GCA} / σ^2_{SCA} variance ratio indicates dominant or epistatic genes' actions more effective than additive genes. Our results do not correspond to those of Mihaljcevic (1998) and Tyagi (1988) who reported that additive gene actions were more effective than non-additive gene actions. The proportion of genetic factors (H) into phenotypic variance was 5.6 % for the days to flowering and 17.4 % for days to physiological maturity. The proportion of additive genetic variance (h^2) into phenotypic variance for both traits was 1.71 % and 2.06 %, respectively.

In the research, plant height for check varieties varied between 147 and 180 cm. That agronomic traits for experimental hybrids were among 138.3 to 194.4 cm. Significant gca effects for plant height were found positive for A_1 (CMS 16 x N42), A_2 (CMS 10 x N11), R_7 (Rha 20) and R_8 (Rha 22) lines, but it was negative for A_5 (H1 CMS 88 X N Record 109) line in all locations (Table 3). Parental lines having positive and significant gca effects for plant height have been reported earlier by Dua and Yadava (1983) and Goksoy et al (2000). In the present study, plant height sca effects were positively significant for A_5 x R_7 and A_5 x R_9 at Tekirdag and Banarli locations whereas it was negatively significant for A_2 x R_8 experimental hybrid at Ferhadanli location (Table 4a).

For the plant height, σ^2_{GCA} / σ^2_{SCA} ratio was higher than 1 at Banarli while it was lower than 1 at the other locations. This case indicated that additive genes were more effective than non-additive genes for this hybrid population at Banarli but non-additive gene actions were found more effective in Ferhandanli and Tekirdag. Previous researchers reported that additive gene actions were more effective than non-additive gene actions for plant height Muruthunjava, 1995, Mihaljcevic, 1988, Goksoy et al, 1999 Joksimovic et al. 2000). The proportion of genetic factors (H) and additive genes (h^2) into phenotypic variance for plant height was 54.4 % and 7.81 %, respectively (Table 5).

The gca effects for 1000 seed weight were found positively significant in A_3 (TTAE 4156) at Banarli and R_8 (Rha 22) line at Ferhadanli, while this effect was negatively significant in R_{10} (Rha 09) at Ferhadanli location. Sca effects for 1000 seed weight were not significant in all locations.

Neither general nor specific combining ability variances were significant for 1000 seed weight. Most of the total genetic variation for this trait was caused by epistatic gene action, since the sca variance was higher than gca variance. On the other hand, negative sca variance for 1000 seed weight indicated that epistatic genes affected 1000 seed weight in decreasing direction (Table 4a). The results of earlier Goksoy and Turan (2004) reported similar findings. Broad-sense (H) and narrow-sense (h^2) heritabilities were estimated as zero because of the very low and negative genotypic variance. Contrary, Pathak (1974) reported that broad-sense heritability ranged between 20 % and (0 % for 1000 seed weight. Also Alza and Fernandez-Martinez (1997) found that heritability for seed weight in sunflower was 84 %.

The gca effects for number of seeds per head were significant on some female lines at Ferhadanli and Banarli locations. A_2 (CMS 10 X N11) had negative gca effect in all locations while A_3 (TTAE 4156) was positive gca effect (Table 3). The sca effects were highly significant for all of hybrid combinations in all locations (Table 4b).

The ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ were found lower at Tekirdag (Center) and Ferhadanli locations except one the other. The ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ indicated that the gca variance was significant at Banarli location but sca variance at Tekirdag. However, both gca and sca variances were no significant at Ferhadanli location (Table 5). These results revealed that additive gene actions were more effective at Banarli but dominant gene actions at Tekirdag for number of seeds per head. On the other hand, epistatic gene actions were more effective at Ferhadanli. Opposite results were obtained in different studies on combining ability in number of seeds per head. Goksoy and Turan (2004) reported that the number of seeds per head was influenced by dominant gene action, as the variances due to sca were highly significant for this character. On the other hand, Goksoy et al. (2000) detected that additive gene action was significant for the number of seeds per head.

Heredity values for the number of seeds per head were estimated 43.2 % for broad-sense and 4.77 % for narrow sense heritability. Fick (1978) noted that heritability for the number of seeds per head was higher than that for seed yield.

Hectoliter weight was measured ranging from 26.3 to 37.8 kg in female lines, and 32.9 to 39.8 kg in male lines. Hectoliter weight of commercial hybrids ranged between 33.7 and 37.6 kg while experimental hybrids had 29.3 kg to 41.3 kg hectoliter weight. Previous researchers reported similar results (Kaya and Atakisi 2004, Marinkovic et al. 2006).

As the ratios of $\sigma^2_{GCA} / \sigma^2_{SCA}$ were lower than 1 for test weight, non-additive gene actions were more effective than additive gene actions on this trait. Our findings do not correspond to those of Marinkovic et al. (2006) who reported that epistatic gene action was more effective than other gene actions on the heredity of hectoliter weight. The sca effects were found significant for all hybrids except A_1 x R_7 hybrid combination in Tekirdag location. The gca effects in parent lines were significant positively for B_{10} (RHA 09) and negatively for B_8 (RHA 22) at Ferhadanli location. However, at Banarli location, gca effects were significant positively for A_2 (CMS 10 X N11) and negatively for A_4 (TTAE BAH 8 A), also.

Environmental and genotype x environment interaction variances were found higher than genotypic variance for hectoliter weight. Therefore, heritabilities for the broad-sense and narrow sense were estimated as 11.80 % and 2.30 %, respectively. The biggest proportion of phenotypic variance was affected from environmental factors. Similar results were also reported earlier by Marinkovic et al. (2006).

In the present study, oil content ranged from 40.5 percent to 42.79 percent for female lines, 37.4 percent to 42.8 percent for male lines, 37 percent to 46 percent for commercial checks and 38.8 percent to 43.6 percent for experimental hybrids (Table 3 and Table 4b).

The gca effects for oil content were only significant in Ferhadanli location while sca effects were no significant for all location. The ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ was found lower than 1 and sca variance was negative and both gca and sca variances were not statistically significant. Therefore, epistatic gene action was more effective than the other gene actions for this trait. Our results support the previous work of Hladni et al. (2007) who reported that non-additive gene actions were more effective than additive gene actions on the heredity of oil percentage, as the ratio of gca:sca variance was lower than 1 for this character.

Heredity values of oil content were estimated 15.30 % for broad-sense and 3.43 % for narrow-sense heritability. Fick (1975) reported that broad-sense heritability for oil content ranged between 52 % and 61 %. On the other hand, Cespedes Torres et al. (1984) found that broad-sense heritability was 26.8 % for oil percentage. In this study, seed yields ranged between 3221 and 3953 kg ha^{-1} for commercial checks and 1971 and 3571 kg ha^{-1} for experimental hybrids over all locations.

The gca effects were found significant in A_2, A_3, A_5 female lines at Ferhadanli and also A_1 and A_4 female lines at Banarli. The gca effects were no significant in the male lines at Tekirdag and Ferhadanli locations while these effects were significant in R_6, R_7 and R_9 male lines at Banarli. The sca effects were only found significant in A_5 x R_7 experimental hybrid at Tekirdag location. Additive gene action was described more effective than non-additive gene action for seed yield at Ferhadanli whereas dominant gene action was more effective than additive gene action, as the ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ was lower than 1 at Tekirdag (Center). In addition, epistatic gene action was more active at Banarli. In the most of previous studies, significant non-additive gene action for seed yield has been reported earlier by Dua and Yadava (1983), Castiglioni et al. (1999), Goksoy and Turan (2004), Jan et al. (2005) and Ortis et al. (2005).

Heredity values for seed yield were estimated as 53 % for broad-sense heritability and 6.54 % narrow-sense heritability. In previous studies, degree of broad-sense heritability was found as 57 % (Pathak 1974), 18 % (Kloczowski 1975), 48.4 % (Cespedes Torres et al. 1984) while degree of narrow-sense heritability was obtained 50.5 % (Mogali and Virupakshappa 1994) and 65 % (Alza and Fernandez-Martinez 1997).

In the present study, oil yields were found between 1259 and 1445 kg ha^{-1} for commercial checks and 693 and 1386 kg ha^{-1} for experimental hybrids over all locations. The gca variance was significant for oil yield at Ferhadanli and Banarli locations whereas sca variance played an important role Tekirdag (Center). These results revealed that additive gene actions were more effective than non-additive genes at Ferhadanli and Banarli whereas dominant gene effects were more active than other gene actions at Tekirdag (Table 5). In earlier researches, Jan et al. (2005) found that non-additive gene actions were significant for oil yield. Contrary to that, Del Gatto et al. (2005) reported that additive gene actions were more effective than non-additive gene actions.

Heredity values were estimated 52.9 % for broad-sense and 7.84 % for narrow-sense. In close agreement with our findings, Mogali and Virupakshappa (1994) reported. Degrees of broad and narrow-sense heritability for oil yield were 64.1 % and 50.7 %, respectively.

The present study showed that A_3 (TTAE 4156) and R_7 (Rha 20) lines were good combiners and they could be evaluated for further cross combinations by present breeding program. Cross combinations A_3 x R_6, A_3 x R_7, A_3 x R_8 and A_4 x R_7 were determined as promising hybrid combinations in terms of higher yield and resistance to broomrape for pre-commercial trials (Table 6).

CONCLUSION

As a results, parental lines A_3 (TTAE 4156A), A_4 (TTAE BAH8 A), R_6 (RHA14) and R_7 (RHA 20) were found as the best-combiners because of their high gca effects for yield and the other characters. It was found that the crosses A_4 x R_7 A_3 x R_7, A_4 x R_8, A_5 x R_6, A_3 x R_9 and A_3 x R_8 were promising hybrid combinations for seed and oil yields. Especially, A_3 x R_6, A_3 x R_7, A_3 x R_{10} and A_4 x R_7 hybrids produced 20-25 % more oil yield compared with the mean of checks varieties in some locations. Among of these experimental hybrids, A_3 x R_6, and A_3 x R_7 might be considered as promising hybrid combinations for higher yield based on their sca effects and resistance to Broomrape.

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Table 3: General Combining Abilities of Parents

Parents		# Days Of Flowering (50 %)						# Days Of Physiological Maturity						Plant Height (Cm)					
		2005		2006				2005		2006				2005		2006			
		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
		Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Female Lines																			
A_1	CMS 16 X N 42	85.3	-0.96	73.0	-0.44	75.0	**-1.16**	114.0	-2.31	101.7	-0.83	103.7	**-2.63**	126.7	**-7.35**	146.6	**-8.80**	126.7	**-11.91**
A_2	CMS 10 X N11	86.0	1.11	76.3	1.29	76.7	0.97	120.7	1.03	111.0	2.17	111.3	0.77	138.3	**-5.68**	150.5	**-12.33**	133.7	-5.51
A_3	TTAE 4156 A	80.0	-0.23	73.0	-0.71	76.0	-0.56	115.0	0.76	108.7	-3.56	109.3	0.91	136.7	**7.72**	153.8	**2.53**	158.0	-0.51
A_4	TTAE BAH 8 A	90.7	-0.89	69.3	-0.51	76.0	-0.29	125.3	1.16	109.0	2.51	105.0	1.64	181.7	**-9.68**	145.7	**5.00**	148.7	5.83
A_5	H1 CMS 88 X N Record (109)	83.3	0.97	73.7	0.36	76.0	**1.04**	114.7	-0.64	105.0	-0.29	107.3	-0.69	176.7	**14.99**	175.5	**13.60**	170.6	**12.09**
Male Lines																			
R_6	RHA 14	79.0	-1.09	74.0	-0.84	74.0	-0.43	117.7	-0.51	112.7	0.71	112.7	0.04	121.7	-2.68	131.1	**1.80**	111.1	-1.44
R_7	RHA 20	83.7	0.97	73.0	0.96	75.3	-0.29	113.0	-0.11	102.3	0.84	104.7	-1.49	133.3	3.32	135.0	**2.20**	117.2	5.49
R_8	RHA 22	81.3	0.77	75.3	**1.29**	76.0	1.11	114.7	0.83	108.7	2.31	109.3	1.04	121.7	4.32	140.0	**6.20**	139.5	**8.83**
R_9	RHA 03	81.3	0.31	73.7	0.63	76.0	0.71	110.7	-0.84	103.0	1.84	105.3	-0.49	113.3	-5.28	129.4	**-2.73**	110.6	-0.44
R_{10}	RHA 09	79.0	0.96	70.7	-2.04	69.7	-1.09	109.3	0.63	101.0	-5.69	100.0	0.91	98.3	0.32	126.1	**-7.47**	111.1	**-12.44**

Parents		1000 Seed Weight (gr)						# Seeds Per Head						Hectoliter Weight (Kg)					
		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
		Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Female Lines																			
A_1	CMS 16 X N 42	55.7	-0.77	50.5	-0.47	47.2	-1.51	564.7	-127.98	883.7	11.88	1085.2	**-138.78**	34.0	10.97	37.8	8.25	35.6	11.05
A_2	CMS 10 X N11	53.9	-2.45	52.6	0.17	57.7	-1.83	1021.9	-28.88	812.1	**-417.67**	836.2	**-221.39**	37.2	11.37	34.0	-3.81	32.3	**18.59**
A_3	TTAE 4156 A	58.3	2.95	55.4	0.25	74.3	**3.63**	711.7	48.33	622.3	**143.82**	603.8	**208.62**	34.5	-4.69	29.0	-2.75	26.3	-12.55
A_4	TTAE BAH 8 A	56.8	1.18	53.7	0.05	48.6	0.06	795.8	73.35	849.7	27.43	1131.2	**160.37**	36.5	-0.96	32.0	-1.15	34.4	**-20.68**
A_5	H1 CMS 88 X N Record (109)	51.5	-0.91	51.1	-0.01	53.0	-0.35	912.3	35.19	1033.3	**234.54**	662.6	-8.82	35.5	-16.69	36.6	-0.55	30.8	3.59
Male Lines																			
R_6	RHA 14	23.5	-0.64	25.0	-0.13	23.3	1.27	542.5	9.76	417.1	107.32	372.7	96.57	38.4	0.24	38.9	1.72	36.2	-4.61
R_7	RHA 20	24.3	-1.12	25.7	0.35	23.9	1.48	273.7	-72.11	384.7	-14.32	358.8	96.76	33.0	-11.63	36.0	-6.88	32.9	-5.81
R_8	RHA 22	27.7	1.44	25.7	**0.67**	22.5	-1.01	355.6	5.75	335.7	21.29	384.8	-54.07	35.0	-4.23	36.3	**-13.15**	35.6	4.25
R_9	RHA 03	24.0	-1.31	25.7	0.19	23.6	-1.22	494.5	-10.45	173.9	-187.56	289.7	-76.83	37.1	9.17	36.1	-1.55	35.7	-2.75
R_{10}	RHA 09	23.6	1.63	24.7	**-1.07**	19.8	-0.52	342.5	67.06	346.1	73.27	329.3	-62.43	37.9	6.44	39.8	**19.85**	38.9	8.92

Parents		Oil Level (%)						Grain Yield (Kg/ha)						Oil Yield (Kg/ha)					
		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
		Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Female Lines																			
A_1	CMS 16 X N 42	41.3	0.78	45.9	0.45	39.3	0.33	1486	-41.25	2125	2.85	2431	**-37.28**	558	-14.60	877	-0.70	855	**-15.34**
A_2	CMS 10 X N11	41.5	-1.48	42.3	**-2.28**	37.9	-1.40	2630	-20.45	2030	**-96.15**	2352	**-59.15**	998	-11.14	767	**-45.61**	776	**-25.89**

A_3	TTAE 4156 A	43.5	2.02	42.7	0.65	39.1	1.61	1978	33.41	1637	**37.92**	1775	**58.05**	776	20.42	978	**22.44**	622	**32.55**
A_4	TTAE BAH 8 A	41.4	0.32	43.8	**1.22**	41.8	-0.04	2155	22.08	2172	-3.35	2619	**39.65**	805	8.20	629	3.82	625	**12.27**
A_5	H1 CMS 88 X N Record (109)	41.5	-1.64	43.9	-0.05	42.7	-0.50	2153	6.21	2488	**58.72**	1594	-1.28	802	-2.88	859	**20.05**	622	-3.60
Male Lines																			
R_6	RHA 14	43.3	-0.15	44.0	0.49	41.1	0.25	1201	-1.52	993	14.32	827	**35.65**	473	-1.72	392	10.05	306	**11.79**
R_7	RHA 20	39.5	0.04	37.1	**-0.88**	37.8	0.37	617	-24.25	940	1.59	795	**33.85**	220	-9.64	292	-4.30	270	**11.86**
R_8	RHA 22	35.9	0.34	42.2	**0.80**	39.0	-0.34	939	8.81	821	12.72	805	-15.41	303	3.44	312	4.65	283	-8.44
R_9	RHA 03	38.5	0.18	37.6	-0.10	35.9	-0.28	1064	-9.12	425	-42.01	653	**-36.68**	369	0.46	142	-12.32	210	-6.84
R_{10}	RHA 09	39.8	-0.40	44.4	-0.31	40.5	0.00	730	26.08	818	13.39	632	-17.41	261	7.46	326	1.92	231	-8.38

Significant at 5 %, Significant at 1 %

Table 4a: Spesific Combining Abilities of Hybrids

Hybrids	# Days Of Flowering (50 %)						# Days Of Physiological Maturity						Plant Height (Cm)						1000 Seed Weight (gr)					
	2005		2006				2005		2006				2005		2006				2005		2006			
	Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
Cross	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**
A_1 x R_6	81.3	1.89	70.3	-4.30	72.0	0.40	116.3	2.11	105.3	**-1.24**	107.0	0.7	146.7	3.01	158.9	-6.67	144.5	-2.36	52.4	-2.56	49.7	0.05	48.9	-0.95
A_1 x R_7	80.0	-1.51	73.0	0.00	71.3	-0.40	113.0	-1.63	106.0	-0.70	104.3	-0.4	151.7	2.01	162.2	-3.73	151.1	-2.96	56.3	1.73	49.9	-0.22	47.9	-2.22
A_1 x R_8	80.0	-1.31	74.7	0.70	73.7	0.50	115.0	-0.56	109.7	**1.49**	108.7	1.4	145.0	-5.65	172.2	1.93	160.5	3.04	62.1	4.99	50.9	0.36	49.9	2.3
A_1 x R_9	81.3	0.49	71.7	-2.30	72.7	-0.10	117.3	1.97	107.7	0.00	108.7	1.5	140.0	-1.05	168.9	7.87	148.9	0.97	51.0	-3.35	49.9	-0.13	47.4	-0.04
A_1 x R_{10}	80.0	0.43	68.7	-4.40	70.7	-0.30	112.0	-1.89	100.7	0.50	102.7	-3.1	148.3	1.68	156.7	0.6	137.2	1.31	56.4	-0.82	48.7	-0.07	49.0	0.92
A_2 x R_6	81.0	-0.51	73.7	-2.00	73.7	-0.10	115.3	-2.23	108.0	**-1.57**	108.0	-1.7	143.3	-1.99	168.9	6.87	148.9	-4.43	54.8	1.46	50.5	0.21	47.4	-2.18
A_2 x R_7	85.7	2.09	74.0	-0.90	74.0	0.10	118.3	0.37	106.7	**-3.04**	106.7	-1.5	153.3	2.01	160.6	-2.2	161.1	0.64	52.0	-0.84	50.9	0.11	49.7	-0.08
A_2 x R_8	82.7	-0.71	72.3	-4.60	75.7	0.40	121.0	2.11	110.7	-0.50	114.0	3.3	146.7	-5.65	157.8	**-8.53**	161.7	-2.03	54.6	-0.81	50.3	-0.81	48.6	1.33

A_2 x R_9	82.0	-0.91	73.3	-2.20	74.0	-0.90	117.7	-1.03	109.0	**-1.71**	109.7	-0.9	151.7	8.95	162.8	5.07	154.4	-0.09	55.2	2.53	50.3	-0.37	49.2	2.14
A_2 x R_{10}	81.7	0.03	73.7	0.20	73.7	0.60	118.0	0.77	110.0	**6.83**	110.0	0.8	145.0	-3.32	151.7	-1.2	148.3	5.91	53.3	-2.33	50.2	0.86	46.6	-1.22
A_3 x R_6	77.7	-2.51	70.3	-4.30	71.0	-1.20	115.7	-1.63	108.3	**4.49**	109.0	-0.8	151.7	-7.05	176.7	-0.33	160.0	1.57	63.2	4.49	50.7	0.27	55.0	-0.03
A_3 x R_7	82.7	0.43	72.3	-0.90	72.3	0.00	120.7	2.97	110.3	**6.36**	110.3	2.0	168.3	3.61	184.4	6.93	170.0	4.64	57.5	-0.76	50.0	-0.87	54.7	-0.58
A_3 x R_8	82.3	0.29	72.3	-2.50	73.3	-0.40	117.0	-1.63	107.0	**1.56**	108.0	-2.8	171.7	5.95	181.1	-0.07	165.0	-4.03	56.7	-4.13	51.6	0.44	53.3	0.49
A_3 x R_9	81.3	0.09	70.8	-3.50	73.9	0.60	117.7	-0.76	107.5	**3.69**	109.8	-0.7	148.7	-7.45	177.4	-3.13	160.0	0.57	61.2	-6.04	50.4	0.29	53.7	1.28
A_3 x R_{10}	82.0	1.69	69.0	-3.90	72.7	1.10	118.0	1.04	107.3	**-16.11**	105.0	2.4	166.7	4.95	164.4	-3.4	144.5	-2.76	67.4	6.44	49.3	-0.12	52.1	-1.17
A_4 x R_6	79.3	-0.17	69.3	-3.30	75.0	2.50	115.3	-2.36	105.3	**-4.57**	111.0	0.4	146.7	5.35	179.4	0.2	163.3	-1.43	52.5	-4.43	50.1	-0.13	54.4	2.87
A_4 x R_7	83.0	1.43	72.3	1.60	73.3	0.70	119.7	1.57	109.0	**-1.04**	110.0	1.0	150.0	2.68	173.9	-6.2	161.1	10.36	56.4	-0.05	50.5	-0.17	54.5	2.87
A_4 x R_8	80.3	-1.04	73.7	1.80	72.0	-2.00	120.0	0.97	113.3	**1.83**	111.7	0.1	146.7	-1.65	188.3	4.47	181.7	6.64	58.3	-0.75	51.6	0.65	48.4	-0.82
A_4 x R_9	81.3	0.43	74.0	3.50	73.3	-0.30	118.3	-0.49	111.0	0.00	110.3	-1.1	138.3	-0.39	171.1	-3.93	178.9	12.91	66.8	10.55	50.6	0.09	46.5	-2.48
A_4 x R_{10}	79.0	-0.64	68.7	-1.90	71.0	-0.80	117.7	0.31	107.3	**3.83**	109.7	-0.4	138.3	-5.99	175.5	5.47	146.1	-7.76	53.9	-5.32	48.8	-0.45	47.3	-2.43
A_5 x R_6	82.7	1.29	72.7	-1.60	72.3	-1.50	120.0	4.11	110.0	**2.89**	109.7	1.4	166.7	0.68	188.4	-0.07	177.8	6.64	55.9	1.04	49.7	-0.41	51.4	0.29
A_5 x R_7	81.0	-2.44	73.7	0.50	73.7	-0.30	113.0	-3.29	105.7	**-1.57**	105.7	-1.0	161.7	**10.32**	193.3	5.2	186.1	8.04	54.3	-0.08	51.8	1.15	51.3	0.02
A_5 x R_8	86.0	2.76	74.0	-0.70	77.0	1.60	116.3	-0.89	104.3	**-4.37**	107.3	-1.9	180.0	7.01	194.4	2.2	177.8	-3.63	57.6	0.7	50.3	-0.63	45.5	-3.3
A_5 x R_9	82.7	-0.11	71.7	-2.70	75.7	0.70	117.3	0.31	106.3	**-1.91**	110.3	1.2	163.3	-0.05	175.5	-5.87	157.8	**14.36**	50.5	-3.69	50.6	0.11	47.7	-0.9
A_5 x R_{10}	80.0	-1.51	70.3	-2.50	72.7	-0.50	115.3	-0.23	105.7	**4.96**	108.0	0.3	171.7	2.68	177.2	-1.47	163.3	3.31	59.2	2.03	49.0	-0.23	53.2	3.9

Significant at 5 %, Significant at 1 %

Table 4b: Spesific Combining Abilities of Hybrids

Hybrids	# Seeds Per Head						Hectoliter Weight (Kg)						Oil Content (%)						Grain Yield (Kg/da)						Oil Yield (Kg/da)					
	2005		2006				2005		2006				2005		2006				2005		2006				2005		2006			
	Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
Cross	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
A_1 x R_6	1070.1	**151.41**	1241.6	**-152.82**	1010.3	**-60.44**	36.3	**-6.04**	39.3	-0.19	34.1	6.88	41.1	0.03	44.3	-0.81	40.3	-0.21	261.8	3.01	294	-6.67	234	-2.36	96.7	**10.56**	117.8	-14.72	84.8	-8.33
A_1 x R_7	557.1	**-279.72**	1450.2	**177.39**	1171.9	**101.01**	36.0	2.16	38.9	4.75	35.0	17.41	41.6	0.38	44.7	0.9	40.4	-0.29	147.8	2.01	345	-3.73	268	-2.96	55.2	**-23.05**	138.7	20.49	97.1	3.86
A_1 x R_8	1004.5	**89.98**	1069.5	**-238.92**	1026.8	**106.70**	37.5	**10.09**	37.1	-6.99	33.3	-9.65	40.5	-0.99	45.2	-0.25	40.5	0.58	289.4	-5.65	260	1.93	240	3.04	105.8	**14.48**	104.5	-22.59	87.0	14.09
A_1 x R_9	1164.9	**266.55**	1189.1	**89.62**	824.8	**-72.55**	38.7	**9.03**	39.0	0.41	34.0	4.35	43.9	2.47	43.6	-0.99	39.3	-0.75	282.6	-1.05	283	7.87	187	0.97	111	**22.69**	110.6	0.45	65.8	-8.75
A_1 x R_{10}	747.6	**-228.22**	1485.1	**124.73**	837	**-74.72**	36.0	**-15.24**	41.3	2.01	32.8	-18.99	38.9	-1.89	45.5	1.16	41.1	0.68	201.4	1.68	344	0.6	198	1.31	70.7	**-24.68**	140.8	16.38	72.1	-0.87
A_2 x R_6	834.6	**-183.09**	1126.1	**161.27**	1003.8	**15.67**	34.4	**-25.44**	37.7	-3.45	34.2	1.01	37.8	-1.04	42.6	0.15	38.3	-0.55	216.1	-1.99	270	6.87	228	-4.43	73.6	**-16.07**	103.9	16.3	77.8	-4.81
A_2 x R_7	849.1	**-86.72**	863.3	**20.11**	754.8	**-233.48**	36.1	**3.43**	37.1	-1.19	33.4	-6.45	38.6	-0.42	39.8	-1.21	37.9	-1.03	204.1	2.01	209	-2.2	178	0.64	72.1	**-9.58**	74.9	1.6	60.8	-21.79
A_2 x R_8	955.7	**-57.88**	817.1	**-61.87**	671.5	**-165.89**	34.8	**-16.97**	38.2	15.75	34.3	-6.85	40.4	1.11	43.0	0.31	39.0	0.81	246.3	-5.65	196	-8.53	154	-2.03	91	**-3.72**	75.8	-6.38	54.1	-8.29
A_2 x R_9	1305.1	**307.65**	712.3	**42.31**	853.1	**38.40**	40.8	**6.96**	38.2	4.81	32.9	-14.52	37.6	-1.5	42.5	0.6	37.8	-0.51	342.8	8.95	170	5.07	200	-0.09	118.4	**26.56**	63.9	-1.41	68.0	4.01
A_2 x R_{10}	1095.1	**20.04**	769.1	**-161.82**	1174.4	**345.30**	35.6	**32.03**	38.3	-15.92	38.2	26.81	40.4	1.84	41.7	0.15	39.8	1.28	278.6	-3.32	186	-1.2	257	5.91	101.6	**2.82**	69.4	-10.11	93.3	30.88
A_3 x R_6	1045.7	**-49.14**	1310.2	**-216.18**	1477.9	**59.83**	33.4	**2.63**	37.5	-7.19	29.0	-19.85	42.4	0.06	45.0	-0.38	43.6	1.75	313.4	-7.05	316	-0.33	387	1.57	121.5	0.27	128	-27.62	151.5	10.52
A_3 x R_7	1014.5	1.50	1469.8	**65.06**	1514.8	**96.51**	36.7	**-7.51**	38.8	14.41	31.9	10.35	44.4	1.91	44.0	0.06	41.5	-0.47	278.7	3.61	350	6.93	386	4.64	111.5	-1.81	138.7	-2.55	144.3	3.22
A_3 x R_8	1136.7	**45.80**	1772.5	**332.14**	1288.9	**21.40**	34.3	**17.43**	35.7	-10.32	30.8	-11.05	42.7	-0.12	45.7	-0.02	40.1	-1.17	308.3	5.95	436	-0.07	327	-4.03	118.4	**-7.92**	179	28.78	118.3	-2.49
A_3 x R_9	1106.4	**-74.66**	1498.8	**-131.48**	1356.9	**-64.72**	36.7	**4.69**	38.3	3.08	32.5	12.61	42.5	-0.13	44.9	0.14	41.6	0.32	268.8	-7.45	379	-3.13	226	0.57	122.9	-0.47	145.6	12.32	129.2	6.81
A_3 x R_{10}	1228.7	**76.50**	1442.8	**-49.54**	1146.1	**-113.02**	35.5	**-17.24**	40.0	0.01	33.2	7.95	40.3	-1.72	44.7	0.19	41.1	-0.43	387.4	4.95	339	-3.4	278	-2.76	140.3	**9.93**	136.6	-10.93	102.8	-18.05

A_4 x R_6	1146.8	**26.91**	1634.9	**224.94**	1315.4	**-54.42**	36.0	**-2.44**	38.6	2.55	29.3	-8.72	37.9	-2.68	45.6	-0.28	36.7	-3.48	285.3	5.35	390	0.2	338	-1.43	97.1	**-11.9**	159.9	22.84	112.7	-8.04
A_4 x R_7	1445.7	**407.68**	1229.5	**-58.76**	1362.6	**-7.44**	34.8	**14.43**	37.8	3.48	29.5	-5.85	41.3	0.5	45.2	0.62	42.4	2.11	390.6	2.68	297	-6.2	349	10.36	144.7	**43.62**	119.1	-3.63	133.4	12.62
A_4 x R_8	1116.4	0.48	1306.8	**-17.14**	1325.9	**106.65**	34.5	**-5.31**	35.7	-11.25	31.7	6.08	43.4	2.3	46.3	0.08	39.4	-0.26	313.5	-1.65	321	4.47	305	6.64	123.8	**9.71**	133.4	1.73	107.9	7.38
A_4 x R_9	756.2	**-343.52**	1010.1	**-105.06**	1246.1	**49.64**	34.5	**-21.04**	37.6	-3.85	29.4	-10.25	39.1	-1.84	46.0	0.74	41.0	1.36	232.2	-0.39	243	-3.93	274	12.91	82.6	**-28.61**	100.3	-14.33	101.0	-1.05
A_4 x R_{10}	1085.6	**-91.56**	1332.1	**-43.98**	1116.4	**-94.43**	37.8	**14.36**	41.1	9.08	33.4	18.75	42.1	1.7	43.9	-1.15	40.2	0.28	278.7	-5.99	309	5.47	247	-7.76	105.3	**-12.81**	122.3	-6.61	89.7	-10.91
A_5 x R_6	1135.6	**53.90**	1599.9	**-17.20**	1240	**39.37**	37.3	**31.29**	39.2	8.28	34.7	20.68	42.2	3.62	46.0	1.32	42.2	2.49	305.8	0.68	379	-0.07	305	6.64	115	**17.14**	156.5	3.21	115.5	10.66
A_5 x R_7	957.1	**-42.73**	1291.6	**-203.80**	1244.3	**43.41**	31.7	**-12.51**	35.4	-21.45	31.0	-15.45	36.5	-2.37	42.9	-0.37	39.5	-0.33	246.9	**10.32**	319	5.2	302	8.04	80.8	**-9.17**	123	-15.92	107.0	2.09
A_5 x R_8	999.3	**-78.39**	1516.8	**-14.21**	981.2	**-68.86**	33.2	**-5.24**	38.2	12.81	35.7	21.48	36.8	-2.3	44.8	-0.12	39.1	0.04	276.4	7.01	363	2.2	210	-3.63	90.5	**-12.54**	146.3	-1.53	73.9	-10.69
A_5 x R_9	905.5	**-156.02**	1426.8	**104.60**	1076.5	**49.23**	35.1	0.36	37.6	-4.45	33.6	7.81	40	0.99	43.6	-0.49	38.8	-0.41	217.5	-0.05	344	-5.87	244	14.36	79.9	**-20.16**	133.9	2.98	85.2	-1.02
A_5 x R_{10}	1362.3	**223.24**	1713.7	**130.61**	978.6	**-63.14**	33.4	**-13.91**	40.7	4.81	30.5	-34.52	38.5	0.06	43.5	-0.34	37.7	-1.79	374.2	2.68	400	-1.47	247	3.31	131.8	**24.74**	156.4	11.27	83.7	-1.05

Significant at 5 %, Significant at 1 %

Table 5: Variance Components and Heredity Degrees

Variance Components	# Days Of Flowering (50 %)			Plant Height (Cm)			1000 Seed Weight (gr)			# Seeds Per Head		
	2005	2006		2005	2006		2005	2006		2005	2006	
	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli
σ^2 GCA	0.051	0.121	0.073	7.334	7.873	8.319	-0.157	0.024	0.266	-633	3946	2156.7
σ^2 SCA	0.322	0.599	0.105	14.976	16.076	3.104	1.135	0.152	-11.813	29929.2	10353.8	-278.7
σ^2 GCA / σ^2 SCA	0.158	0.202	0.695	0.49	0.49	2.68	0.138	0.158	0.022	0.021	0.4	7.7

σ^2_A	0.102	0.242	0.146	14.668	15.746	16.638	-0.314	0.048	0.532	-1266	7892	4313.4
σ^2_D	0.322	0.599	0.105	14.976	16.076	3.104	1.135	0.152	-11.813	29929.2	10353.8	-278.7
$\sqrt{(\sigma^2_D / \sigma^2_A)}$	1.777	1.573	0.848	1.01	1.01	0.432	1.901	1.779	4.712	4.8	1.1	0.3
$H = \sigma^2_G / \sigma^2_F$	5.64 %			54.40 %			0.0 %			43.20 %		
$h2 = \sigma^2_A / \sigma^2_F$	1.71 %			7.81 %			0.84 %			4.77 %		

Variance Components	Hectoliter Weight (Kg)			Oil Level (%)			Grain Yield (Kg/da)			Oil Yield (Kg/da)		
	2005	2006		2005	2006		2005	2006		2005	2006	
	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli
σ^2_{GCA}	4.5	8.04	10.29	0.047	0.125	0.023	-5.21	218.864	185.099	3.188	43.882	5.824
σ^2_{SCA}	185.2	53.02	67.82	1.892	-0.708	0.826	2416.02	106.13	586.153	291.67	128.33	70.01
$\sigma2_{GCA} / \sigma2_{SCA}$	0.02	0.15	0.15	0.024	0.176	0.028	0.002	2.06	0.316	0.01	0.34	0.08
σ^2_A	9	16.08	20.58	0.094	0.25	0.046	-10.42	437.728	370.198	6.376	87.76	11.65
σ^2_D	185.2	53.02	67.82	1.892	-0.708	0.826	2416.02	106.13	586.153	291.67	128.33	70.01
$\sqrt{(\sigma^2_D / \sigma^2_A)}$	4.53	1.81	3.29	4.486	1.683	4.237	15.23	0.492	1.258	6.76	1.21	2.45
$H = \sigma^2_G / \sigma^2_F$	11.80 %			15.30 %			53.30 %			52.90 %		
$h2 = \sigma^2_A / \sigma^2_F$	2.30 %			3.43 %			6.54 %			7.84 %		

Table 6: Combined Results Comparison Of Hybrid And Commercial Checks For All Traits.

Genotypes			Days of flowering (50%)	Days of physiological maturity	Plant height (cm)	Tem girth (mm)	Head diameter (Cm)	1000 seed weight (gr)	# seed per head	Hectoliter weight (kg)	Oil level (%)	Seed yield (Kg/da)	Oil yield (Kg/da)	Frequency (%)	Intensity	Degree of Attack
1	C70165	C70165	**77**	112	159	23	19	**56**	1233	36	**44**	322	143	68	**4**	**2.7**
2	Sanbro	Sanbro	**71**	113	170	25	21	51	**1407**	**37**	40	340	138	64	3	1.9
3	P4223	P4223	73	112	160	25	22	53	**1566**	35	41	**395**	**162**	**2**	**1**	**0.0**
4	A_1 x R_6	CMS 16 X N 42 / RHA 14	72	110	150	24	17	50	1107	**37**	42	263	111	46	**2**	**0.9**
5	A_1 x R_7	CMS 16 X N 42 / RHA 20	**71**	108	155	24	21	51	1060	**37**	42	254	108	47	**2**	**0.9**
6	A_1 x R_8	CMS 16 X N 42 / RHA 22	74	111	159	25	21	54	1034	36	42	263	111	51	**2**	**1.0**
7	A_1 x R_9	CMS 16 X N 42 / RHA 03	73	111	153	23	20	**49**	1060	**37**	42	251	107	52	**2**	**1.0**
8	A_1 x R_{10}	CMS 16 X N 42 / RHA 09	**71**	**105**	**147**	24	21	51	1023	**37**	42	248	105	49	3	1.5
9	A_2 x R_6	CMS 10 X N 11 / RHA 14	74	110	154	23	20	51	988	35	40	238	95	58	**2**	1.2
10	A_2 x R_7	CMS 10 X N 11 / RHA 20	74	111	158	25	21	51	822	36	**39**	**197**	**77**	47	3	1.4
11	A_2 x R_8	CMS 10 X N 11 / RHA 22	76	**115**	155	24	21	51	**815**	36	41	**199**	**81**	51	3	1.5
12	A_2 x R_9	CMS 10 X N 11 / RHA 03	74	112	156	25	**24**	52	957	**37**	**39**	238	92	58	**4**	2.3
13	A_2 x R_{10}	CMS 10 X N 11 / RHA 09	74	113	148	23	20	50	1013	**37**	41	240	97	57	3	1.7
14	**A_3 x R_6**	**TTAE 4156 A / RHA 14**	**71**	111	163	24	19	**56**	1278	33	**44**	339	**148**	**29**	**2**	**0.6**
15	**A_3 x R_7**	**TTAE 4156 A / RHA 20**	72	114	174	**26**	21	54	1333	36	**43**	338	**146**	**26**	**2**	**0.5**
16	**A_3 x R_8**	**TTAE 4156 A / RHA 22**	73	111	173	**26**	19	54	**1399**	34	**43**	**357**	**154**	43	**2**	**0.9**
17	A_3 x R_9	TTAE 4156 A / RHA 03	74	112	162	24	20	55	1321	36	**43**	291	126	60	3	1.8
18	A_3 x R_{10}	TTAE 4156 A / RHA 09	73	110	159	24	21	**56**	1273	36	42	335	141	47	3	1.4
19	A_4 x R_6	TTAE BAH 8 A / RHA 14	75	111	163	**26**	22	52	**1366**	35	40	338	137	50	**4**	2.0
20	**A_4 x R_7**	**TTAE BAH 8 A / RHA 20**	73	113	162	**26**	22	54	**1346**	34	**43**	**345**	**148**	49	**2**	**1.0**
21	A_4 x R_8	TTAE BAH 8 A / RHA 22	72	**115**	172	**26**	22	53	1250	34	**43**	313	135	69	**2**	1.4
22	A_4 x R_9	TTAE BAH 8 A / RHA 03	73	113	163	**26**	**17**	55	1004	34	42	250	105	63	**4**	2.5
23	A_4 x R_{10}	TTAE BAH 8 A / RHA 09	**71**	112	153	25	21	50	1004	**37**	42	278	117	61	**2**	1.2
24	A_5 x R_6	H1 CMS 88 X N Record (109) / RHA 14	72	113	178	23	**23**	52	1178	**37**	**43**	330	144	64	3	1.9
25	A_5 x R_7	H1 CMS 88 X N Record (109) / RHA 20	74	108	180	24	20	52	1325	**33**	40	289	115	63	**2**	1.3

26	A_5 x R_8	H1 CMS 88 X N Record (109) / RHA 22	**77**	109	**184**	25	19	51	1164	36	40	283	116	**78**	**4**	**3,1**
27	A_5 x R_9	H1 CMS 88 X N Record (109) / RHA 03	76	111	166	**21**	18	50	1166	35	41	268	110	69	3	2,1
28	A_5 x R_{10}	H1 CMS 88 X N Record (109) / RHA 09	73	110	171	24	22	54	1136	35	40	340	137	61	**2**	1,2

RECENT MOLECULAR STUDIES ON DOWNY MILDEW DISEASE

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ABSTRACT

Downy mildew is a common sunflower disease and appears as yellow to white patches on the upper surfaces of older leaves. *Plasmopara halstedii*, which is a fungal parasite, causes that disease. Downy mildew has been spread to the whole world from North America and this disease causes more yield lost in temperate climate than subtropical climate. Antifungal chemicals are used for struggling with the disease. As a choice, the resistance hybrid cultivars are also preferred. In some cases these solutions cannot be enough due to development of fungicide or resistant gene resistance pathogens. Thus, molecular studies on downy mildew disease have been conducted. In this presentation, recent development in molecular studies on downy mildew disease will be introduced.

Key words: Downy mildew, sunflower, disease

INTRODUCTION

Sunflower, the forth wildly produced oil crop in the world, right after soybean, rapeseed, cotton seed with 41.3 million tonnes production in 2013/14, is a valuable oil crop (FAO, 2014). The market value of sunflower is provided with its oil content rather than the meal. The oil concentration of the sunflower seeds might range from 260 g/kg to 720 g/kg among the genotypes (Hu et al., 2010). Sunflower oil is exceptional having high unsaturated linoleic fatty acid and low linoleic acid concentration (Dorrell and Vick, 1997).

Downy mildew (*Peronospora halstedii*) disease was spread from North America, where is the gene center of sunflower, to Europe (Anonymous, 1984). The first determination of the disease was in 1922 in North America, later on the disease was spread to Europe in 1966 starting from France (Sakr, 2014). The disease could cause yield loss until 80%, thus struggling with the disease is very important for sunflower breeders (Molinero-Ruiz et al., 2003). The symptom of the disease could be in two ways: systematic and secondary symptoms. The systematic symptoms are usually observed at rooting stage of the seedling and this situation causes killing of the seedlings. Hence, these will be some blank spots in the field, where seedlings are supposed to be. However, if seedlings survive, the systematic symptoms of the disease occur on the cotyledons or first true leaves with thickness and yellow parts. Another symptom is white cottony appearance underside of the leaves, which is caused by fungal mycelium and spores. Besides, the infected plants are dwarfed and have very less amount of seeds when they reach maturity stage. Secondary symptom of the disease is small angular lesions on the upper surface of leaves. Usually, secondary symptoms do not cause systemic symptoms or yield loss (Friskop et al., 2009; Gascuel et. al., 2015).

The management of the disease has been obtained by two methods; fungicide application and production of hybrid plants. Metalaxyl and mefenoxam were used until end of 1990s. However, by the finding resistant *P. halstedii* isolates, azoxystrobin and fenamidone have been started to use for downy mildew disease. Development of resistant *P. halstedii* lines is still possible. Thus, using

fungicide is not the most effective method for management of downy mildew disease. The other method is production of hybrid sunflower lines. It is, therefore, resistance genes, which are called *PI* genes, are used. Until 1980s resistant sunflower lines carried Pl_1 and Pl_2 genes, after 1980s these genes were replaced with Pl_6 and Pl_7 genes (Tourvieille de Labrouhe et al., 2010).

History of *Plasmopara halstedii*

The pathogen was discovered by Farlow in 1882 as *Peronospora halstedii.* After modification of Peronospora genus, the fungus was named *Plasmopara halstedii* in 1888 (Sackston, 1981). Over the years, many fungus, which caused downy mildew disease in sunflowers, were detected and classified as *Plasmopara halstedii* due to their sporangiophores and spoangia morphology (Stevens, 1913). According to Novotelnova's observation, the pathogen in Europe was different form the North America and she renamed the fungus as *Plasmopora helianthi;* however, the observations of Novotelnova were just based of morphological. Thus, this name is not accepted today.

Geographical distribution of *Plasmopara halstedii*

The first downy mildew syndrome in *Helianthus annuus* were detected in 1890s and it became a serious problem for sunflower production in 1920s in North America (Henry and Gilbert, 1924; Young and Morris, 1927). In the middle of the 20th century the disease was spread to the Europe (Novotelnova, 1966). The distribution map of *P. halstedii* is shown in Figure 1. As it seen in the figure, the pathogen has been distributed all over the world.

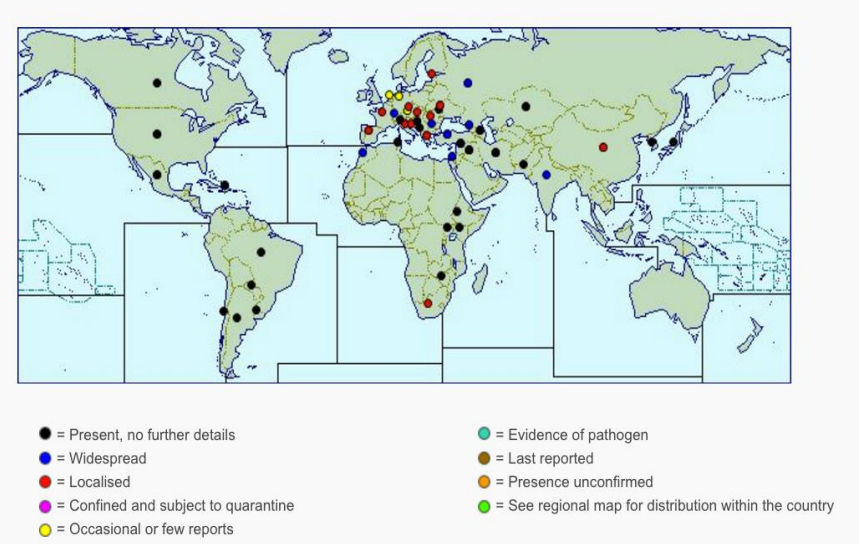

Figure 7: Geographical distribution of *Plasmopara halstedii*

Apart from the figure, the distribution map is given in Table 1 in detailed.

Table 1: Geographical distribution of *Plasmopara halstedii*

Region	**Countries**
EPPO (European and Mediterranean Plant Protection organization)	Albania, Austria, Bulgaria, Czech Republic, Egypt, Estonia, France, Germany, Hungary, Italy, Moldova, Morocco, Poland, Romania, Slovakia, Spain, Switzerland, Turkey, Russia, Ukraine, Yugoslavia
Asia	Azerbaijan, China, Georgia, India, Iran, Iraq, Israel, Japan, Kazakhstan, Pakistan, Russia, Turkey
Africa	Egypt, Ethiopia, Kenya, Morocco, Zimbabwe, Uganda
North America	Canada, USA (California, Kansas, Minnesota, North Dakota, South Dakota)

Region	Countries
Central America and Caribbean	Dominican Republic
South America	Argentina, Brazil, Chile, Uruguay, Paraguay

Evolution of *Plasmopara halstedii* in sunflower cultivated zones

Plasmopara halstedii is native to North America, then it spread to Europe. According to its physiological properties, the organism is spread into two races; race 300 in North America and race 100 in Europe. Later on, race 710 was introduced into sunflower zones in Europe from USA in 1980s (Tourvieille de Labrouhe et al. 2000, Delmotte et. al. 2008, Ahmed et. al. 2012). Recombination between races was occurred and new races were appeared and 14 races in Europe and 35 races in the world have been identified (Delmotte et. al. 2008, Ahmed et. al. 2012, Gulya 2007).

New generation races are able to beaten *Pl* loci, were discovered over the years all over the world as listed in Table 2. Although hybrid sunflowers having Pl_6-Pl_7 gene are grown in all Europe, 304, 307, 314, 334, 704 and 714 races have never been recorded out of France.

Table 2: Virulence of 15 *Plasmopara halstedii* races on sunflower differential lines

Race	Differential lines								
	D1 Ha-304[a]	D2 Rha-265[a]	D3 Rha-274[a]	D4 PMI3[b]	D5 PM-17	D6 803-1[c]	D7 HAR-4[a]	D8 QHP1[b]	D9 Ha-335[a]
100	S	R	R	R	R	R	R	R	R
300	S	S	R	R	R	R	R	R	R
304	S	S	R	R	R	R	R	R	S
307	S	S	R	R	R	R	S	S	S
314	S	S	R	S	R	R	R	R	S
334	S	S	R	S	S	R	R	R	S
700	S	S	S	R	R	R	R	R	R
710	S	S	S	S	R	R	R	R	R
703	S	S	S	R	R	R	S	S	R
704	S	S	S	R	R	R	R	R	S
707	S	S	S	R	R	R	S	S	S
714	S	S	S	S	R	R	R	R	S
717	S	S	S	S	R	R	S	S	S

Race	Differential lines								
	D1	D2	D3	D4	D5	D6	D7	D8	D9
	Ha-304[a]	Rha-265[a]	Rha-274[a]	PMI3[b]	PM-17	803-1[c]	HAR-4[a]	QHP1[b]	Ha-335[a]
730	S	S	S	S	S	R	R	R	R
770	S	S	S	S	S	S	R	R	R

[a] USDA genotypes (USA), [b] INRA genotypes (France), [c] IFVC genotypes (Yugoslavia), * S: susceptible, R: resistant (Tourvieille de Labrouhe et. al. 2000)

Pl gene has vital importance on sunflower production. Unfortunately, the gene has a short life period, which is not enough to obstruct virulence emerge of *P. halstedii* (Sakr 2014).

Apart from morphological observation, the development in biotechnology has given an opportunity for classification of *P. halstedii* in detailed by molecular techniques. First of all, 21 RAPD markers were used to differentiate 77 samples from 12 different countries at low levels in 2003 by Roeckel-Drevet et. al. 2003. In a study, conducted by Spring et. al. (2006), ITS regions were partially sequenced by molecular markers. By this study, the polymorphism between 100, 310 and 330 was detected, along with populations, which are exemplified by 700, 701, 703, 710 and 730. Giress and colleagues (2007) identified high genetic variability between isolates from French and Russia by SNP markers. Ahmet et. al. (2012) defined that recombination facilitated by multiple introductions are reasons for appearing new races of *P. halstedii*.

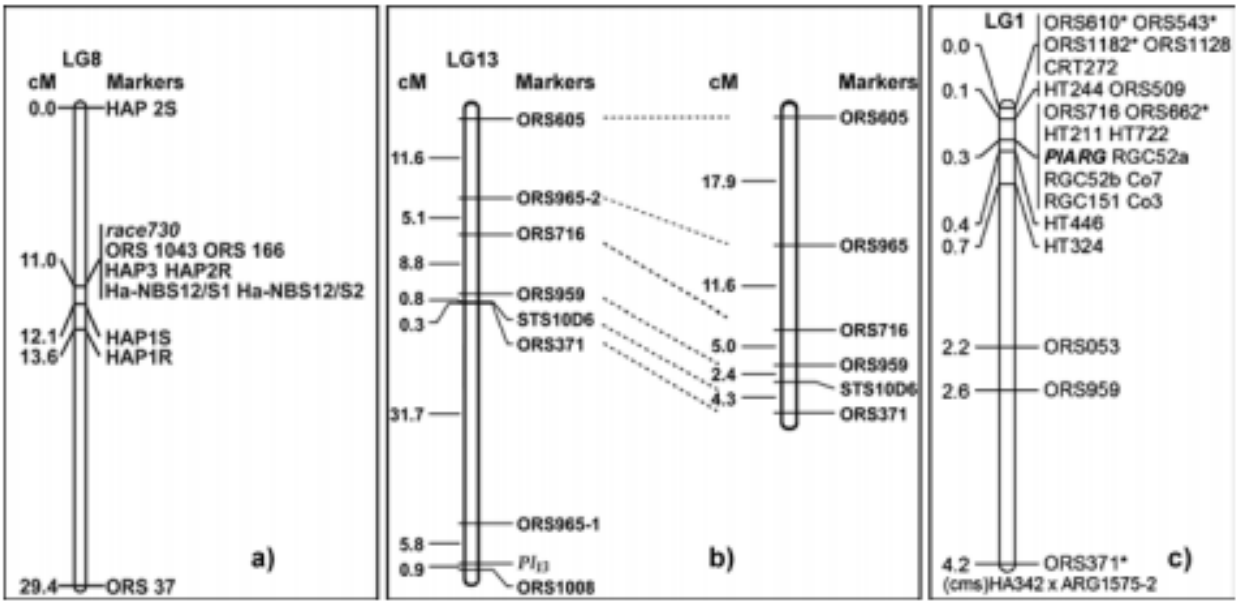

Figure 8: Mapping of *PI* genes (Jocic et. al., 2012)

Genetic background of sunflower (*Helianthus annus* L.)

Cultivated sunflower is diploid crop (2n=2x=34) (Rieseberg and Seiler 1990). On the point of having 14 annual ad 39 perennial, there are 53 wild species of genus *Helianthus* (Moyers and Roisberg 2013, Marek et al. 2014). Wild annual *Helianthus* species are all diploid (2n=2x=34), just

like s cultivated sunflower. On the other hand, perennial varieties could be diploid (2n=2x=34), tetraploid (2n=4x=68) or hexaploid (2n=6x=102) (Qi et. al. 2016).

Introducing new genes to cultivated sunflowers is one of the struggling methods to downy mildew disease. Having gain resistance of pathogens to resistance genes is the reason of the introducing different genes at different times. Inbred lines HA 335, HA336 (*Pl*$_6$), HA 337, HA 338, HA 339 (*Pl*$_7$) and RHA 340 (*Pl*$_8$) have been broadly used all over the world as a defense to downy mildew disease (Miller and Gulya 1988,1991). However, at least eight races , resistant to *Pl*$_6$ and *Pl*$_7$, were identified at France by Gulya et. al. (2011). e. Up to now, 20 downy mildew resistance genes (*Pl*) have been discovered (*Pl*$_1$-*Pl*$_{18}$, *Pl*$_{21}$ and *Pl*$_{Arg}$) (Table 3). Fourteen of these genes (*Pl*$_1$, *Pl*$_2$, *Pl*$_5$-*Pl*$_8$, *Pl*$_{13}$-*Pl*$_{18}$, *Pl*$_{21}$ and *Pl*$_{Arg}$) have been introduced to specific linkage groups (LGs) of cultivated sunflowers (Figure 2) (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997; Molinero-Ruiz et al. 2003; Yu et al. 2003, Mulpuri et al. 2009, Romano et al 2010, Bachlava et al. 2011; Liu et al. 2012; Qi et. al. 2015; Qi et. al. 2016).

Table 3: *PI* genes that are used for Downy mildew disease resistance

Name of Gene	Gene source	Inbred line	LG region	Introducing Year	Referance
Pl$_1$	-	RHA265, RHA266	LG8	1970	Kinman
Pl$_2$	-	RHA274	LG8	1975	Fick et. al.
Pl$_5$	-	DM-2	LG13	1984	Miller and Gulya
Pl$_6$	*H. annuus*	HA335, HA336	LG8	1991	Miller and Gulya
Pl$_7$	*H. praecox*	HA337, HA338, HA339	LG8	1991	Miller and Gulya
Pl$_8$	*H. argophyllus*	RHA340	LG13	1991	Miller and Gulya
Pl$_{13}$	*H. annuus*	HA-R5	LG1	2009	Mulpuri
Pl$_{14}$	-	-	LG1	2011	Bachlava et. al.
Pl$_{15}$	RNID	RNID	LG8	2010	Romano et. al.
Pl$_{16}$	*H. annuus*	HA-R4	LG1	1996	Roeckel-Drevet
Pl$_{17}$	*H. annuus*	HA 458	LG4	2015	Qi et. al.
Pl$_{18}$	*H. argophyllus*	HA-DM1	LG2	2016	Qi et. al.
Pl$_{21}$	-	PAZ2	LG13	2012	Vincourt
Pl$_{Arg}$	*H. argophyllus*	Arg1575-2	LG1	1991	Seiler

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MOLECULAR STUDIES OF SUNFLOWER RESPONSES TO ABIOTIC STRESSES

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is the third major crop for vegetable oil production worldwide among *Asteraceae* species. Mutant resources or routine protocols to transform genes to sunflower are not available as sunflower genome has not been completed yet. Abiotic stress conditions like drought, extreme temperatures, and high salt causes series of biochemical, physiological and morphological changes. These conditions lead to the production of excess reactive oxygen species (ROS) and osmotic imbalances that limit the productivity and growth of plants. In a scientific literature search, it was found that several genes were characterized in abiotic stress tolerance of sunflower. For instance, sunflower *HaWRKY6* shows functional response in temperature stress, and it is regulated by a miRNA. Sunflower HD-Zip I members *HaHB4*, *HaHB1* and *HaHB11* have critical functions in drought, freezing, and submergence tolerance, respectively. The functions of many sunflower regulatory genes and transcription factors in abiotic stresses are still unclear due to divergent genes encoding for transcription factors. For further studies, outstanding experimental strategies can be applied to overcome difficulties of studying divergent genes encoding for transcription factors in sunflower in abiotic stress tolerance. Understanding of plant responses to abiotic stresses is essential for structural and functional characterization of environmental stress-induced genes. Here we present the current molecular studies of sunflower responses to abiotic stresses.

Keywords: sunflower, abiotic stress, drought, salinity, heat, low temperature

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the significant oil crops in the world and is North America's native crop. It is used in medicine ad as food and is first domesticated by Indians (Kaya, Jocić, & Miladinović, 2012). Sunflower is also used as an ornamental plant and grown commercially. High oil and protein containing commercial sunflower hybrids are used for oil crop breeding (Cvejić, 2016). It is cultivated area is over 22 million ha and annual production of sunflower is over 9 million tonnes in the world (Fernández-Martínez et al., 2009). Sunflower production is mainly concentrated in Ukraine, Russia, Argentina and India (Gulya 2014). According to Turkish Statistical Institute, sunflower production is generated as 2.6% and became 1.7 million tonnes in Turkey (Tuik, 2015). Development of varieties including high oil content became a milestone in oil seed sunflower breeding in the world. Another milestone in the development of sunflower was the discovery of cytoplasmic male sterility. This highly reliable method paved the road to the production of commercial hybrid seeds with inherent advantages (Leclercq, 1969). In early efforts, breeders tried to cope with parasitic weeds (broomrape, *Orabanche cumana*) and insects (Homeosome electellum, sunflower moth) (Fick, 1997) by genetic control. In 1910-1912, Krasnodar by Vasilii Stepanovich Pustovoit started a scientific sunflower breeding program from locally developed varieties. Sunflower is more tolerant to abiotic stresses compared to other field crops because its main organs such as stem, leaves, head and roots have developed specific

structures able to grow under negative conditions or in marginal soils in semiarid zones. To increase the genetic tolerance of cultivated sunflower against abiotic stresses, diversity of the wild *Helianthus* species has been used with good reactions (Škorić, 2009). *H. argophyllus* and *H. paradoxus* showed the best results as wild sunflower species in sunflower breeding against drought and salinity, respectively. Integration of molecular breeding techniques is essential to provide the genetic tolerance mechanisms of wild Helianthus species towards enhancing the abiotic stress tolerance in sunflower breeding program. More progress has been carried out about heat tolerance compared to cold tolerance in sunflower breeding. On the other hand, special breeding programs are needed to be develop in sunflower to deal with mineral toxicities and deficiencies.

Under abiotic stress conditions, transcription factors (TFs) (bZIP, MYC, MYB and DREB), protein kinases and proteases are essential for the regulation of transcriptional changes under adverse environments such as abiotic stress conditions (Pradeep et.al. 2006). Transcription factors are induced by abiotic stress conditions to activate transcription machinery (Figure 1). Cold stress, salinity and drought cause production of reactive oxygen species (ROS) in photosynthesis pathway, limit the availability of CO_2 for the dark reaction and this, in turn, leaves oxygen as the main reductive product of photosynthesis (Mitter, 2002). Abiotic stresses such as drought, salt and cold lead to the accumulation of hydroxyl radicals, hydrogen peroxide, and superoxide in the cells (Hasegawa et.al. 2000). Because of the accumulation of these products along the oxidative stress, many expressed sequence tags (ESTs) from leaf and stem cDNA libraries express catalases, thioredoxins, oxygen-evolving enhancer proteins and peroxidases (Kawasaki et. al., 2001). Due to oxidative stress and accumulation of ROS, most of those proteins are up-regulated in stress conditions (Kawasaki et al., 2001).

In literature, several TFs of *Asteraceae* species are defined as essential in abiotic stress tolerance. During the last five years, characterized *Asteraceae* TFs include sunflower *HaWRKY6* that is regulated by a miRNA in temperature response; chrysanthemum *DgWRKY3* that is involved in salt tolerance; sunflower HD-Zip I members *HaHB4*, *HaHB1* and *HaHB11* that are functional in drought, freezing and submergence tolerances, respectively; chrysanthemum DREB subfamily member of the AP2/ERF family *CgDREBa* and the bHLH member *CdICE1* that are essential in freezing, salt and drought stress tolerance; chrysanthemum MYB TF, *CmMYB2* that is involved in salinity and drought stress; chrysanthemum NAC *DgNAC1* that confers salt tolerance; chrysanthemum zinc finger protein *DgZFP* bringing about salt tolerance (Table 1).

Table 1. *Asteraceae* genes encoding transcription factors under abiotic stress conditions.

Gene name and source	**Function**	**Reference**
Sunflower *HaWRKY6*	High temperature tolerance	Giacomelli et al., 2012
Chrysanthemum *DgWRKY3*	Salt tolerance	Liu et al., 2013
Sunflower *HaHB4*	Drought tolerance	Dezar et al., 2005
Chrysanthemum *CgDREBa*	Freezing, salinity and drought tolerance	Chen et al., 2012
Sunflower *HaHB1*	Freezing and drought tolerance	Cabello et al., 2012
Chrysanthemum *CdICE1*	Freezing, salt and drought tolerance	Song et al., 2014
Chrysanthemum *CmMYB2*	Salinity and drought tolerance	Shan et al., 2012
Chrysanthemum *DgNAC1*	Salinity and drought tolerance	Liu et al., 2011

From drought and salinity stress samples, microsatellites located within ESTs in *H. annuus* are analyzed from populations from arid desert and salty areas. Test statistics of lnRV and lnRH were used to select candidate genes that have a wide variety of functions. 17 significant loci of included genes were analyzed based on BLAST hits with homology search. According to the results, genes were categorized as five transcription factors, three cellular components, four genes with catalytic or metabolic functions, four genes of unknown homology or function and one DNA-repair gene (Kane et al. 2007).

A large quantity of ESTs from *Helianthus* spp. are available in public databases, but they are not studied well (Giacomelli, 2010). Giacomelli et al. (2010) estimated 97 sunflower WRKY members derived from EST databases. They report that *Asteraceae* WRKY family can be the source of specific new functions with a particular diversification. Additionally, they suggest that the sunflower *WRKY*s can be used as markers of tolerance to necrotrophic pathogens because they could have a significant function in biotic stress response. Furthermore, specific features of the sunflower WRKY family are identified. For instance, they suggest that *HaWRKY4* may function in senescence (Giacomelli, 2010).

Flooding is one of the environmental abiotic stress conditions that affect food production negatively (Boyer, 1982). Cabello et al. (2016) studied on sunflower transcription factor *HaHB11*, which is a member of the sunflower homeodomain-leucine zipper I subfamily of transcription factors. According to their results, overexpression of HaHB11 in transgenic Arabidopsis plants led to larger rosettes, wider stems and significantly increased biomass compared to wild type plants. Transgenic Arabidopsis plants expressing *HaHB11* showed enhanced tolerance to flooding stress. Additionally, transgenic plants produced twice the amount of seeds that the wild type plants produced (Cabello, 2016).

DROUGHT TOLERANCE IN SUNFLOWER BREEDING

Quartacci and Navari-Izzo (1992) indicated that sunflower seedlings exposed to water deficiency accumulated lower levels of soluble proteins, chlorophyll, and total and polar lipids compared to control plants. Under water stress, root growth extension is observed into moist soil regions. To escape from desiccation tolerance, there are available mechanisms, pathways and reactions, including the accumulation of intracellular proteins such as late embryogenesis-abundant (LEA) proteins. They stabilize other proteins and membranes against drying. Dehydrins are among drought stress induced proteins in D-11 subgroup of LEA family (Giordani, 1999).

Mayrose et al. (2011) analyzed protein phosphatase 2C and the HD-Zip transcription factor ATHB8 under drought stress conditions. Protein phosphatase 2C gene is from a group of serine /threonine protein phosphatases. These proteins are negative regulators in plant responses under abiotic stress conditions such as drought (Schroeder et al. 2001; Tahtiharju&Palva, 2001). HD-Zip transcription factor ATHB8 induces developmental reactions to the environmental conditions. *ATHB8* expression decreased under drought stress such that 1.3 fold repression in native plants, 2.6 fold repression in weeds were observed, while the highest repression was found in crops as 3.2 (Mayrose et al., 2011). Interestingly, they showed that no control plant has the expression of the *ATHB8* gene. Additionally, *ATHB8* transcription factor is available in reduced growth and weedy plants under drought conditions.

Members of the sunflower (or other *Asteraceae* species) WRKY family are not clear completely so far. HaWRKY76 is a sunflower transcription factor whose biological role is not found yet because the WRKY family is highly diversified in the *Asteraceae* (Giacomelli et al. 2010). Raineri et al. (2015) indicated that HaWRKY76 is a divergent sunflower WRKY transcription factor. It enhances the dehydration and submergence tolerance in Arabidopsis when expressed in transgenic plants. It is suggested that HaWRKY76 could be potential tool to make drought tolerant plants (Raineri, 2015).

SALINITY TOLERANCE IN SUNFLOWER BREEDING

Mineral salt accumulation in global arable lands leads to abiotic stresses. After moisture stress, salinity is in the second rank in causing agricultural problems. Accumulation of excess amount of soluble salts, mineral toxicity or deficiency may cause this stress (Singh, 2006). Salinity stress limits plant growth and productivity (Khan et al., 2014). Khan&Asim (1998) evaluated that limited cell division resulted from salt stress causes cell volume reduction. Salt stress negatively affects biochemical and physiological changes, placement of solute dissolved proteins, nutrient uptake, ion-uptake and carbon assimilation (Schroeder et al., 2013; Naz&Bano, 2015). Selectivity of root membranes is impaired by excess amount of Na^+ and Cl^- that are predominant ions causing high ionic imbalances (Bohra&Dörffling, 1993). To examine the comparative differences of salinity effect, different physiological characters such as compartmentation of Na^+ and Cl^- ions, osmotic adjustment, selectivity for K^+ ahould be taken into consideration regarding to the salt tolerance in crops (Wyn Jones&Storey, 1981). Ahmed et al. (2005) explained that sunflower cultivars grown in saline environment show crucial reductions in height, leaf area and stem girth. These growth limitations cause oil percentage reductions. In salinity conditions, plant cell turgor pressure is reduced and then this causes stomatal closure, which limits carbon fixation and photo-assimilation rate (Gale & Zeroni 1984).

Fernandez et.al (2008) studied eighty genes isolated from organ-specific cDNA libraries under salinity (NaCl) and low temperature conditions. They looked at microarray profiling of chilling and NaCl-treated sunflower leaves, and indicated significant changes in transcription factors, defense/stress related proteins, transcript abundance and effectors of homeostasis under both stresses. They categorized results of differentially expressed genes according to their functions (Table 2). In Table 2, down-regulated and up-regulated number of genes in categorized metabolism are given under salinity stress.

Table 2. Number of genes involved in different functional categories (Fernandez, 2008).

	Cold			**Salinity**		
Functionally classified proteins	**NC**	**Up**	**Down**	**NC**	**Up**	**Down**
Central metabolism/Photosynthesis	1	2	7	2	2	6
Translation machinery	2	3	1	1	3	2
Transcriptional machinery	2	2	-	2	2	-
Signaling machinery	-	1	1	-	1	1
Protein turnover/folding/interactions		3	2	2	1	2
Transport	-	3	-	-	2	1
Secondary metabolism	1	-	2	-	1	2
ROS machinery	-	5	2	2	3	2
Total	6	19	15	9	15	16

NC: No change.

First genetic map of sunflower was constructed by the help of quantitative traits controlling physiological characters regarding to the oil yield and the adaptive responses of sunflower to abiotic stresses (Tang, 2003). This type of genomics-based approach allows the development of low-cost

procedures that will be used further by researchers in breeding programs whose goals are enhancing sustainability and yield stability under abiotic stress conditions.

Fernandez et al. (2008) analyzed that EST T411, similar to a plastidic aldolase is up-regulated under salinity stress. Plastidic aldolase genes are indicated in *Nicotiana* plants and are grouped as AldP1 and AldP2. Yamada et.al. (2000) firstly reported that AldP2 was up-regulated under salt stress while AldP1 was suppressed in salt stress conditions. EST H136 (similar to a chloroplast drought-induced stress protein) is down-regulated under chilling and salinity stresses (Fernandez et al., 2008). CDSP (CHLOROPLAST DROUGHT-INDUCED STRESS PROTEIN) is a type of thioredoxins, which play role in oxidative stress (Broin et al., 2000)

It is found that salinity induces transcription of the *MIPS* (*MYO-INOSITOL-1-PHOSPHATE SYNTHASE*) during biosynthesis pathway of myo-inostol and its derivatives (Nelson, 1998; 1999). Myo-inositol-1l-phosphate synthase (MIPS) is functional in *de novo* inositol biosynthesis pathway (Loweus and Loweus, 1983). In *M. crystallinum*, salinity stress induces higher expression of *MIPS* mRNA as 5-folds, resulting in free inositol accumulation of 10-folds (Ishitani et al., 1996).

Understanding of genetic mechanism to salty environment will improve plant responses to changing conditions and develop insights to long-standing questions. Edelist et al. (2009) reported constitutively under- or over-expressed genes regarding to potassium and calcium transport (homologues of *KT1, KT2, ECA1*) in hybrid species of *H. paradoxus.* They found that salinity treatment induced over-expression of homologues of the potassium transporter *HAK8* and its transcriptional regulator.

In sunflower, a small family of three genes (*HAS1*, *HAS1.1* and *HAS2*) encodes asparagine synthetase (AS; EC 6.3.5.4) (Herrera-Rodríguez et al., 2007). They are regulated differentially by nitrogen, carbon and light availability. Gene specific probes are used in Northern analysis under osmotic stress, heavy metal stress and salt stress. They reported that stress treatments did not induce any changes in the expression of *HAS2*. Osmotic and salt stresses decreased the expression of *HAS1* and *HAS1.1* genes in light conditions (Herrera-Rodríguez et al., 2007).

SALT OVERLY SENSITIVE2 (SOS2) and PLASMA MEMBRANE PROTEIN3-1 (PMP3-1) are functional in homeostasis. They were analyzed in two salinity-contrasting sunflower lines, Hysun-38 (salt tolerant) and S-278 (moderately salt tolerant) (Saadia, 2013). In sunflower root tissues from both tolerant and moderately tolerant lines, *SOS2* expression showed gradual increase under salt stress. A gradual increase of *SOS2* expression was observed in leaf tissues of tolerant variety compared to moderately tolerant one. They observed highest level of *PMP3-1* expression in the roots of tolerant sunflower line in the post-salinity level (6 and 12 h of stress treatment). Higher expression of *PMP3-1* was observed in moderately tolerant line at 12 and 24 h of salt treatment (Saadia, 2013).

NAC family transcription factors in plants are functional in abiotic stress responses (Jeong, 2010). In tolerance to abiotic stresses, only a few stress-responsive NAC proteins are characterized (Nakashima, 2011). Manjunath et al. (2013) developed a simple and effective screening methodology to identify transformants under salt tolerance. They created leaf discs of *EcNAC1* gene transformants. They analyzed *EcNAC1* gene with *HPT II* specific primers and *Sac I* restriction enzyme is used to digest the amplified *EcNAC1* gene product. They suggest that initial identification of promising transformants result from *in vitro* screening strategy at plant level based on the target gene (Manjunath, 2013).

HEME OXYGENASE1 (HO1) is functional in protecting mechanisms against environmental stress responses (Zhu, 2014). It is a stress responsive antioxidant enzyme that cleaves heme to biliverdin IXα (BV). BV functions in concominant release of carbon monoxide (CO) and production of free iron (Fe^{2+}) (Shekhawat 2010). Zhu et al. (2014) cloned sunflower *HaHO1* gene, which is required for sunflower salinity acclimation. They showed the induction of *HaHO1* was closely associated with the sunflower salinity acclimation.

HEAT TOLERANCE IN SUNFLOWER BREEDING

Heat stress is defined as high temperature lasting in enough duration that cause important yield reduction compared to control plants (Singh, 2004). Emissions of heat stress in environment resulting from automobiles, industry and urbanization cause temperature increase that endangers diversity of fauna and flora (Singh et al. 2006). High temperature causes heat stress in plants that affects physiological, morphological and physiological traits negatively (Table 3).

High temperatures may cause stomatal closure, a rise in respiration rate, leaf, or canopy temperature, cell membrane injuries, disruption of the photosynthetic apparatus, and the induction of stress-specific growth regulators, which shorten the total growth period due to changes in crop phenology, biomass, fruiting sites, gamete sterility, seed fruit, seed size, and seed quality (Moriondo & Bindi 2006; Moriondo et al. 2011).

In the growing environment, plants are more vulnerable to heat stress in their flowering stages. Under such conditions, large quantities of pollens are selected by breeders. To obtain the best pollination and seed formation, it is necessary to maintain pistil, stigma or disk flowers that are tolerant to high temperature (Škorić, 2012).

Table 3. Effects of heat stress on sunflower.

Traits	**Effects**	**LITERATURE**
Leaf growth period (d)	Decreased by 1.04 days per °C above 36°C	Rawson and Hindmarsh (1982)
Grain weight /yield (g)	Grain weight was reduced up to 21% and final grain yield reduced by 10% at 38°C	Ploschuk and Hall (1995)
Grain-filling duration (d)	Reduced by 2–6 days at 38°C	Ploschuk and Hall (1995)
Grain weight (g)	40% decrease when temperature is >35°C during early grain development	Rondanini et al. (2003, 2006)
Respiration rate (mmol m^{-2} s^{-1})	Increased 19% when night temperature 5°C higher than control	Manunta and Kirkham (1996)
Oleic acid (%)	Increased oleic acid production at the expense of linoleic acid	Harris et al. (1978); Fernández-Moya et al. (2002)
Leaf temperature	1–2°C higher than ambient air temperature (42°C) in susceptible lines	Kalyar et al. (Forthcoming 2013)
Heat stress injury (%)	Decreased 10–65% in sunflower germplasm with variable resistance evaluated at 40°C	Kalyar (2013)

WRKY transcription factors are functional in plant stress responses. The sunflower *HaWRKY6* contain a target site for the binding of miR396. Giacomelli et al. (2012) analyzed the possible post-transcriptional regulation of *HaWRKY6* by miR396 in the *Asteraceae*. They found that the silencing of *HaWRKY6* due to miR396 accumulation is responsible for high-temperature protection in sunflower (Giacomelli, 2012).

LOW TEMPERATURE TOLERANCE IN SUNFLOWER BREEDING

Low temperature limits crop productivity in many environments. When the temperature is above freezing level (> 0 °C), it is called as chilling; if it is below 0 °C, it is called as freezing. Kalaydzhyan et al. (2009) developed sunflower genotypes that are tolerant to cold after mutagenizing the plants by dimethyl sulfate (DMS) as chemical mutagen. 44.000 seeds of about 2.000 mutagenic progenies were screened under low temperatures by planting them in early and late winter. 499 plants from 72 mutagenic progenies were able to grow under harsh winter and low temperature conditions (down to −20°C).

HaF455 involved in ribosomal activity is induced by cold and salinity stresses in sunflower (Fernandez et al. 2008). Fernandez et.al. (2008) showed that the expression of EST H123 [GenBank: BU672069] having high identity with myo-inositol phosphate synthase (MIPS protein, isomerase involved in inositol metabolism) was decreased by chilling and salinity stresses.

CONCLUSION

There are many reports on molecular mechanism of sunflower abiotic stress tolerance. However, molecular attempts to sunflower abiotic stress tolerance have not been enough as compared to the molecular studies performed with other crops. Especially, the use of molecular techniques such as QTL identification and associating mapping will enable a faster and more efficient breeding program in sunflower abiotic stress tolerance. For further studies, the application of different molecular methods such as transcriptomics will help development of new sunflower cultivars that are more tolerant to abiotic stress conditions. In further studies, array based cDNAs will contribute to the understanding of functions of more sets of genes functioning in sunflower abiotic stress tolerance.

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MOLECULAR STUDIES INVOLVED IN SUNFLOWER RESPONSES IN DROUGHT STRESS

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ABSTRACT

Sunflower is a major oil seed crop worldwide that shows adaptations to diverse environmental conditions, such as water and salt stresses. It is adapted to grow in semi-arid conditions. To provide expansion of cultivated area, it is crucial to determine and create drought tolerant sunflower genotypes. There are diverse physiological and biochemical responses against water stress by up- or down-regulating stress tolerance genes and other mechanisms, including hormones and secondary metabolite accumulations. For instance, an increase in the expression of sunflower aquaporin gene *HaTIP7* was observed in guard cells and root phloem under drought conditions. Overexpression of a drought-responsive dehydrin gene, *HaDHN1*, makes the sunflower line tolerant to water-limited conditions. For further studies, outstanding transcriptomic strategies can be applied to examine the genes encoding for transcription factors in sunflower in drought tolerance. Here, we present recent molecular studies associated with drought stress tolerance in sunflower genotypes.

Keywords: sunflower, drought stress, molecular studies, tolerance

INTRODUCTION

Sunflower (*Helianthus annuus* L. var. *macrocarpus* Ckll.) oil is the fourth most significant vegetable oil after palm, canola and soy in the world. Planted all over the world, sunflower is used for a wide range of purposes including oil production from oil seed sunflower, consumption of seed directly from confectionary sunflower and cosmetics production from ornamental sunflower. Sunflower production is mainly concentrated in Ukraine, Russia, Argentina and India (Gulya 2014). According to Turkish Statistical Institute data, sunflower production is increased 2.6% and became 1.7 million tonnes in Turkey (Tuik, 2015). It is cultivated in over 22 million ha land, and annual production of sunflower is over 9 million tonnes in the world (Fernández-Martínez et. al., 2009). Geographical, archeological, morphological and molecular evidences suggest that sunflower was first domesticated in eastern North America (Rieseberg, 2001). Sunflower breeding programs date back to early 20th century. In 1910-1912, Krasnodar by Vasilii Stepanovich Pustovoit started a scientific sunflower breeding from locally developed varieties. In first efforts, breeders tried to cope with parasitic weeds (broomrape, *Orabanche cumana*) and insects (*Homeosome electellum*, sunflower moth) (Fick, 1997) by genetic controlling. Development of varieties including high oil content became a milestone in oil seed sunflower breeding in the world. Another milestone in the development of sunflower was the discovery of cytoplasmic male sterility. This highly reliable method paved the road to the production of commercial hybrid seeds with inherent advantages (Leclercq, 1969). In recent years, sunflower breeding programs have started to concentrate on the development of varieties tolerant to environmental stresses.

Drought stress is the most significant abiotic stress factor that affects the plant production because around one third of the soils are affected by drought worldwide. All plant organs show reactions to drought stress with morphological, physiological, and metabolic alterations. To

minimize yield reduction due to drought stress, there are various mechanisms that are categorized in three groups of (1) dehydration avoidance, (2) drought escape, and (3) dehydration tolerance (Singh, 2000). In the slowly dehydrated attached leaves of drought-stressed plants, dehydration avoidance by osmotic adjustment is improved (Levitt, 1985). The activation of drought escape mechanism in plants is a significant process in early maturation that provides suitable environment for late-season drought stress conditions (Singh, 2000). Water uptake and consumption is minimized by soil drought. In order to withstand drought conditions, plants decrease the transpiration rate by stomatal closure, which in turn leads to overheating in the leaves due to local high temperature. Because of water loss, photosynthesis rate also decreases gradually (Žolkevič, 1968). Increasing the water retention capacity is a protection mechanism in plants to escape from water loss. Reduction in transpiration rate and enhancement of water uptake from soil are together referred to as water or turgor potential under drought conditions. In drought stress, dehydration tolerance increases with leaf number. (Levitt, 1985)

Several morphological and physiological parameters are used to examine the drought tolerance levels in sunflower (Škorić, 2009). They include leaf water potential, yield stability, root xylem diameter, root growth, stomatal conductance, osmotic adjustment, canopy temperature, abscisic acid (ABA) accumulation, seedling recovery after stress, growth under stress, proline accumulation and leaf rolling. These parameters were used to evaluate the differences in drought tolerance levels among sunflower varieties in attempts to develop drought tolerant sunflower genotypes. In an early attempt, Škorić (1992) evaluated over 30 different parameters to study the drought tolerance in sunflowers. In another attempt, Petrović et al. (1992) evaluated the free-proline accumulation rate and nitrate reductase activity under conditions of water stress, and found large amounts of differences between sunflower varieties. Therefore, proline accumulation and nitrate reductase activity levels were proposed to be used as indicators for the estimation of drought stress in sunflower varieties. Unlike other plant species such as Arabidopsis, wheat, rice and soybean, water stress tolerance mechanism is still not completely comprehended in molecular basis in sunflower even though most of drought-related genes are cleared in other plant species (Cellier et al., 1998).

MOLECULAR STUDIES OF DROUGHT TOLERANCE IN SUNFLOWER

Morphological, physiological, and metabolic modifications respond to drought stress in whole plants. At the cellular level, water deficit causes cell damage in all plant organs. In adaptive processes, other responses occur (Ingram and Bartels, 1996). In sunflower, there are several management mechanism to coper with drought stress. Water deficit reduces root proliferation, leaf size and stem extension by disturbing water relations. A variety of physiological and biochemical responses are controlled at cellular and whole-organism levels start to manage drought stress (Farood, 2009). CO_2 assimilation in the leaves is reduced by membrane damage, stomatal closure and disturbed activity of various enzymes that function in adenosine triphosphate synthesis and CO_2 fixation. Oxidative load generating reactive oxygen species (ROS) is increased by enhanced metabolite flux through the photorespiratory pathway. ROS cause injury to biological macromolecules under drought stress (Farood, 2009). This is among the major obstacles for growth. Plants display some mechanisms to cope with the drought stress. The major mechanisms that curtail the effects of water loss include the diffusive resistance, enhanced water uptake by deep and prolific root systems, and succulent and smaller leaves to limit the transpirational loss. Moreover, potassium ions provide osmotic adjustment while silicon develops root endodermal silicification and enhances the cell water balance. To sustain cellular functions under drought conditions, low-molecular weight osmolytes including proline, glycine-betaine and other amino acids, polyols and organic acids have significant emerging roles. Plant growth substances such as auxins, gibberrellins, salicylic acid, ABA and cytokinins regulate the plant responses toward drought stress. To reduce the adverse effects of drought stress, citrulline polyamines and several enzymes act as antioxidants (Farood, 2009).

In breeding programs, development of tolerant cultivars and selection of drought tolerance is achieved by the help of physiological trait improvements or genetic modifications. More recently, molecular markers were effectively applied to select for enhanced drought tolerance among sunflower varieties (Škorić, 2009). At molecular level, several drought responsive genes and transcription factors have been characterized in sunflower. However, the studies to identify drought responsive genes, such as the genes encoding for late embryogenesis abundant (LEA) proteins, dehydration-responsive element-binding proteins and aquaporins in sunflower have been limited under drought stress conditions. Although a lot of physiological studies have been carried out in sunflower, molecular studies and genetic modifications over drought stress tolerance are limited.

Expressions of many genes are up- and down-regulated by water deficit (Table 1). Liu et al., (2003) analyzed the structural and functional characterization of environmental stress-induced genes under drought and salinity stresses in sunflower. Differential display was used to compare overall differences in gene expressions between drought- or salinity-stressed and unstressed (control) plants of sunflower. Guanylate kinase (signal transduction), *lytB* (antibiotic/drug resistance), selenium-binding protein (heavy metal stress), polyprotein (reverse transcriptase), and AC-like transposable element were identified from sequence analysis of used clones under drought and salinity stress conditions. To regulate water fluxes in plants, aquaporins are one of the major functional transporters in plants. Under water deficit conditions, sunflower aquaporin gene *HaTIP7* accumulated in the roots inducing stomatal closure (Aguado, 2014). In drought and exogenous ABA conditions, sunflower *HaABRC5* of ABI5-Interacting Proteins (AFP family) was up-regulated in roots, seedling shoots and leaves (Liu et al. 2004). This gene is predicted to be an ABA-responsive nuclear protein playing a role in plant stress responses in sunflower. In drought conditions, hydrophilin and LEA proteins are essential as soluble proteins to provide maintenance of cellular integrity. Drought induced transcripts of *HaELIP1*, *HaDHN1*, and *HaDHN2* accumulated in leaves of tolerant sunflower variety under progressive drought (Cellier, 1998). Water stress induced *HaELIP1* gene expression and accumulation of dehydrins in sunflower leaves (Ouvrard et al. 1996). Dehydrins are from D-11 subgroup of LEA proteins that are functional as water stress-induced proteins. Dehydrins accumulate in desiccation tolerant seed embryos during water stress conditions (Close, 1997). Actually, the function of dehydrins in drought stress tolerance mechanism is not clear, but their accumulation in drought stress was shown to be increased in previous studies (Giordani et al., 1999; Hundertmark and Hincha, 2008). Interestingly, the expression of the *HaDHN1A* dehydrin gene was under the control of two possible mechanisms of ABA-dependent or ABA-independent pathways. The expression level of *HaDHN1A* transcript was lower in ABA-deficient sunflower mutant compared to ABA-sufficient non-mutant under water limited conditions, suggesting the involvement of ABA-dependent tolerance mechanism in sunflower responses under drought stress (Giordani, 1999). In ethylene synthesis, ACCO (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE) is a main regulatory enzyme. *HaACCO2* transcript expression increased in sunflower leaves (Liu et al. 1997). Drought and exogenous ABA application induced the expression of this gene as well (Ouvrard et al. 1996).

Gago et.al. (2002) reported that the transcript abundance of a homeodomain-leucine zipper protein, *Hahb-4*, *was* controlled by water stress conditions in sunflower whole seedlings, roots, stems and leaves. Additionally, ABA was proved to function as a component in signal transduction pathway that regulates *Hahb-4* expression under water stress conditions. ABA is involved in various regulations in plant physiological and developmental stages, and in pathways to cope with drought and salinity stresses (Skriver & Mundy 1990; Leung & Giraudat 1998). The functional analysis of the promoter sequences of genes involved in sunflower drought tolerance identified *ABRE* (ABA responsive elements) consensus sequences indicating the involvement of ABA signaling in drought tolerance in sunflower.

Table 1. Genes involved in sunflower drought tolerance.

Genes	Results	Reference
HaELIP1, HaDHN1,* and *HaDHN2	drought-induced genes	Ouvrard et al. 1996
HaTIP7	transcript accumulation by water deficits	Sarda et.al. 1997
HaACCO2	induced by drought and exogenous ABA application	Ouvrard et al. 1996
ABI5-Interacting Proteins (AFPs)	involved in ABA response	Garcia et al. 2008
LTP genes	induced by water deficit and ABA application	Colmenero-Flores et al, 1997

Water deficit stress and ABA application induce *LTP* genes encoding for Lipid Transfer Proteins. These proteins are functional as epidermal cell wall proteins that are essential for the secretion of extracellular lipophilic substances (Martin and Brewbaker, 1971). Ouvrard et. al. (1996) showed that *HaLTP* gene expression increased in ABA treatment and drought stress conditions in sunflower. Mitotic activity and DNA synthesis activity is constrained by drought stress and ABA treatment (Robertson et al., 1990). Liu et.al (2003) showed that *HaRPS28* expression in different organs of sunflower decreased under drought and salinity stresses. Additionally, they realized that *ABRE* repeats in 3' UTR of *HaRPS28* mRNA are expressed in low levels, suggesting a new comprehension and study about ARE-mediated decay pathway under drought stress conditions in sunflower (Liu, 2003).

Giordani et al. (2011) analyzed 8 genes, namely *NAC1*, *DREB*, *ABA-C5*, *ABP1*, *DHN*, *HSP*, *LTP* and *DES*, which are functional in drought responses in eight sunflower inbred lines with phenotypic characters. Gene expression analyses proved that these genes are putatively essential in drought stress responses. *NAC1* gene belongs to the NAC family of transcription factors functioning in morphogenesis and stress responses (Ooka et al. 2003). Drought-responsive-element-binding (DREB) protein encoding genes are transcription factors, which bind DRE cis-elements of drought-responsive genes (Shinozaki and Yamaguchi-Shinozaki 2007). ABA-responsive-C5 (ABAC5) encoding gene was involved in ABA-mediated drought response, and there are two copies in the sunflower genome (Liu and Baird 2004). A sunflower specific gene named *AUXIN-BINDING PROTEIN* (*ABP1*) was shown to be involved in the auxin transport within the cell and was predicted to be the auxin receptor (David et al. 2007). Induction of *ABP1* under drought stress in sunflower suggests the involvement of auxin signaling in drought tolerance mechanism. In addition to these genes, genes encoding for heat shock proteins (HSP) and desaturase enzyme are also induced under drought conditions in sunflower. The nucleotide diversity values of four genes (*NAC*, *ABA-C5*, *DREB*, *ABP1*) were shown to be lower than the other four, although they were highly functional genes encoding for proteins involved in the regulation of transcription or signaling cascades under stress conditions (Giordani, 2011).

Drought tolerance of sunflower has not been studied in details although different plant characters have been analyzed and numerous attempts have been carried out to understand the mechanisms involved in drought tolerance in sunflower. Wild sunflower species provide high level of drought tolerance by controlling various sets of genes to create new drought tolerant sunflower lines (Škorić, 2009). Appropriate screening techniques, controlling genetic backgrounds and analyzing physiological mechanisms of drought tolerance can be developed by the help of selection methods and breeding programs (Škorić, 2009).

Molecular Markers in Sunflower Breeding Against Drought

In crop breeding programs, molecular markers play a crucial role in detection of characters. Molecular marker tools can be applied successfully to oil seed crops such as sunflower, soybean and groundnut to control the seed quality or other characters affected by abiotic and biotic stresses (Sujatha, 2009). Ali et al., (2009) analyzed physico-chemical attributes of sunflower seeds under drought stress at different growth stages, i.e. vegetative and reproductive stages. From results of their comprehensive studies, it was concluded that drought affected some constituents of sunflower seed oil in different cultivars. Distinctive parameters such as fatty acid composition, oil yield, iodine value and oil tocophenol content are significant factors that are the most vulnerable to water deficit (Ali et al., 2009). Studies conducted in unfavorable conditions, especially drought showed that it affects the seed composition and seed numbers (Nel, 2001; Anwar et al., 2006). In quantitative trait loci (QTL) identification studies, several physiological traits were associated with genomic locations (Hervé 2001). Hervé et.al. (2001) analyzed traits related to photosynthesis including internal CO_2 concentration, net photosynthesis rate, leaf chlorophyll content, and water status traits including transpiration, stomatal conductance, relative water potential and leaf water potential in recombinant inbred sunflower lines. Analyzed traits showed a correlation between water potential and transpiration, and between transpiration and photosynthesis rates. This study was the first one about the identification of genetic characters involved in water status and photosynthesis in sunflower under drought conditions. Genetic markers associated with these physiological characters were also identified in sunflower inbreed lines, and their utilization in future breeding programs was evaluated.

Haddadi et.al. (2011) detected genomic regions associated with leaf related traits and yield components in recombinant inbred sunflower lines under water stress. This study is a suggestive work for the development of future marker-based approaches in sunflower. This study can develop improved understanding of positional cloning of related genes in development, and improvement of near-isogenic lines in sunflower varieties. Kiani et al. (2007) detected QTLs related to water status and osmotic adjustment in sunflower with two water treatments in greenhouse conditions.

In a recent study, Abdi et.al. (2013) compared the relative water content and chlorophyll concentration in 70 recombinant sunflower inbred lines under drought and control conditions. By using 210 simple sequence repeats (SSRs), 11 genes were placed in 17 linkage groups. A total of 10 and 8 QTLs were identified for chlorophyll levels and relative water content, respectively. Utilization of SSR markers to develop an association mapping gives a greater precision in decoding the genetic map. Identification of genomic regions related to drought tolerance phenotypes will develop a future understanding of marker-based approaches in drought stress conditions in sunflower. These molecular studies give new insights to the development of drought tolerant sunflower varieties in molecular ways.

CONCLUSION

Although a large number of drought-induced genes have been characterized in other plant species, molecular basis of sunflower tolerance to water deficit has not been completely understood. There are many reports on molecular mechanism of sunflower drought stress tolerance. Sunflower drought tolerance can be managed by the use of molecular techniques such as marker-assisted selection, QTL identification and associating mapping. At the molecular level, several drought responsive genes and transcription factors have been characterized in sunflower drought stress tolerance. However, the application of different molecular methods such as transcriptomics will help the development of new sunflower cultivars that are more tolerant to drought stress conditions. Applications such as mass-screening and breeding, exogenous application of hormones and osmoprotectants to growing plants are also ongoing studies in sunflower. Additionally, array based

cDNAs will contribute to the understanding of functions of more sets of genes functioning in sunflower drought stress tolerance.

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DETERMINATION THE GENETIC CHARACTERIZATION OF DIFFERENT LINES OF SUNFLOWER (HELIANTHUS ANNUUS L.)BY USING GENETIC RESOURCES BASED ON SSRS (SIMPLE SEQUENCE REPEAT)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is one of the most important sources of oil crops in the world and Turkey. The oil of sunflower seed is known as high qualified oil due to having unsaturated acid like oleic and linoleic acid. Using conventional plant breeding methods for determine a high oleic acid sunflower lines are laborious and time consuming but the process of new breeding system is now being accelerated and carried out with more precision and fast-track manner than the classical breeding techniques: also utilization of DNA markers especially by SSR markers has many advantages for example recently, in many plant species marker system has successfully undertaken for identification the genetic resources of different plant species: it also has an important place in sunflower.These markers are used to measure the differences in DNA level. İn Turkey it can be found infrequent researches regarding DNA identification, therefore in this research it was decided to use SSR molecular markers method to provide more genetic levels information about sunflower for further studies. In this research, 10 SSR primers and 41 oleic sunflower lines were used as a material in order to estimation of genetic diversity among high oleic sunflower lines. Sunflower lines were obtained from Trakya Agricultural Research Institute, Turkey. As a result maximum genetic similarity among 41 high oleic asid lines was obtained betweenG3 K6 ASN and K7 RSN 7/13; YDAH SN 2/13 and K7 RSN 7/13; YDAH SN 3/13 and K7 RSN 7/13.A minimum genetic similarity was observed between (K7 RSN 7/13) and (YDAH SN 1 /13). It was identified a total number of 79 alleles. t he number of alleles per locus ranged from 2 (ORS598) to 12 (ha4136). Based on physiological analysis among sunflower lines G3K6 7/13 ASN, ASN G3K8 YDAH SN 3/13 and 1/13, ASN G5K8 YDAH SN 5/13 and 4/13 YDAH SN 2/13 and G3K6 7/13 ASN was performed the best.

Key words: Sunflower (*Helianthus annuus* L.), DNA, SSR**,** oleic acid

INTRODUCTION

Sunflower is one of the most important oil crops in the world due to higher adaptation capability. (Kaya et al., 2012, Skoric, 2012; Kaya, 2014b). The sunflower oil quality is determined by the saturated and unsaturated fatty acid ratio. The sunflower oil is a high qualitative one, due to very high percentage of poly-unsaturated fatty acids which can reach 90% from the total (Kinman and Earle, 1964; Vrânceanu, 1974, 2000; Skoric, 1989; Schuster, 1993). Among unsaturated fatty acids, the linoleic one is dominant in classical sunflower. There is an important genetic variation regarding the fatty acid composition of the sunflower oil (Cummins et al., 1967; Simpson and George, 1985).
Using conventional plant breeding methods for determine a high oleic acid sunflower lines are laborious and time consuming but the process of new breeding system is now being accelerated and carried out with more precision and fast-track manner than the classical breeding techniques: using of DNA technology in agricultural research has progressed rapidly over the last two decades. The

procedure of DNA extraction should also be quick, simple and cheap. Molecular markers are powerful tools to study genetic variation and relate them to phenotypic variation (Varshney et al., 2005). SSRs (Simple Sequence Repeats) show high reproducibility and genomic covering, co-dominance, neutrality and they are highly polymorphic (Spooner et al., 2005). In sunflower (Helianthus annuus L.), microsatellites and SSR method have been particularly useful in the studies of phylogenic relationships, genotype identification and calculation of genetic relationship between inbred lines(Tang et al. 2002; Poormohammad Kiani et al 2007; Darvishzadeh 2012; Grandon et. al, 2012; Singchai, et. al., 2013). SSR is a powerful technique for assessment of genetic diversity at molecular level. This method aleso (SSR) takes less time, reliable and gives good quality of DNA even without using RNas.

MATERYAL AND METHOD

This research was conducted in Laboratory of The Institute of Biotechnology at Ankara University. In this research, 41 sunflower lines (19 female and 22 male) i.e. G3K6, G5K8, YDAH, K6, K7 were used as a plant material. The seeds were obtained from National Sunflower Breeding Project conducted by Trakya Agricultural Research Institute.

DNA Extraction:

Leaves were harvested from sunflower lines in the field conditions, freeze-dried and ground to powder. DNA extraction was performed according to the cetyl-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). The extracted DNA content was measured using DNA standards in agarose gel (0.8 % w/v).

The PCR reaction contained 20 ng DNA, 1X reaction buffer, 1.5 mM Mgcl2, 0.2 mM of each of dNTP, 0.5 uM of each forward and reverse primer, 0.3 IU Taq DNA polymerase. DNA amplification was performed in a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems Inc., Foster city, CA) with 10 uL reaction volume. DNA samples were denatured initially at 94 °C for 3 min, then subjected to the following 20 cycles: 94 °C for 30 s, 63 °C for 30 s with a decrement of 0.5 °C per cycle, and 70 °C for 1 min. This was followed by another 20 cycles of 94 °C for 15 s, 55 °C for 30 s, and 70°C for 1 min. A 10 min extension was performed at 72 °C as the last step. Amplified products were analyzed using 1.5 % agarose gel. Electrophoresis was performed at 120 volts DC for 2.5 hrs in a submarine electrophoresis system (Maxi sub XL). After electrophoresis, remove the gel from the tank and view the gel under UV illumination and photograph using gel documentation system (Table 1).

Table 1. Sequences of the SSR primers, Fluorescent Dye and references used in the investigation

NO	SSR loci	Forward primer 5'-3'	Revers primer 5'-3'	Fluorescent Dye	References
1	ORS 149	Gctctctatctcccttgactcg	tgctctaagatctcaggcgtgc	D3	Darvishzadeh. 2012
2	ORS 154	Gcacctttggtgaggagata	tgcatcagtagctattgtctat	D3	Darvishzadeh. 2012
3	ORS 1068	Aatttgtcgacggtgacgatag	ttttgtcatttcattacccaagg	D3	Tang et al. 2002
4	ORS 488	Cccattcactcctgtttcca	ctccggtgaggatttggatt	D4	Tang et al. 2002
5	ORS 598	Ccaaatgtgaggtgggagaa	atagtccctgacgtggatgg	D4	Tang et al. 2002
6	ha4136	Cctattcctgataattcactaagc	ggtagcatgcttacattaagatg	D4	Poormohammad Kiani et al 2007
7	ha3513	Tgacccattcaacttcttaa	tcatggttcctgatgagaat	D4	Poormohammad Kiani et al 2007
8	ha1604	Gcaaatgcactaaaggcccc	ccctactcaaaccttacctc	D3	Poormohammad Kiani et al 2007

The quality and quantity of the extracted samples was estimated by using Spectrophotometer (NanoDrop ND-1000) and by 0.8% Agarose Gel Electrophoresis. Polymerase chain reaction (PCR) amplifications were performed as described by Şelli et al. (2007). Forward primers of each pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green), and D4 (blue) (Proligo, Paris, France).

The PCR products were first separated on a 2% (w/v) agarose gel and visualized under UV light. For further determination of polymorphisms, the PCR products were run on CEQTM 8800 XL capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA). Allele sizes were determined for each Sunflower SSR loci using the Beckman CEQ Fragment Analysis software.

Genetic Analysis

Identical cultivars, number of alleles, allele frequency, expected (HE) and observed (HO) heterozygosities, estimated frequency of null alleles (r), and probability of identity (PI) were calculated for each loci using the IDENTITY 1.0 program (Wagner and Sefc, 1999) according to Paetkau et al. (1995). Similarity matrix and Dendrogram was constructed with the unweight pair-group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973), using the Numerical Taxonomy and Multiware Analysis System software (NTSYS-pc) (version 2.0) (Rohlf, 1988).

RESULT AND DISCUSSION

SSR Analysis

10 SSR primers were successfuly amplified and electrophoresed by CEQTM 8800 XL capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA). The size of the markers are varied among 300 bp to 400 bp in 1% agarose gels (Figures 1).

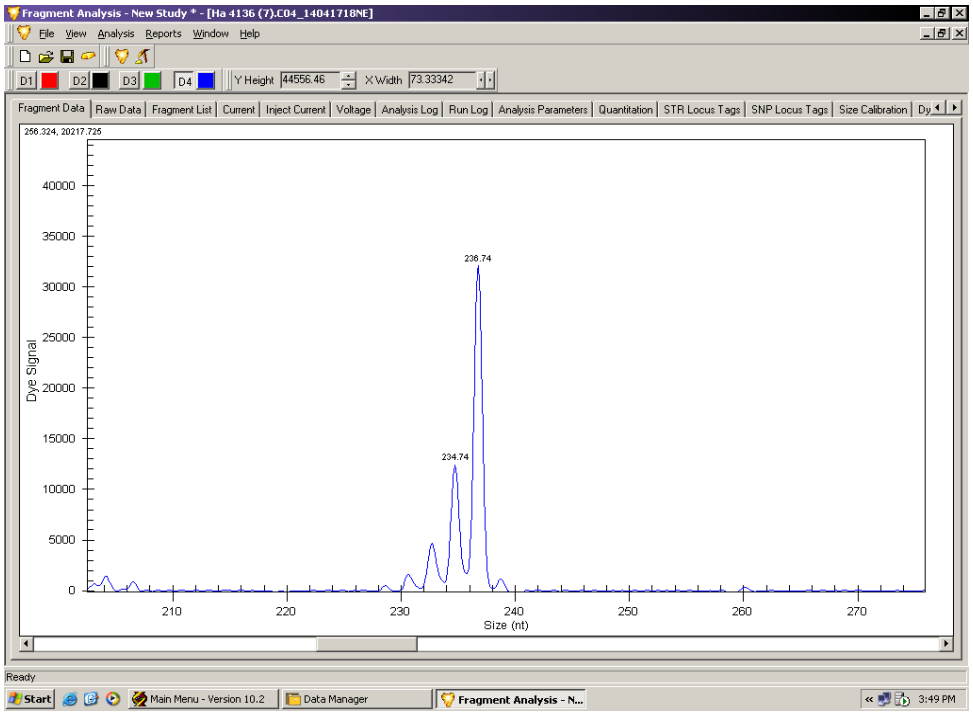


Figure. 1. Homozygote pic profile (allele) at Ha4136 SSR loci

The image of PCR products in agrose jel was observed (Figure 2).

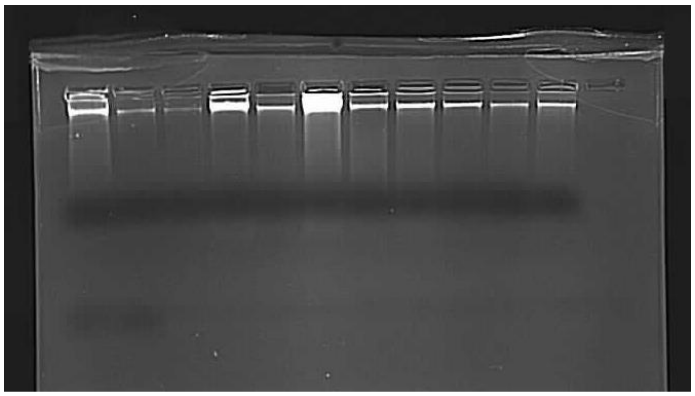


Figure 2. High resolution agarose gel images of SSR markers in Sunflower line.

It was observed that a total number of 57 alleles. the number of alleles per locus ranged from 2 (ORS598) to 12 (ha4136) Table 3. In this research the maximum allel frequency was observed on 3513 lokusuna (536) aittir. Daha sonra OR598 (378), ORS 1068 (364), Ha 4136 (288), ORS 546 (228), ORS 154 (223), ORS 488 (177), ORS 78 (162), ORS 149 (142) and Ha 1604 (121) primers.

Table 3. Number of alleles (*N*A), expected heterozygosis (*H*E), observed heterozygosis (*H*O), probability of identity (PI), and the frequency of null alleles (r)

Loci	Number of alleles (n)	Expected heterozygosity (He)	Observed heterozygosity (Ho)	Probability of identity (PI)	Null alleles (r)
ha1604	3	0.392	0.048	0.549	0.246
ha4136	12	0.740	0.634	0.129	0.060
ha3513	7	0.713	0.926	0.221	-0.124
ORS546	5	0.674	0.975	0.243	-0.179
ORS598	2	0.195	0.170	0.704	0.020
ORS488	4	0.320	0.024	0.507	0.224
ORS78	4	0.433	0.000	0.424	0.302
ORS1068	8	0.762	0.951	0.171	-0.106
ORS154	7	0.660	0.585	0.209	0.044
ORS149	5	0.617	1.000	0.355	-0.236
Total	57	5.506	5.313	-	-
Mean	5.7	0.550	0.531	-	-

Genetic Similarity

Overalls, the values for genetic distances ranged from % 40 - % 90 (Figure 3). The average of genetic similarity was % 48, depicting a high level of genetic variation among studied sunflower genotypes. Among the sunflower genotypes "34 (K7 RSN 7/13)" showed significant distinction, being grouped in a different branch than the other genotypes.

The results of similarity revealed a low genetic similarity was observed as follows:

G3 K6 ASN & K7 RSN 7/13 : YDAH SN 2/13 & K7 RSN 7/13 : YDAH SN 3/13 & K7 RSN 7/13 : G5K8 ASN 6/13 & K7 RSN 7/13 : G5K8 ASN 2/13 & K7 RSN 7/13 with % 16 genetic similarity. The high similarity among sunflowers lines were observed among G5K8 ASN 5/13 & YDAH SN 3/13: G5K8 ASN 5/13 & YDAH SN 1 /13 lines with % 94 (0.94).

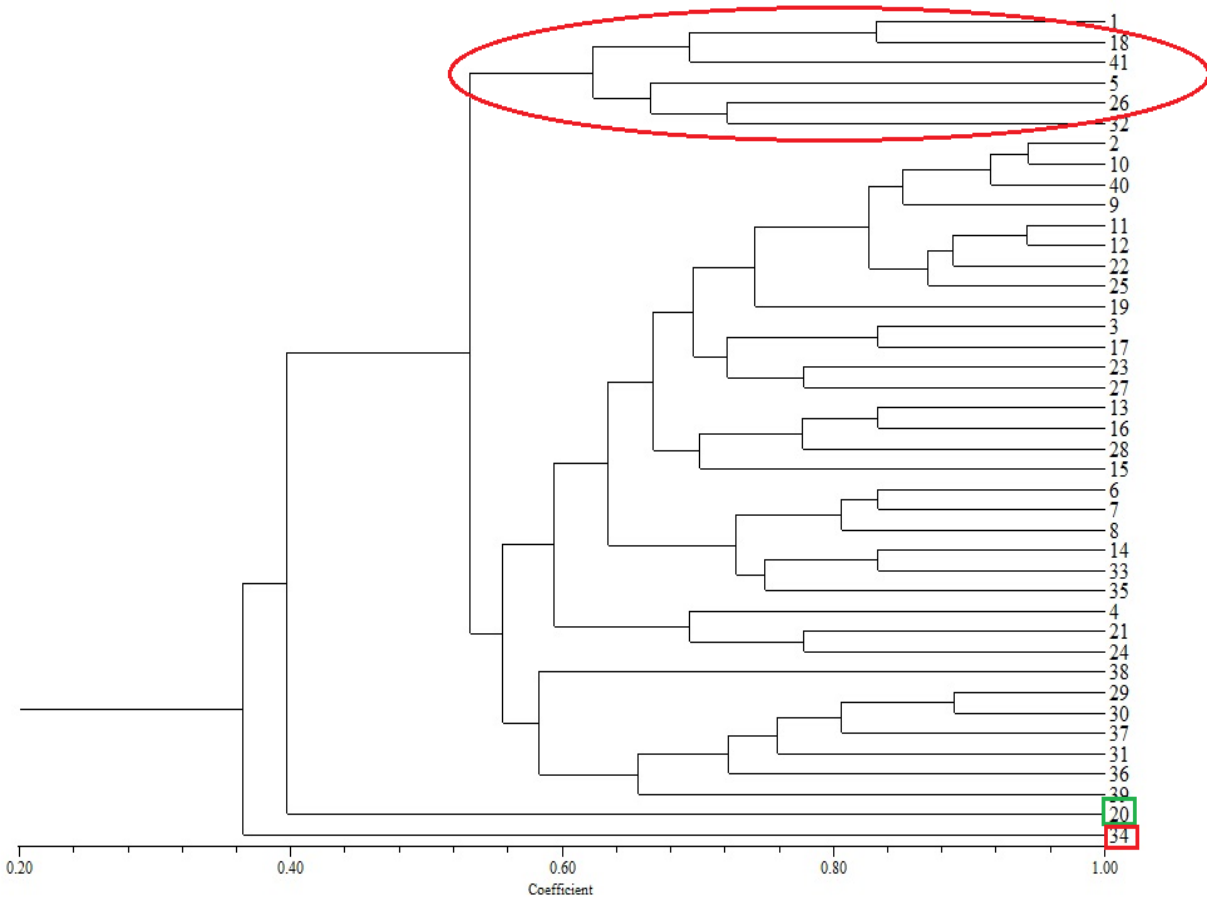


Figure 3: Dendrogram showing the genetic relationship among 41 Sunflower Lines

As sunflower is a highly cross pollinated crop therefore, a high number of alleles per locus could be a result of the natural out crossing among the parental material and also due to having a broad genetic base (Zia et. al, 2014). The average number of alleles found in our study is comparable to those reported in other studies on sunflowers. For instance, Darvishzadeh 2012, used 38 markers for15 sunflower genotypes; the average allele frequency in his study was 2.32. but in our study we detected an average allele frequency 5.7. İt shows that the markers selected in this present study have high polimorphic content.

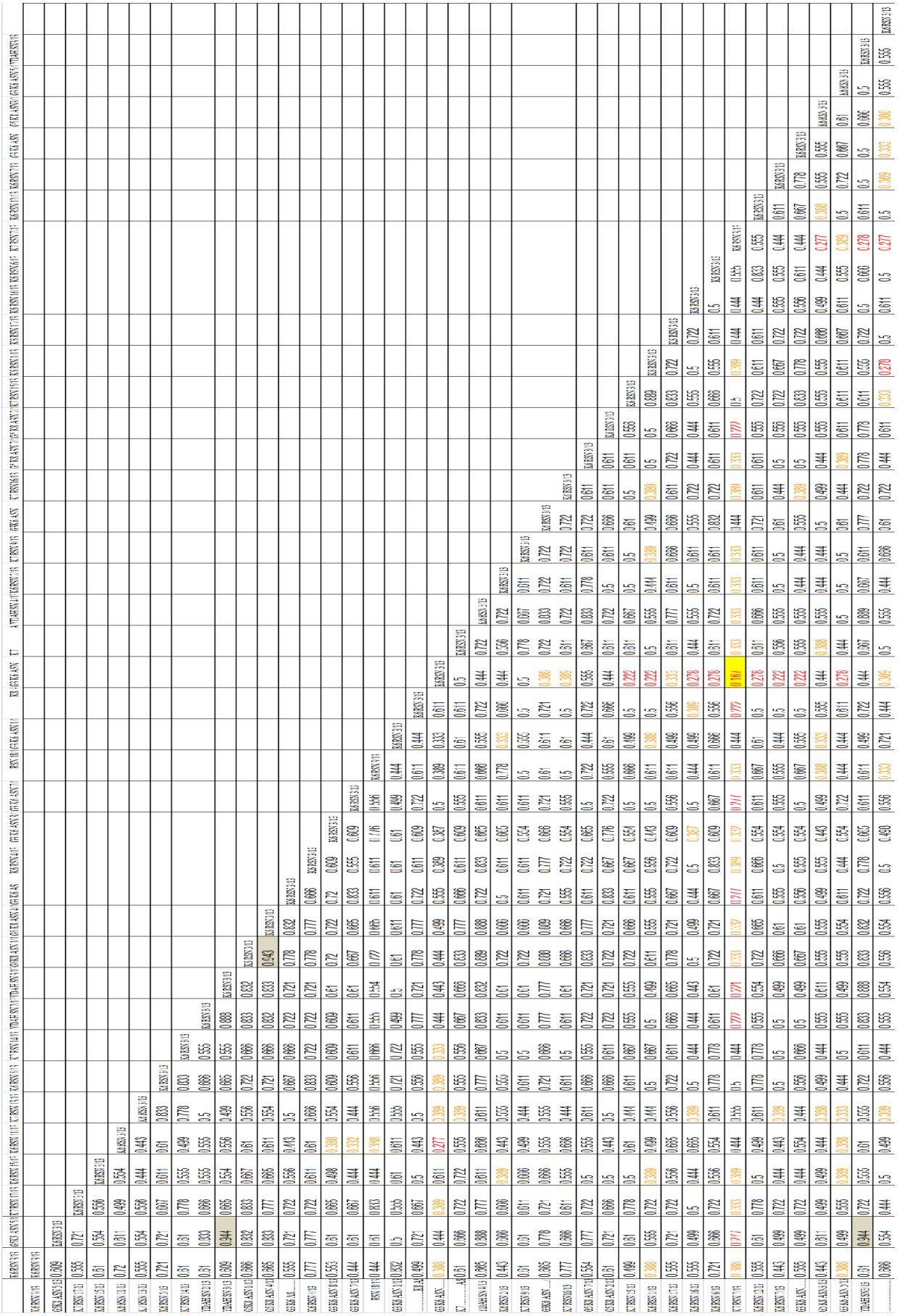
In the other study which was carried out by Darvishzadeh and his colleagues in 2010, 38 SSR locus was used for the characterization of 28 sunflower cultivars. It was reported that the loci ORS 598 gave high PI but surprisingly in our study it was observed that the ORS598 loci gave the lowest number of alleles. Duca and his colleagues (2013) used 13 pairs of SSR primers were used for genotyping of sunflower lines, in their study ORS78 SSR primer were excluded from the analysis due to unclear profiles with stutter bands. This outcome was different in our research means ORS78 SSR primer gave 4 alelle and showed 0.433 expected heterozygosity (H_e).

The present study, using molecular markers and morphological traits, investigated the genetic relationships of 41 sunflower lines from Turkey. Among the sunflower cultivars analyzed in this study, no identity, synonym and homonym genotypes were found.

The SSR technique used here was found to be quite effective in determining the genetic relationships among sunflower lines.

it is expected that the results of this study will assist current sunflower breeding efforts in Turkey as well as maintain the genetic integrity of the genetic resources.

Table 4: Genetic distances (GD) between different lines of sunflower



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GENETIC DIVERGENCE IN SUNFLOWER ACCESSIONS

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Importance of sunflower-

Sunflower (*Helianthus annuus* L.), belonging to the family '*Asteraceae*' (*Compositae*) and genus '*Helianthus*', is a diploid species (2n = 2x = 34) and native to southern parts of USA and Mexico. Sunflower is an important oilseed crop and is the preferred source of oil for domestic consumption and cooking worldwide (Hu *et al*., 2010). Sunflower was introduced to India during 1969 and gained popularity during 1980's with the development of first sunflower hybrid BSH-1 (Seetharam, 1980). In the oilseed scenario, sunflower competes with other three major oilseeds, *i.e.* soyabean, groundnut and rapeseed mustard at global level. Sunflower has a great potential in bridging the gap between the demand and supply of edible oil in future. Sunflower holds great promise because of its short duration, wider adaptability, photo-insensitivity, drought tolerance and higher amount of superior quality oil. The oil and protein content in sunflower ranges from 35-45 per cent and 18-20 per cent, respectively. Higher oil yield is an ultimate objective of sunflower researchers as this oil is considered as a good quality oil from health point of view, due to presence of polyunsaturated fatty acids which are known to reduce the risk of cardiac related problems (Monotti, 2004). Additionally, due to the possibility of using its oil as raw material for manufacturing biodiesel, it is arousing the interest of farmers, agriculture professionals and companies in the world.

Importance of genetic divergence in crop improvement-

In plant breeding, genetic diversity in parental lines is a pre-requisite for developing genetically superior hybrids. More diverse the parents, greater are the chances of obtaining heterotic expression in F1 with possibility of broad spectrum of variability in segregating generations. Yield is the combination of numerous components which are influenced by environmental instabilities. It is highly recommended to explore configuration of yield via breeding approaches. Sunflower being a highly cross-pollinated crop has a great scope for increasing productivity by diversifying hybrid base. In downsizing the breeding lines to be maintained, assessment of genetic divergence also helps a lot. The concept of D^2 statistics, based on measurements of morphological characters is frequently used as a tool for estimating genetic divergence by the plant breeders (Mahalanobis, 1936; Rao, 1952). Tracing D^2 as a generalized statistical distance, genotypes are grouped on the basis of minimum genetic distance using Tocher's method as described by Rao (1952). Varieties from different localities are generally included in the hybridization programmes assuming genetic diversity and greater likelihood of recovering promising segregants. However, Murthy and Anand (1966) noted that there is no parallelism between geographical and genetical diversity. Genetic diversity existing within and between groups of germplasm is important, and particularly, useful in proper choice of parents for realising higher heterosis and obtaining useful recombinants. D^2 statistic is a useful tool for estimating the genetic divergence in plant breeding experiments.

The knowledge about the magnitude and nature of variability present in a population due to the genetic and non-genetic causes is an important prerequisite for a breeding programme to improve the yield potential of genotypes, as greater variability among the genotypes leads to better chance for further improvement in the crop.

Methods to measure genetic divergence-

Evaluation of genetic divergence is performed through methods based on agronomic, morphology and molecular characteristics. Mahalanobis (1936) outlined a statistical procedure 'D^2 statistic' to measure the genetic divergence among the test genotypes involving quantitative characters in a given population. This concept is based on the technique of utilising measurement in respect of an aggregate of characters. Clustering methods objectify to separate a group of original observations on different subgroups in order to obtain homogeneity within and heterogeneity between subgroups. Among these methods, optimization and hierarchical ones are employed on a large scale by plant breeders. Visualization and interpretation of distances may be facilitated by the use of a clustering method and/or graphical dispersion. Multivariate analysis has been considered as an important tool in quantifying the degree of genetic divergence in different crops (Rao, 1952).

Genetic divergence in sunflower-

Genetic divergence is a process of one species diverging over time into more than one species, *i.e*., passing small random changes over time, from one generation to next generation. Varieties from different localities are generally included in the hybridization programmes assuming genetic diversity and greater likelihood of recovering promising segregants. However, Murthy and Anand (1966) noted that there is no parallelism between geographical and genetical diversity. Several investigations that evaluated the genetic divergence in sunflower crop were conducted by using morphoagronomic characters (Arshad *et al*., 2007). Genetic divergence estimation between different sunflower genotypes has been studied, aiming to develop parents for hybrids constitution or even the formation of new segregating populations, from the intercross of divergent genotypes with complementary agronomic characteristics.

Reddy and Devasenamma (2004) studied 61 genotypes of sunflower and grouped them into 19 clusters based on their genetic diversity. Based on inter cluster distance value and *per se* performance of genotypes, the genotypes namely, EC-376211, EC-399318, RHA-344 and BLC-P6 were selected which could be intercrossed to obtain high heterotic expression and also to recover desirable transgressive segregants.

Reddy *et al*. (2005) assessed genetic divergence among 102 genotypes and grouped them into 12 clusters. Based on the inter cluster distances and *per se* performance, the genotypes namely, GMU-4, GMU-11, GMU-14, GMU-16, GMU-25, GMU-40 and GMU-70 were selected which could be intercrossed to obtain high heterosis and also to recover desirable transgressive segregants. Seed yield/plant contributed maximum to divergence (40.2%) which was followed by number of leaves/ plant (25.8%) and 100-seed weight (17.0%).

Loganathan *et al*. (2006) conducted multivariate analysis of divergence among 50 genotypes of sunflower which led to their grouping into 14 clusters. Seed yield contributed maximum towards genetic divergence, followed by 100-seed weight and plant height.

Mahalakshmi *et al*. (2006) studied 29 genotypes of sunflower for their genetic divergence by D^2 analysis. The genotypes were grouped into 7 clusters. The character, days to first flowering contributed more towards genetic divergence.

Sridhar *et al*. (2006) assessed genetic divergence using D^2 statistics among 44 sunflower genotypes and grouped them into 9 clusters. Plant height and oil content contributed more towards genetic divergence.

Binodh *et al*. (2007) studied genetic divergence of 24 breeding lines for 8 traits in sunflower. The genotypes were grouped into 10 clusters where cluster I was the largest containing 13 genotypes, followed by cluster IV with 3 genotypes. The inter-cluster distance was the maximum between cluster VI and cluster VIII, followed by cluster IV and cluster VI and cluster VI and cluster IX. The study revealed that plant height contributed maximum towards divergence (45.29%),

followed by seed yield per plant (25.72%) and oil content (15.94%). Based on the inter-cluster distance and *per se* performance, the genotypes *viz.*, 17A, 47A, CSFI 5325, CSFI 5415, CSFI 5436 and CSFI 5013 were identified as suitable parents which could be intercrossed to obtain high heterosis.

Camarano *et al*. (2010) investigated genotypic divergence among 10 sunflower populations using Mahalanobis' D^2 statistics and canonic variables to identify more similar and/or divergent groups. The results of the individual variance analyses pointed out significant differences for the initial flowering, final flowering, plant height, oil content, moisture content and yield in all the experiments. Very high genetic variability was noted among the populations for these traits. The traits, stem diameter, head diameter, 1000-seed weight and number of seeds per head presented differences, which were sometimes significant and sometimes not, indicating that these traits show genotype-environment interaction.

Punitha *et al*. (2010) assessed genetic diversity among 17 sunflower genotypes using 9 agronomic characters and indicated the presence of substantial genetic diversity. The genotypes were grouped into 4 clusters. Among the investigated characters, seed yield, plant height, oil content and oil yield exhibited high contribution towards genetic divergence. It was observed that the inclusion of CSFI 5076, CSFI 5162, CMS 47A, CSFI 5005, CMS 17A, CMS 47A, CSFI 5069, CSFI 5422, CSFI 5109, CSFI 5155, CSFI 5002, COSF 1A , CSFI 5161 and CSFI 5015 in future breeding programs could result in the development of superior sunflower cultivars.

Mandel *et al*. (2011) conducted population genetic analysis of the primary gene pool of sunflower based on a broad sampling of 433 cultivated accessions and 24 wild sunflower populations. Gene diversity across the cultivars was 0.47, as compared with 0.70 in the wilds, indicating that cultivated sunflower harbours roughly two-thirds of the total genetic diversity present in wild sunflower.

Kumari and Sheoran (2012) evaluated 80 sunflower genotypes for genetic divergence using D^2 analysis. The genotypes were grouped into 10 clusters. Cluster I was the largest one with 22 genotypes, followed by cluster II (18), IV (17), VI (11), III (7) and V, VII, VIII, IX and X with one genotype each. The genotypes, DRSF-120 R, P70R, Nandyal-1 and RHA-586 were identified as divergent and superior performers. Likewise, genotypes from different sources were grouped in the same cluster, thus, suggesting that geographical diversity does not necessarily represent genetic diversity.

Reddy *et al*. (2012) studied genetic divergence in 64 genotypes of sunflower and grouped the genotypes into 9 clusters. The pattern of distribution of genotypes into various clusters was random and indicated that the geographical and genetic diversity were not related. Plant height contributed maximum towards genetic divergence, followed by stem diameter and head diameter.

Ayaz *et al*. (2014) evaluated seventeen sunflower hybrids and fifteen inbred lines including ten Cytoplasmic male sterile lines and five restorer lines for flower initiation days, full flowering days, full developmental days, height of plant, disk diameter, stem thickness, leaves per plant, hundred achenes weight, achenes yield and oil content percentage. The maximum achenes yield was contributed by Hysun-33 2119 kg/h followed by SMH-0924 and SMH-0925. SMH-1028 and SMH-0926 were suggested as potential significant hybrids for future breeding plans to incorporate maximum achenes yield and oil content percentage. The CMS-11, CMS-25 and CMS-10 were long statured with vigorous stem and all the restorers were early maturing recommended for including in hybridization program to generate high heterotic factions.

Chandirakala *et al*. (2014) assessed genetic divergence of 38 sunflower genotypes using Mahalanobis D2 statistics. These genotypes were grouped into 13 clusters, among which the cluster IX with 9 genotypes was the largest. Maximum inter cluster distance was recorded between cluster XII and XIII (39.58) followed by clusters II and XII (38.18). Hence hybridizing between these

divergent groups may lead to higher variation in segregating population. In this study, the genotypes *viz.*, GMU 322, COSF3B and COSF4B in the cluster II, the genotypes *viz.*, GMU 503, GMU 1074, GMU 1108 in the cluster XII and the genotype COSF1B in the cluster XIII are widely divergent and crosses may be effected among the genotypes of these clusters to get more heterosis among the hybrids.

Pandya *et al.* (2014) evaluated forty genotypes of sunflower [*Helianthus annuus* (L.)] for seed yield and its components and grouped them in 5 clusters. The clustering pattern of genotypes was independent of their geographical distribution. Taking into account cluster mean for important seed yield components, the various clusters which can provide the desired parents like GMU-1033, GMU-411 for hybridization for improvement of characters.

Masvodza *et al*. (2015) used 16 cytoplasmic male sterile (CMS) lines and 10 male restorer (R) lines and characterised them for ten morphological variables namely, days to 50% flowering, head diameter, leaf length, leaf width, petiole length, nodding, lodging, number of leaves, plant height, stem diameter and uniformity. The genetic base of the collection was observed as narrow and would need more diversification.

Sunflower improvement in relation to genetic divergence-

Genetic diversity existing within and between groups of germplasm is important, and particularly, useful in proper choice of parents for realising higher heterosis and obtaining useful recombinants. D^2 statistic is a useful tool for estimating the genetic divergence in plant breeding experiments. To get more heterotic F_1's and large number of desirable transgressive segergants, selection of parents for hybridization should be properly based on genetic diversity rather than geographic diversity. The mating systems in any field crop determine the gene flow and hence the propensity with which reference population can be improved through genetic amelioration. Sunflower is predominately a cross- pollinated crop and the pollination is by and large insect-mediated, though some degree of self pollination cannot be ruled out in some genotypes for the reasons of hermophroditism and some homogamy. This necessitates that heterozygosity *per se* be maintained in sunflower populations.

Sunflower improvement strategies include; development of heterotic hybrids, elite composites and/ or improved open-pollinated populations developed through random mating (hand pollination or male sterility mediated), followed by selection (various recurrent selection procedures). All these methods would necessarily entail in their objectives for accumulation of gene constellations for intra and inter allelic interactions in genotypic background(s) of agronomic significance. In hybrids, dominance deviation of alleles, and in improved populations, accumulation of additive genes with greater complimentary effects are harnessed for better trait expression and hence higher economic yield.

The success of any chosen breeding programme would depend upon the extent of heritable genetic variation, response of selection pressure exerted, the magnitude and direction of associations among various yield contributing traits and selection indices used in reference population(s). Hence , analysis of genetic diversity among inbred accessions is of vital importance in Sunflower breeding.

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COMBINING ABILITY AND GENETIC COMPONENTS FOR SEED YIELD IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Being one of the most important oil crops in the world main goals in sunflower breeding are increased seed and oil yield per hectare. Bearing in mind breeding direction and global importance of this oil crop objective of this study was to evaluate general combining ability (GCA) of six sunflower genotypes and specific combining ability (SCA) of their crosses as well as to estimate components of genetic variability for seed yield/plant. Genotypes were crossed according to incomplete diallel method (without reciprocals) and fifteen F1 progenies were derived. Both, additive and non-additive, genetic components were significant in seed yield expression but according to GCA/SCA ratio additive component was more important. The highest GCA value was recorded in G1 genotype, while the highest SCA value was recorded in combination G2xG3, for seed yield. Analysis of components of genetic variability revealed that dominant gene effects (H1) were more important than additive (D) and frequency of dominant genes was greater than recessive ones. Dominant and recessive genes were not equally distributed among parents as presented by the $H_2/4H_1$ ratio which was different than 0.25 (0.17). According to average degree of dominance (1.14) superdominance was the case in seed yield expression.

Key words: seed yield, combining ability, dominant and recessive genes

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in the World and main crop for production of edible oil in Serbia. Consequently, main goals in sunflower breeding are increased seed yield and thus oil yield per hectare. Sunflower seed yield, as a complex trait, requires the most effort in breeding process because all other goals in breeding through improving individual properties are in a function of increasing seed yield and thus oil yield. The success in creating new, superior, genotypes largely depends on the possession of adequate genetic variability in the parental material because the greater are the chances for obtaining superior F1 plants. Furthermore, for sucessful breeding it is necessary to dispose information about mode of inheritance and combining ability. Obtained information helps in breeding proces through selection of perspective parental lines with the aim of creating genotypes that will improve production. There are many papers that deal with this topic which differ in the results. Previous results have found that both, additive and non-additive, components are important in the inheritance of seed yield (Škorić et al., 2007). Some authors emphasize the larger importance of additive component of genetic variance for seed yield (Putt, 1966; Sindagi et al., 1979; Petakov, 1992 and Karasu et al., 2010). Contrary to them, larger role of non-additive component on the inheritance of seed yield was determined in previous research by many authors (Marinković, 1984; Mihaljčević, 1989; Joksimović, 1992; Lande et al., 1997; Rather et al., 1998; Goksoy et al., 2000; Cecconi et al., 2000; Jocić, 2002, 2012; Sakthivel, 2003; Farrokhi et al., 2008; Gvozdenović et al., 2008 and Hladni et al., 2010). Combining abilities are divided into general (GCA), which represent the value of the parent used in crosses, and specific (SCA), representing the value of certain crossing. The most reliable method for testing combining abilities of genotypes is diallel (Živanović et al., 2006). Diallel crossing was proposed by the zoologist-geneticist Dr. Schmidt (1919) and firstly applied in plants by Sprague and Tatum (1942). Masood et al. (2005) used 8x8 diallel to test combining ability in sunflower and

found greater importance of non-additive effects in controlling seed yield. Vice versa Mijić et al. (2008) in 6x6 diallel of sunflower inbred lines found greater significance of additive component. Investigating the GCA/SCA ratio in inbred lines of sunflower using the method line x tester Andarkhor et al. (2013) have found greater importance of non-additive genetic components in controlling seed yield, considering that GCA/SCA ratio was less then 1 (<1).

Objective of this study was to evaluate combining abilities of sunflower genotypes through their crossings and to obtain information about components of genetic variance.

MATERIAL AND METHODS

Six sunflower genotypes were crossed according to incomplete diallel (without reciprocals). F1 progeny and parents were sown in three replicates in a randomized block design at Rimski šančevi experimental field of the Institute of Field and Vegetable Crops from Novi Sad. Experimental plot size was 10 m^2 with four, 3.6 m long rows and 70x30 cm plant spacing. The data were recorded on 10 plants in each replicate from middle rows. Harvest was done at the stage of physiological maturity and seed yield/plant was recorded in laboratory on a technical scale with an accuracy of 0.01 g. General combining abilities (GCA) of parents and specific combining abilities (SCA) of F1 were tested according to diallel method 2 by Griffing (1956). The assumption of this method is that there are no differences in reciprocal crosses.

Mathematical model for analysis of combining abilities as follows:

$Y_{ij}=m+g_i+ g_j+s_{ij}+1/bc\sum\sum e_{ijkl}$

Analysis of components of genetic variance was performed according to the method suggested by Mather and Jinks (1971).

RESULTS AND DISCUSSION

Analysis of variance of combining ability for seed yield/plant showed statistically highly significant differences in the general (GCA) and specific (SCA) combining abilities between parents used in this experiment (Tab. 1). As GCA and SCA provide information for additive and non-additive gene actions considering that GCA/SCA ratio was higher than 1 it can be concluded that additive gene action played greater importance in the inheritance of seed yield/plant. In earlier studies Putt (1966) and Sindagi et al. (1979) found that general combining ability for seed yield in sunflower is more important than specific combining ability, indicating that additive component is more important than non-additive. In contrast, a significant impact of non-additive component of genetic variance in the inheritance of seed yield/plant in sunflower was observed by many authors (Marinković, 1993; Bajaj et al., 1997; Kumar et al., 1998; Chandra et al., 2011 and Andarkhor et al., 2012). The highest and statistically significant and positive GCA value was calculated for the G1 genotype so it can be concluded that this genotype represents the best general combiner for improving this trait (Tab. 2). Parental genotypes G4 and G5 also demonstrated positive GCA values but without statistical significance. In other parent genotypes were established negative GCA effects. Significant and positive value of SCA effect was recorded only in crossing combination G2xG3. In other crossing combinations SCA values were not statistically significant, while in five crossings were found negative SCA values.

Table 1. Analysis of variance of combining abilities for seed yield/plant in sunflower.

Source	Df	SS	MS	F-value
GCA	5	2718.59	543.72	11.31**
SCA	15	2664.86	177.66	3.70**
Error	40	1922.97	48.07	

Table 2. GCA (diagonal) and SCA (above diagonal) effects for seed yield/plant in sunflower

Genotypes	G1	G2	G3	G4	G5	G6
G1	13.62**	-21.39	-0.04	-1.83	-9.65	10.48
G2		-5.83	17.59*	-10.48	3.22	1.92
G3			-3.78	13.45	11.96	1.26
G4				3.81	14.29	-14.33
G5					1.61	5.52
G6						-9.43

$LSD_{0.05}$ GCA= 7.00 $LSD_{0.05}$ SCA= 17.16
$LSD_{0.01}$ GCA= 9.37 $LSD_{0.01}$ SCA= 22.96

The analysis of components of genetic variance revealed that the dominant component (H1) was greater than the additive (D), which shows that most of the genetic variation in the inheritance of seed yield/plant makes the non-additive component (Tab. 3). According to the calculated F value dominant genes prevailed in relation to recessive ones, as confirmed by the calculated frequency of dominant (u) and recessive (v) genes. Furthermore, calculated value of the H2/4H1 ratio indicated the unequal representation of dominant and recessive genes in parents. From the KD/KR ratio, which is greater than one, is also evident that the dominant genes prevailed in respect to the recessive ones, in the inheritance of seed yield/plant. Average degree of dominance $\sqrt{H_1}/D$ (1.14) indicated that superdominance was the case in expression of seed yield/plant, considering all crossings.

Table 3. Components of genetic variance for seed yield in sunflower.

Components	Value
D	535.98
H_1	698.17
H_2	473.63
F	544.60
E	48.07
u	0.78
v	0.22
$H_2/4H_1$	0.17
$\sqrt{H_1}/D$	1.14
K_D/K_R	2.60

CONCLUSIONS

Main objective in breeding programe is development of superior synthetics or hybrids. To achieve this aim it requiers estimation of gene action in various traits in order to design an efficient breeding plan for further genetic improvement of the initial material. Sunflower seed yield is one of the most important traits considering this crop and information about combining abilities and components of genetic variance are necessary for improving this valuable trait. In this research we found that both, additive and non-additive, genetic components were important in expression of seed yield/plant but additive genetic component prevailed. According to GCA value genotype G1 is the best general combiner for improving this trait and that genotype will be used in further hybrid combinations in order to obtain highly productive hybrids of sunflower. Crossing combination G2xG3 had the highest and significant SCA value and that combination will be tested with other

perspective hybrid combinations. Analysis of components of genetic variance revealed that dominant genes were prevalent in expression of seed yield/plant, as confirmed by the frequency of dominant genes. Dominant and recessive genes were not equally distributed among parents as presented by the $H_2/4H_1$ ratio which was different than 0.25 (0.17). According to average degree of dominance (1.14) superdominance was the case in seed yield expression.

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RECOMBINATION AND SELECTION IN SUNFLOWER POPULATIONS FROM EEA PERGAMINO INTA

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ABSTRACT

The achievement of new sources of genetic variability is central for the development of breeding programs. At Pergamino EEA (33º 53'S, 60º 35'), in the 2014-15 , the following populations were evaluated: P7xBulkVert C3/Bulk AO C2(C1), Bulk AO / Bulk Vert(C1),P7xBVert C3/Bulk AOC2(C2),Bulk AO/ Bulk Vert (C2) ,Bulk A.O(C2), Bulk AO(C3) and the hybrid Paraíso 20. The characters were flowering days, seed yield and *Verticillun* resistance. In the populations Bulk AO / Bulk Vert (C1); Bulk AO/ Bulk Vert (C2) , P7BulkVert(C1) , P7BulkVert(C2), P7BulkVert(C3), Bulk AO(C2), Bulk AO(C3), P7xBulkVert C3/Bulk AO C2 (C1), P7xBulkVert C3/Bulk AO C2(C2), PGRK (C0), PGRK(C2), Bulk AO(C1), BulkVert(C3), BulkVert (C4) and P7 (C0) were also evaluated percentage of oil content, plant height, head diameter, weight of 100 seeds, number of seeds/ head and percentage of kernel. The objective was to describe the variability obtained in germplasm of different origin and the advance achieved in the improvement of characters from recombination and selection cycles. The combination of different origin germplasm allowed to achieve yields similar to the hybrid. In P7xBulkVert C3/Bulk AO C2(C2), the cycles of selection and recombination improved *Verticillium* resistance. The highest variability was obtained in number of seeds /head and the slowest was observed in weight of 100 seeds. The variability of germoplasm in different characters would allow to obtain improved genotypes

Key words*: Helianthus annuus; breeding , variability*

INTRODUCTION

The achievement of new sources of genetic variability is central for the development of breeding programs .The first step in a cultivars development program me is to form a population with genetic variability for the characters of interest.

The potential advantages of a complex population (involving hundreds of parents) are that the number of alleles for each locus increases with the number of parents used and the probability of heterozygote in multiple loci is greater. Mass selection has been effective in sunflower breeding for precocity oil and diseases resistance (Pustovoit 1964). Open pollination sunflower populations would be a good source for the development of inbred lines (Eberhart 1967)

Verticillium wilt, caused by pathogen *Verticillium dahliae* (Kleb), is one of the main diseases of sunflower (*Helianthus annuus* L.) in Argentine; thus obtaining of cultivars of good performance against the pathogen is a priority in breeding programs. Pereyra et al, 1999, reported that this disease causes up to 73% yield losses. The search of germplasm with diseases resistance, industrial quality, yield and oil content led to improvement to obtain populations that meet these characters. The sunflower Group of INTA Pergamino Experimental Station (EEA) develops as part of its work methodology the formation of populations of different origin with traits related to the quality, disease resistance and yield to be used as a source of new cultivars.

The objective was to describe the variability obtained in germplasm of different origin and the advance achieved in the improvement of characters from recombination and selection cycles.

MATERIALS AND METHODS

The populations studied were the following:**P7**: Recombination and selection of lines with outstanding agronomic characteristics and good seed yield. **Bulk Vert :** Obtained by recurrent selection of germplasm of good performance in seed yield and resistance to *Verticillium dahliae.* **Bulk AO**: It is obtained from recombination lines of high oleic acid content. **P7xBulkVert**./ **Bulk AO.** It is obtained from the crossing and recombination of populations (P7xBulk Vert) x Bulk AO. **PGRK:** Obtained by recurrent selection from Ruso x Klein. **Bulk AO/ Bulk Vert:** It is obtained from the crossing and recombination of populations Bulk AO xBulk Vert

At EEA Pergamino INTA (33º 57' S, 60º 34' W) were planted a trial with the statistical design of a randomized complete block with 3 replications and plots of 3 rows of 6.0 m, row spacing of 0.7 m. The germplams evaluated were the populations (P7xBVert C3/Bulk AO C2)C1, Bulk AO / Bulk Vert C1,(P7xBVert C3/Bulk AO C2)C2,Bulk AO/ Bulk Vert C2 ,Bulk A.OC3 y Bulk A.Oc2 and the commercial hybrid Paraíso 20. The analyzed characters were: days to flowering, yield (kg ha-1) and *Verticillium* resistance. Seedling inoculation method was applied to evaluate *Verticillium* resistance. The scale was R (resistant), MR (moderately resistant), MS (moderately susceptible), AS (very susceptible)

In Bulk AO / Bulk Vert C1; Bulk AO/ Bulk Vert C2 , P7BVc1 , P7BVc2, P7BVc3, Bulk A.Oc2, Bulk A.OC3, (P7xBVert C3/Bulk AO C2)C1,(P7xBVert C3/Bulk AO C2)C2PGRK Cycle 0, PGRK Cycle 2, Bulk A.OC1, BVert C3, BVert C4, P7 Co, Paraíso 20, Olisun 4 and ACA 885 were analyzed oil content, plant height, head diameter, 100-Seed weight (g); Seed nº/head and kernel content

RESULTS AND DISCUSSION

Table 1 Yield, days to flowering and *Verticillium* resitance in populations of Pergamino INTA

Population	Days to flowering	Yield (kg/ha)	Verticillium(*)
Paraiso 20(commercial hybrid)	66	1938	1,4
(P7xBVert C3/Bulk AO C2)C1	65	1619	2,03
Bulk AO / Bulk Vert C1	68	1555	2,4
(P7xBVert C3/Bulk AO C2)C2	68	1450	1,8
Bulk AO/ Bulk Vert C2	69	1183	2,17
Bulk A.OC3	64	947	--
Bulk A.Oc2	67	706	--
Mean	67	1342	
C.V.%	2,25	16,03	
LSD 5%	3,00	444	

(*) (1-1.9) moderately susceptible , (2-2.9): susceptible

Table 1 analyzes days to flowering, yield adjusted by oil content and Verticillum resistance in populations of the Pergamino EEA. We observed that P7xBVert C3/Bulk AO C2)C1 y Bulk AO / Bulk Vert C1 didn't have significant yield differences with Paraíso 20. The longest cycles was found in Bulk AO / Bulk Vert and the shortest was found in Bulk A.OC3. The most advanced selection cycles improved *Verticillium* resistance.

The different origin germplasm combinations allowed to reach similar yields to the commercial hybrids. Concerning *Verticcillum* resistance, it was observed that even though (P7xBVert C3/Bulk AO C2) C2 performance was inferior to the commercial hybrids, selection and recombination allowed to improve that character.

Table 2 Characters in different origins populations of Pergamino INTA

Population	Plant height (cm)	Head diameter (cm)	Seed nº/head	100-Seed weight (g)	Oil content (%)	Kernel content (%)
Bulk AO / Bulk Vert C1	156	19	1295	5,7	37,4	72,9
Bulk AO/ Bulk Vert C2	157	15	996	5,1	36,1	73,1
P7BVc1 (w 3153)	147	18	1228	5,1	35,7	70,8
P7BVc2	164	20	1521	5,1	39,5	73,8
P7BVc3	162	18	1288	4,9	39,6	75,8
Bulk A.Oc2	106	13	-	4,3	36,9	75,4
Bulk A.OC3	118	16	-	5,2	40,4	78,3
(P7xBVert C3/Bulk AO C2)C1	128	16	801	4,6	39,5	77,2
(P7xBVert C3/Bulk AO C2)C2	145	15	689	4,5	37,5	73,2
PGRK Ciclo 0	179	15	-	4,5	31,8	63,1
PGRK Ciclo 2	205	16	996	5,4	34,3	64,3
Bulk A.OC1	144	18	-	5,7	46,1	73,5
Paraiso 20(commercial hybrid)	140	16	1705	4,6	42,1	79,0
Olisun 4 (commercial hybrid)	141	16	1125	5,5	45,8	75,1
ACA 885(commercial hybrid)	173	17	1263	5,7	42,7	72,7
BVert C3	173	15	1081	5,2	40,6	75,0
BVert C4	172	16	-	4,6	36,0	74,5
P7 Co	147	12	573	3,3	34,1	71,2
Promedio	153,0	16,2	1120	4,9	38,7	73,3
C.V.(%)	12,2	24,1	36,0	8,6	12,9	8,6
LSD 5%	12,6	2,7	480	1,1	4,3	5,5

Table2 shows the analyzed characters of populations from Pergamino INTA. Highest plant height was found in PGRK and the smallest in Bulk A.Oc2 and Bulk A.Oc3. Highest head diameter was found in in P7BulkVertC2 and the smallest in P7 C0. Paraiso 20 had the highest seed nº/head., P7BVc2, Bulk AO / Bulk Vert C1 and P7BVc3 had also high values. Highest **100**-seed weight values were found in Bulk AO / Bulk Vert and ACA 885 , and the smallest was found in P7C0. Bulk A.OC1, Olisun 4, ACA 885 and Paraíso 20 had the highest oil content and PGRK C0 had the smallest. Highest kernel content was found in Paraíso 20, Bulk A.OC3 and (P7xBVert C3/Bulk AO C2)C1 and the smallest was found in PGRK.

Table 3 Characters in populations composed and original

Population	Plant height (cm)	Head diameter (cm)	Seed nº/head	100-Seed weight (g)	Oil content (%)	Kernel content (%)
Bulk AO / Bulk Vert	156	17	1145	5,4	36,7	73
Bulk AO	123	16	sd	5,1	41,2	73,5
Bvert	173	16	1081	4,9	38,3	74,8
P7BVert	158	19	1345	5,0	38,3	73,5
P7 Co	147	12	573	3,3	34,1	71,2
Bvert	173	16	1081	4,9	38,3	74,8

Table 3 compares the average values of composed populations with values of original populations. Bulk AO / Bulk Vert showed medium plant height value and an increase in the head diameter, 100 seed weight and seed nº/head, on the other hand there was a diminution of oil and kernel content values with those of the original populations. P7BVert showed medium plant height value and an increase in the head diameter , 100 seed weight and seed number/head, on the other hand the oil content remained the same for the original population with the value more high (BVert).

Table 4 Characters in recombination cycles Bulk AO/Bulk Vert population

	Bulk AO / Bulk Vert C1				Bulk AO/ Bulk Vert C2			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	156	110	192	21,8	157	125	182	13,8
Head diameter(cm)	18,9	12,5	24,7	3,9	15,1	9,3	18,5	2,8
Seed nº/head	1287	649	1612	552,6	993	725	1388	255,9
100 seed weight (gr)	5,7	2,5	8,4	1,7	5,2	3,5	8,7	1,4
Kernel content(%)	72,8	52,4	82,9	8,6	73,0	67,9	78,3	3,6
Oil content (%)	37,2	27,6	42,6	4,4	36,1	28,8	43,7	3,4

Table 4 compares two recombination cycles of the Bulk AO/Bulk Vert population. In cycle 2 there was a diminution in the averages of all the characters, except for plant height and kernel content.

Table 5 Characters in recombination cycles of P7xB Vert population

	P7BVc1				P7BVc2				P7BVc3			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	147	125	170	15,5	164	125	192	15,0	162	145	178	7,8
Head diameter(cm)	18,0	12,5	23,4	3,4	20,3	10,5	27,4	4,6	17,9	12,5	23,5	3,5
Seed nº/head	1207	884	1555	279,7	1529	1125	1761	350,9	1287	766	1610	362,4
100 seed weight (gr)	5,0	2,6	6,2	1,2	5,1	2,8	6,5	1,3	4,82	3,5	6,5	0,9
Kernel content(%)	71,0	61,7	81,0	6,4	73,8	64,4	85,0	6,8	75,9	69,3	83,2	4,4
Oil content (%)	35,0	24,9	44,9	6,2	39,8	33,1	50,3	5,7	39,4	33,4	48,4	4,6

Table 5 shows the values of the three recombination cycles of the P7xB Vert population. The highest values of plant height, head diameter, seed number/head, 100 seed weight and oil content were obtained in cycle 2. Cycle 3 had the highest kernel content.

Table 6 Characters in cycles of recombination of the population Bulk AO

	Bulk A.OC1				Bulk A.Oc2				Bulk A.OC3			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	146	140	160	7,2	106	100	120	5,4	118	90	170	16,0
Head diameter(cm)	18,3	13,5	26,2	4,8	13,4	9,2	20,1	3,0	15,6	7,3	20,0	3,9
100 seed weight (gr)	5,4	4,4	6,5	0,8	4,2	2,4	6,0	1,3	5,1	4,0	7,1	1,1
Kernel content(%)	74,1	70,1	78,9	3,8	75,6	65,0	81,7	5,1	78,5	77,6	79,5	0,7
Oil content (%)	45,6	41,3	48,0	3,0	36,4	31,4	42,9	3,8	40,1	35,8	45,0	3,1

Table 6 analyzes three recombination cycles of the Bulk AO population. The highest 100 seed weight and oil content values were obtained in cycle 1. The highest kernel content was obtained in cycle 3.

Table 7 Characters in recombination cycles of the P7xBVert C3/Bulk AO C2 population

	(P7xBVert C3/Bulk AO C2)C1				(P7xBVert C3/Bulk AO C2)C2			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	128	100	165	15,8	145	110	190	21,1
Head diameter(cm)	16,5	9,2	28	4,6	15,4	10,5	21,0	2,9
Seed nº/head	789	532	1420	368,6	697	401	1545	360,2
Peso 100 Aq. (gr)	4,7	2,4	6,9	1,5	4,5	2,4	7,0	1,4
Kernel content(%)	77,1	65,0	94,0	9,0	73,2	67,3	79,3	4,3
Oil content (%)	39,4	34,4	50,8	5,3	37,5	27,4	45,7	5,5

Table 7 compares two recombination cycles of the P7xBVert C3/Bulk AO C2. Cycle 1 showed the highest head diameter, seed number/head, 100 seed weigh, kernel content and oil content . Cycle 2 reached the highest plant height.

Table 8 Characters in recombination cycles of the PGRK population

	PGRK Ciclo 0				PGRK Ciclo 2			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	179	160	202	12,7	205	160	245	19,2
Head diameter(cm)	15,1	8,5	27,2	5,0	16,7	8,5	22,5	3,7
Seed nº/head	-	-	-	-	1010	449	1617	349,6
Peso 100 Aq. (gr)	4,5	2,9	5,8	1,0	5,5	3,2	7,2	1,1
Kernel content(%)	63,2	59,5	67,1	2,6	64,2	57,8	72,4	4,9
Oil content (%)	32,2	25,8	39	5,4	34,4	26,7	41,5	4,5

Table 8 compares the cycles of the PGRK population. In all characters , cycle 2 reached the highest values. The method applied was the recurrent selection and it was possible to improve the values of all characters.

Table 9 Characters in recombination cycles of BVert the population

	BVert C3				BVert C4			
	Media	Mín	Máx	D.E.	Media	Mín	Máx	D.E.
Plant height(cm)	173	130	203	19,8	172	150	190	10,8
Head diameter(cm)	14,9	6,0	22,1	5,1	16,3	9,0	23,8	4,1
Seed nº/head	1058	742	1585	367,3	953	865	1040	123,7
Peso 100 Aq. (gr)	5,2	3,6	7,7	1,3	4,5	3,2	6,4	1,0
Kernel content(%)	75,1	70,0	80,1	3,5	74,6	64,8	88,5	7,4
Oil content (%)	40,9	34,5	44,1	3,2	38,4	26,2	46,4	7,0

Table 9 shows two recombination cycles of the BVert. population. Plant height values were similar in both cycles. The highest seed number/ head, 100 seed weight, kernel and oil content values were achieved in cycle 3 . The average of head diameter was higher in cycle 4.

In all the analyzed populations the character that showed most variability was seed number/head. The character that showed least variability was 100 seed weight.

In general, composed populations allowed to improve yield components and, in some cases, to reach similar yields to commercial hybrid.

The existent variability in most of the studied germplasm would allow to keep and obtaining improved genotypes

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AN EMS MUTATION ALTERING OIL QUALITY IN SUNFLOWER INBRED LINE

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ABSTRACT

The main objective of this research was to increase genetic variability of sunflower in terms of oil quality and productivity using induced mutations. A preliminary sensitivity test was performed to establish optimal ethyl-methane-sulphonate (EMS) doses for seed treatement. The results showed that high EMS concentrations (0.5-2.5%) caused low survival rates, therefore lower EMS doses were used. Thousand seeds of the sunflower high-oleic inbred line L31 were treated with 0.1% solution of EMS to induce mutations. In the M_2 generation, seeds were screened for fatty acid composition and alterations occured in individual plants. In the next generation a putative mutant line, ML31-1, was isolated with significantly lower oleic acid content compared to the wild type L31 grown in the same year. We assumed that heterozygous mutation occurred, manifested by changing a dominant allele *Ol* to the recessive *ol*. After self-polination in the next generation the segeregation of oleic acid was from 346.6 to 949.1 g/kg and of linoleic acid from 39.9 to 339.3 g/kg. In subsequent generations, individual selection and evaluation of progenies continued in several directions depending on the content of oleic acid: low, increased or high. The stable progenies were evaluated in micro-plot tests for seed yield and other agronomic traits in comparison with their respective wild type.

Key words: sunflower, induced mutation, ethyl-methane-sulphonate, fatty acids, oleic acid

INTRODUCTION

Sunflower oil has been traditionally appreciated as a high-quality commodity in the world oil market (Fernandez-Martinez et al., 2009). Standard sunflower oil is liquid at room temperature due to high content of unsaturated fatty acids. The most abundant is polyunsaturated linoleic acid (C 18:2), about 550-700 g/kg, followed by monounsaturated oleic acid (C 18:1) with 200-250 g/kg. Keeping up with the trends of the food and other industries, sunflower breeders have been able to significantly change the quality of the oil (Cvejić et al., 2014). The high-oleic sunflower hibrids have increased content of oleic acid 800 g/kg and more, compared to a standard type of sunflower. Oil of the high-oleic hybrids has excellent nutritional properties, is a suitable raw material for many derivatives of the chemical industry and for the production of high quality biodiesel, is more favorable because of higher oxidative stability, more resistance to heating and heart-healthy properties (Haddadi et al., 2011). Sunflower breeders have developed a large number of high-oleic hybrids because of the rapidly increasing interest of oil industry (Škorić et al., 2008). However, selection pressure to one particular trait can influence variability of other traits.

Sunflower genetic variability is often limited, as its genetic base of available inbred lines is narrowed. Genetic variability can be broadened by interspecies hybridization with wild species and mutation breeding (Cvejić et al., 2015). The great variability arising after mutagen treatment offers breeders unique challenge for the development of new genetic combinations (Velasco et al. 1999). Induced mutations have been applied for the past 40 years to produce mutant cultivars in sunflower by changing plant characteristics that significantly increase seed yield and quality (Cvejić and Bado, 2009). The first high-oleic sunflower variety Pervenec was obtained by induced mutagenesis by

seed treatment of the variety VNIIMK 8931 with the solution of dimethyl sulfate (DMS) and selection for increased content of oleic acid over 840g/kg (Soldatov 1976). Worldwide, Pervenec is used as a high-oleic trait donor in breeding programs. There are publications about other sources of high-oleic mutants with 800g/kg of oleic acid (Ivanov et al. 1992) and with 900g/kg of oleic acid (Andrich et al., 1992). Recently, the new high oleic sunflower mutant was obtained which ultra-high oleic content was not affected by temperature during grain filling, representing an advantage over the high oleic Pervenets and traditional genotypes (Leone et al. 2013, Alberio et al. 2016). The mode of inheritance of oleic acid content proved to be complex and has been studied by numerous authors, but there is no unanimity among scientists over the number of genes which control this trait (Fick, 1984; Urie, 1984; 1985; Miller et al., 1987; Fernandez-Martinez, 1989; Fernandez et al., 1999; Demurin and Škorić, 1996; Velasco et al., 2000; Lacombe and Berville, 2001; Lacombe et al., 2002; Perez-Vich et al., 2002; Vares et al., 2002; Schuppert et al., 2006). The common conclusion of all studies is that the presence of gene *Ol* is crucial for creating high oleic sunflower genotypes, while number and function of genes controlling this inheritance of this trait remain to be determined.

The main objective of this research was to increase genetic variability of sunflower inbred line in terms of oil quality and productivity. The first step was to assess the efficiency of ethyl-methane-sulphonate (EMS) mutagenic treatments, while the second is to detect mutant lines with different (changed) oil quality; this would provide new genetic variability and better crop productivity and stability.

MATERIAL AND METHODS

Plant material: Sunflower inbred line L31 (wild type) was used for mutagenesis. Line was developed in Institute of Field and Vegetable Crops in Novi Sad, Serbia. This line has over 800 g/kg oleic acid and has potential for further improvement of productivity and stability.

Mutagenic treatment: Ethyl-methane-sulphonate (EMS) mutagenesis of seeds from line L31 was performed in the Joint FAO/IAEA Laboratories in Seibersdorf, Austria. In order to determine the survival rate, fifty seeds were treated with 5 concentrations of EMS solution, 0.5, 1.0, 1.5, 2.0 and 2.5% (v/v), respectively; treatment concentrations were based on studies of other species (Kodym and Afza, 2003). Before the treatment, seeds were transferred to nylon meshes and pre-soaked in distilled water for 24 hours at room temperature. Seeds were then incubated in 200 ml of sodium phosphate buffer (0.1 M, pH 7.4) with gentle shaking (100 rpm) and different EMS concentrations were added. Incubation lasted 4 h. After the EMS treatment, the seeds were washed in distillated water several times. The control, non-mutagenized seeds were treated similarly, except for exposure to the mutagen. All treated seeds and the controls were sown in boxes using the flat method (Gaul, 1963) in a glasshouse under controlled environmental conditions (22-35ºC, lighting of 12h photoperiod). The parameter used to assess the dose response was the survival rate. The number of viable seedlings were calculated after a week of sowing and survival rate was determined by calculating number or survived seedlings per total number of planted seeds. Based on these results, batches of seeds were treated with two concentration of EMS, 0.1% (v/v) and 0.25% (v/v), respectively, and planted in the field.

Selection method: After the mutagenesis, M_1 seeds were planted in the nursery field of the Institute of Field and Vegetable Crops in Rimski Šancevi, Novi Sad, Serbia and after self-pollination of M_1 surviving plants, M_2 seeds were harvested. Seeds from each head were screened for fatty acid composition. Seeds of the wild type were grown and screened at the same time/. Mutants with altered fatty acid content were selected by screening. Seed from selected plants were planted next year in the field and after self-pollination, the M_3 seeds were collected. In next

generations plants were selected by pedigree method and seeds were screened for fatty acid composition. Fatty acid composition was measured by gas chromatography.

Agronomic evaluation: Selected mutants (M_6) and wild type were planted in comparative trial. The trials were organized in randomized block design with three replicates. Following traits were analyzed: days to flowering (from plant emergence to full flowering - UPOV - stage F3.2), plant height (10 plants per replication), seed yield per plant, thousand seed weight, oil content (NMR) and fatty acid composition by gas chromatography (AOCS Official Method Ce 1-62, 1993).

Statistical analysis: The statistical data analysis of mutant generation was performed using Statistica 12 (StatSoft, DEL, USA). The selection progress in successive generations is illustrated in table and figures. Statistically significant differences between examined traits was determined by of t-test? In order to compare distributions of oleic and linoleic acid among mutant generations it was necessary to make corrections for their fluctuation over the years (Spasibionek, 2006).

RESULTS AND DISCUSSION

In order to obtain optimal concentration of EMS solution, seeds were treated with five different doses. The effect of treatment was evaluated by calculating the survival rate. The survival rate varied from 25% (2.5% EMS solution) to 32% (0.5% EMS solution) in the glasshouse (Table 1). This drastical reduction of survival rate showed that all five doses were too high for mutagenic treatment. For that reason further bulk treatments were adjusted with 0.1 and 0.25% of EMS. Depending on the concentration of EMS treatment, the survival rate was 86% (by use of 0.1% EMS solution) and 31% (by use of 0.25% EMS solution) of the M_1 seedlings growing in the field (Table 1). Since the plants treated with 0.25% EMS solution had poor seed set (19.2%), further analysis were based on plants treated with 0.1% EMS solution. In general, results of the sensitivity test showed high frequency of lethality leading to the conclusion that less drastic EMS concentrations should be used for sunflower inbred line L31 seed mutation induction. Generally, optimal EMS concentration for mutation induction differs not only between plant species, but also between different genotype of the same crop. Osorio et al. (1995) reported that EMS concentration of 70m*M* (0.87% EMS) was used to obtain mutagenic sunflower line CAS-3. In *Arabidopsis thaliana*, the LD50 rate determined for Ler and Cor-0 seeds was 0.2% EMS for 16h and 0.13-0.25% for 12.5h, respectively (Jander et al., 2003). The LD50 rates for sugar beet seed balls were 1% EMS for 12h (Hohmann et al., 2005).

Table 1. Results from EMS treatment.

EMS Treatments	No of treated seeds	M_1 seedlings - Survival (%)	Sterility (%)	Seed set (%)
Glasshouse				
0.5%	50	32		
1%	50	30		
1.5%	50	28		
2%	50	27		
2.5%	50	25		
Total	250			
Field				
0.1%	500	86.0	0.0	75.6
0.25%	500	31.0	0.4	19.2
Total	1000	58.5	0.2	47.4

Since no mutant selection is recommended in M_1, as mutation may remain masked or undetectable due to chimerism presence (Bado et al. 2015), M_2 generation of 0,1% EMS-mutagenized population was developed. To isolate the mutants, 378 individually harvested M_2 seed stocks were screened for fatty acid composition and alterations occured in individual plants. These individual plants were planted in the next generation and the mean content of oleic acid in the seed oil decreased from 867 to 603 g/kg while mean linoleic content increased from 40 to 305 g/kg. Mutant line, designated ML31-1, was identified (Table 2). Mutant line had significantly changed oleic acid content compared to the wild type, L31, grown in the same year. We assumed recessive mutation occurred, manifested by changing a dominant allele *Ol* to the recessive *ol*, especially due to the fact that the effect of recessive gene is manifested in the later generations (Knowles, 1989). For that reason, seeds were collected from each ML31-1 plant and used as a source of segregating mutant plants.

Table 2. Oleic and linoleic acid concentrations (g/kg) in the seed oils of mutants (ML31-1, ML-31-11, ML31-12, ML31-13) and the wild type (L31) of sunflower in five M generations.

Gen eration	No of plants	Fatty acid (g/kg)	Mutants				Check	CV			
			ML31-1	ML31-11	ML31-12	ML31-13	L31	ML31-1	ML31-11	ML31-12	ML31-13
M_2	378	Oleic	790.0** (590.0[a])				821.0	22.32			
		Linoleic	144.0** (325.0[a])				94.0	25.01			
M_3	45	Oleic	603.0**				867.0	40.82			
		Linoleic	305.0**				40.0	45.84			
M_4	163	Oleic		519.9**	649.9**	863.6	850.8		12.25	22.55	8.24
		Linoleic		339.3**	231.5**	39.9	40.1		19.88	20.01	12.32
M_5	275	Oleic		514.7**	624.0**	855.0	832.5		12.26	11.22	4.86
		Linoleic		359.3**	269.0**	57.0	50.9		15.48	12.83	8.38
M_6	308	Oleic		482.0**	613.0**	887.0	867.0		9.62	10.21	8.66
		Linoleic		391.3**	270.0**	13.0	40.0		8.29	10.11	8.73

*,**significant at P=0.05 and P=0.01, respectively

[a]meaen value of individual plants

In the next generation (M_3), it was convenient to maintain mutant selection as in segregating population. After harvesting seeds were screened for fatty acid composition. The content of oleic and linoleic acid was significantly changed comparing to the wild type (Table 2). The segregation of oleic acid ranged from 346.6 to 949.1 g/kg and of linoleic acid from 39.9 to 339.3 g/kg (Fig. 1 and 2). In subsequent generation (M_4), individual selection and evaluation of progenies continued in several directions depending on the content of oleic acid: low, increased or high. We identified three subsequent mutants, designated ML31-11, ML31-12 and ML31-13.

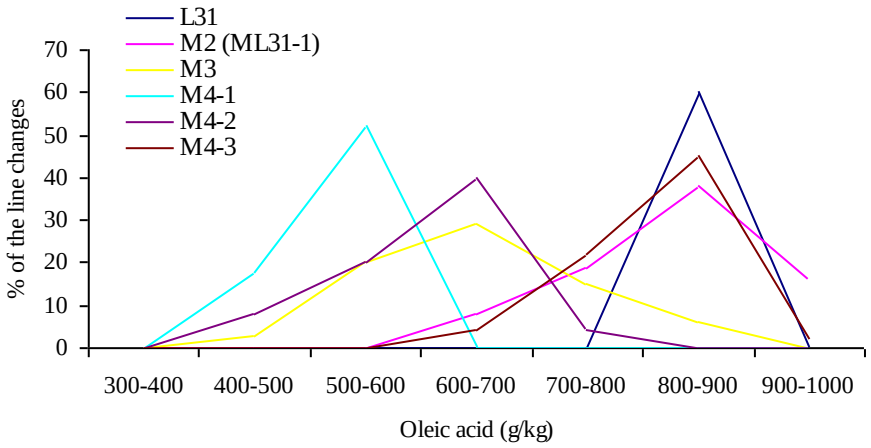

Fig.1. Distribution of oleic acid content (g/kg) in four M generations of sunflower mutant ML31-1 and subsequent mutants ML31-11 (M4-1), ML31-12 (M4-2) and ML31-13 (M4-3) compare to wild type L31

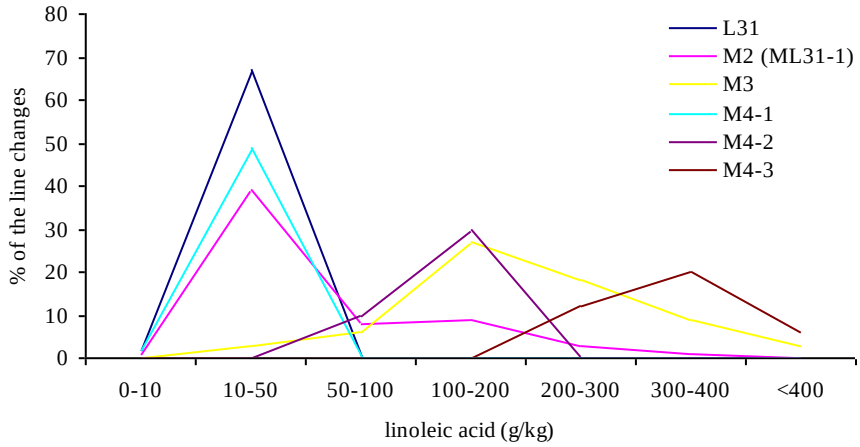

Fig.2. Distribution of linoleic acid content (g/kg) in four M generations of sunflower mutant ML31-1 and subsequent mutants ML31-11 (M4-1), ML31-12 (M4-2) and ML31-13 (M4-3) compare to wild type L31

In M_6 generation subsequent mutants ML31-11, ML31-12 and ML31-13 were evaluated and showed significant differences in one or more characteristics in regards to wild type (Table 3). Due to fatty acid content, mutant lines ML31-11 and ML31-12 had significantly lower concentration of oleic acid and significantly higher concentration of linoleic acid compared to the wild type. The content of oleic acid was higher in ML31-12 mutant line than in ML31-11. The thousand-seed-weight of these mutant lines was significantly higher than of the wild type. With respect to oleic acid content, values obtained were similar between the wild type and the mutant ML31-13, however, other examined traits such as oil content, thousand-seed-weight and seed yield were significantly higher in mutant line than the wild type (Table 3). This improvement represents the progress of wild type (line L31) thought mutation breeding since the seed yield and its components are the most important traits in sunflower production. Two mutant lines (ML31-12, ML31-12) exhibited highly significant increase in seed yield compared to the wild type. The oil content in the seed is closely linked to seed yield, which is the main purpose of sunflower growing (Škorić, 2012). Significant increase in oil content was observed in the mutant line ML31-13. This obtained increase is a very notable result, since no drastic mutation has been reported for seed oil content in sunflower (Vranceanu and Iuoras, 1991, Cvejić et al., 2015).

Table 3. Comparison between mutants ML31-11, ML31-12, ML31-13 and wild type L31 for some agronomic traits and fatty acid composition investigated in the field trials.

Traits	Mutants			Wild type
	ML31-11	ML31-12	ML31-13	L31
Full flowering (days)	58.0(±0.33)	57.0(±0.33)	58.0(±0.67)	57.0(±0.01)
Plant height (cm)	134.8(±2.10)	126.6(±0.62)	133.2(±1.21)	133.6(±0.15)
Seed yield (g/plant)	25.9(±0.13)	29.5**(±0.08)	30.1**(±0.32)	24.7(±0.05)
Thousand-seed-weight (g)	63.61**(±0.13)	63.13**(±0.11)	64.79**(±0.15)	59.5(±0.12)
Oil content (%)	50.56(±0.13)	50.09(±0.14)	54.1**(±0.13)	50.4(±0.10)
Palmitic acid (g/kg)	54.3(±0.20)	48.2(±0.08)	34.8(±0.01)	39.2(±0.01)
Stearic acid (g/kg)	59.5(±0.41)	57.6(±0.02)	50.8(±0.10)	49.6(±0.08)
Oleic acid (g/kg)	482.0**(±1.13)	613.0**(±2.23)	887.0(±2.40)	867.0(±3.08)
Linoleic acid (g/kg)	391.3**(±2.14)	270.0**(±0.21)	13.0**(±0.01)	40.0(±0.70)
Linolenic acid (g/kg)	1.1(±0.00)	1.0(±0.00)	1.2(±0.01)	1.0(±0.00)

*,**significant at P=0.05 and P=0.01, respectively

Induced mutagenesis lead to genetically inherited variability of sunflower inbred lines in terms of oleic and linoleic acid content, which will be more suitable for use in breeding programmes. Further studies will include identification of molecular changes that led to changes in oleic acid content in new mutant lines.

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SUNFLOWER GENETIC GAIN IN ARGETINA

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ABSTRACT

Genetic gain studies help breeders to refine and/or change their breeding programs in desired directions and to make estimates of future progress. There are two methods to estimate genetic gains: comparing an historic set of cultivars with uniform management or from the trial data collected by breeding programs. Using the first approach, a set of historical hybrids released to the Argentinian market by a private breeding program between 1984 and 2015 were evaluated in a four location experiment during 2014/2015 season. We included hybrids of four segments: conventional (CONV), herbicide tolerant (HT), high oleic (HO) and herbicide tolerant high oleic (HTHO). Genetic gain for oil yield in CONV hybrids was 25.5 $kgha^{-1}year^{-1}$. For the HT hybrids, the genetic gain was 63.6 $kgha^{-1}year^{-1}$. In the case of the HO, genetic gain was 49.6 $kgha^{-1}year^{-1}$. For the HTHO hybrids genetic gain was 29.1 $kgha^{-1}year^{-1}$. When the HT hybrid was launched to the market in 2003, the oil yield compared with the best CONV hybrid was 25.6% lower. The HO hybrid was launched to the market in 2004; the oil yield compared with the best CONV hybrid was 19.5% lower. In the case of the HTHO, the oil yield compared with the best CONV hybrid was 26.3% lower. The gaps have been closed for HT and HO and reduced for HTHO. Once the gaps are closed the genetic gain will depend on the level of resources dedicated to each segment.

Key words: *Helianthus annuus* L., Genetic Gain, Breeding, Sunflower, Yield drag

INTRODUCTION

Maximize the genetic gain is one of the main objective of most of the commercial breeding programs. The genetic gain can be defined as the yield increase divided by the time consumed to develop the higher yielding cultivars.

There are two different ways to evaluate the genetic gain. One is to analyze a dataset of different hybrids during a given period of time using linear mixed models (de la Vega et al., 2007). The other approach is to produce an historic set of cultivars and evaluate them in a trial network (López Pereira et al., 1999; Sadras et al., 2000; Vear et al., 2003). However, this approach has a couple of sources of inaccuracies related with the weather, the diseases and the weather by management interactions (Bell et al., 1995).

Sunflower (*Helianthus annuus* L.) crop is one of the main edible oil crops in Argentina. Cultivation of Sunflower varieties began in the early 30s by immigrants who brought seeds from Europe. In the 60s, new varieties were obtained by official breeding programs coming from the combination wild species that improved disease tolerance (Bertero and Vazquez, 2003). In 1969, Leclercq discovered the cytoplasmic male sterility and several restorer genes were discovered after, allowing a rapid spread of hybrid production by public and private companies.

The germplasm evaluated in this study is coming from the same breeding program and will be restricted to hybrids developed by Dekalb from 1984 to 1999, by Monsanto from 2000 up to 2009 and from that year to the present by Syngenta. The breeding activities started in the mid-70s in Ines Indarht, then it continues in Bragado and now it is based in Camet, all in Buenos Aires province, Argentina.

The main objectives of this breeding program have been grain yield and oil content. Also some diseases like Verticillium wilt (*Verticillium dahliae* Klebahn) and Downy mildew (*Plasmopara halstedii (Farl.) Berl. & De Toni*) have been very important breeding traits for selection.

From the 90s, new type of hybrids appeared in the market and today the Argentinean market of oil type sunflower can be separated in four segments: conventional hybrids (CONV), herbicide tolerant hybrids (HT), high oleic hybrids (HO), and herbicide tolerant- high oleic hybrids (HTHO).

After the discovery of Imazethaphyr resistance in wild sunflower in 1998 by Al-Khatib et al.; seed companies incorporated this source of tolerance to create the IMISUN hybrids with resistance to different imidazolinone herbicides. This technology that allows better weed control was rapidly adopted by farmers in Argentina. It was reported that some linkage drag around IMISUN gene coming from wild sunflower produced a decrease in oil content in the seed (Sala et al., 2012).

As a request from the industry to get better quality of sunflower oil, the high oleic market was developed. Breeding programs incorporated the Pervenets mutation to increase the percentage of oleic acid content in the seed. The hybrids containing this mutation produce a different profile of fatty acids with high percentage of oleic acid and industry pays a premium price to the farmers for high oleic content above 80%. Depending of the donor of this mutation and the quality of the conversion, more or less yield drag has been observed in this type of hybrids compared with the CONV hybrids.

The newest market segment in Argentina is the HTHO that combine herbicide tolerance and high oleic acid content. Hybrids from this segment presented the largest yield drag.

The objectives of this work were: To quantify the genetic gain of this breeding program considering hybrids developed from 1984 up to 2015 and to calculate yield drags between market segments and the evolution they had.

MATERIAL AND METHODS

SITES AND CROP MANAGEMENT

Rainfed trials were conducted in five locations: Quemú Quemú, América, Olavarría, Camet and Necochea. All of them were located within the sunflower production area and the planting dates where the same as farmers (Table 1). Rainfalls during growing season were adequate for crop development. A final plant density of 50000 plants ha-1 was achieved planting higher densities and then doing manual thinning at the stage of V2 (Schneitter and Miller, 1981). Herbicides were applied after planting and remaining weeds were controlled manually. Insecticides were applied for insect control. Fungicides were applied in R1, no pressure of disease was observed.

Table 2. Location, planting dates and coordinates.

Location	planting date	GPS coordinates
America	10-Oct-2014	35°30'S 63°30'W
Quemú Quemú	14-Oct-2014	36°30'S 63°35'W
Olavarría	2-Oct-2014	36°41'S 60°22'W
Camet	16-Nov-2014	37°46'S 57°53'W
Necochea	22-Nov-2014	38°33'S 58°53'W

PLANT MATERIAL

Hybrids were produced during summer 2013-2014 in Syngenta sunflower breeding station in Camet. Most of the materials were chosen by the year of registration, performance and farmer adoption level. Table 2 summarizes hybrids, year of registration and market segment.

Table 3. Hybrids included in the trials, year of release to the market and market segment.

Hybrid name	year of release	market segment	Hybrid name	year of release	market segment
DK G100	1984	CONV	DK 3880CL	2003	HT
DK G105	1990	CONV	DK 4000CL	2003	HT
DK 3881	1993	CONV	DK 3910CL	2008	HT
DK 4100	1994	CONV	DK 3948CL	2008	HT
DK 3878	1997	CONV	SYN 3970CL	2012	HT
DK 4040	1997	CONV	SYN 4070CL	2012	HT
DK 3915	1997	CONV	DK_OILPLUS3845	2004	HO
DK 4050	1999	CONV	DK_OILPLUS3945	2004	HO
DK 3920	2002	CONV	SYN 3950HO	2011	HO
DK 3820	2003	CONV	SX132397HODM	2015	HO
SPS3150RDM	2004	CONV	DK 3955CLHO	2009	HTHO
DK 3810	2004	CONV	SYN 3965CLHO	2013	HTHO
DK 4045	2005	CONV			
DK 3940	2006	CONV			
DK 4065	2009	CONV			
SYN 3825	2013	CONV			

EXPERIMENTAL DESIGN AND MEASUREMENTS

A randomized complete block design with two repetitions was used for trials. Plot size was 7 meters by 4 rows. Interrow distance was 0.7 meters. Only the 2 central rows were harvested with a combined harvester machine. Samples were collected to measure oil content with nuclear magnetic resonance equipment. Days from planting to R 5.5 were measured in Quemú Quemú and Camet.

CALCULATIONS

Genetic gain was calculated as the slope of the regression between the trait and year of release. To make it comparable with other studies the genetic gain was also expressed as the % of the average yield for the considered period. Oil yield was calculated as the product of grain yield and percentage of oil content. Oil yield gap between market segments was calculated by the difference among best yielding CONV hybrid and traited hybrid expressed as the percentage of the CONV.

RESULTS AND DISCUSSION

YIELD PERFORMANCE OF CONV MARKET SEGMENT

When genetic gain of oil yield in each location was analyzed, a similar slope in Camet, America and Necochea and Olavarría was observed (Table 3). Yield data from Quemú Quemú was excluded from the analysis due to poor quality.

Table 4. Slope and determination coefficient for the linear regression analysis between oil yield and year of release for trial locations.

Location	Slope	r^2
América	21.14	0.391
Camet	25.58	0.398
Necochea	24.20	0.505
Olavarría	31.08	0.638

The genetic gain derived from the combined analysis of 4 locations was 25.51 kg year^{-1} for oil yield (Fig. 1). That rate represents 1.77 % of the average oil yield of the period considered. De la Vega et al. (2007) also found a positive trend in oil yield using a linear mixed model to calculate the best linear unbiased predictor. The reported genetic gain was up to 14.4 kg year^{-1} for oil yield in a dataset from central Argentina from 1983 to 2005.

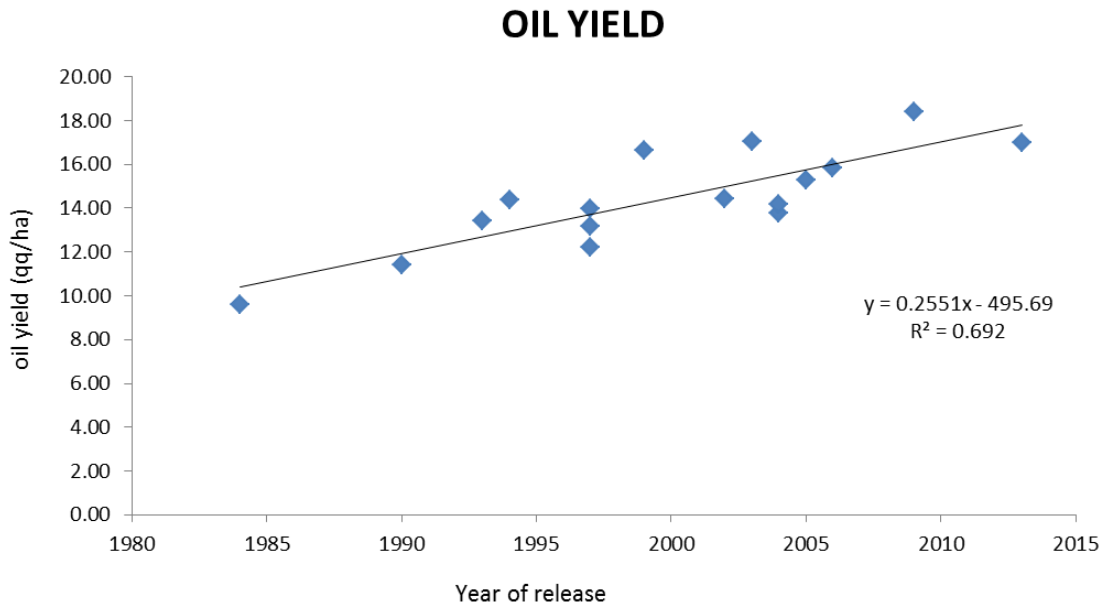

Figure 9. Linear regression between oil yield and year of release combining four locations.

Sadras et al. (2000) found a positive correlation between oil yield and year of release when cultivars from 1963 to 1998 were evaluated. However, no genetic gain was calculated. López Pereira et al. (1999) studied set of historical cultivars released between 1930 and 1995 and found positive association between grain and oil yield and year of release. This association has not been constant, a clear turning point was observed during the seventies with the introduction of hybrid cultivars. No significant improvement has been found after that point. These authors postulate that the lack of improvement might be related with "defiency" breeding (Richards et al., 1997). This means that breeding for disease tolerance, grain quality and a narrow genetic base might have restrained genetic gain for yield.

In France, a genetic gain study using a similar approach of this one but using cultivars released from 1960 up to 2000 found an improvement that represents 1.3% for grain yield per year (Vear et al., 2003). In a more recent study in the United States of America, Hulke and Kleingartner (2014) found a genetic gain of 0,698% for cultivars released from 1975 up to 2013. The main reasons for this level of genetic gain were related with the focus on defensive breeding. In this study, a clear progress in oil yield was found. The difference in the results of this study and the others found in literature might be explained by the fact that other assessment of the genetic gain for sunflower used different set of hybrids, different periods and different methodologies.

The genetic gain in oil yield for this particular study was only explained by grain yield and not by oil content. When components of oil yield were analyzed separately, the grain yield genetic gain was 46.92 kg year^{-1} but no clear tendency for oil content was observed (Fig. 2). The lack of association between oil content and year of release in this germplasm could be because the first released hybrids already have similar levels of oil content to the recently released hybrids. An example of this is the hybrid G100 registered in 1984 with an average oil content of 52.5%.

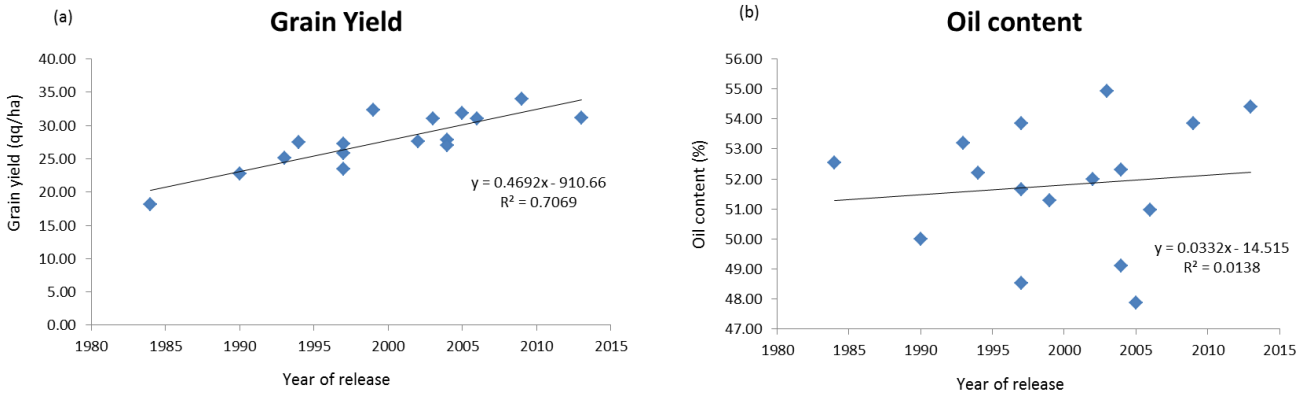

Figure 10. (a) Grain yield and (b) oil content linear regression with year of release.

De la Vega et al. (2007) found a bi linear function for the description of the relation of grain yield and year of release with a clear improvement from 1983 until 1995 and no improvements after that. Two reasons are postulated to explain this plateau. First, that in Argentina, during that period, a change in breeding process resulted in an increase of oil yield mainly due to the higher grain oil concentration rather than grain yield. Second, from 1991 to 2005 the explosive growth of soybean in central Argentina pushed the sunflower industry toward more marginal, lower rainfall western environment giving as result the declining of grain yield genetic gain.

COMPARISON BETWEEN MARKET SEGMENTS

Genetic gain for different market segments are presented in Fig. 3. Trendlines for each market segment are shown. Genetic gain for oil yield was 25.5 kg year^{-1}, 63.6 kg year^{-1}, 49.6 kg year^{-1} and 29.1 kg year^{-1} for CONV, HT, HO and HTHO respectively. It is important to point out that only few hybrids were used to calculate genetic gain for segments HT, HO and HTHO.

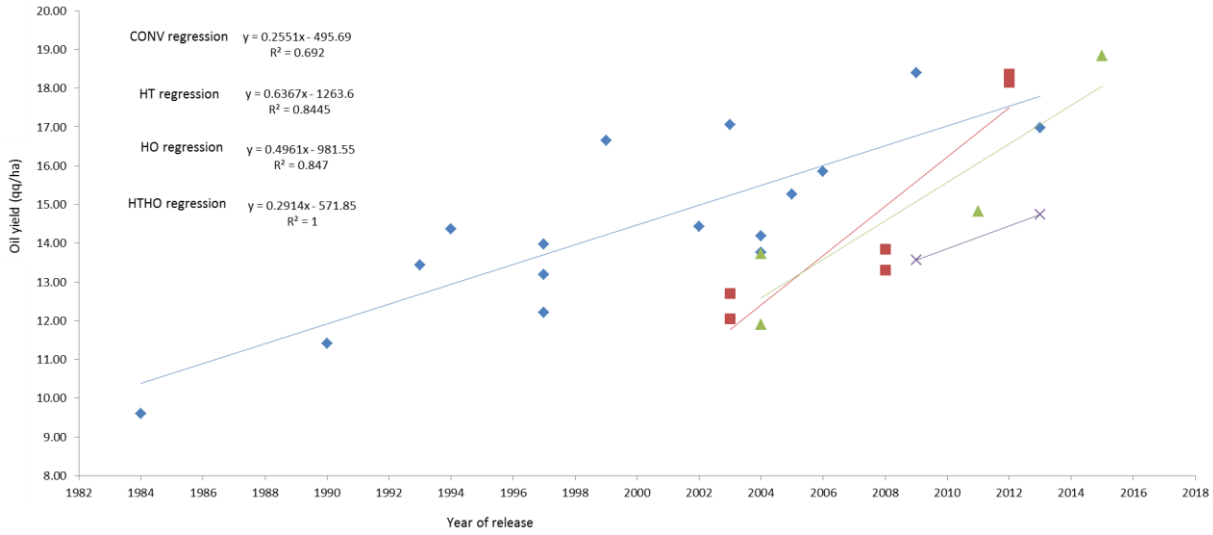

Figure 11. Linear regression between oil yield and year of release for four market segments. Symbols: diamond = CONV market, squares = HT market, triangles = HO market, cross = HTHO market.

As expected, HT, HO and HTHO have higher genetic gain than CONV segment mainly due to the fact that the first hybrids registered including the new traits presented a yield gap compared with the best yielding CONV hybrids. The reason of that gap might be related with the introduction of undesired alleles coming from the donor lines used as source of the new traits. The other reason for that gap could be related with the conversion process that had few backcrosses and the lack of tools like molecular markers for trait detection and background recovery.

First HT hybrids were released in 2003 with an oil yield gap of 25.52%. By 2012 this gap was closed reaching similar levels of performance with the CONV segment. A total of 10 years were needed to close this gap. The genetic gain in oil yield in HT market segment was explained by both, an increase in grain yield and oil content (Fig. 4). This situation differs from CONV segment where genetic gain in oil yield was explained only because of grain yield. This is an indicator of oil content linkage drag with of the introduction of IMISUN trait, as IMISUN gene comes from wild sunflower.

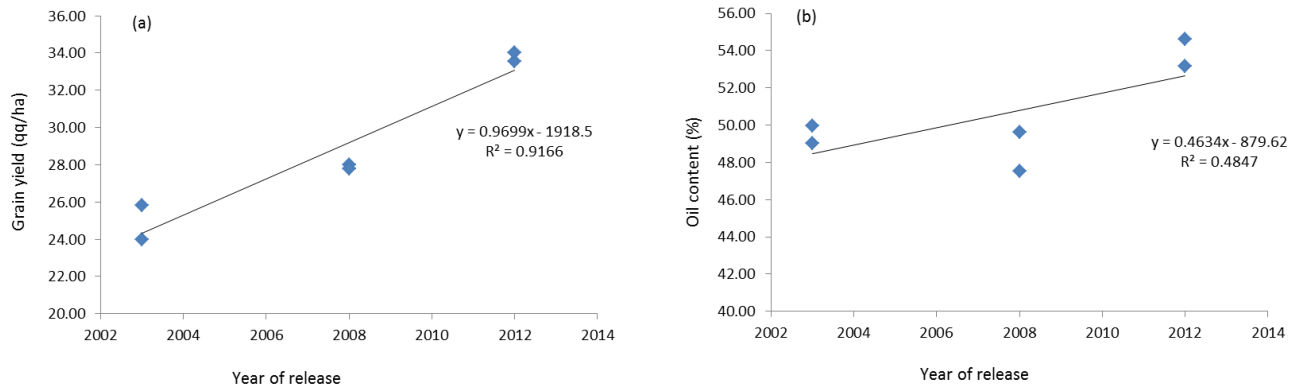

Figure 12. (a) Grain yield and (b) oil content linear regression with year of release for HT market segment.

First HO hybrids were released in 2004 with an oil yield gap of 19.5% and by 2015, after 12 years of breeding, this gap was closed and now HO hybrids have similar performance to CONV hybrids. The genetic gain in oil yield in HO segment was explained because of grain yield, as there is no tendency in oil content (Fig. 5).

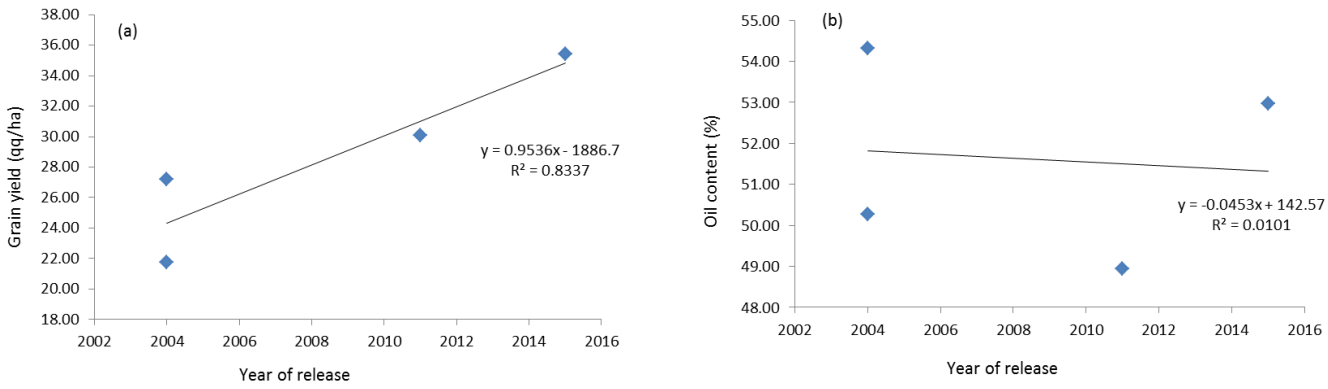

Figure 13. (a) Grain yield and (b) oil content linear regression with year of release for HO segment.

In the HTHO segment only two hybrids were included as this is the newest market segment. First HTHO hybrid was released in 2009 with an oil yield gap of 26.3% compared with the best CONV at that time. The second release in 2013 reduced the oil yield gap to 19.9%.

CYCLE LENGTH.

Cycle length defined as days from planting to R 5.5 showed a positive correlation with the year of release in all market segments (Fig. 6 a-b-c). Considering harvest moisture as indirect measurement of cycle length also presented positive correlation with year of release (Fig. 6 c-d-e). The same positive correlation was found comparing cycle length and oil yield (Fig. 7).

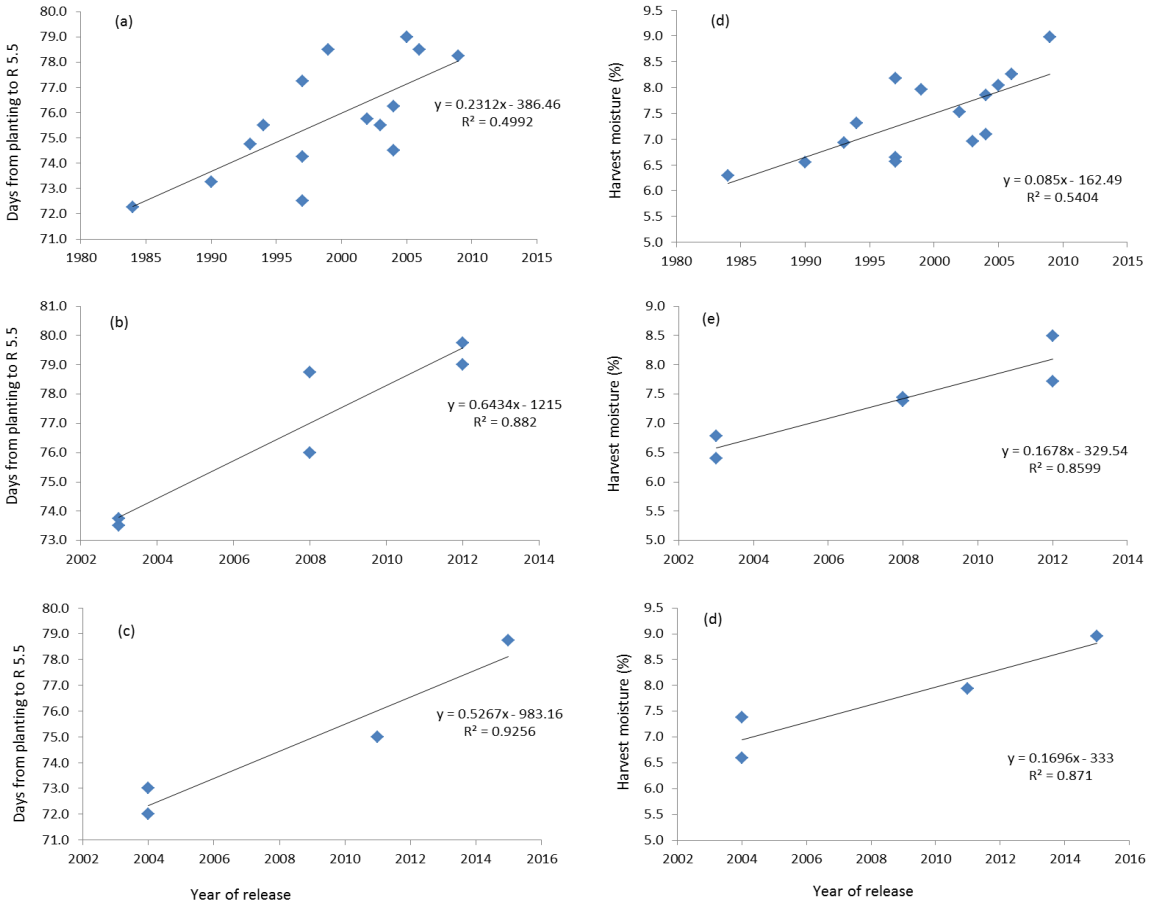

Figure 14. Relationship between cycle length and year of release. Cycle length as days from planting to R 5.5: (a) CONV market segment, (b) HT market and (c) HO market. Cycle length as harvest moisture: (d) CONV market segment, (e) HT market segment and (f) HO market.

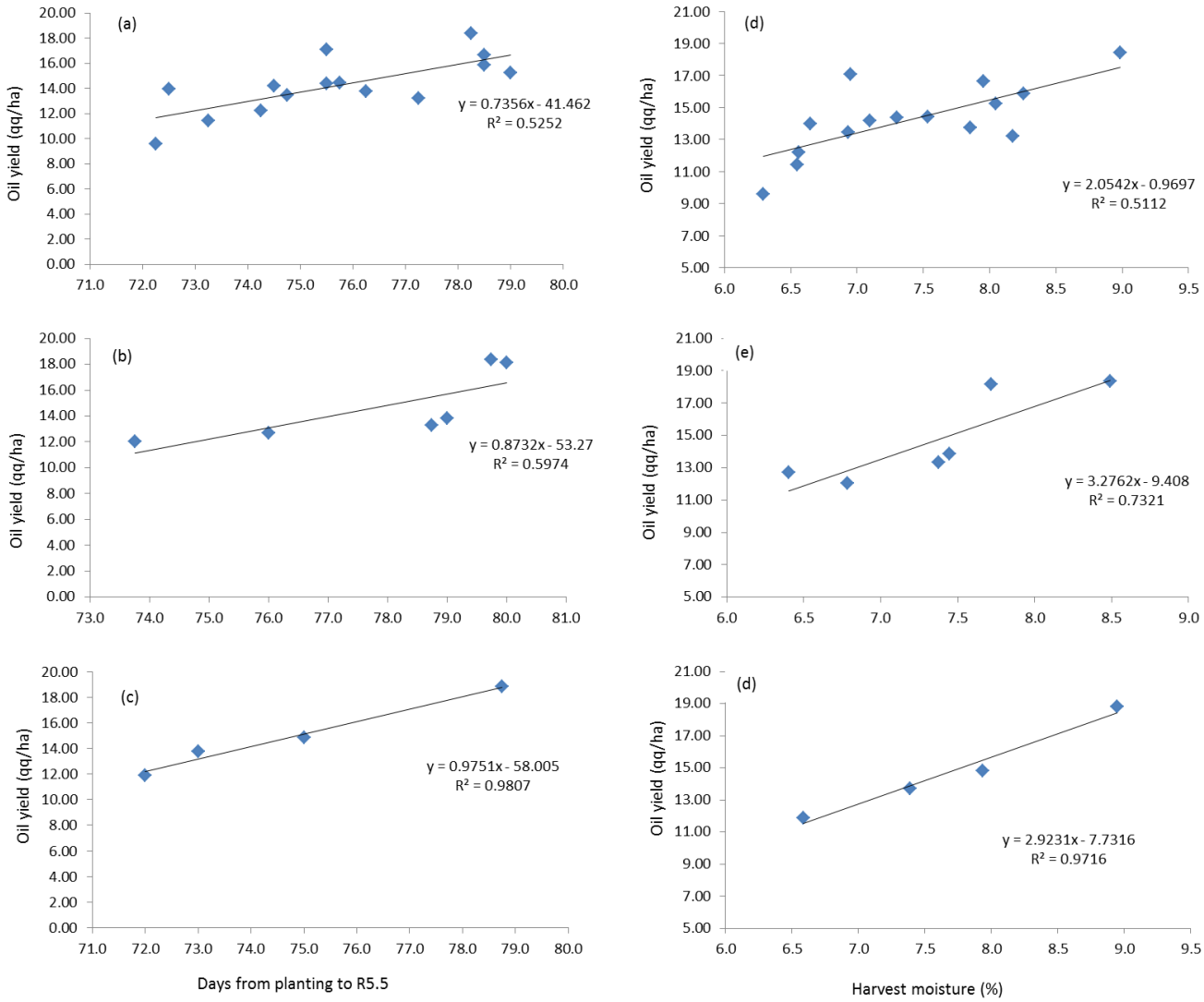

Figure 15. Relationship between oil yield and cycle length. Cycle length as days from planting to R 5.5: (a) CONV market segment, (b) HT market segment and (c) HO market segment. Cycle length as harvest moisture: (d) CONV market segment, (e) HT market segment and (d) HO market segment.

Earlier studies indicated no relation between days to anthesis and year of release (De la Vega et al., 2007; Sadras et al., 2000). Even more, Lopez Pereira et al., 1999 reported that breeding and selection shortened cycle length during the period 1930 and 1995 and that most of this reduction in cycle length was accounted for a reduction in time to anthesis.

CONCLUSIONS

Oil type sunflower breeding programs objective is to increase oil yield but strategies to achieve this could be different. In this study, and for the hybrids included, we reported different tendency in grain yield, oil content and time to anthesis compared with other studies that included a different set of Argentinean or foreign cultivars.

As breeding strategies could be different among breeding programs, studies that mix many pools of germplasm could be undercovering specific effects or tendency for different traits in each germplasm. Genetic gain studies done by specific germplasm might allow detecting these tendencies that each breeding program improved to get the final objective of increasing oil yield.

This particular germplasm is in active growth as no plateau was detected either for grain or oil yield. The increase in cycle length has reached the maximum value for suitable season growth in Argentina. The challenges for this germplasm will be to maintain the same rate of genetic gain in oil yield.

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PRODUCTION POTENTIAL OF NEW SUNFLOWER HYBRIDS DEVELOPED AT DOBRUDZHA AGRICULTURAL INSTITUTE – GENERAL TOSHEVO

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ABSTRACT

Sunflower breeding at Dobrudzha Agricultural Institute – General Toshevo (DAI) is traditionally carried out at a high level showing very good results. The working collection includes over 6 000 inbred lines. In the past decade, many new materials with very good combining ability and valuable properties were developed here. The new released hybrids possess high production potential. Over 1400 hybrid combinations are annually being tested in Bulgaria and abroad. Work is primarily focused on two-linear simple hybrids with full fertility restoration. A large part of the new genotypes were included in official varietal testing networks in Bulgaria and abroad; having demonstrated high performance, they were registered in the varietal lists of EU and other countries. These are hybrids Alpin, Veleka, Vokil, Velko, Gabi, Mihaela, Dea, Sevar. A number of foreign companies included our hybrids in their catalogs and are now promoting them. The testing of already released hybrids is ongoing with a view of their distribution on larger territories under variable environments. The developed sunflower lines and hybrids are the result from the efforts of the entire research team of the Sunflower Breeding Department at DAI who are working on several important projects aimed at higher productivity and oil content, variable chemical composition of oil, early maturity, drought tolerance and resistance to economically important diseases and the parasite *Orobanche*. The aim of this investigation was to present a brief characterization of the new DAI hybrids, demonstrating their productivity and adaptability potential.

Key words: Sunflower, New hybrids and lines, Production potential, Official varietal testing

INTRODUCTION

Sunflower is an oil seed crop of primary importance in Bulgaria. The country is one of the greatest exporters of sunflower worldwide (Christov et al., 2009). In 2013, the export reached a peak – over 1 000 000 tons, which was 17.8 % of the global export. Almost two thirds of the production from this crop are being annually exported.

During the last decade the volume of the produce increased twice, from 788 000 tons in 2003 to 1 697 000 tons in 2015. This is a result from both the larger areas sown with sunflower and the higher mean yield, which was about 1200 kg/ha in 2003, and 2120 kg/ha in 2015. Annually 700 000 – 800 000 ha of sunflower are sown in Bulgaria. It has been noted that during the recent years the normal crop rotation is not being observed and sunflower is sown at an interval of 2-3 years, some times even only one year. Although profitable, this practice should be limited because it can cause the distribution of a number of diseases and pests, which, on its turn, can compromise irreversibly the sunflower crops.

Under the contemporary conditions characterized with certain variations of the abiotic and biotic environmental factors, adequate response is needed; such a response requires development of new

breeding materials with enhanced productivity and resistance to stress influences of various nature (Ivanova and Mihova, 2012, Marinković et al, 2011; Mihova, 2011; Gonzáles et al, 2013; Khan et al., 2013). Specific methods for evaluation of the genetic variability and selection of genotypes with high adaptability potential are applied (Mihova, 2013). The year 1917 can be considered the beginning of sunflower breeding in Bulgaria (Stoyanova et al., 1977). In 1963, the breeding and improvement work on development of hybrids started, using the method of inter linear hybridization (Petrov et al., 1994). The practical application of the methods for heterosis breeding became possible after the discovery of a stable CMS source by Leclercq (1969) and fertility restorer genes for this type of CMS (Enns et al.; Kinman, 1970; Leclercq, 1971; Vranceanu and Stoenesko, 1971). There are several schemes for hybrid seed production developed at DAI (Velkov and Stoyanova, 1974), but only one of them is used in practice – a simple hybrid with full restoration of fertility.

In 1979, the first Bulgarian hybrid Start was released and distributed on the territory of the entire country (Gotsov et al., 1981; Ivanov et al., 1988). A new page of Bulgarian sunflower breeding was opened in 1988, when the new hybrid Albena was registered in France, followed by registration in Bulgaria as well on the next year. During the years to come it became the most widely distributed hybrid in Bulgaria, while in France it occupied as much as 42 % of the sunflower production areas. The hybrid was grown very successfully in other European countries as well. The high results of hybrid Albena in Bulgaria and abroad made it a world standard. More than 20 own and joint hybrids involving the mother line of Albena have been registered. Up to now mainly early hybrids have been developed at DAI. The best known among them are Super Start, Dobrich, Mussala, Maritsa, Rada, Merkuryi, Perfekt, Diamant, and the large-seeded variety Favorit.

During the 1990's, hybrid San Luka was released, which occupied about 90 % of the sunflower areas in Bulgaria till 2008. For more than a decade now, the invasion of foreign hybrids in Bulgaria has increased considerably (Georgiev et al., 2009). Almost all major sunflower seed companies introduced new products in the domestic market and aided by their enormous financial potential logically displaced our own hybrids from the sunflower production areas. We were unprepared economically for this situation. However, efforts were made and opportunities were found for distribution of our products abroad. Now our hybrids are successfully produced and traded on the Ukrainian, Russian, Romanian, Moldovian and other markets. The testing of our hybrids with the aim of their registration is ongoing.

At DAI, the breeding of sunflower is carried out in three main directions:

Higher productivity;

Higher resistance to the economically important diseases, the parasite *Orobanche*, some herbicides, soil and air drought; Higher oil percent in seed и and higher variability of its chemical composition.

The work in these directions is in accordance with the present reality of sunflower production, the scientific achievements in this field, the conventional and bio technology methods applied in breeding, the human resources and last, but not least, the financial means available in our system. Our work is focused on the use of heterosis by developing and investigating inbred lines, testing of experimental hybrids and production of seeds from parental lines of already developed and registered hybrids (Petrov et al., 1994).

The aim of this investigation was to present a brief characterization of the new developed and registered hybrids of DAI – General Toshevo, revealing their production and adaptability potential.

MATERIAL AND METHODS

The main investigations related to this study were carried out at DAI – General Toshevo. They are the result from the implementation of a long-term program which encompasses several 4-year periods. The main purpose of this program is to develop sunflower hybrids with enhanced production potential, resistant to economically important diseases and the parasite *Orobanche* by combining conventional and biotechnology methods.

Breeding material was used, which includes Bulgarian and foreign direct and hybrid varieties, landraces and foreign populations, our old sterility maintainer lines, their sterile analogues and fertility restorers, wild species of genus *Helianthus*, and species from other genera of *Compositae* family. To obtain new forms, lines and hybrids, the following methods are applied: hybridization (interspecific, interlinear, intraspecific and intergeneric), experimental mutagenesis, selection, gamma-induced parthenogenesis, embryo culture, somaclonal variation, combined use of *in vitro* methods and physical mutagenesis.

Eight new male fertile two-linear sunflower hybrids were developed through the method of interlinear hybridization. These are Alpin, Veleka, Vokil, Velko, Gabi, Mihaela, Dea and Sevar. The mother components of these hybrids are lines 2607, 217, 3607 and 807. They possess very good general and specific combining ability and resistance to economically important diseases; with the exception of line 2607, they also possess resistance to the parasite *Orobanche*, races A-F.

Using these mother lines, other new hybrids registered abroad have also been developed, as well as hybrids which are now in the process of official testing. The fertility restorers involved in the new hybrids are 10681R, 166R, 340R, 105R, 127R, 10671R, 509R and 626R. They are all resistant to the above diseases and the parasite *Orobanche* and have excellent combining ability. All are branched and rich in pollen.

Each new sunflower hybrid goes through three-year testing in the trial fields of DAI according to a growing technology approved for this crop (Georgiev et al., 1997). The standards used in this testing were the most widely distributed hybrids in Bulgaria – San Luka and Maritsa, as well as the most highly productive and most marketed foreign hybrids Brio, Diabolo, Meldimi, Clarica, PR64F50, LG5665, PRLE19, PRLE25, etc.

Having demonstrated very good results, the new hybrids were provided to our partners from Saaten Union – Romania to produce and distribute them. Following one more year of testing in their experimental fields, they were subjected to a three-year official testing at 10 locations within the system of the State Institute for Variety Testing and Registration. Having once again demonstrated very good results, the new sunflower hybrids were officially registered. They are enlisted in the European catalog of field and vegetable crops. The trait seed yield kg/ha was read. The observations and the evaluation of the morphological characters were made in accordance with the UPOV protocol (2002). The phytopathological characterization of the hybrids was made at DAI – General Toshevo. The resistance to downy mildew (*Plasmopara halstedii*) was determined according to the standard methodology (Vear and Tourvieille, 1987) adapted to the working conditions of the institute. The response of the hybrid to races 700 and 731 of the pathogen was presented as percent of resistance.

The resistance to grey spots on sunflower (*Phomopsis helianthi*) was done according to the method of Encheva & Kiryakov (2002) under field conditions against artificial infection background. The type of attack was read one week after full flowering and at stage milk maturity according to the following scale: 0 – no symptoms; 1 – necrotic spot up to 5 cm in diameter; 2 – necrotic spot with diameter more than 5 cm; 3 – several merged necrotic spots on stem; 4 – stem broken at the place of infection.

The testing for black spots on sunflower (*Phoma macdonaldii*) was carried out under field conditions in an artificial infection field. Inoculation was done at stage budding – beginning of flowering according to the method of Maric et al. (1981). The response of the plants was read at stage yellow-brown maturity according to a 4-degree scale: 0 – no symptoms; 1 – necrotic spot localized around the petiole; 2 – several merged necrotic spots on stem; 3 – entire stem covered with necrotic spots or broken.

The resistance to the parasite broomrape (*Orobanche cumana*) was determined by the method of Panchenko (1975). The evaluation was made under greenhouse conditions using the index percent of resistance. The experimental data were analyzed by ANOVA 3. The applied statistical model was:

$$Yijk = Y.. + Gi + Yj + Rk + (GY)ij + (GR)ik + (YR)jk + (GYR)ijk + Eijk$$

where Gi is the factor genotype , Yj is the factor climatical conditions, and Rk - the factor location.

The Additive Main Effects and Multiplicative Interaction (AMMI) model has developed a new statistical method for analyzing the genotype by environment interaction. In the AMMI method, first the main additive effects of genotype and environment are considered by variance analysis, then are analyzed by principal characteristics of remain value from variance analysis model (Gauch et al, 1996; Dias et al., 2003, Lee, 2004). Totally, AMMI follows three basic purposes: first, this is an appropriate method for primary analysis of performance tests. Second, it explains the effect of the genotype × environment interaction. Third, performance estimate is done with greater accuracy. This method is applied to estimate the ecological stability and plasticity of the hybrids. Data were analyzed with the help of the software SPSS, version 19.0.

RESULTS AND DISCUSSION

Following the official testing, all new hybrids underwent a two-year test for distinctness, uniformity and stability at the State Institute for Variety Testing and Registration – Romania. They were acknowledged as distinct, uniform and stable. Their morphological descriptions were done using the methodology of UPOV (2002).

BIOLOGICAL AND ECONOMIC PROPERTIES

The eight new Bulgaria hybrids presented here are from the group of the medium early varieties, with growth season 110 – 120 days; however, the earliest among them are Alpin, Mihaela and Sevar. They all have linoleic type of oil, with oil percent within the range 46-50 %. The number of seeds per plant can reach up to 1200 – 1300, which makes their production potential very high, exceeding sometimes 4300 kg/ha. The stems are medium high and resistant to lodging. All hybrids possess very high plasticity with regard to the growing conditions. Hybrids Velko and Dea demonstrated highest drought resistance.

The possibility of sowing both parental forms simultaneously is a great advantage of all presented hybrids. The father lines are strongly branched and very rich in pollen, which, on its part, allows planting design of 10:2 female to male lines. This design should naturally be provided with at least 3-4 good bee hives per ha.

PRODUCTIVITY

All eight new hybrids were subjected to three-year official testing at the State Institute for Variety Testing and Registration – Romania. The testing was carried out at 10 locations representative for regions with various soil and climatic conditions from all over the country. The first three, Alpin, Veleka and Vokil were tested during 2009 – 2011 (Table 1).

The three years of investigation were with comparatively similar climatic conditions as evident from the given mean yields, which were with similar values and low variation. Averaged for the investigated period, all new hybrids exceeded the standard. Hybrid Alpin demonstrated highest mean results with an average yield for the entire period of testing 3572 kg/ha, followed by Vokil and Veleka.

Table 1. Mean results from 10 locations of official testing of hybrids Alpin, Veleka and Vokil

Hybrids	Yield kg/ha	% from standard	Yield kg/ha	% from standard	Yield kg/ha	% from standard	Averaged for 3 years kg/ha	Relative yield according to the standard, averaged for 3 years
	2009		2010		2011			
Standard	3108	100	3240	100	3659	100	3336	100
Alpin	3489	112	3619	112	3609	99	3572	108
Veleka	3272	105	3368	104	3485	95	3375	101
Vokil	3259	105	3356	104	3635	99	3417	103

The group of hybrids including Velko, Mihaela and Gabi were tested during 2011 – 2013 (Table 2). In this case, again, the mean results of the three hybrids were above the mean standard of the entire investigated period. The exceeding was within the range 4 – 9 %, hybrid Velko being the highest yielding. It was also the hybrid with highest mean results out of all eight according to the index seed yield during the entire official testing - 3578 kg/ha. Hybrid Velko also gave best results during the less favorable year 2012.

Table 2. Mean results from 10 locations of official testing of hybrids Velko, Mihaela and Gabi

Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Averaged for 3 years kg/ha	Relative yield according to the standard, averaged for 3 years
	2011		2012		2013			
Standard	3659	100	2648	100	3642	100	3316	100
Velko	3694	101	3072	116	3969	109	3578	109
Mihaela	3618	99	2934	111	3757	103	3436	104
Gabi	3689	101	2879	109	3947	108	3505	106

The last two hybrids, Sevar and Dea, were officially tested during 2012 – 2014 (Table 3). They also exceeded the standard, averaged for 3 years. Dea showed better results, especially in 2012, the year with lowest precipitation, which defines this hybrid as drought resistant.

Table 3. Mean results from 10 locations of official testing of hybrids Sevar and Dea

Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Averaged for 3 years кг/ха	Relative yield according to the standard, averaged for 3 years
	2012		2013		2014			
Standard	2648	100	3642	100	3234	100	3175	100
Sevar	2650	100	3959	109	3210	99	3273	103
Dea	2954	112	3783	104	3424	106	3387	107

The results from the dispersion analysis are presented in Table 4.

Table 4. Dispersion analysis for the index ***productivity***

	Mean of square	df
G	11089,8 [c]	8
Y	15793 [c]	2
R	7726,6 [c]	7
G x Y	1416,1 [c]	16
G x R	438,1 [a]	56
Y x R	4061,7 [c]	14
G x Y x R	474,3	112
Error	221,4	278

The applied dispersion analysis proved with a high degree of significance (p=0.001) the differences in the genetic potential of the selected hybrids according to the index ***productivity***. The effects of the factors climatic ***conditions*** and ***location*** were with highest significance.

The interaction between the indices ***genotype x climatic conditions*** and ***genotype x location*** was also determined statistically. The lower significance of /G x R/ showed that the predominant part of the genotypes responded in a similar way to the various locations of testing. Their response to the year conditions /G x Y/ was specific. This fact allowed applying AMMI (1, 2) models for evaluation of the ecological plasticity and stability of the investigated hybrids with regard to the index productivity (Table 5).

Five of the investigated Bulgarian hybrids had ASV < 0.200 indicative of high ecological stability and plasticity. Hybrids Alpin, Veleka and Vokil showed lower response to the changeable environments with regard to the index productivity. Hybrids Sevar, Dea and Gaby had more susceptible reaction.

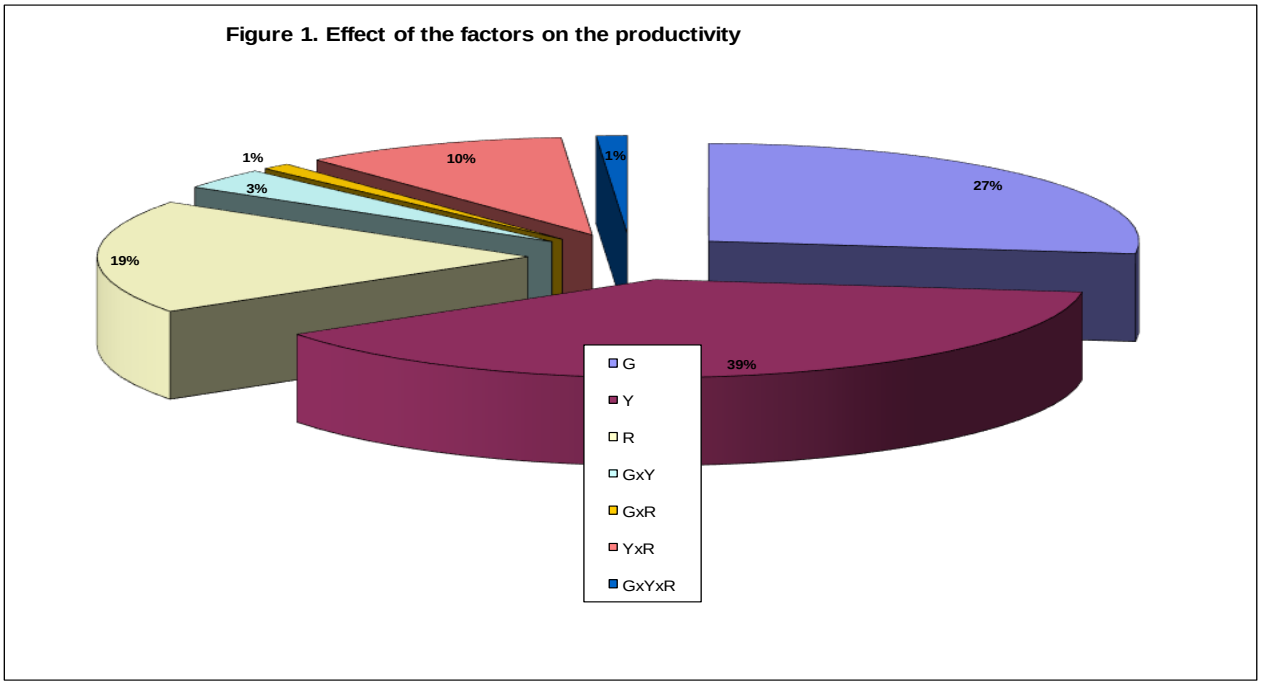

Table 5. Ranking of investigated hybrids by the index ***productivity*** according to their ecological plasticity and stability

Hybrid	**Parameters**		
	Rank	**ASV**	**I**
Alpin	1	0,022	2,432
Veleka	2	0,084	2,213
Vokil	3	0,111	1,945
Velko	4	0,165	1,929
Standard	**5**	0,180	1,896
Mihaela	6	0,187	1,854
Gabi	7	0,208	1,805
Dea	8	0,233	1,725
Sevar	9	0,276	1,664

All eight Bulgarian sunflower hybrids were officially registered and included in the European catalog of field and vegetable crops. Because of the demonstrated very good productivity potential, they were included also in the systems of official varietal testing of non-EU countries such as Ukraine, Serbia, Kazakhstan, Russia, etc

In 2013 the testing of our hybrids continued in Hungary as well, at three locations in the central and southern parts of the country (Table 6). Seed yield varied from 3150 до 5304 kg/ha, most of the results being within the range of the higher values and demonstrated a very good level of the index ***productivity***. Only hybrids Velko and Dea exceeded the standard at all three locations, and the mean value of hybrid Mihaela also exceeded it. Hybrid Dea demonstrated highest mean results - 4441 kg/da, showing once again that it is hybrid with high plasticity and productivity.

Table 6. Results from testing in Hungary

	Bekecsaba		Lanycsok		Cegled		Average	
Hybrids	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from strd
STANDART	4381	100	3892	100	3621	100	3965	100
ALPIN	3679	84	3301	85	3438	95	3473	88
VELEKA	3714	85	3411	88	3435	95	3520	89
VOKIL	3559	81	3458	89	3617	100	3545	89
MIHAELA	4926	112	3800	98	3924	108	4217	106
VELKO	4628	106	3969	102	3772	104	4123	104
GABI	3403	78	3219	83	3503	97	3375	85
SEVAR	4118	94	3554	91	3150	87	3607	91
DEA	5304	121	3922	101	4098	113	4441	112

Table 7. Results from the testing in Ukraine

Hybrids	KHARKIV		ODESSA		ZAPOROGYE		KIROVOGRAD		AVERAGE	
	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std
STANDARD	3445	100	817	100	1823	100	3075	100	2290	100
Neoma CL	3376	98	670	82	1422	78	3290	107	2190	96
Adagio CL	3796	110	1006	123	1977	108	2970	97	2437	106
ALPIN	3397	98	930	114	1792	98	3670	119	2447	107
VELEKA	3108	90	980	120	1802	99	2520	82	2103	92
VOKIL	3498	101	1007	123	2226	122	3210	104	2485	109
MIHAELA	3671	106	630	77	2029	111	3360	109	2423	106
VELKO	3985	116	500	61	2482	136	3600	117	2642	115
GABI	3434	99	840	103	2039	112	2910	95	2306	101

In 2014, at four locations in the central, southern and eastern part of Ukraine, some of the most recent hybrids of DAI – General Toshevo were tested and compared to the most widely distributed

and highly productive commercial hybrids (Table 7). In the regions of Odessa and Zaporogye, where the climate is dryer, the results were naturally lower, while in Kharkiv and Kirovograd the hybrids revealed their true potential. The maximum yields obtained were 3985 kg/ha from Velko in Kharkiv region and 3670 kg/ha from Alpin in Kirovograd. In general, all tested Bulgarian hybrids, with the exception of Veleka, exceeded the standard at the four locations, and Alpin, Vokil, Mihaela and Velko were at the productivity level of the foreign hybrids currently dominant on the market.

PHYTOPATHOLOGICAL CHARACTERISTICS

The phytopathological characterization was done under laboratory conditions and in the infection field of DAI where all newly developed materials of the Sunflower breeding department are subjected to testing for the economically important diseases and the parasite *Orobanche*. The results from these investigations are given in Table 8.

Table 8. Phytopathological evaluation of sunflower hybrids in artificial infection field at DAI – General Toshevo.

Hybrid	*Phomopsis helianthi*		*Phoma macdonaldi*		*Plasmopara helianthi*		*Orobanche cumana*
	Attacking rate	#	Attacking rate	#	Resistance to race 700, %	Resistance to race 731, %	Resistance to races A-F, %
San Luka	3/3(3)	3	1/3(1)	1	100.0	92.9	100.0
Perfekt	1/3(1)	1	1/3(1)	1	84.5	-	100.0
Diabolo	2/3(2)	2	1/3(1)	1	100.0	90.5	100.0
Brio	1/3(1)	1	0	0	100.0	100.0	100.0
Meldimi	2/3(2)	2	1/3(1)	1	100.0	90.0	100.0
PR64F50	1/3(1)	1	0	0	100.0	100.0	100.0
Valin	2/3(2)	2	1/3(1)	1	100.0	95.0	100.0
Alpin	2/3(2)	2	1/3(1)	1	100.0	100.0	100.0
Veleka	1/3(1)	1	0	0	100.0	100.0	100.0
Vokil	1/3(1)	1	0	0	100.0	90.0	100.0
Mihaela	2/3(2)	2	1/3(1)	1	100.0	100.0	100.0
Gabi	1/3(1)	1	0	0	100.0	100.0	100.0
Velko	1/3(1)	1	0	0	100.0	100.0	100.0
Dea	1/3(1)	1	0	0	100.0	70.0	100.0
Sevar	1/3(1)	1	0	0	100.0	100.0	100.0

Attacking rate - what part of the plant stem was covered with spots of the pathogen (1/3, 2/3, 3/3). In brackets – number of spots. Rank: 0 – immune; 1 – resistant; 2 – moderately resistant; 3 – moderately susceptible; 4 – susceptible.

Hybrids Veleka, Vokil, Gabi, Velko, Dea and Sevar were resistant to the fungal pathogen *Phomopsis helianthi*, similar to other foreign high-yielding hybrids widely distributed in Bulgaria, such as Brio and PR64F50. In comparison to San Luka and the other hybrids presented in this study, they performed as more tolerant to this disease. With regard to the other important leaf pathogen *Phoma macdonaldi*, these six hybrids demonstrated immune reaction. Although with one degree only, the other hybrids were below their resistance to this disease.

With the exception of Perfekt, the other hybrids demonstrated 100 % resistance to downy mildew (*Plasmopara helianthi*) race 700. To the most recent race 731, full resistance was demonstrated by Brio, PR64F50, Alpin, Veleka, Mihaela, Gabi, Velko and Sevar. In the greater number of Bulgarian hybrids, this resistance came from the male parent. The resistance to the parasite *Orobanche cumana* was 100 % due to the contribution of both parental components.

CONCLUSION

The new Bulgarian sunflower hybrids were officially registered in Romania and were enlisted in the European catalog of the field and vegetable crop varieties. They are distinct, uniform and stable. They demonstrated high and stable yields under variable environments in Bulgaria and abroad, which makes them hybrids with high adaptability potential. They also possess high field resistance to the economically important diseases and the parasite *Orobanche*.

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HYBRIDIZATION BETWEEN CULTIVATED SUNFLOWER AND WILD ANNUAL SPECIES HELIANTHUS NEGLECTUS HEISER

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ABSTRACT

Interspecific hybridization was carried out between sterile analogues of cultivated sunflower lines with normal cytoplasm and wild annual *Helianthus neglectus* accession E-017 from collection of DAI-General Toshevo. The obtained F1 progenies were characterized from morphological, biochemical and phytopathological point of view. They were distinguished with diversity of seed oil content. The most variable phenological phases of hybrid plants from all crosses were duration of flowering period and germination. The hybrid plants from crosses 325 A x E-017, 818 A x E-017 and 3482 A x E-017 were characterized with higher seed oil content than the other studied crosses. Hybrid forms distinguished with resistance to stem canker, downy mildew and the parasite broomrape were obtained. The hybrid plants, carriers of Rf genes, could be used in sunflower breeding programs for developing restorer lines.

Key words: Hybridization, *Helianthus neglectus,* Resistance

INTRODUCTION

Helianthus species were not only the material, the sunflower varieties originated from, but also they continue to be the source of useful characters in sunflower improving work. (Thompson et al., 1981; Atlagic, 2004). Wild species are adapted to a wide range of habitats and possess a considerable amount of genetic diversity that might be a rich source of alleles for continued improvement of the cultivated sunflower (Seiler and Rieseberg, 1997; Burke et al., 2002; Škorić, 2009). The narrow genetic base of domesticated sunflower is a main concern of breeders worldwide. Wild *Helianthus* species offer a significant amount of genetic diversity, including important traits, such as disease resistance, fertility restoration, cytoplasmic male sterility, various seed-oil characteristics, protein content, fatty acid composition, tolerance to abiotic stress factors (Seiler and Rieseberg, 1997). They have been included in the breeding process as sources of valuable characters for the cultivated sunflower, but their use was attended with some difficulties, such as poor crossability and frequent F_1 sterility in interspecific hybrids, abortion of the hybrid embryo and long period of dormancy. This fact limited the usefulness of many wild Helianthus species. The wild annual *Helianthus neglectus* was the subject of research investigations, carried out by Atlagic (2004), Christov (2008), Škorić (2009), etc. They found that this wild sunflower species provided valuable genetic diversity for the improvement of cultivated sunflower. According to Christov (1993) in the annual species, differentiation of a genotype is possible, because of self-pollination of a single plant in natural or artificial conditions. In that way, a different number of stabilized genotypes, which, in total, expressed the diversity of the given species, represented each species. It was known, that within each *Helianthus* species, there is a great diversity in form and the value of individual traits.

The aim of this investigation was to obtain hybrid plants, carriers of Rf genes, resistant to some diseases (*Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii*) and to the parasite broomrape, with varied seed oil and protein content, suitable for including in sunflower breeding programs for developing diverse restorer lines.

MATERIAL AND METHODS

The investigation was carried out in Dobrudzha Agricultural Institute, General Toshevo. The accession E-017 of wild annual species *H. neglectus* was included in this study. The cultivated sunflower was represented by five CMS lines– 325 A, 818 A, 3482 A, 828 A and 846 A. The classical methods of interspecific hybridization were applied for obtaining of hybrid seeds. Interspecific crosses *cultivated sunflower x wild species* were performed and the obtained hybrid plants were grown in field conditions. As paternal component in the realized crosses was the accession of wild *Helianthus neglectus*. The sterile analogues of fertile sunflower lines with normal cytoplasm were used as maternal parents.

Phytopathological evaluations of F1 hybrid progenies were carried out in laboratory conditions and in artificial infection plot. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berlese et de Toni) was carried out on the method of Vear and Tourvieille (1987). Evaluation for resistance to grey spots on sunflower (*Phomopsis /Diaporthe helianthi* Munt.-Cvet. et all.) was carried out on the method of Encheva and Kiryakov (2002) in field conditions on artificial infection plot. Evaluation for resistance to black spots on sunflower (*Phoma macdonaldii* Boerema / *Phoma oleracea var. helianthi-tuberosi* Sacc) was carried out on the method of Fayralla i Maric (1981) in field conditions on artificial infection plot. The seed oil and protein content was determined on the method of Rushkovskii (1957). The weight of 1000 seeds was measured on three samples, where each sample consists of 25 or 50 seeds. Ten plants from the accession, grown in field conditions, were used for this investigation in aim to collect a sufficient pollen quantity. The follow phenological characters, conformed to UPOV characteristics, were determined: germination (days), beginning of button formation (days from germination), beginning of flowering (days from germination), period of flowering (days), beginning of maturity of central inflorescence (days from germination), vegetation period (days). Germination was reviewed at cotyledons emergence of 75% of the sown seeds. Beginning of button formation was reviewed at inflorescence formation of the central stem, and beginning of flowering – beginning of flowering of central head for 25% of the plants. For the end of vegetation period was accepted the withering of stems and leaves of all plants. The seed set (%) was calculated as a correlation between a number of inseminated disk florets to the total number of disk florets in one inflorescence. The analysis of experimental data was done by the statistical package BIOSTAT 6.0. For analyzing the obtained results, consistent to the aims of investigation, the follow quantities were analyzed: arithmetic mean ($\bar{x}$) and the coefficient of variation (VC), which showed the relative uniformity or variability of the studied characters.

RESULTS AND DISCUSSION

Plants from one population of annual species *H. neglectus* was crossed with sterile analogues of cultivated sunflower lines. All obtained F1 interspecific hybrid plants were fully branched with or without central head. Cultivated lines were not branched. The branching was typical character for wild species. Anthocyanin coloration was observed on stems, leaves and rarely on the petioles of some hybrid plants. The presence of anthocyanin pigmentation and branching on F1 plants were the suitable markers for early establishment of the hybrid type of obtained plants. The obtained hybrid plants were 120 cm to 160 cm tall. Some differences in plant height, color of disk florets and leaves, length of branches were observed among plants from the same cross and between different crosses. This was due to the fact, that the paternal parent was a population. Leaves were mostly alternate, ovate and slightly serrate on the margins, truncate at the base. The crossability between *H. neglectus,* accession E-017, and cultivated sunflower was 53,3% (Table 1). This was because both species were close from the taxonomic point of view (Seiler G.J., 1992). The lower crossability was determined for the crosses 828 A x E-017 and 846 A x E-017. Seeds were obtained from all pollinated inflorescences. Similar results were obtained by Hristov M. (1990) and Nikolova L.

(1998). The difficulties, connected to the low crossability of this species, resulted by obtaining of seeds with immature endosperm and sterility of the hybrid plants.

The results from hybridization showed that the seed set was low and varied from 2,9 % for the hybrid 828 A x E-017 to 11,7 % for the hybrid 325 A x E-017. Some differences in viability of hybrid seeds were established. The percentage of viable F1 plants varied from 20 % for the hybrid 846 A x E-017 to 58,3 % for hybrid 325 A x E-017, which was distinguished also with the biggest number of seeds per head. The crosses 325 A x E-017, 818 A x E-017 and 3482 A x E-017 were characterized with better crossability and the biggest number of obtained hybrid seeds originated from them.

Table 1. Crossability between wild species *H. neglectus* (E-017) and cultivated sunflower lines (*H. annuus*).

Hybrid combination	Pollinated inflorescences			Obtained seeds			Obtained hybrid plants	
	Total number	With seeds		Average per head	Total number	Seed set %	Total number	Average per seeds, %
		number	%					
325 A x E-017	3	2		12	24	11,7	14	58,3
818 A x E-017	3	2		11	22	10,8	12	54,5
828 A x E-017	3	1		3	3	2,9	1	33,3
846 A x E-017	3	1		5	5	4,9	1	20
3482 A x E-017	3	2		12	24	11,6	10	41,7
***H.annuus* x E-017**	**15**	**8**	**53,3**	**8,6**	**78**	**8,4**	**54**	**78,46**

The phenological observations were done and the main phenological phases were studied. The mean values of the studied phenological phases and their variation for the parents and the obtained hybrid F1 progenies were presented on table 2. Hybrid plants were distinguished with higher variation than their parents regarding all studied phenological phases were. Differences were observed both between plants from the different crosses and among plants from the same cross. This was because the accession E-017 was maintained as population. Vegetation period of hybrids was shorter than that of wild species *Helianthus neglectus* and varied from 85 to 110 days for the earlier to 120-130 days for the other progenies. The duration of flowering period of hybrid plants was determined as the most variable phenological phase for all studied progenies, followed by variation in germination and vegetation.

Variation was also observed in some biochemical characters connected to seed oil and protein content of parental forms and the obtained progenies. The average values of the studied biochemical characters were presented on figure 1. Seeds of wild *H. neglectus* were characterized with low 1000 seeds weight and seed oil content, and high content of hull. The seeds of sunflower hybrid plants were with higher content of oil in seeds and kernels. The oil content of hybrids in kernel varied from 64,5% to 71% and in seeds – from 35,8% to 49%. The highest seed oil content (49%) was determined for the crosses 325 A x E-017, 818 A x E-142 and 3482 A x E-017.

Table 2. Mean values and variation coefficients of the studied phenological phases of the parents and their F_1 hybrids.

Phenological phases	P_1		P_2		F_1	
	cultivated sunflower (*H. annuus* L.)		*Helianthus neglectus* Heiser		*H. annuus x H. neglectus*	
	$\bar{x}$	VC	$\bar{x}$	VC	$\bar{x}$	VC
Germination, days	9,6	3,4	13,7	7,32	12,5	14,9
Beginning of button formation, days from germination	41,9	3,3	49,5	3,9	46,5	7,7
Beginning of flowering, days from germination	53,9	5,2	72,2	2,9	58,6	10,3
Flowering, days	6,3	13,6	48,2	4,5	12,8	18,7
Beginning of maturity of central inflorescence, days from germination	94,5	6,16	104	5,9	94,5	11,9
Vegetation period, days	112,5	10,3	155,5	4,8	120,5	12,9

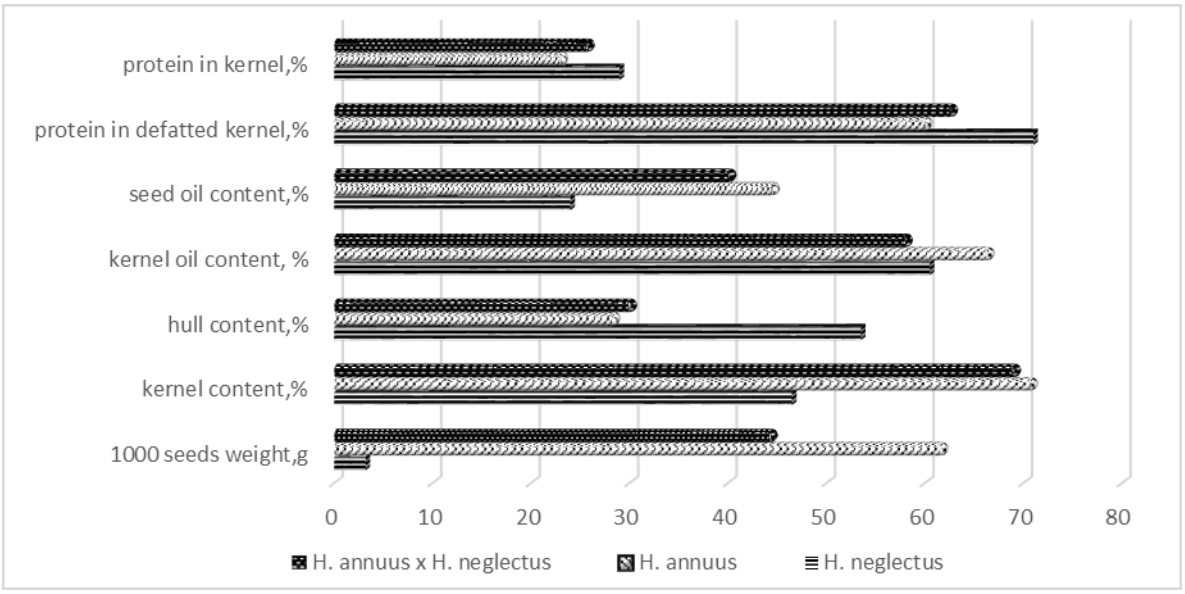

Fig. 1. Average values of characters, connected to seed oil and protein content of hybrid plants and their parents.

The hybrid seeds, originated from wild sunflower species have lower oil content compare to the cultivated sunflower, but it could be changed quickly to acceptable level by backcrossing with cultivated sunflower lines (Seiler and Rieseberg, 1997). The kernel content (%) was of importance for the total oil yield per hectare. Increasing of kernel content gave the opportunity for accumulation of bigger oil quantity in it and lead to increasing the seeds oil content, respectively, to increasing the seed yield. Seed oil content was a character, which reflected simultaneously on the kernel content and the oil content in it. Therefore, the sunflower seed oil content of hybrid plants was set up by the relative portion of kernel to the whole seeds, and the content of oil in it. The seed oil content of the new registered hybrids and cultivars was increased due to decreasing of hull content predominantly than increasing the other characters (Nikolova V., 1987). According to this dependence, hybrid plants with the lowest hull content and highest oil content were selected.

The reaction of hybrid materials to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with aim to establish the sources for resistance to these pathogens (Tables 3-4).
The hybrid combinations 3482 A x E-017 and 818 A x E-017 were resistant (100%) to downy mildew and the parasite broomrape. They were characterized with immune type of reaction to the pathogens caused grey and black spots on sunflower. Their vegetation period was 115-118 days. The other crosses also demonstrated a certain resistance to *Pl. helianthi* and *Orobanche cumana*. They could be successfully included in the sunflower breeding programs for developing new resistant lines.

Table 3. Phytopathological evaluation of F_1 hybrid progenies for resistance to *Pl. helianthi* and *Orobanche cumana.*

Resistance, %	Hybrid combination	
Resistance 100 % to *Pl. helianthi* Novot. and 76-99% to *Orobanche cumana* Wallr.	3482 A x E-017	818 A x E-017
Resistance 76-99% to *Pl. helianthi Novot*. and *Orobanche cumana* Wallr.	828 A x E-017 846 A x E-017	325 A x E-017

Resistant type of reaction to the pathogens caused grey and black spot on sunflower showed three hybrid forms -828 A x E-017, 846 A x E-017 and 325 A x E-017.

Table 4. Phytopathological evaluation of F_1 hybrid progenies for resistance to *Phomopsis helianthi Munt.-Cvet. et all.* and *Phoma macdonaldii Boerema.*

Type of reaction	Hybrid combination	
Immune to *Phomopsis helianthi* and *Phoma macdonaldii*	3482 A x E-017	818 A x E-017
Resistant to *Phomopsis helianthi* and *Phoma macdonaldii*	828 A x E-017 846 A x E-017	325 A x E-017

CONCLUSION

Wild *Helianthus* species have been included in sunflower breeding programs mainly as donors for resistance to diseases and to the parasite broomrape. Transfer of genes, controlling the resistance, into cultivated sunflower lines gave the opportunity for diversification of cultivated sunflower. Resistance 100% to downy mildew and immune type of reaction to the pathogens *Phomopsis helianthi* and *Phoma macdonaldii* was established for some crosses, obtained with participation of E-017 accession.
Plants from the hybrid combinations 325 A x E-017, 818 A x E-017, 828 A x E-017, 846 A x E-017 and 3428 A x E-017 could be used as donors for resistance. They also carried Rf genes and distinguished with varied seed oil content.
The obtained hybrid materials were useful initial materials for application in sunflower breeding programs for producing restorer lines with high seed oil content and resistance to the main diseases with economic importance.

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COMPARATIVE INVESTIGATION OF IMMATURE EMBRYOS GROWING OF INTERSPECIFIC SUNFLOWER HYBRIDS

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ABSTRACT

Comparative investigation of immature embryos growing of interspecific sunflower hybrids was carried out in vitro conditions. Four hybrid combinations, in which wild annual and perennial sunflower species participated, were used. These were the hybrid combinations 807A x E-131 (*Helianthus argophyllus*), 3A x E-130 (*H. argophyllus*), 807A x M-129 (*H.divaricatus*) and 3A x M-146 (*H.tuberosus*). In in vivo conditions the embryos necrotized and died because of incompatibility between species. In in vitro conditions, the method of embryo rescue was applied. On the base of this method, different number of hybrid embryos was isolated. The tissue culture of Azpiroz et al. (1987) was applied, when some modifications of the tissue were used. The results of the investigation showed that using embryo rescue the hybrid plants could be grown. The seed set of hybrids, originated from perennials was too low than that of hybrids, originated from annual species. The obtained hybrid plants were cultivated in greenhouse conditions and sufficient quantity of seeds was obtained. Next generations were grown in field conditions. They were used as initial material for developing sunflower lines with valuable agricultural characters, resistant to biotic and abiotic stress factors.

INTRODUCTION

For the breeding of most crops was necessary to accelerate or shorten the process repeatedly. That was due to the need of fast developing of hybrids and uniformed lines, fast transfer of new genetic material, valuable for sunflower breeding programs. Shortened vegetation period, improving the quality of seed production, overcoming of new pathogens races and overcoming of incompatibility of cultivated and wild forms were the subjects of the main research work. The method of embryo culture, used in this connection, allowed faster creation of lines. This method led to reducing the duration of lines obtaining. Six generations could be produced in one year as contrasted with classical methods (Alissa et al., 1986; Aspiroz et al., 1987). Applying the embryo rescue method was a precondition for developing the effective method for decreasing the sterility and obtaining of hybrid forms in *Helianthus* genus (Chandler & Bear, 1983; Bohorova *et al*., 1985; Krauter & Fried, 1991 and etc.). Paul & Barthou (1994) suggested method for cultivation of embryos from commercial hybrids in unsterile conditions. Special equipment, preliminary treatment of embryos and different chemical substances were necessary for their cultivation in "in vitro" conditions. In this study we presented the data of successful obtained crosses between cultivated sunflower and annual and perennial species of genus *Helianthus* using embryo rescue method and classical breeding methods.

MATERIAL AND METHODS

Plant material

The investigation was carried out in Dobrudzha agricultural institute (DAI), General Toshevo. The embryos were isolated from preliminary crosses, obtained using classical methods in sunflower breeding – isolation, pollen collecting, pollination. Cultivated sunflower lines 807A and 3A, developed in DAI were used as maternal parent. Some accessions of wild sunflower species were used as paternal component. The annual species *Helianthus argophyllus*, (accession *E-131*)

and perennial species *H. divaricatus* (accession *M-129*) and *H.tuberosus* (accession *M-146*) were used as pollinators.

Explants and sterilization

The immature embryos are isolated from the inflorescence from the 3rd to the 14th day after crossing. The day of performing embryo isolation is different for each cross. Depending on the used genotype, the embryo size is from 2 to 7 mm. The formed young embryos are removed from the inflorescence and placed in a lint bag. Sterilization is done with commercial bleaching solution (without diluting it) for 20 min, then the bags are transferred to a laminar box and washed with sterile distilled water. Using scalpel and pincers, the husk is removed from the not well formed seed, and the embryo is separated from the endosperm and immediately placed on a nutrition medium.

Nutrition media, sterilization and cultivation

For the separated 2-3 mm large embryos the nutrition media suggested by Chander and Beard (1983) are used. The initial medium on which the embryos are cultivated is B5 with added amino acids as follows: L-alanine – 100 mg/l, L-glutamine – 800 mg/l, L-serine – 160 mg/l, L-tryptophane – 5- mg/l, L-cysteine – 10 mg/l and NOK – 0.05 mg/l. Sucrose is 120 g/l, and the agar is 7g/l; pH of the medium is 5.7. The nutrition medium is distributed in 10 cm Petri dishes after autoclaving, and 10 embryos are plated on each dish. Five to seven days after chlorophyllization of the cotyledons, the embryos are transferred to solid agar nutrition medium. The nutrition medium contains only B5 – salts and 10 g/l sucrose.

For cultivation of embryos bigger than 3-4 mm, the methodology of Azpiroz et al (1987) is applied, which is simpler and with a shorter cycle. The medium for cultivation of the isolated embryos is MS, the macrosalts being reduced in half, the vitamins are of medium B5, the sucrose is 20 g/l, with 100 mg/l of inositol, pH being 5.7.This medium is distributed in 5 Petri dishes each with diameter 10 cm. Ten embryos are placed in each dish.

The cultures are placed in a phytosanitary room at temperature 24±2^{o} C and illumination 2500 – 3000 lux, with photoperiod 18/8 h. The plants are grown in the cultivation constructions till beginning of budding stage at temperature 18-20^{o} C, and the next stages occur at temperature 22-26^{o} C. When roots reach length 2-3-4 cm, the plants are transferred to a soil under non-sterile conditions, covering them with glass cover for about 4-6 days to ensure successful rooting and acclimatization.

RESULTS AND DISCUSSION

The process of creating of sunflower lines, parental lines of a hybrid, was long, difficult, and needed at least 10-12 years. For acceleration of that process, some alternative ways in the field of plant biotechnology were searched. Different theoretical opportunities and in vitro technics existed for intensification of the breeding process, but not all of them could be applied, because of their low effectiveness in sunflower. One of the methods, which could be successfully used, was embryo cultivation. This method was used in sunflower for quick obtaining of lines, restorers of fertility, as well as sterile analogues (Plotnicov, 1983). In perfect conditions this method allowed vastly shortening the breeding process and obtaining 6 generation in one year, which was impossible to be done by classical methods (Alissa *et al*., 1986; Azpiroz *et al.,* 1987). This method was easy and cultivated sunflower embryos could be grown on simple synthetic tissue with small quantity of hormone supplements. The cultivation of interspecific embryo rescue was disparate. Our investigations showed that they had small size and aborted prematurely. In this case the tissues were more complicated and at least one preliminary investigation had to be done and determined when

exactly the embryos died. It depended on type of interspecific crosses – crosses with annuals and perennials (Drumeva, M. & Nenova, N., 2012; N. Nenova *et al.*,2014; Valkova D. *et al.*,2014) described in the previous chapter. описани в предишния раздел.

Based on our previous investigations, in this study we included interspecific hybrids obtained with participation of annuals and perennials. Hybrid combinations, seed set and number of obtained plants, grown in the soil were presented on table 1.

Table 1. Number of isolated embryos and obtained plants grown in the soil from F_1 interspecific sunflower hybrids.

№	Hybrid combination	Seed set, %	Number of embryos	Number of plants
1	*807 A x H.argophyllus E- 131*	53	62	57
2	*3A x H.argophyllus E -131*	71	117	66
3	*3A x H.tuberosus M-146*	0.2	5	1
4	*807 A x H.divaricatus M-129*	0.2	5	2

The results showed, that the highest seed set was determined for the hybrid combination *807 A x E-* 131 (*H. argophyllus*). The species *H.argophyllus* (acc. E- 131) was included in other cross too but the difference in seed set was 18%, which showed that the maternal component also had an effect for the successful crossability. In this combination there were 55 more isolated embryos, which was precondition for surviving of bigger number of plants after sowing in the soil. The embryos from both crosses with participation of *H.argophyllus* (E- 131) were with different size. The embryo size of combination *807 A x E-131* (*H. argophyllus*) was 4-7 mm, and for the other hybrid combination *807 A x E-131* (*H. argophyllus*) - 3-4 mm. It was suggested that larger embryos possessed more endosperm than the others. They contained more nutrients and their survival mechanisms were better.

The testing results of hybrids, obtained with participation of perennials were quite different. The seed set was too low 0.2%, and five embryos were isolated from both hybrids - *3A x M-146 (H. tuberosus)* and *807A x M-129* (*H. divaricatus*). Obviously, the incompatibility of wild annuals and cultivated sunflower was lower than that of perennial species.

These three species, *H. argophyllus*, *H. divaricatus* and *H. tuberosus*, included in the investigation possessed valuable economic characters and could be used as sources of new genetic material to be transferred to the genome of cultivated sunflower. The barriers of incompatibility between most of wild species and cultivated sunflower were hardly overcome using conventional methods. This enforced using of rescue embryo method. All F_1 plants were planted and grown in nursery conditions and after that in the field. Each plant was isolated separately. Selection on morphological characters, evaluation for resistance to downy mildew, phoma, phomopsis and broomrape were carried out. The obtained plants from the cross *cultivated sunflower x perennial species* died during their vegetation. The presented results concerned the plants from hybrid combination *cultivated sunflower x annual species*.

The results of evaluation for resistance to diseases and parasite broomrape were presented on table 2.

Table 2. Resistance of interspecific hybrids to diseases and parasite broomrape.

Resistance, %	Hybrid combination
Resistance 100% to *Pl.helianthi* Novot. and 76-99% to *Orobanche cumana* Wallr.	807 A X E-131
Resistance 76-99% to *Pl.helianthi* Novot. and *Orobanche cumana* Wallr.	3A X E-131
Type of reaction	Hybrid combination
Immune to *Phomopsis helianthi* and *Phoma macdonaldi*	807 A X E-131
Resistant to Phomopsis helianthi and *Phoma macdonaldi*	3 A X E-131

One of the main purposes of interspecific hybridization was directed to transfer of genetic material from wild *Helianthus* species into the genome of cultivated sunflower. The obtained materials with participation of wild annual species *H. argophyllus* (accession E-131) possessed resistance to *Pl. helianthi* from 76% до 100%. The resistance to broomrape varied from 76% до 99%. The type of reaction to the leaves pathogens (*Phomopsis helianthi* and *Phoma macdonaldii*) varied from immune to resistant. The present resistance was transferred from wild accessions because the cultivated sunflower was susceptible regarding the studied pathogens.

Seed oil content was an important character in developing new sunflower forms for including in breeding programs. Wild sunflower species were characterized with low seed oil content. Hybrid combinations were distinguished with higher seeds oil content. The highest seed oil content 41.7% was determined for the cross 3 A x E-131, followed by 39.5% for hybrid combination 807 A x E-131. The pointed values oil content in hybrid seeds were comparatively low, but after backcrossing and self-pollination it could be increased.

CONCLUSIONS

On the base of the pointed results in this investigation, some conclusions could be made:

- Wild annual species were characterized with lower incompatibility with cultivated sunflower than wild perennial species. The improved by us embryo rescue method was suitable for overcoming the incompatibility of wild annual *Helianthus* species and cultivated sunflower.

- The species *H.argophyllus*, accession E-131 was a source of Rf genes and genes for resistance to economically important diseases and parasite broomrape. Transfer of these genes was determined by evaluation of hybrid material.

- Seed oil content was low, but in next generations after backcrossing and self-pollination it could be increased.

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DEVELOPMENT OF SUNFLOWER HYBRIDS RESISTANT TO HERBISIDES

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ABSTRACT

Broomrape (*Helianthus annuus*) and weed control are the main problems in sunflower seed production in Bulgaria. Surmount these problems conducted to development and spreading of hybrids, tolerant to herbicides. CL Plus source was used for creation of hybrids, resistant to herbicide from the imidazolinone group. During the period of study, 112 IMI resistant hybrids were tested. Seed yield, oil yield and seed oil content were tested. Seed yield above the mean standard of 51 hybrids possessed gene for resistance to herbicides Pulsar 40 + Stomp 330 EC at variety testing trails-1 and 2 was reported. Best results at variety testing trails-3 for seed yield were reported for combinations 1111 A x 185 (128.1%) and 1111 A x 437 R (124.8%). Exceeding the mean standard for the character oil yield was reported for 50 hybrid combinations at variety testing trails-1 and 2. Hybrid combination 1111A x 51/2/2 was relieved with the highest oil content 51.4% in air-dry seeds. Best results at variety testing trails-3 for oil yield were reported for combinations 1111 A x 185 R (132.7%) and 1111 A x 437 R (128.5%).

Key words: *Helianthus annuus*, Sunflower, Hybrids, IMI herbicides resistance

INTRODUCTION

Cultivated sunflower is a main crop for oil production in Bulgaria. Significant problems for its cultivation in Bulgaria were *Xanthium strumarium* L., creeping thistle (*Cirsium arvense* L.), wild hemp (*Cannabis sativa* L.), black nightshade (*Solanum nigrum* L.) and others. There were not so many available herbicides for sunflower, as for example in cereals. Some of registered herbicides were not effective enough in the aforementioned weeds (weed mention above). An additional problem in climate change becomes more significant is the lack of effect of soil herbicides under conditions of prolonged drought.

In recent years, they created conditions for widespread parasite broomrape in Bulgaria. There was a rapid change in the population of the parasite, namely the appearance of new more virulent races (Shindrova, 1994; Shindrova, P., 2006). A similar process was observed in other countries such as Turkey, Spain, Romania and others. (Pacurenu-Joita *et al*. 1998, Kaya *et al*, 2014, Fernandez-Martinez *et al*, 2000). This situation requires the search for means to control and reduce losses that broomrape causes. At this stage, control is carried out by sowing of broomrape resistant hybrids. IMI herbicides used with imidazolinone (IMI) tolerant hybrids on the other hand control of both broomrape and key weeds in sunflower.

Al-Khalib *et al.*, 1998 reported for transferring of resistance to imidazolinone into cultivated sunflower and developing "IMISUN" line. Meanwhile, Alonso (1998) first reported for 100% chemical control of broomrape in sunflower resistant to imazethapyr. New source for IMI (Imazethapir) resistance (CLPlus) created by induced mutagenesis (Ethyl methane sulfonate) in wild type *H. annuus* was published by Sala *et al*., 2008. Biochemical studies in various field conditions indicate that CLPlus delivers a higher level of tolerance to IMI compared to the IMISUN (Sala *et al*., 2012).

MATERIAL AND METHODS

In 2008 started a work on transfer of genes for resistance to herbicides from the group of imidazolines in restorer lines of DZI- Toshevo. They were characterized by morphological uniformity, good economic performance and very good combining ability. Some of them are resistant to *Plasmopara helianthi*. To create sustainable materials a source of resistance - CL Plus was used. The best time for testing sunflower plants for resistance is in phase 3-5 pair of true leaves. The dose of the herbicides from the group of imidazolinone was 120 ml/da (Pulsar 40) and 230 ml/da (Stomp 330 EU). After 15 days of treatment, the hybrids were characterized phenotypically in terms of herbicide activity and selectivity according to 9-point system of EWRS- (European Weed Research Society). Three trials were performed: Variety testing trials-1 included 31 hybrid combinations tested for second year; Variety testing trials-2 included 65 hybrid combinations, tested for first year; Variety testing trials-3 - 16 hybrids combinations.

Hybrids were tested in 2015 at the experimental breeding fields of DZI-General Toshevo in a randomized block method in three repetitions, as the area of each repetition is 10 m2 (Barov and Shanin, 1965). Aim of the experiment: testing of experimental imidazolinone (IMI) tolerant hybrids

RESULTS AND DISCUTIONS

Sunflower is the main crop for oil production in Bulgaria. The sunflower production fields have been increased during the recent years and spread on more than 700 000 ha. It needed to work on reducing the impact of poor environmental condition. Creation and implementation in practice of herbicide-tolerant hybrids would enable farmers to cope with the problems that cause weeds and parasite broomrape.

Since 2008 the scientists at the department on sunflower breeding in G. Toshevo were working on the task of creating hybrids tolerant to herbicides from the group of imidazolinone (IMI). There were created 112 hybrid combinations in which allele called *Ahasl1-3* is in the homozygous state. This new traits present better stability of the herbicide tolerance in different environmental condition, permit developing new herbicide formulations providing more flexible and reliable weed control, higher oil content, etc. than previous IMISUN trait (Sala *et al.,* 2008 a, b; Weston *et al*., 2012).

After 15 days of treatment the hybrids were characterized phenotypically (Fig 1) in terms of herbicide activity and selectivity according the 9 point system of EWRS- (European Weed Research Society). The character seed yield was evaluated**.** The standards for comparison of the new hybrids in terms of seed yield are Neoma and LG 5661 CL. Thirty-one hybrid combinations were tested in variety testing trials 1 for second year. Fourteen of the hybrids exceeded the mean standard by seed yield. In table 1 were presented 8 hybrids with the highest values. Two hybrids possessed seed yield over 4,000 kg/ha. These are 1111 A x 67/1 R - 4390 kg/ha and 1111 A x 102/2/3 R - 4270 kg/ha.

Table 1. Seed yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC from the class of imidazolinones, increasing adjacent mean standard. Variety testing trials 1. Tested for 2th year

№	Cross	Seed yield kg/ha	% of mean St
82	1111 A x 14/1/1 R	3670	113.6
83	1111 A x 14/1/2 R	3560	110.2
84	1111 A x 14/1/2 R	3570	110.5
85	1111 A x 14/1/3 R	3490	108.0
92	1111 A x 16/2/2 R	3550	109.9
93	1111 A x 16/2/3 R	3400	105.3
St	***NEOMA***	***3400***	***105.3***
St	***LG 5661 CL***	***3060***	***94.7***
	Mean standard	***3230***	***100.0***
108	1111 A x 67/1 R	**4390**	115.1
115	1111 A x 102/2/3 R	**4270**	111.9
St	***NEOMA***	***3970***	***104.1***
St	***LG 5661 CL***	***3660***	***95.9***
	Mean standard	***3815***	***100.0***

In 2015, 65 IMI tolerant hybrid combinations in variety testing trials-2 were tested for the first year. The best results were presented in table 2. The commercial hybrids Adagio, Alego, Neoma and LG 5661 CL were used as standards. Thirty-six hybrids exceeded the mean standard on seed yield. For eight of them the exceeding was more than 10 %. The best results were obtained for the crosses 1111 A x 53/3 R (126.8%) and 1111 A x 77/1/1 R (124.5%). Two hybrids had seed yield more than 4000 kg/ha. These were 1111 A x 77/1/1 – 4110 kg/ha and 1111 A x 40/2/2 – 4070 kg/ha.

The results of Variety testing trials-3 were presented on table 3. All hybrids, included in table 3, exceeded the mean standard of the used Neoma and LG 5661 CL. Ten combinations, from the tested 16 (62.5%), exceeded the mean standard from 0.1 to 28.1 %. The highest seeds yield was obtained from hybrids 1111 A x 185 R (128.1%), 1111 A x 437 R (124.8 %) and 1111 A x 410 R (119.0 %).

Table 2. Seed yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC from the class of imidazolinones, increasing adjacent mean standard. Variety testing trials 2. Harvest year 2015

№	Cross	Seed yield kg/ha	% of mean St
124	1111 A x 25/1 R	3990	111.8
128	1111 A x 40/2/2 R	**4070**	114.0
St	***ADAGIO***	***2790***	***78.2***
St	***ALEGO***	***3570***	***100.0***
St	***NEOMA***	***3960***	***110.9***
St	***LG 5661 CL***	***3960***	***110.9***
	Mean standard	***3570***	***100.0***
147	1111 A x 51/2/2 R	3600	115.8
150	1111 A x 53/3 R	3940	**126.8**
155	1111 A x 57/1 R	3700	119.0
156	1111 A x 57/2 R	3630	116.8
St	***ADAGIO***	***3170***	***102.0***
St	***ALEGO***	***3260***	***104.9***
St	***NEOMA***	***3330***	***107.1***
St	***LG 5661 CL***	***2670***	***85.9***
	Mean standard	***3108***	***100.0***
162	1111 A x 77/1/1 R	**4110**	**124.5**
181	1111 A x 20/2 R	3670	111.2
St	***ADAGIO***	***3250***	***98.5***
St	***ALEGO***	***3340***	***101.2***
St	***NEOMA***	***3730***	***113.0***
St	***LG 5661 CL***	***2880***	***87.3***
	Mean standard	***3300***	***100.0***

Table 3. Seed yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC from the class of imidazolinones, increasing adjacent mean standard. Variety testing trials 3. Harvest year 2015

№	Cross	Seed yield	
		kg/ha	% of Mean St
3	1111 A x 100/2/3 R	2995	103.7
5	1111 A x 100/3/3 R	3272	113.3
6	1111 A x 175 R	3197	110.7
7	1111 A x 414 R	2944	102.0
8	**1111 A x 185 R**	**3698**	**128.1**
9	1111 A x C 61	2891	100.1
10	1111 A x 514 R	2960	102.5
13	1111 A x 462 R	2917	101.0
14	1111 A x 410 R	2997	103.8
15	**1111 A x 437 R**	**3603**	**124.8**
St	***Neoma***	***3051***	***105.7***
St	***LG 5661 CL***	***2723***	***94.3***
	Mean standard	***2887***	***100.0***

Biochemical study of sunflower hybrids, tolerant to herbicides Pulsar 40+ Stomp 330 EC

The testing results of 31 imidazolinone (IMI) tolerant hybrid combinations in Variety testing trials-1 in respect of the trait oil yield per hectare are presented in Table 4. The oil content in air-dry seeds of new hybrids ranged from 44.1% to 46.8%. At table 4 were presented hybrid combinations with the greatest increasing in compared to the adjacent mean standard. These are 1111 A x 67/1 R - 122.8%, 1111 A x 16/2/2 R - 120.4% and 1111 A x 102/2 / 3 R - 119.4%.

Our results confirmed the conclusion of Weston *et al*., (2012) that absence of genes from a wild source around *Ahasl1-3* determines that the oil content in the hybrids carrying the CLPlus trait show the same oil yield per hectare as those of their conventional counterparts (Weston *et al*., 2012).

Table 5 presented the hybrid combinations at Variety testing trials-2 with the highest increase compared to the adjacent mean standard in terms of oil yield. In 30 hybrid combinations (46%) oil content in the seed is higher than the mean standard. The oil content of the new hybrids ranged from 42.0% to 51.4%. Hybrid combination 1111 A x 51/2/2 R stand out with 7.8% excess oil content in the seeds compared to the mean standard. Very good results in terms of oil yield were reported in combinations 1111 A x 53/3 R- 141.7%, 1111 A x 51/2/2 R - 139.8% and 1111 A x 77/1/1 - 136.7%.

Table 4. Oil yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC, increasing adjacent mean standard. Variety testing trials 1**.** Tested for 2th year

№	Cross	Oil content %	Oil yield	
			kg/ha	% of mean St
82	1111 A x 14/1/1 R	44.1	1618	117.2
83	1111 A x 14/1/2 R	46.1	1641	118.9
85	1111 A x 14/1/3 R	45.5	1588	115.1
92	1111 A x 16/2/2 R	46.8	1661	**120.4**
93	1111 A x 16/2/3 R	46.8	1591	115.3
St	***NEOMA***	***45.8***	***1557***	***112.8***
St	***LG 5661 CL***	***39.3***	***1203***	***87.2***
	Mean standard	**42.6**	**1380**	**100.0**
108	1111 A x 67/1 R	46.8	2055	**122.8**
112	1111 A x 99/1 R	46.6	1831	109.4
114	1111 A x 102/2/1 R	46.3	1810	108.2
115	1111 A x 102/2/3 R	46.8	1998	**119.4**
St	***NEOMA***	***47.3***	***1878***	***112.3***
St	***LG 5661 CL***	***40.1***	***1468***	***87.7***
	Mean standard	***43.7***	***1673***	***100.0***

Table 5. Oil yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC, increasing adjacent mean standard. Variety testing trials 1. Harvest year 2015

№	Cross	Oil content in seeds %	Oil yield	
			kg/ha	% of mean St
147	1111 A x 51/2/2 R	**51.4**	1850	**139.8**
150	1111 A x 53/3 R	47.6	1875	**141.7**
155	1111 A x 57/1 R	47.8	1769	133.7
156	1111 A x 57/2 R	**49.3**	1790	135.3
St	***NEOMA***	***48.1***	***1602***	***121.1***

St	***LG 5661 CL***	***39.1***	***1044***	***79.0***
	Mean standard	***43.6***	***1323***	***100.0***
162	1111 A x 77/1/1 R	47.4	1948	**136.7**
St	***NEOMA***	***45.6***	***1693***	***118.8***
St	***LG 5661 CL***	***38.3***	***1157***	***81.2***
	Mean standard	**41.9**	**1425**	**100.0**

Table 6. Oil yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC, increasing adjacent mean standard. Variety testing trials 3. Harvest year 2015

№	Cross	Oil content in seeds,%	Oil yield	
			kg/ha	% of mean st
3	1111 A x 100/2/3 R	43.1	1291	108.7
5	**1111 A x 100/3/3 R**	**42.7**	**1398**	**117.7**
6	1111 A x 175 R	40.8	1209	101.8
7	1111 A x 414 R	44.0	1296	109.1
8	**1111 A x 185 R**	43.1	**1527**	**128.5**
9	1111 A x C 61	42.5	1228	103.4
10	1111 A x 514 R	43.0	1275	107.3
13	1111 A x 462 R	43.7	1275	107.3
14	**1111 A x 410 R**	**44.8**	**1342**	**113.0**
15	**1111 A x 437 R**	43.7	**1576**	**132.7**
St	***Neoma***	***44.6***	***1361***	***114.6***
St	***LG 5661 CL***	***37.2***	***1014***	***85.4***
	Mean standard	***40.9***	***1188***	***100.0***

The results for the oil content in the seed and oil yield per hectare for imidazolinone (IMI) tolerant hybrids, included in the Variety testing trials-3 are presented in Table 6. Ten hybrid combinations (62.5%) demonstrated excess over adjacent mean standard. Values above 105% were reported for 9 hybrids. The highest values were reported for crosses 1111 A x 437 R (132.7%), 1111 A x 185 R (128.5%), 1111 A x 100/3/3 R (117.7%) and 1111 A x 410 R (113.0%).

CONCLUSIONS

During the period of study, 112 imidazolinone (IMI) tolerant hybrid combinations were tested. All hybrid combination possessed allele *Ahasl1-3* in the homozygous state.

Seed yield, seed oil yield and seed oil content were determined. Seed yield above the mean standard at Variety testing trials 1 and 2 were observed in 51 hybrids.

Best results for seed yield at Variety testing trials 3 are reported for combinations 1111 A x 185 R (128.1%) and 1111 A x 437 R (124.8%). Hybrids exceeded the mean standard by oil yield per hectare were demonstrated in 50 hybrid combinations at Variety testing trials 1 and 2. Hybrid combination 1111 A x 51/2/2 R was stand out with the highest oil content- 51.4% in

air dry seeds. Best results for oil yield at Variety testing trials 3 are demonstrated in combinations 1111 A x 185 R (132.7%) and 1111 A x 437 R (128.5%).

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RESPONSE TO WATER STRESS INDUCED BY PEG 6000 ON GROWTH OF PLANTLETS IN SOME SUNFLOWER GENOTYPES RESULTED FROM INTERSPECIFIC HYBRIDISATION

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ABSTRACT

In 2008 at NARDI Fundulea a breeding program was initiated to improve sunflower for drought resistance by genes introgression from the *H. argophyllus* species into cultivated sunflower. Test of new genetic material was represented by hybrid combinations resulting from a selection process that took place over a period of 7 years including all stages of the breeding (hybridization: *H. annuus* x *H. argophyllus*, backcross, self pollination and selection). For this study, the testing of genotypes was performed under laboratory conditions to water stress induced by the presence of PEG 6000 in 5 concentrations and 3 replications/variant. In contrast with plantlets from backcross 7, at higher concentrations of PEG (15% and 20%) many plantlets from maternal lines did not survive until the end of the experience. The 10% PEG 6000 concentration permitted the best analysis of the tested material and it will be maintained in our further studies. For most studied lines were pointed out backcross descendents that exceed their maternal lines and combinations that are bellow the initial levels especially at low stress levels. A number of eight backcross 7 descendents were selected for the final stage of improving for drought resistance, in order to obtain new hybrids.

Key Words : Helianthus argophyllus, interspecific hybridization, self pollination, backcross, water stress, PEG 6000, growth

A NEW BULGARIAN SUNFLOWER HYBRID DEA

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ABSTRACT

Sunflower Dea was developed at Dobrudzha Agricultural Institute – General Toshevo (DAI). It is a male sterile two-linear hybrid derived through interlinear hybridization. The mother is line 217 possessing cytoplasmic male sterility, and the father is line 626 R, a branched fertility restorer. Both parental forms have excellent general and specific combining ability. Hybrid Dea is of medium maturity, growth period 116 – 119 days, plant height 160-165 cm and head diameter 20-23 cm. Absolute seed weight is 58-62 g and oil content is 48-49%. Flowering is 12-14 days. The new hybrid successfully underwent 3-year testing at DAI according to the standard testing practice. The maximum seed yield was 5313 kg/ha, while oil content reached 50 %. It is resistant to economically important diseases and to the parasite *Orobanche*. In 2012 hybrid Dea was submitted for official testing within the structures of the Romanian Varietal Commission at 10 locations. In the first three years it exceeded the Romanian standard with averagely 7,3 % by seed yield. The mean seed yield per ha for the three years of testing was 3387 kg. The hybrid was officially registered in Romania and was enlisted in the European Catalog of Field and Vegetable Crop Varieties.

Key words: Hybrid, Seed yield, Oil yield, Growth period

INTRODUCTION

The main task and priority of contemporary sunflower breeding are the development of high-yielding hybrids resistant to the economically important diseases and parasites. This tendency imposes the necessity to introduce new hybrids meeting the demands of the market. The good hybrid, apart from being high-yielding, should also be adaptive to the changeable biotic and abiotic environmental factors thus revealing its maximum potential under specific conditions. The wide use of sunflower justifies the necessity of increasing the improvement work on this crop, determining the various directions and specificity of tasks in its breeding of (Encheva and Georgiev, 2009; Encheva et al., 2014; Encheva V. *et al.*2014; Valkova D. *et al.* 2014). The breeding of a new hybrid is a long process which involves collecting and developing of initial material, selection and choice of parental components, testing of the new forms, their registration and distribution. The successful outcome of each breeding project is highly productive genotypes which not only possess valuable properties from a research point of view, but which are also able to find good realization in production (Chamurliyski and Tsenov, 2013; Chamurliyski et at., 2011).

The aim of this investigation was to present a detailed morphological, biological and economic characterization of a modern Bulgarian hybrid (Dea) which meets the requirements for high productivity, resistance and adaptability. It was registered and enlisted in the European Varietal List of 2015. The hybrid is medium early, high-yielding and possesses very good drought resistance.

MATERIAL AND METHODS

Hybrid Dea was developed by the method of inter linear hybridization. It is a male fertile two-linear hybrid. The mother component is line 217 which possesses cytoplasmic male sterility and which has been developed through hybridization between the Bulgarian candidate variety No 72 and line 246 originating from Russian cultivars. By using the methods of selfing and selection, the line was developed as morphologically and genetically homogenous. It is characterized with very good general and specific combining ability.

Its successful use as the mother component in the most recent Bulgarian sunflower hybrids Veleka, Vokil, Divna, Vyara and Gabi confirmed its excellent properties. Its phytopathological evaluation determines it as resistant to the parasite *Orobanche* up to race F, moderately resistant to phoma and alternaria and moderately susceptible to phomopsis.

The father component is line 626 possessing Rf fertility restorer genes, which was developed through hybridization of lines 654 R and 620 R, selfing and selection. The line is strongly branched, with specific lemon yellow coloration of the ray flowers and rich in pollen. It has very good general and specific combining ability. It is resistant to downy mildew up to race 731 and to the parasite *Orobanche* up to race F, moderately resistant to phoma, phomopsis and alternaria. The hybrid cross was developed in 2009 and was tested for two years in a competitive varietal trial and in a unified varietal trial, where it exceeded the standard with over 6 %. The testing was carried out after predecessor wheat applying agronomy practices suitable for growing of this crop. The trial plots were each 12 m with standard block design, in three replications of two rows. The plant density was 61220 plants/ha. Three standards were used – San Luka, Brio and PR 64F50. During the growth season all morphological and phenological characters of the hybrid cross were determined according to the UPOV Protocol (2002). The main elements of yield were taken into account: seed yield kg/ha, oil content in seed, %, and oil yield, kg/ha. Phytopathological evaluation of the hybrid and the parental lines was done at DAI – General Toshevo. The resistance to downy mildew (*Plasmopara halstedii*) was determined according to a standard methodology (Vear F., Tourvieille D., 1987) adapted to the working conditions of DAI. The percent of resistance was expressed in the response of the hybrid to races 700 and 731.

The resistance to the parasite broomrape (*Orobanche cumana*) was determined by the method of Panchenko (1975). The evaluation was done under greenhouse conditions using the index percent of resistance. The resistance to gray spots on sunflower (*Phomopsis helianthi*) was determined by the method of Encheva and Kiryakov (2002) under field conditions against artificial infection background. The type of attack was determined one week prior to flowering at stage milk maturity. The following scale was used: 0 – no symptoms; 1 – necrotic spot with diameter up to 5 cm; 2 – necrotic spot with diameter more than 5 cm; 3 – several merged necrotic spots on the stem; 4 – stem broken at the place of infection.

The testing for black spots on sunflower (*Phoma macdonaldii*) was carried out under field conditions against artificial infection background. The inoculation was done at stage budding – beginning of flowering by the method of Maric et al. (1981). The reaction of the plants was read at stage yellow-brown maturity according to a 4-degree scale as follows: 0 – no symptoms; 1 – necrotic spot localized around the petiole; 2 – several necrotic spots on stem; 3 – entire stem covered with necrotic spots or broken.

RESULTS

In 2012 the hybrid cross 217 A x 626 R was provided to Saaten Union – Romania as a candidate hybrid for registration in the European Catalog of Field and Vegetable Crops and for testing within the system of the State Institute for Variety Testing and Registration – ISTIS Romania. Following a three-year testing during 2012 – 2014, it was officially released with certificate No 4935/09.06.2015 under the trade name Dea.

Morphological description

The morphological description was done according to the methodology of UPOV (2002) and is presented in (Table 1).

Table 1. Morphological characteristics of sunflower hybrid DEA

№	Traits	Expression	Degree
1.	Hypocotyl:anthocianin coloration	Absent	1
2.	Hypocotyl:anthocianin coloration	Absent	1
3.	Leaf: size	Large	7
4.	Leaf: green color	Medium green	5
5.	Leaf: blistering	Weak	3
6.	Leaf: serration	Medium	5
7.	Leaf: shape of cross section	Concave	1
8.	Leaf: shape of distal part	Acuminate	8
9.	Leaf: auricules	Large	7
10.	Leaf: wings	Absent	1
11.	Leaf: angle of lowest lateral veins	Right or nearly right angle	2
12.	Leaf: height of the tip of the blade compared to insertion of petiole (at 2/3 height of plant)	High	7
13.	Stem: intensity of hairiness at the top	Very strong	9
14.	Time of flowering	Medium	5
15.	Ray flower: density	Dense	7
16.	Ray flower: shape	Narrow ovate	2
17.	Ray flower: disposition	Flat	1
18.	Ray flower: length	Medium	5
19.	Ray flower: color	Oringe yellow	4
20.	Disk flower color	Orange	2
21.	Disk flower: anthocyanin coloration of stigma	Absent	1
22.	Disk flower: intensity of anthocyanin coloration of stigma	-	-
23.	Disk flower: presence of pollen	Present	9
24.	Bract shape	Rounded	3
25.	Bract: length of the tip	Long	7
26.	Bract: green color of the external part	Medium	5
27.	Bract: attitude in relation to head	Not embracing or very slightly embracing	1
28.	Plant: natural height	Medium to tall	6
29.	Plant: branching	Absent	1

30.	Plant: type of branching	-	-
31.	Plant: natural position of closest lateral head to the central head	-	-
32.	Head: attitude	Half-turned down with straight stem	4
33.	Head: size	Medium	5
34.	Head: shape of grain side	Weakly concave	2
35.	Seed: size	Medium	5
36.	Seed: shape	Narrow ovoid	2
37.	Seed: thickness relative to width	Thin	3
38.	Seed: main color	Dark brown	6
39.	Seed: stripes on margin	Strongly expressed	3
40.	Seed: stripes between margin	Strongly expressed	3
41.	Seed: color of stripes	Brown	3

Biological and economic properties

The sunflower hybrid Dea is medium early, with duration of the growth season 116-119 days. Plant height is within the range 160-165 cm, with head diameter 20-23 cm. The absolute weight of seeds is 58-62 g and oil content is 49-50 %. The oil is of linoleic type. The percent of kernel in seed reaches up to 74-75 %, and the protein in seed is 19-20 %. Seed weight per plant is 78-84 g, and seed number is 1150-1300. The duration of flowering is 12-14 days. The maximum yield obtained in the experimental fields of DAI was 4545 kg/ha, and in neighboring Romania – 5313 kg/ha.

The seed production of the new hybrid allows simultaneous sowing of the two parental lines because their flowering coincides. This is a great advantage with a view of the necessary agronomy practices. The father line 626 R is strongly branched and rich in pollen. The most suitable seed production scheme is 10:2 (mother to father lines), with at least 3-4 well developed bee colonies available per ha.

Preliminary testing at DAI – General Toshevo

Hybrid Dea was subjected to three-year testing in the trial fields of DAI, involving two-year testing in a competitive varietal trial and one-year testing in a unified competitive trial (Table 2).

Table 2. Testing of hybrid DEA at DAI - General Toshevo

Hybrids	Seed yield, kg/ha	% from mean standard	Oil percent, %	Oil yield, kg/ha	% from mean standard
2009 – competitive varietal trial					
Dea	4039	115,5	48,2	1947	121,4
San Luka (st.)	3031	86,7	44,6	1352	84,3
Klarisa (st.)	3317	94,9	49,3	1635	101,9
Brio (st.)	4139	118,4	44,1	1825	113,8
Mean standard	3496	100,0	46,0	1604	100,0
2010 – competitive varietal trial					
Dea	4097	114,5	50,8	2081	118,6
San Luka (st.)	3528	98,6	46,8	1651	94,1
Klarisa (st.)	5319	92,8	53,6	1779	101,4
Brio (st.)	3886	108,6	47,2	1834	104,5
Mean standard	3578	100,0	49,2	1755	100,0
2011 – unified varietal trial					
Dea	3767	106,1	49,1	1850	106,6
San Luka (st.)	3189	89,9	47,1	1502	86,5
Klarisa (st.)	3553	100,1	52,3	1858	107,0
Brio (st.)	3906	110,0	47,3	1848	106,5
Mean standard	3549	100,0	48,9	1736	100,0

During the period of testing, hybrid Dea exceeded the mean standard by seed yield with 6.1 – 15.5 %. The exceeding was highest in 2009 both by seed yield (15.5 %) and oil yield (21.4 %). The exceeding by oil yield for the three years of testing was within 6.6 – 21.4 %.

Both yields were highest in 2010: 4097 kg/ha seed yield and 2081 kg/ha oil yield.
In the unified varietal trial, hybrid Dea was compared to the most promising and most productive hybrids of DAI, showing the following results: 6.1 % above the mean standard by seed yield and 6.6 % above the mean standard by oil yield.

The oil content of this hybrid reached 50.8 % and was higher than the standards San Luka and Brio.

Official testing

In 2012 hybrid Dea was provided to Saaten Union – Romania for official three-year testing on the territory of Romania and for registration. The results are given in (Table 3).

Table 3. Results from the official testing of hybrid “DEA”

Region	Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Relative yield according to the standard, averaged for 3 years
		2012		2013		2014		
1.Troian	Standard	1999	100	3821	100	1868	100	
	Dea	2030	102	3683	96	2366	127	108
2.Tecuci	standard	3154	100	4316	100	3393	100	
	Dea	3125	99	3511	81	3227	95	92
3.Rm.Sarat	standard	2124	100	4531	100	3292	100	
	Dea	2906	137	5313	117	3292	112	122
4.Portaresti	standard	2492	100	3487	100	3763	100	
	Dea	3437	138	4564	131	4614	123	131
5.Peciu Nou	standard	2873	100	3363	100	3230	100	
	Dea	3567	110	3470	103	3421	119	111
6.Negresti	standard	3057	100	4408	100	4201	100	
	Dea	2553	84	4043	92	3882	92	89
7.Mircea Voda	standard	2204	100	2698	100	4580	100	
	Dea	3123	142	3172	118	4958	108	123
8.Inand	standard	2441	100	3507	100	2495	100	
	Dea	2529	104	3578	102	2658	107	104
9.Dalga	standard	3801	100	4055	100	3518	100	
	Dea	3777	99	4367	108	3767	107	105
10.Cogealac	standard	1981	100	2240	100	2716	100	
	Dea	2496	126	2135	95	2059	76	99
Средно от 10 пункта	standard	2648	100	3642	100	3234	100	
Averaged from 10 locations	Dea	2954	112	3783	104	3424	106	109

The official testing of new hybrids in Romania is carried out at ten locations representative for almost all soil-and-climate regions suitable for growing of field crops.

Two Romanian hybrids were involved as standards. During the second and third year hybrid Daniel was used as a standard, and during the first year – hybrid Alex.

During the first year of official testing the new hybrid Dea gave a mean yield from all locations 2954 kg/ha, which was a 12 %-exceeding, the highest during the three-year testing. The low yield of kg per da was due to the unfavorable conditions in 2012 related to very high air temperatures for a long period of time and to the long-lasting drought during almost the entire growth season. Nevertheless, hybrid Dea demonstrated the best results among all tested hybrids in that year and performed as resistant to drought and high air temperatures.

During the second year the exceeding of the standard was with 4 % at seed yield 3783 kg/ha, which was the highest result from the official three-year testing. During the third year the exceeding was 6 %, the seed yield being 3424 kg/ha.

The highest seed yield was 5313 kg/ha from location Rm.Sarat in 2013, and the lowest - 2030 kg/ha from Troian in 2012. The highest exceeding of the standard with 12 % was at location Mircea Voda in 2012.

In seven out of ten locations hybrid Dea showed results exceeding the standard with up to 31 %. For the three years of testing, Dea exceeded the standard with 9 % and this was the main reason for its official registration and enlisting in the European catalog of field and vegetable crops.

Phytopathological characterization

The evaluation of the resistance of the hybrid to economically important diseases and the parasite *Orobanche* were carried out in the infection fields of DAI. The results from them are presented in (Table 4).

Table 4. Phytopathological evaluation of sunflower hybrids in artificial infection field at DAI – General Toshevo

Hybrid	Phomopsis helianthi		Phoma macdonaldi		Plasmopara helianthi		Orobanche cumana
	Attacking rate	Rank	Attacking rate	Rank	Resistance to race 700, %	Resistance to race 731, %	Resistance to races A-F, %
San Luka	3/3(3)	3	1/3(1)	1	100.0	92.9	100.0
Diabolo	2/3(2)	2	1/3(1)	1	100.0	90.5	100.0
Brio	1/3(1)	1	0	0	100.0	100.0	100.0
PR64F50	1/3(1)	1	0	0	100.0	100.0	100.0
Dea	1/3(1)	1	0	0	100.0	60.0	100.0

Hybrid Dea was resistant to the fungal pathogen *Phomopsis helianthi.*
To the other important leaf pathogen *Phoma macdonaldi* the hybrid demonstrated immune reaction.

The resistance of hybrid Dea to downy mildew on sunflower *Plasmopara helianthi*, race 700 was 100 %, and to the most recent race 731 its resistance was moderate.

To the parasite *Orobanche cumana* the resistance was 100%.

CONCLUSIONS

- Hybrid Dea is clearly distinct, uniform and stable.
- It was officially registered in Romania and was enlisted in the European catalog of the field and vegetable crop varieties.
- It possesses very good adaptability and realizes its high potential under variable soil-and-climatic conditions.
- The hybrid is resistant to drought and high temperatures.
- It is also resistant to the economically important diseases and the parasite *Orobanche*.

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INVESTIGATION ON SUNFLOWER LINES AND HYBRIDS (*HELIANTHUS ANNUUS* L.) FOR EXPRESSION OF HETEROSIS AND DOMINANCE RATE OF IMPORTANT ECONOMIC TRAITS IN F_1 UNDER THE CONDITIONS OF NORT-EAST BULGARIA

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ABSTRACT

The investigation wascarried out during 2013–2014 in the trial field of Dobrudzha Agricultural Institute (DAI). Nine hybrid combination of oil seed sunflower were investigated, which were obtained from the crossing of three sterile lines to three fertility restorers. The following traits were studied: plant height, head diameter, and 1000 kernel weight. Hypothetical and actual heterosis and heritability rate were determined in all hybrid combinations. For statistical processing of the results, two factor dispersion analysis and variation and correlation analyses were applied using software XLSTAT Pro. ver 7.0.1.

It was demonstrated that 1000 kernel weight was affected most by the head diameter in the tested lines and hybrid combinations. Lower was the effect on 1000 kernel weigh by plant height according to the performed correlation analysis. The variation analysis showed highest variation of the traits 1000 kernel weight and head diameter, while plant height was with the lowest value of variation among the investigated traits. Highest values of hypothetical and actual heterosis of the trait plant height were found in the cross 89R x 217A. For the trait head diameter, highest values of hypothetical and actual heterosis and heritability rate were determined in the cross 89R x 813A.

Key words: sunflower, heterosis, heritability rate, hybrid,

MATERIAL AND METHODS

The investigation was carried out during 2013 – 2014 in the trial field of Dobrudzha Agricultural Institute – General Toshevo (DAI). Nine hybrid combinations of oil seed sunflower were investigated, which were derived from the crossing of three sterile lines to three fertility restorers. The hybrid combinations were tested in a trial of three replications designed by the Latin square method. The size of the plot was 7.35 m^2. The standards used were the Bulgarian hybrid San Luka and some of the most productive and well established in Bulgaria foreign hybrids Meldimi, Clarissa and P64LE19.

The traits plant height, head diameter and 1000 kernel weight were studied. Hypothetical heterosis, real heterosis and heritability rate were determined for all hybrid combinations. Two-factor dispersion, variation and correlation analyses were applied for statistical processing of results using the software XLSTAT Proversion 7.0.1.

The data on the investigated traits were subjected to genetic analysis to determine the theoretical (the mean value of the two parents) and the real (the value of the better parent)

heterosis in F_1, and the percent of heritability of the investigated traits (according to Omarov, 1975). The dominance rates in the heritability of the traits in F_1 were determined using the methodology of Romero and Frey, 1973.

RESULTS AND DISCUSSION

Highest value of hypothetical heterosis in (Table 1) for the trait plant height was determined in the cross 2003A x 89R (58.22%) while real heterosis with highest index was determined in the hybrid combination 217A x 85R. Highest heritability rate of this trait, averaged for two years, was found in the cross 217A x 85R.

The results presented in (Table 2) characterize the expressions of heterosis in F_1 and the rate of dominance in F_1 generation for head diameter. In cross 813A x 89R, a clearly expressed positive heterosis was observed in the heritability of this trait both with regard to the mean value of the two parents and with regard to the parent with higher value regardless of the genotype and the year of growing. The mean values from the two years of investigation on the hypothetical heterosis in generation F_1 were different and varied from 27.67 % in cross 217A x 85R to 52.34 % in cross 813A x 89R. In cross 2003A x 89R a high heritability rate was determined for this trait – 1.75 %, averaged for the two years.

Highest hypothetical heterosis for the trait 1000 kernel weight (Table 3) was determined in cross 2003A x 84R - 85.40 %. Positive real heterosis according to the better parent was observed in hybrid combination 813A x 84R – 71.58 %, averaged for two years. High mean value was obtained in this combination for dominance rate in F_1, averaged for two years. The dominance rates in generation F_1 for the three traits had positive values higher than 1 implying super dominance. In (Table 4) it was proved that 1000 kernel weight was influenced most by the head diameter of the tested lines and hybrid combinations. Lower was the effect of 1000 kernel weight in comparison to plant height according to the correlation analysis carried out. Head diameter was influenced by plant height.

It becomes clear from the variation analysis in (Table 5) that highest was the variation of the traits 1000 kernel weight and head diameter, while plant height had lowest value of variation among the investigated traits.

CONCLUSIONS

Highest value for hypothetical heterosis of the trait plant height was determined in the cross 2003A x 89R (58.22%) while real heterosis with highest index was found in the hybrid combination 217A x 85R. Highest was the heritability rate of this trait in the cross 217A x 85R, averaged for two years.

Highest hypothetical heterosis of the trait 1000 kernel weight was determined in the cross 2003A x 84R - 85.40%. Positive real heterosis and heritability rate of the trait 1000 kernel weight according to the better parent was observed in hybrid combination 813A x 84R – 71.58 %, averaged for two years.

In cross 813A x 89R, a clear positive heterosis was observed in the heritability of the trait with regard to both the mean value of the two parent and to the parent with higher value regardless of the genotype and the year of growing. In cross 2003A x 89R, high heritability rate of this trait was determined – 1.75 %, averaged for two years.

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Table 1. Heterosis (%) and dominance rate in F_1 of the trait plant height

		Heterosis %		Dominance rate in F_1
Hybrid	Year	Hypothetical	Real	
217A x 84R	2012	24.35	8.64	1.74
	2013	22.74	6.44	1.43
	mean	**23.55**	**7.54**	**1.59**
217A x 85R	2012	46.43	48.45	36.23
	2013	34.81	37.29	27.33
	mean	**40.62**	**42.87**	**31.78**
1017A x 89R	2012.00	50.72	45.72	17.32
	2013.00	27.29	23.15	9.39
	mean	**39.01**	**34.44**	**13.36**
813A x 84R	2012	12.76	8.27	18.3
	2013	7.39	0.72	10.61
	mean	**10.08**	**4.50**	**14.46**
813A x 85R	2012	15.49	3.79	1.36
	2013	23.75	14.16	2.82
	mean	**19.62**	**8.98**	**2.09**
813A x 89R	2012	18.84	8.01	1.87
	2013	26.17	11.66	1.77
	mean	**22.51**	**9.84**	**1.82**
2003A x 84R	2012	36.68	30.07	8.27
	2013	31.72	19.70	5.70
	mean	**34.20**	**24.89**	**6.99**
2003A x 85R	2012	57.40	39.34	4.42
	2013	43.39	36.60	8.72
	mean	**50.40**	**37.97**	**6.57**
2003A x 89R	2012	58.90	42.62	5.16
	2013	57.53	37.60	6.00
	mean	**58.22**	**40.11**	**5.58**

Table 2. Heterosis (%) and rate of dominance in F_1 of the trait head diameter

		Heterosis %		Dominance rate in F_1
Hybrids	Year	Hypothetical	Real	
217A x 84R	2012	31.73	4.76	1.23
	2013	42.76	3.33	1.12
	mean	**37.25**	**4.05**	**1.18**
217A x 85R	2012	25.74	0	1
	2013	29.6	1.19	0.77
	mean	**27.67**	**0.59**	**0.88**
1017A x 89R	2012	40.12	4.76	1.18
	2013	48.27	2.38	1.07
	mean	**44.20**	**3.57**	**1.13**
813A x 84R	2012	27.27	2.38	1.11
	2013	34.86	0	0.89
	mean	**31.06**	**1.19**	**1**
813A x 85R	2012	34.73	4.76	1.09
	2013	44.73	4.76	1.17
	mean	**39.73**	**4.76**	**1.13**
813A x 89R	2012	43.31	8.12	1.3
	2013	61.37	11.42	1.36
	mean	**52.34**	**9.77**	**1.33**
2003A x 84R	2012	27.48	2.5	1.13
	2013	53.28	16.66	1.69
	mean	**40.38**	**9.58**	**1.41**
2003A x 85R	2012	29.62	5	1.26
	2013	50.36	14.44	1.6
	mean	**39.99**	**9.72**	**1.43**
2003A x 89R	2012	31.57	2.5	2
	2013	57.69	7.89	1.5
	mean	**44.63**	**5.20**	**1.75**

Table 3. Heterosis (%) and dominance rate in F_1 for the trait 1000 kernel weight

		Heterosis %		Dominance rate in F_1
Hybrids	Year	hypothetical	real	
217A x 84R	2012	50.8	0.62	1
	2013	78.38	64.68	4.71
	mean	**64.59**	**32.65**	**2.86**
217A x 85R	2012	30.52	2.2	1.1
	2013	39.95	9.59	1.44
	mean	**35.23**	**5.89**	**1.27**
1017A x 89R	2012	39.54	7.58	1.33
	2013	109.4	61.61	3.69
	mean	**74.47**	**34.60**	**2.51**
813A x 84R	2012	39.4	43.6	2.65
	2013	104.8	99.56	19.3
	mean	**72.1**	**71.58**	**10.97**
813A x 85R	2012	38.4	30.8	6.25
	2013	54.47	26.5	2.46
	mean	**46.44**	**28.65**	**4.36**
813A x 89R	2012	37.6	41.1	6.25
	2013	66.9	50	5.93
	mean	**52.25**	**45.55**	**6.09**
2003A x 84R	2012	38.1	32.9	12.7
	2013	132.7	82.08	4.77
	mean	**85.40**	**57.49**	**8.74**
2003A x 85R	2012	37.1	37.6	14.09
	2013	57.6	42.74	4.59
	mean	**47.35**	**40.17**	**9.34**
2003A x 89R	2012	36.3	44.5	10
	2013	68.9	50.25	4.75
	mean	**52.60**	**47.38**	**7.38**

Table 4**.** Correlation analysis

	Plant height	*Head diameter*	*1000 kernel weight*
Plant height	1		
Head diameter	0.44	1	
1000 kernel weight	0,58**	0,76***	1

Table 5. Variation analysis

	Plant height	*Head diameter*	*1000 kernel weight*
Mean value	122.2	17.3	48.9
Standard Error	4.4	1.0	2.8
VC % Variation	177.8	249.5	242.5

CORRELATIONS AND PATH COEFFICIENT ANALYSIS OF CONFECTONERY SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

The most important criterion for introducing new confectionary hybrids into production is high protein yield. Breeding for increase of kernel protein content results in increased protein yield. Path coefficient analysis was performed to separate direct and indirect effects of studied traits on seed kernel protein content, and to identify traits which could be used as selection criteria in sunflower breeding. The research was conducted during three vegetation seasons on 22 NS high-protein two-line confectionary sunflower hybrids produced within the breeding program at IFVCNS, Novi Sad, Serbia. Strong and very strong correlations were found among the largest number of examined traits. Based on the analysis of simple correlation coefficients, strong negative correlation was determined between kernel protein content and kernel ratio (-0.516*). A weak negative interdependence was determined between head diameter, seed protein content, and kernel protein content. Positive but weak correlation was determined between kernel protein content and thickness of seed, length of seed, width of seed, and 1000 seed weight. Path coefficient analysis for kernel protein content at phenotypic level showed that the thickness of seed had a strong positive direct effect on kernel protein content (DE=382*). Kernel ratio and width of seed had a very strong direct negative effect on kernel protein content (DE=-0.990**; DE=0.600**). A weak direct positive effect of head diameter, seed protein content and length of seed was established, whereas 1000 seed weight had a weak direct negative effect on kernel protein content. This indicates that thickness of seed has high influence on kernel protein content.

Key Words : confectionary sunflower, correlations, kernel protein content, path coefficient analysis, quantitative traits

MORPHOLOGICAL CHARACTERIZATION OF UGA-SAM1 SUNFLOWER ASSOCIATION MAPPING POPULATION

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ABSTRACT

In order to use germplasm collections more efficiently and effectively, it is important to characterize the diversity of the germplasm. The objective of this study was to assess morphological diversity of a sunflower association mapping population UGA-SAM1 composed of 286 accessions and obtained from the USDA sunflower collection. Accessions were characterized for 10 traits to determine available morphological variability. The Shannon-Weaver diversity index (*H'*) was used to determine allele richness according to the frequency of genotypes in each nominal class. Phenotypic variation was found for all evaluated traits with *H'* values ranging from 0,45 to 0,90. The highest diversity was found for leaf lateral veins angle and height of the tip of the leaf blade compared to insertion of petiole, while least diversity was found for seed color and leaf shape. Homogeneity analysis by means of alternating least squares (HOMALS) grouped accessions to three major clusters: 1) RHA-Oil, 2) RHA-Oil and RHA-non Oil and 3) a mix of remaining accessions including Oil and non Oil accessions. The presented results confirm usefulness of UGA-SAM1 as a rich source of variability and as such a valuable resource for sunflower research.

Key Words : Characterization, diversity index, Germplasm, morphological, UGA-SAM1

HIGH OLEIC SUNFLOWER HYBRID OXY WITH CHANGED SEED TOCOPHEROL CONTENT

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ABSTRACT

Single cross sunflower hybrid Oxy was developed in VNIIMK, Krasnodar and registered by Russian State Commission on testing and protection of breeding achievements in 2014. Hybrid Oxy is considered an innovation from biochemical genetics to breeding for oil quality. All of the hybrid, female and male parent lines are homozygotes for a dominant high oleic mutation *Ol*, as well as recessive tocopherol mutation *tph1*, *tph2* and *tph3*. The main breeding character of the hybrid Oxy is increased oil oxidative stability up to 14 times due to high content of oleic content, gamma- and delta-tocopherols. Tocopherol mutations in sunflower seeds were originated from spontaneous mutagenesis within a gene pool of cultivated plants of *Helianthus annuus* L. All *tph1*, *tph2* and *tph3* mutations are monogenic recessive and non-lethal. The recombination of these mutations allows producing nearly any types of tocopherol profiles with four known alpha-, beta-, gamma- and delta-homologues. Mid-ripening hybrid Oxy possesses acceptable seed yield potential about 3 t/ha, broomrape resistance to race A-E, phomopsis tolerance and stay-green stem. Seed oil content averages 48%, hull content – 23%. Hybrid Oxy is not a GMO. The oil of the hybrid is intended to be used in the area of native oil with maximum level of oxidative stability i.e. for frying purposes.

Key words: Oil, Stability, Breeding, Oleic Acid, Tocopherol, Mutation

INTRODUCTION

Genetic research has led to an entirely new level in the global plant breeding for oil quality associated with overcoming interspecific barriers in genetic variation of seed lipid composition between different oil crops. For example, sunflower can produce oil similar to olive in fatty acid composition and flax - to sunflower. Plant varieties are developed with industrial-commodity targeting.

Sunflower breeding for oil quality based on the current trends to transfer the individual steps of industrial technology in the cells of living organisms. This process allows obtaining the desired substances of natural origin with ecological clean biosynthetic approach. Breeding strategy in this case is to create varieties with new types of oil determined by its use. There is selection of genotypes both on the extreme manifestation of the trait, i.e. minimum or maximum and the optimum content of desired substances. It is obvious that each type of oil has individual quality parameters.

Unlike breeding for yield increase, when traits of productivity, resistance to diseases and abiotic stresses are formed and implemented in the field during harvesting, breeding for improved quality deals with so-called “cross-cutting” characters of chemical composition of the seeds. These traits are formed in the plant in the field conditions and pass to the raw

materials and products of technological processing, i.e. they are realized in the industrial or consumer sectors.

The quality of the oil, i.e. its nutritive, biological and technological properties, depends on composition of fatty acids of a triacylglycerol molecule, and presence of related compounds. One of the important problems in improving of oil quality is to increase its resistance to oxidation for preventing toxic products of rancidity during storage and use.

The degree of unsaturation of fatty acids which correlated positively with the ability to oxidation and the presence of natural antioxidants, especially tocopherols, protecting against the free radical accumulation are the main factors of oil oxidation (Velasco *et al*., 2003, 2004; Warner *et al*., 2008).

HYBRID OXY DEVELOPMENT

The first major achievement in sunflower breeding for oil oxidative stability was held with high oleic variety of Pervenets in VNIIMK, Krasnodar (Soldatov, 1976). This variety has become a unique donor of the high oleic mutation in breeding programs worldwide.

Found in further studies the effect of synergism in the joint action of fatty acids and tocopherols on the stability of oil to oxidation has opened up opportunities in sunflower breeding by combining the desired genes (Demurin, 1993; Demurin *et al*., 1996). As a result of this work sunflower commercial hybrid, named Oxy, was developed in VNIIMK. It has both traits combined of high oleic acid and the high content of powerful antioxidants such as gamma- and delta-tocopherols (Table 1).

Table 1. Composition of fatty acids and tocopherols in the oil of sunflower hybrid Oxy

Hybrid	Fatty acid composition, %				Tocopherol composition, %			
	palmitic	stearic	oleic	linoleic	α	β	γ	δ
Standard	5,1	3,5	31,2	60,2	100	<1	<1	0
Oxy	4,3	3,8	86,2	5,7	<1	<1	60	40

Single-cross sunflower hybrid Oxy was obtained in the framework of breeding and genetic program to improve the quality of oil by the crossing inbred lines VK876 A × VK195. All parent forms, including female CMS analogue and maintainer, as well as a male fertility restorer are homozygous on four genes controlling the trait of high oleic acid content (dominant mutation *Ol*) and high content of powerful antioxidants such as gamma- and delta-tocopherol (triple homozygote for recessive mutations of *tph1*, *tph2* and *tph3*). The main valuable character of the hybrid Oxy is increased to 14-fold oxidative stability of the oil compared to the normal genotype due to the simultaneous change in the composition of fatty acids and tocopherols, which gives this hybrid world priority (Table 2).

Table 2. Breeding characteristics of the sunflower hybrid Oxy

Trait	Standard	Oxy
Vegetation period, days	94	94
Seed yield, t/ha	3,3	3,1
Seed oil content, %	51,8	47,8
Oil yield, t/ha	1,5	1,3
Oil type	linoleic, α-tocopherol	high oleic, γ- and δ-tocopherols
Oxidative stability, hours (Rancimat-test, 120 °C)	3,1	44,3

Hybrid Oxy belongs to the middle ripening group. The seed yield does not differ from the standard. The hybrid is resistant to broomrape (A-E), downy mildew, tolerant to phomopsis (stay-green). The vegetation period from germination to biological ripeness is 94 days, the achenes oil content of 48% and hull content of 23%. It is obvious that this hybrid is designed to produce special oil, i.e. for long shelf-life or frying. Hybrid Oxy has been included in the Russian state register of admitted and protected varieties since 2014.

CONCLUSIONS

Thus, sunflower hybrid Oxy was developed with common breeding methods without the use of transgenic techniques. The seed oil possesses the highest level of oxidative stability by combining high oleic acid content with the increase concentration of gamma- and delta-tocopherols asstrong endogenous antioxidants. This natural oil without any chemical modification and addition of exogenous ingredients can be useful in the industries with high demands for resistance to oxidation.

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CYTOGENETIC STUDY OF HELIANTHUS STRUMOSUS AND ITS F_1 AND BC_1F_1 HYBRIDS WITH CULTIVATED SUNFLOWER

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ABSTRACT

Helianthus strumosus L. is represented in Novi Sad collection of wild sunflower species with large number of accessions (14 with seed reserves and 20 in the field collection). It is often used as a source of resistance to disease-causing agents in the breeding of cultivated sunflower. Interspecific crosses with cultivated sunflower lines were performed using 17 accessions of this species. Six F1 hybrid combinations were obtained using two*H.strumosus* accessions with a total of 48 plants, while in backcrossing 51 BC1F1 plant was obtained. Nine originated from crossing F1 and 42 from crossing F1OP with cultivated sunflower. Cytogenetic analysis showed 3 levels of ploidy in the examined accessions of *H.strumosus* (n = 17, 34 and 51) and high pollen viability ranging from 83.13- 98.93%. F1 hybrids exhibited reduced pollen viability (26.83 - 55.34%), and there were occurrences of male sterility. Analysis of chromosomal association of F1 hybrids showed that chromosome number was 68, and that most commonly observed associations were 25-34 bivalents with the occurrence of quadrivalents, hexavalents and univalents. BC1F1 hybrids also had male sterile plants, while pollen viability ranged from 5.66 - 80.85%. Analysis of chromosomal associations in diakinesis showed a varying number of chromosomes (55 - 70), while the number of bivalents was 15-27, trivalents 0-3, quadrivalents 0-4, hexavalents 0-1 and univalents 1-5. In addition to irregular patterns of chromosome pairing in diakinesis, F1 and BC1F1 hybrids also exhibited irregularities like fast, lagging chromosomes and chromosome bridges in other stages of meiosis. Cytogenetic analyses show the difficulties in obtaining progenies of interspecific hybrids that will contain the desirable genes from *H.strumosus*.

Key Words : Sunflower, *Helianthus strumosus* L., Interspecific crosses, Cytogenetic analyses

VALIDATION OF SCAR-MARKER FOR RESTORATION FERTILITY GENE IN UKRANIAN INITIAL MATERIAL OF SUNFLOWER

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ABSTRACT

The breeding lines, lines of mutant origins, samples of interspecific hybrids and varieties of sunflower (in general 105 sample) for the presence of HRG01 locus, which linked with the Rf_1 gene have been analyzed. The HRG01 locus has always identified when this gene is present in the plant material. In the result of amplification band 426 bp has been synthesized. In the absence of the Rf_1 gene the specific band in the samples of sunflower was not found. Therefore, amplified product in male sterility restoration lines was not synthesized, while its synthesis was in male restoration lines. Percentage of samples with HRG01 locus in samples obtained from interspecific hybrids was 62. It was found that the allele of HRG01 locus occurred in 30 % of sunflower varieties. Its frequency was varied from 0.053 to 0.263.

Key words: Sunflower, SCAR marker, *Rf* gene

INTRODUCTION

The different types of DNA markers widely used in genetic and breeding research of sunflower. A significant amount of information about variability of markers is accumulated by RAPD, AFLP, SSR and SNP analyses. It has been constructed detailed genetic maps of sunflower including resistance genes to pathogens, morphological and biochemical traits, which are relative to a particular type of molecular markers (Jan et al., 1998; Lai et al., 2005; Heesacker et al., 2008; Tang et al., 2002). It was possible not only with the development of molecular methods, but detailed studies of genetics of resistance, morphological and biochemical traits of sunflower that studying the effects of most genes (Sharypina et al., 2008). Therefore, information on linkage marker and gene can be used in marker-assisted selection, which is widely used in many countries to intensify the breeding process (Popov & Kirichenko, 2010).

Restoration of pollen fertility in sunflower is controlled by Rf_1 gene with the possible interaction between at least two or three *Rf* genes. Features of genetic control of pollen fertility restoration are summarized in review articles and monographs (Gavrilova & Anisimova, 2003; Vedmedeva & Tolmachev, 2006; Popov & Kirichenko, 2010). The main step of creation of the sunflower restorer lines is determination of the ability of these lines to fully restore fertility of pollen in their crosses with male sterile lines. This selection process is laborious and time consuming (Popov & Kirichenko, 2010). Therefore, the creation of inbred sunflower lines should involve different DNA markers for screening the presence of *Rf* genes in various initial material that optimizes the breeding process.

Currently, the details of mapping of restoring fertility pollen gene Rf_1 has been gathered (Jan et al., 1998; Horn et al., 2003; Kusterer et al., 2005; Schnabel et al., 2008). Thus, based on polymorphic of RAPD fragments two SCAR markers – HRG01 and HRG02 were developed (Horn et al., 2003). One of the closely linked markers TRAP was converted to STS marker (Yue et al. 2010). Distance between STS and Rf_1 was 0.4 cM. Construction of a genetic map based on SSR-markers revealed that the *Rf1* gene is located in 13 linkage group (Tang et al., 2002). Also the molecular mechanisms of interaction between mitochondrial and nuclear genes were clarified (Moneger et al., 1994; Horn et al., 1999).

For efficient use of molecular markers in the breeding process their initial material of various origins should be validated. The purpose of this study was to establish the presence of SCAR-marker (HRG01) linkage with the gene Rf_1 in various breeding material of sunflower.

MATERIAL AND METHOD

Thirty seven inbred lines of sunflower created in the laboratory of breeding and genetics of sunflower of the Plant Production Institute named after V. Ya. Yuriev of NAAS (Kharkiv, Ukraine) were involved, including 11 male sterile lines, 19 male sterility restoration lines and 7 mutant lines. In addition, the study used 29 sunflower samples that obtained from interspecific hybrids. Also we involved 39 sunflower varieties of different origins.

SCAR-marker identification was performed by PCR with a pair of primers that flank certain areas of genomic sunflower DNA. The nucleotide sequences of primers to locus HRG01 were as follow: F: TATGCATAATTAGTTATACCC and R: ACATAAGGATTATGTACGGG (Horn et al., 2003).

PCR was performed using reagent kit GenePak PCR Core of LLC "Laboratory Izogene" (Russia). The final volume of the reaction mixture was 20 μl and contained 20 ng of genomic DNA with the addition of 0.2 mM of each primer. In test tubes of reaction mixture 20 μl of mineral oil was added. PCR was performed in thermocycle "Tertsyk" (Russia) using program according (Kusterer et al., 2005).

PCR products were run on 2 % agarose gel with high resolution and the addition of ethidium bromide in the low-molarity buffer. The amplified products were visualized using photography in UV light with photosystem NikonD50. For determination of the lengths of PCR products DNA ladders 50 bp and Mcombi (LLC " Laboratory Izogene", Russia) were used.

RESULT AND DISCUSSION

Cytoplasmic male sterility (CMS) always is in use for creation of high yields sunflower hybrids. On the base of CMS the inbred lines of three types – male-sterile lines ($cyt^S rf_1 rf_1$), sterility fixing lines ($cyt^N rf_1 rf_1$) and male sterility restoration lines ($cyt^N Rf_1 Rf_1$) create. The creation of inbred lines of sunflower is based on the interaction between classical cytoplasm PET1 with gene *Rf*.

At the first stage of the testing of marker HRG01 the inbred lines of sunflower (male-sterile and male sterility restoration lines) from collection of initial material of the laboratory of sunflower breeding and genetics of Plant Production Institute named after V. Ya. Yuriev of NAAS were involved. These lines are used to create single cross and three-way cross hybrids of sunflower in Plant Production Institute (Kirichenko et al., 2014). According to literature PCR product with size 426 bp indicates the presence of HRG01 locus and as a consequence of *Rf1* gene presence in sunflower genotypes. Involvement of inbred lines of Kharkiv breeding in the research allowed us to conduct the validation of HRG01 markers. Thus, absence of amplicon has been observed in all male-sterile lines, while in male sterility restoration lines

were identified PCR product with size of 426 bp. These results confirm the diagnostic ability of the marker to identify gene *Rf1* in the plant genotypes (fig.1).

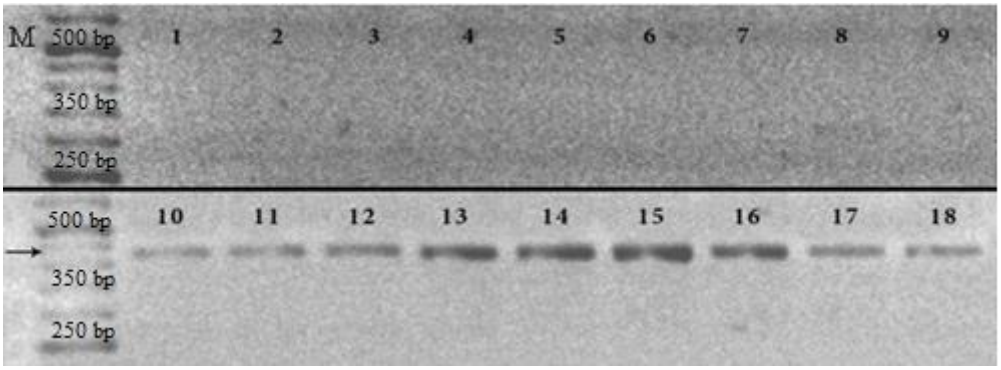

Fig.1 Electrophoregram of separation of amplified products of SCAR-marker HRG 01. 1–9 – male-sterile lines; 10–18 – male sterility restoration lines. The arrow shows the PCR product of the size of 426 bp.; M – DNA ladder «Mcombi».

Six lines of mutant origin were also tested with a pair of primers to HRG01. In the three lines of sunflower – Mkh1829, Mkh4 and Mkh42 the amplified product with size of 426 bp was found. It was absent in the lines Mkh2122, Mkh108, Mkh1091.

For the molecular genetic analysis samples obtained from interspecific hybrids of different origin have been involved. They were created with using annual wild species of sunflower *H. annuus*, *H. argophyllus* and *H. debilis*. It should be noted, that these samples are not analyzed for the presence of *Rf1* genes using classical plant breeding methods. As a result, molecular analysis revealed that 17 samples had specific PCR product. The size of this product was 426 bp, which corresponds with male sterility restoration lines, in which the presence of *Rf1* gene has been clearly identified by hybridization with male-sterile lines. The frequency of such lines was 0.586. In 12 samples obtained from interspecies hybrids PCR product with size of 426 bp (frequency 0.414) was not observed. The results allow to differentiate experimental material into two groups – the samples male-sterile lines type (absence of amplicon 426 bp) and the samples of male sterility restoration lines type (presence of amplicon size 426 bp).

Sunflower varieties were further involved to test the marker HRG01. The varieties of sunflower are the source of initial material for a complex of traits, including genotypes with genes *Rf1*. However based on the genetic structure the most varieties are fixers of sterility. This means that the populations consists mainly of genotypes $cyt^{N}rf_1rf_1$. Therefore, for intensification of creation of male fertility restorer lines from sunflower varieties DNA markers linked to the gene *Rf1* are need to use. Using pairs of primers to HRG01 locus amplified product with size of 426 bp was obtained. Results of separation of amplified products are shown on figure 2. It should be noted that amplified product 426 bp was not identified in all varieties-populations because most genotypes of these varieties are fixers of sterility.

In the studies of the molecular genetic structure of Ukrainian varieties the 426 bp allele of HRG01 locus were identified only in four varieties – Zaporiz'kii konditerskii, Mistsevii 1, Mistsevii 2, Mistsevii 15, ChaS. The frequency of allele 426 bp for these varieties was 0.105, 0.263, 0.579, 0.684 and 0.316, respectively. In other varieties this band was not detected.

However 426 bp allele of HRG01 locus was not identified in a sample of sunflower varieties of Russian breeding. In the varieties of sunflower breeding of Greece only variety Rodopi detected allele of 426 bp size with frequency 0.053. In other varieties allele of this size is not identified.

In French variety Nain noir and varieties Slovenska siva and Bucianska olejna from Czechoslovakia we also not found band 426 bp.

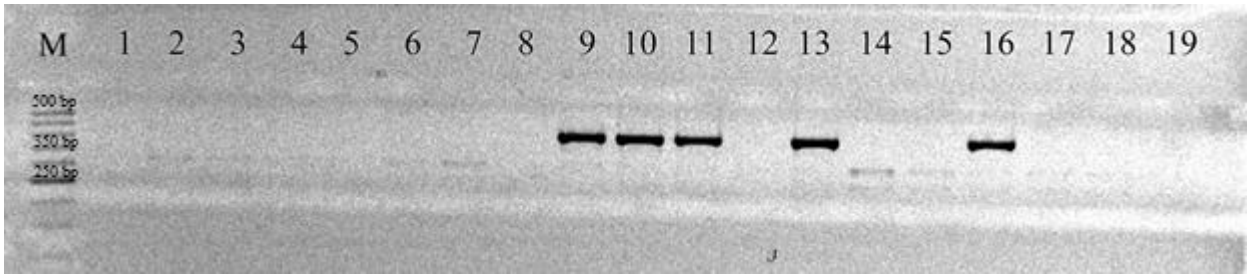

Fig. 2. Electrophoregram of separation of amplified product of SCAR-marker HRG 01 in sunflower variety Mennonite (Canada). 1–19 – genotypes of variety Mennonite; M – DNA ladder 50 bp.

Analysis of the distribution of frequency of allele 426 bp of HRG01 locus for the Hungarian varieties revealed that allele was present only in varieties Mezoeheguesi and Lovaszpatonai. The allele frequency was 0.053 in both varieties. In two other varieties of Hungarian breeding allele was not detected.

According to the analysis of HRG01 locus allele 426 bp was identified in three out of four USA varieties. These are the varieties Untitled (PI 432515), Arrowhead, Ghray Mammoth. The frequencies of allele in these varieties were 0.105, 0.158 and 0.211 respectively. In variety Mingren allele 426 bp is not detected.

In varieties of Canadian breeding the allele of size 426 bp of HRG01 locus was identified only in variety Mennonite. Its frequency was 0.263. In other varieties allele is not detected.

In general, it should be mentioned that the 426 bp allele of HRG01 locus in 12 (30 %) of sunflower varieties is distributed and in 27 varieties (70 %) is not identified.

CONCLUSION

SCAR-marker (locus HRG01) clearly identified in breeding lines, which are shown of the presence or absence of a gene Rf_1. Using samples of sunflower, created with the involvement of annual wild species and varieties has proved the presence in their genotypes of PCR products with size of 426 bp, which indicates also the presence of the Rf_1 gene. The results make it possible to conduct targeted selection of male lines from interspecific hybrids and varieties.

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THE PUBLIC SUNFLOWER ASSOCIATION MAPPING POPULATION

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ABSTRACT

In recent years, major steps have been made toward generating genetic and genomic resources in sunflower; however, the tools for associating phenotype with genotype have remained largely undeveloped. To help fill this gap, we developed a permanent, publicly-available association mapping resource for sunflower. The Sunflower Association Mapping (SAM) population consists of 271 diverse inbred lines and captures nearly 90% of the genotypic diversity in cultivated sunflower. We have grown the SAM population in replicate at three locations and phenotyped it for a number of agronomically important traits including days to flower (DTF) and plant architecture. All individuals within the SAM population were sequenced to a minimum of 8-10x depth with the Illumina platform. Using custom bioinformatics pipelines developed in collaboration with the software company SAP, we extracted 613,011 high confidence SNPs to be employed for genome-wide association study (GWAS) analyses. To identify the alleles associated with phenotypes characterized in the SAM population, we have completed development of a GWAS pipeline which includes imputation, population structure calculation, kinship, mixed linear model, p-values adjustment, meta-analysis across replicates and environments, tests for GxE, and calculation of variance components and effect sizes at each SNP position. We implemented this custom pipeline on the SAM phenotypic data for DTF and total branching which was previously analyzed using genotypes from a 10K Illumina SNP array. We present details of the SAM population including phenotypic and genetic diversity, results from the newly developed custom GWAS pipeline, and comparisons to previous association results in sunflower employing SNP arrays.

Key Words : association mapping, whole genome sequencing, GWAS, flowering time, genetic diversity

FH-586- A SHORT DURATION HIGH YIELDING SUNFLOWER HYBRID UNDER SEMIARID CONDITIONS

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ABSTRACT

Oilseeds Research Institute, Faisalabad Pakistan is working on the development of high yielding sunflower hybrids, resistant to insect pest and diseased suitable in existing cropping pattern of variable agro climatological condition of Pakistan.

17 hybrids viz., FH- 533, FH-557, FH-558, FH-572, FH-583, FH-585, FH-586, FH-587, FH-592, FH-593, FH-594, FH-595, FH-596, FH-598, FH-600 and two checks FH-331 and Hysun-33 were evaluated at this Institute for their performance under semi-arid conditions of Pakistan during autumn (August to November) 2014. The data depicted highest seed yield of 1950 kg per ha for the hybrid FH-586 and followed closely by Hysun-33 the check the seed yield of which was 1930 kg per ha. The 2nd check FH-331 yielded 1825 kg per ha. The important aspect shown by FH-586 was its early physiological maturity (14 days) than imported hybrid Hysun-33. The former matured in 77 days as compared to later (91) days. Early maturity helps farmers to prepare land for wheat crop, the best sowing time of which in Pakistan is 1st fortnight of November. It is also supportive in mitigating the import bill incurred on edible oil on one hand and maximizing wheat seed yield on the other hand. The entire advantage reaped due to its sowing during autumn is not only for Pakistan but for the entire humanity as the wheat grain produced in the process may be helpful for eradicating hunger in the world.

Key Words : Short duration, Physiological maturity, Import bill, High yield, Hunger eradication.

CYTOMORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF INTERSPECIFIC HYBRIDS BETWEEN *HELIANTHUS ANNUUS* AND H. *ARGOPHYLLUS* T. & G.

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ABSTRACT

Successful interspecific hybrids were obtained through sexual hybridization between cultivated *Helianthus annuus* (ARM-243B; 2n=34) and a wild *Helianthus* species [*H. argophyllus;* 2n=34*;* PI-468649], using the later as pollen parent for transferring desirable traits like downy mildew resistance, oil content and hopper resistant from wild species into cultivated background. Morphological, cytological and simple sequence repeats (SSR) based molecular analyses were carried out to confirm the hybrid nature of F1 plants. The hybrids exhibited morphological features intermediate to both the parents for few attributes and more related to wild *Helianthus* species like, leaf and stem hairiness, flower colour, stem size, branching, disc floret pigmentation, plant height, seed size and seed shape, etc. A reduction (89.9%) in pollen fertility was recorded in F1 plants as compared to both the parents. Meiotic analysis revealed a mixture of univalents, bivalents, trivalents and quadrivalents in all the pollen mother cells (PMCs) analysed. In addition to bivalents and univalents, a trivalent was also observed in few PMCs, indicating segmental homology between chromosomes. Higher level of chromosome configurations like quadrivalents was also observed in 42 out of 50 PMCs. Frequently observed chromosome configurations in diakinensis were 15 II + 1 IV and 13 II + 2 IV. The results suggested that the species *H. argophyllus* and *H. annuus* differ by 1-2 translocations and 1-2 inversions. Hybridity of interspecific hybrids was confirmed through sunflower specific molecular markers. Primers ORS-05, ORS-896 and ORS-908 were found to reveal highly polymorphic bands in the parents and were used for confirmation of hybridity of the F1s. The informative SSR markers screened in the study will be useful marker resources for tracking the flow of *H. argophyllus* genetic material among the progenies that may be produced by future backcrosses to *H. annuus*. Results show that the classical method of crossing is applicable in sunflower breeding programs for obtaining interspecies hybrids.

Key Words : sunflower, wild species, prebreeding, inter-specific hybridization

BROADENING THE GENETIC BASE OF CULTIVATED SUNFLOWER (*HELIANTHUS ANNUUS* L.) IN INDIA THROUGH PREBREEDING

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ABSTRACT

In India, sunflower production is severely constrained by heavy yield losses due to diseases like *Alternaria* ster leaf spot (*Alternaria ster helianthi*), downy mildew (*Plasmopara halstedii* (Farl.), powdery mildew (*Golovinomyces cichoracaerum*), rust (*Puccinia helianthi* Schw.) and sunflower necrosis disease. Further, susceptibility to water stress in rainfed cultivation results in low yields. Hence, recent years have witnessed a decline in the acreage under the crop mostly in the traditional sunflower growing regions. Among the various approaches to manage these stresses, host plant resistance is the most reliable and economical to the end users. With its large potential for export as confectionary or non-oilseed, there is a need to develop genotypes with specific quality characteristics. However, plant breeding efforts to develop varieties/hybrids with the desired economic characteristics are constrained by the narrow genetic base of the cultivated sunflower. Concerted efforts are required to incorporate additional genetic variability from reliable sources by integrating modern biotechnological tools and conventional breeding methods. Wild *Helianthus* species are rich sources of genetic variability in terms of resistance to biotic and abiotic factors, altered plant architecture, high yield, oil content, maturity duration, oil quality and continue to serve as sources of cytoplasm and fertility restorer genes. Prebreeding is required to broaden the genetic material potential for increased heterosis and to integrate useful genes such as resistance to biotic and abiotic stresses, better oil quality and higher yield performance into developed inbred lines. Successful introgression of desirable genes from the distantly related wild *Helianthus* into cultivated sunflower requires a clear understanding of the genome relationships of the wild *Helianthus* species and the cultivated sunflower through extensive genetic, cytogenetic, and molecular investigations. This review is mainly focused on the current status of the ongoing prebreeding work and the utility of the prebreeding materials developed in India for sunflower improvement.

Key Words : Sunflower, prebreeding, Helainthus species

MOLECULAR BREEDING FOR MAJOR DISEASES OF SUNFLOWER IN INDIA: PRESENT STATUS AND FUTURE NEEDS

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ABSTRACT

Sunflower is an important sources of vegetable oil in the world. The adaptability and versatility of the crop is being demonstrated by its cultivation from subtropical to sub arctic areas. Asia accounts for nearly 20–22% of the global sunflower and contributes to about 18% of the production. The productivity of sunflower in Asia is about 1.0 t/ha which is lower than the world average. India is the second largest grower of sunflower in the Asian continent. The major problems confronting sunflower productivity in India is the vulnerability of the crop to various biotic stresses. Climate change has lead to the evolution of minor diseases into epidemic status in sunflower. The major diseases of sunflower in India include *Alternaria* leaf spot (*Alternaria helianthi*), downy mildew (*Plasmopara halstedii* (Farl.), powdery mildew *(Golovinomyces cichoracaerum*), Sclerotinia rot (*Sclerotinia sclerotiorum*) sunflower necrosis disease (SND) caused by *Tobacco necrosis virus*, rust (*Puccinia helianthi* Schw.) and dry head rot (*Rhizopus* spp). Polygenic inheritance of resistance is reported in case of *Alternaria* leaf spot and powdery mildew. The resistance to *P. halstedii* is known to be controlled by dominant *Pl* genes, grouped in clusters (*Pl*1-*Pl*13 & *Pl*Arg) each conferring resistance to different races. Resistance for *P. helianthi* is reported to be controlled by seven genes *R*1-*R*5, *R*Adv and *Pu*6. Nature of resistance to Sclerotinia is described as partial, quantitative and mostly additive. Promising resistance sources for these fungal diseases have been found in wild sunflowers and exotic germplasm derived through interspecific hybridisation. Fine mapping of these diseases will aid in precision breeding. SND being a virus disease is transmitted by aphid *Myzus persicae* and*Capitphorus elaegni*, breeding for resistance against these vectors is known to reduce incidents of SND. In this review, emphasis is mainly focused on the current status of knowledge related to 'R' genes controlling resistance against major diseases of sunflower in India and their sources as well as markers associated with these genes.

Key Words : sunflower, diseases, breeding, markers

GENE EFFECTS AND COMBINING ABILITIES OF SUNFLOWER YIELD AND MORPHOLOGICAL TRAITS BY LINE X TESTER MATING DESIGN

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ABSTRACT

Recent changes in vegetable oil production in Uganda has realized that sunflower is now the main oilseed crop for producing vegetable oil. However the oil production is not enough for domestic needs and as such, much of the seed in production is basically from the imported sunflower hybrids. The development of sunflower hybrids with high genetic potentials for seed yield and other seed yield components requires information on the GCA and SCA for agronomically important traits. Understanding the genetic basis and mode of gene action for grain yield and important agronomic traits of sunflower would facilitate the improvement of sunflower production in Uganda. Choosing suitable lines for breeding as a parental component of a hybrid variety is of great importance. Seven CMS inbred lines used as females and six restorers used as males were crossed in a line x tester mating design to produce 42 single cross hybrids. Planting was done at the National Semi-Arid Resources Research Institute (NaSARRI) in 2013. The design was alpha-lattice (7 x 8) with three replications. One experimental hybrid (Belmonte) was used as a check. The traits recorded were days to 50% flowering, days to maturity, head diameter, plant height, number of seeds per head, and weight of seeds per head. The objective was to investigate the GCA and SCA effects of the F1 hybrids to the expression of the mentioned morphological characters.

Key words: Combining ability, gene effect, Line x tester design, Sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) has become the main oilseed crop in Uganda especially in the eastern and northern districts of the country. It is a source of livelihood for a number of resource poor farmers in these areas as the main immediate source of income. It is still the only main source of edible oil in the country followed by palm oil which was introduced into the country recently. By late 1980, Uganda was importing 98% of the total edible oil in the country. Considering the high oil content compared to other oilseeds, sunflower shows the greatest potential in reducing Uganda's dependence on imported edible oil. Its oil is used mainly as cooking oil and for soap making while the seed cake is being used as livestock feed. Most rural poor farmers and commercial producers in the sunflower growing areas obtain much lower yields than the expected potential yields due to lack of improved varieties and poor agronomic practices. There is severe lack of sufficient seed of acceptable varieties to meet the demand for the required plantings. Most of the released hybrids that have been released so far are being imported from South Africa. Their prices are expensive and not affordable by the poor resource farmers in Uganda.

Production of sunflower in a country needs genotypes that are widely adaptable so that it can be grown in a wider area and becomes of economic importance to the country. Breeders usually attempt to identify superior varieties for most of the characters of economic interest. A variety might be high yielding in a geographic region for which the breeder is evolving varieties but when employed as a parent in crosses, this variety may emerge as a poor combiner. In other words, this line does not appear to transmit desirable genes for the better

performance to its progeny. Such a behavior could result from intra- and/or inter-allelic interaction of genes concerned with the character. Thus superior performance of a variety is not always reflected in its combining ability.

The concept of combining ability is a measure of gene action. Combining ability analysis is used in the breeding programme for testing the performance of lines in hybrid combinations and also for characterizing the nature and magnitude of gene action involved in the expression of quantitative traits. General combining ability (GCA) of a line/variety refers to the average value of that line/variety estimated on the basis of its performance when crossed with other lines (Falconer, 1989). General combining ability is largely due to additive genetic effects and additive x additive epistasis. Meanwhile specific combining ability (SCA) is used to designate in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines crossed. Specific combining ability is largely a function of non additive dominance and other types of epistasis. The concept of general and specific combining ability is of practical importance to the breeders. It is therefore, in the interest of breeders to know how the two combining abilities are related to various components of heritable variations.

A successful breeding programme depends on the variability present among the different genotypes and in-depth understanding of the underlying gene action and genetic architecture of traits related to yield. Selection of parents based on their performance *per se* alone may not always be a sound procedure, since phenotypically superior genotypes may yield inferior hybrids and/or poor recombinants in the segregating generations. It is very important to identify parents with high general combining ability (GCA) value for the trait to be improved (Banerjee and Kole, 2009).

Information on gene action and combining ability helps in the choice of suitable parents for hybridization programmes for developing superior F_1 hybrids so as to exploit hybrid vigour and building genotypes to be used in the breeding programme. The objectives of the present study were to assess the nature and magnitude of gene action controlling the inheritance of seed yield and yield characters in selected sunflower genotypes.

MATERIALS AND METHODS

The sunflower lines were introduced from USA, Canada and Australia. Seven CMS inbred lines were used as females and six restorers used as males in a line x tester mating design to produce 42 single cross hybrids. Planting was done at the National Semi-Arid Resources Research Institute (NaSARRI), Serere in 2013. The design was alpha-lattice (7 x 8) with three replications. One experimental hybrid (Belmonte) was used as a check. Each plot had four rows and two middle rows were used for data recording. The spacing was 75 x 30 cm at a length of 4 m long. No fertilizer was applied since most farmers do not use fertilizer in their fields. Yield data was obtained from the two middle rows during harvest. The traits recorded were days to 50% flowering, days to maturity, head diameter, plant height, number of seeds per head, and weight of seeds per head.

RESULTS AND DISCUSSIONS

Analysis of variance is presented in Table 1. There was highly significant difference among the female lines for days to 50% flowering, days to maturity and plant height. No significant

difference was recorded for head diameter, number of seeds per head and seed weight per head. Similar observations were recorded for males whereby high significant differences were observed for days to 50% flowering, days to maturity and plant height. Female x male interaction only showed significant difference for days to 50% flowering and days to maturity.

Table 1: Mean square variances for the different traits studied

SoV	d.f	Days to 50% flowering	Days to maturity	Head diameter (cm)	No of seeds per head	Plant height (cm)	Seed weight per head (gm)
Rep	2	11.90***	6.16	10.89	127586	185	553
Female	6	9.47**	5.76***	9.34	73216	772***	352
Male	5	25.48***	4.32***	6.78	39936	987***	165
F x M	30	3.67***	0.74***	8.51	49920	231	226
Residual	60	1.20	3.20	8.05	83735	155	
Total	124	3.61		8.22		240	

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

Table 2 shows the mean performance of the single cross hybrids evaluated. Days to 50% flowering ranged from 57 to 63 days while days to maturity ranged from 88 days in Cms850 x RHA346 to 99 days in Cms850 x RHA374-1, Cms850 x RHA373 and Cms412 x RHA374-1. The highest number of seeds per head was recorded in Cms383 x RHA447 with 1291 mean number of seeds per head followed by Cms404 x RHA447 and Cms383 x RHA271. The tallest hybrid was cms403 x RHA374-1 which recorded 153 cm in height followed by Cms404 x RHA373. For seed weight per plant, Cms383 x RHA271 and Cms383 x CM632 had the highest seed weight per plant with 79 gms.

Table 2. Mean performance of the single crosses evaluated at Serere, 2013

Hybrids	Days to 50% flowering	Days to maturity	Head diameter (cm)	No of seeds per head	Plant height (cm)	Seed weight per head (gm)
Cms383x CM632	58	91	19	1112	129	79
Cms 402x CM632	61	95	14	704	109	46
Cms 403x CM632	60	96	13	662	112	44
Cms 404x CM632	60	98	16	959	111	67
Cms 412x CM632	61	95	20	905	133	72
Cms 433x CM632	60	91	15	870	119	53
Cms 850x CM632	58	91	16	1057	144	66
Cms383XRHA271	60	95	18	1208	136	79
Cms 402x RHA271	61	98	15	990	113	63
Cms 403x RHA271	61	98	15	691	116	45
Cms 404x RHA271	61	98	15	707	128	51
Cms 412x RHA271	60	96	18	941	141	66
Cms 433x RHA271	62	95	16	685	119	57
Cms 850x RHA271	58	89	16	1174	150	68
Cms383x RHA346	61	92	12	690	114	41
Cms 402x RHA346	60	96	15	880	130	50

Cms 403x RHA346	60	92	16	889	117	77
Cms 404x RHA346	60	97	17	1196	134	77
Cms 412x RHA346	62	96	14	703	129	37
Cms 433x RHA346	61	91	15	966	122	57
Cms 850x RHA346	59	88	14	615	121	47
Cms383x RHA373	61	98	15	719	142	57
Cms 402x RHA373	61	98	15	621	122	45
Cms 403x RHA373	63	98	14	612	115	37
Cms 404x RHA373	61	98	15	955	148	64
Cms 412x RHA373	61	95	16	778	139	48
Cms 433x RHA373	61	91	14	795	116	47
Cms 850x RHA373	63	99	18	1107	141	72
Cms383X RHA374-1	65	98	17	794	139	49
Cms 402x RHA374-1	63	98	14	750	133	44
Cms 403x RHA374-1	63	99	16	852	153	51
Cms 404x RHA374-1	62	98	16	939	145	70
Cms 412x RHA374-1	63	99	16	925	146	64
Cms 433x RHA374-1	63	98	14	643	137	43
Cms 850x RHA374-1	60	93	14	760	147	44
Cms383x RHA447	60	92	16	1291	141	70
Cms 402x RHA447	61	97	13	705	104	37
Cms 403x RHA447	60	97	16	951	138	61
Cms 404x RHA447	62	97	17	1284	136	72
Cms 412x RHA447	61	98	14	840	134	44
Cms 433x RHA447	60	91	16	969	128	65
Cms 850x RHA447	57	91	13	610	122	33

The GCA effects for females and males are presented in Table 3. For days to 50% flowering, Cms850 was the only female line that recorded significant negative GCA effect. In order to reduce the time for days to 50% flowering, HA850 could be useful. No male showed any significant difference in days to 50% flowering however, RHA374-1 could be used for increasing days to 50% flowering as days to 50% flowering is positively correlated to yield. All the female lines recorded highly significant difference ($P<0.001$) for days to maturity. Since days to maturity is positively correlated to yield, the females with positive GCA effects such as Cms402, Cms403, Cms404 and Cms 412 would be useful. In areas with less rainfall, Cms433 and Cms850 could be useful in the breeding programme. Among the males, highly significant difference ($P<0.001$) was also recorded. RHA373 and RHA374-1 had high positive GCA effects which could also improve yield. CM632 and RHA346 had high negative GCA effects which would be good for rainfall areas. No significant GCA effects were recorded for head diameter for the females and the males. However, Cms383, Cms404 and Cms412 among the females and CM632 and RHA271 among the males could be useful in improving the head diameter in the breeding programme. For number of seeds per head, only Cms404 had a significant positive GCA effect. For plant height, Cms402 was the only female line with a significant negative effect GCA effect. It could be useful in decreasing the plant height against lodging. Meanwhile, among the males, RHA374-1 had high significant positive ($P<0.001$) GCA effect. No any genotype among both the female and male lines had any positive significant GCA effect for seed weight per head. However, among the female lines, Cms383, Cms404 and among the male lines, CM632 and RHA271 could be useful in improving the yield performance of the sunflower lines.

Table 3. General combining ability effects for various yield components in sunflower at Serere

	Days to 50% flowering	Days to maturity	Head diameter (cm)	Number of seeds per head	Plant height (cm)	Seed weight per head (gm)
Females						
Cms383	0.096	-0.92***	0.68	99.82	3.5	6.4
Cms402	0.270	1.61***	-0.93	-94.13	-11.3**	-8.6
Cms403	0.253	1.59***	-0.48	-93.12	-4.7	-3.6
Cms404	0.123	2.50***	0.52	137.7*	3.8	10.4
Cms412	0.430	1.10***	0.96	-20.4	7.2	-1.0
Cms433	0.443	-2.46***	-0.54	-47.93	-6.2	-2.4
Cms850	-1.615*	-3.42***	-0.21	18.07	7.6	-1.2
SE	0.365	0.153	0.945	67.98	4.2	5.0
Males						
Cm632	-0.993	-1.59***	0.76	26.36	-7.4	5.0
RHA271	-0.487	0.37*	0.65	44.52	-0.6	5.1
RHA346	-0.733	-2.17***	-0.67	-20.81	-6.1	-0.9
RHA373	0.828	1.53***	-0.15	-70.90	1.8	-3.5
RHA374-1	2.041	2.39***	-0.20	-60.07	13.1***	-4.0
RHA447	-0.656	-0.53***	-0.39	80.90	-0.8	-0.5
SE	0.337	0.14	0.88	62.94	3.8	4.6

The SCA effects are presented in Table 4. SCA effects are indicators of dominance gene effect. For days to 50% flowering, positive SCA effects were observed in the crosses Cms433 x RHA271, Cms850 x RHA373, Cms383 x RHA374-1 and Cms404 x RHA447. For negative SCA effect, this was recorded in Cms383 x CM632, Cms403 x RHA374-1 and Cms850 x RHA447. This results in early flowering hybrids. For days to maturity, Cms850 x RHA373 had the highest positive SCA indicating that it had the highest maturity period. Meanwhile, Cms403 x RHA374-1 had the highest negative SCA effect indicating that it was the earliest hybrid in maturity. For number of seeds per head, Cms850 x RHA373 had significant ($P<0.05$) positive SCA effect followed by Cms850 x RHA271 and Cms383 x RHA 447. For plant height, negative significant SCA effect were recorded in Cms404 x CM632, Cms403 x RHA 374 and Cms850 x RHA 447 while positive significant SCA effect for seed weight per head was recorded in Cms403 x RHA346 and Cms850 x RHA373.

Conclusion

A number of genotypes have shown variability with desirable GCA and SCA effects that can be used in the sunflower breeding programme in Uganda. High variability is recorded especially in days to maturity.

Table 4: Estimates of specific combining ability effects for yield and yield components in sesame at Serere

Hybrids	Days to 50% flower	Days to maturity	Head diameter (cm)	No of seeds per head	Plant height (cm)	Seed weight per head (gm)
Cms383x CM632	-1.47*	-1.87***	2.41	116.2	2.8	11.7
Cms 402x CM632	0.50	-0.73**	-1.31	-97.5	-1.9	-6.7
Cms 403x CM632	-0.48	0.89**	-2.75	-140.4	-5.5	-13.2
Cms 404x CM632	0.53	1.65***	-0.42	-74.0	-15.5*	-4.6
Cms 412x CM632	0.64	-0.24	3.13	29.8	3.7	12.0
Cms 433x CM632	0.15	-0.31	-1.04	22.3	3.2	-5.6
Cms 850x CM632	0.13	0.62*	-0.02	143.7	13.9	6.3
Cms383XRHA271	-0.67	-0.17	1.19	194.6	3.4	11.8
Cms 402x RHA271	0.08	0.92***	-0.20	170.8	-4.7	10.4
Cms 403x RHA271	0.12	1.07***	-0.99	-130.0	-8.4	-13.2
Cms 404x RHA271	0.69	0.01	-1.65	-344.3**	-4.9	-20.8*
Cms 412x RHA271	-1.13	-0.54*	0.57	47.9	5.0	5.8
Cms 433x RHA271	1.48*	1.74***	0.74	-181.2	-3.7	-1.6
Cms 850x RHA271	-0.58	-3.03***	0.34	242.1*	13.3	7.6
Cms383x RHA346	0.54	-0.03	-3.49*	-258.5*	-13.6	-20.6**
Cms 402x RHA346	0.19	1.12***	1.45	125.5	17.6*	3.6
Cms 403x RHA346	-0.61	-2.45***	2.00	133.3	-2.4	25.6*
Cms 404x RHA346	-1.18	1.46***	1.68	210.4	6.5	11.0
Cms 412x RHA346	1.08	1.40***	-1.44	-124.7	-2.2	-17.6*
Cms 433x RHA346	0.56	0.24	0.39	165.5	4.7	4.5
Cms 850x RHA346	-0.58	-1.73***	-0.59	-251.5*	-10.8	-6.6
Cms383x RHA373	-0.59	2.36***	-0.68	-178.9	6.5	-2.5
Cms 402x RHA373	-1.14	-0.32	0.93	-82.7	1.7	0.8
Cms 403x RHA373	0.98	-0.13	-0.85	-92.8	-12.5	-11.8
Cms 404x RHA373	-0.62	-1.07***	-0.52	19.5	12.5	0.5
Cms 412x RHA373	-1.23	-2.64***	-0.63	0.4	0.2	-3.5
Cms 433x RHA373	-0.77	-3.42***	-1.13	44.0	-9.9	-3.6
Cms 850x RHA373	3.37*	5.22***	2.88	290.3*	1.5	20.1*
Cms383X RHA374-1	2.49***	1.35***	0.69	-115.0	-7.8	-9.6
Cms 402x RHA374-1	-0.24	-1.11***	-0.03	35.2	1.2	0.5
Cms 403x RHA374-1	-60.42***	-95.74***	-14.62***	-733.0***	-115.0***	-53.9***
Cms 404x RHA374-1	-1.05	-1.98***	-0.14	-8.0	-1.3	7.1
Cms 412x RHA374-1	0.06	-0.36	0.09	136.4	-4.3	12.6
Cms 433x RHA374-1	-0.52	3.06***	-0.74	-118.0	0.4	-6.5
Cms 850x RHA374-1	-1.07	-0.62*	-0.74	-66.7	-3.1	-6.5
Cms383x RHA447	-0.30	-1.63***	-0.12	241.6*	8.6	9.2
Cms 402x RHA447	0.61	0.12	-0.84	-151.4	-13.9	-8.7
Cms 403x RHA447	-0.35	0.96***	1.72	93.7	14.0	10.2
Cms 404x RHA447	1.63*	-0.08	1.05	196.4	2.6	6.8
Cms 412x RHA447	0.58	2.38***	-1.72	-89.8	-1.9	-9.4
Cms 433x RHA447	-0.90	-1.31***	1.77	67.3	5.2	12.7
Cms 850x RHA447	-1.27*	-0.45	-1.88	-358.0**	-14.8*	-20.8*
SE	0.63	0.26	1.64	117.8	7.2	8.7

SOURCE-SINK RATIO EFFECTS ON THE EXPRESSION OF GENES ASSOCIATED WITH GRAIN GROWTH IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Grain size is the result of the coordinated growth of the embryo, endosperm and maternal tissues. Understanding the clues of the development and growth of these tissues is essential for increasing grain weight, a key component of sunflower yield and quality. This research was aimed at evaluating the effect of pre-anthesis shading (source-sink ratio reduction) on grain growth and the expression of genes associated with grain size between R3 and physiological maturity in sunflower. Two sunflower genotypes contrasting in grain weight were sown in a split plot design with three replicates. Shading treatments (nets intercepting 80% of incident radiation) were set over the plots from R3 to R5 stage. Ovaries and grains (the last divided in pericarp and embryo) were sampled from R3 to R9 stage. RNA was extracted from ovary and grain tissues. The time-course of the expression of putative orthologous genes for sunflower of HaGW2 (RING-type E3 ubiquitin ligase-like) and HaAP2 (EREBP-like), were assessed by qPCR. Grain weight was affected ($P < 0.05$) by both genotype and shading treatments. The lower source-sink ratio decreased final grain weight. Interestingly, the expression of HaGW2 and HaAP2 genes was affected by the genotypes and the source-sink ratio in flowers and grains tissues across the developmental stages. Results presented here suggest that HaGW2 and HaAP2 genes act in the pericarp and might be involved in driving the growth of grains in this crop.

Key words: Sunflower, grain weight, grain size, genetic control, AP2, GW2.

INTRODUCTION

Grain weight in crop plants is an important agronomic trait and a key component of sunflower yield. Grain development requires a double fertilization event generating two products within the embryo sac: the embryo and the endosperm (Lopes and Larkins, 1993). The embryo is surrounded by the endosperm, which, in turn, is enclosed within the maternal seed coat. The grain size is regulated by the coordinated growth of the embryo, endosperm, and maternal tissues (Fang et al., 2012; Xia et al., 2013).

In recent years, the knowledge about grain development improved considerably, and some genetic and molecular mechanisms are now known, mainly in model plants (Sundaresan, 2005; Sun et al., 2010; Li and Li, 2015; Orozco-Arroyo et al., 2015). However, in grain crops like sunflower this knowledge is still partial. Grain size is affected by the

maternal and/or zygotic tissues. It is known that in various crops the grain weight and grain size has a polygenetic control (Zhang et al., 2012; Kesavan et al., 2013). In *Arabidopsis* APETALA 2 (AP2) encodes a member of the AP2/EREBP (ethylene responsive element binding protein) may restrict grain growth by limiting cell proliferation in the integuments (Jofuku et al., 2005; Ohto et al., 2005). Ap2 mutant grains exhibit delayed cellularization of the endosperm resulting in larger embryo sacs and bigger embryos that show increased cell number and size and this larger grain trait was passed through the maternal sporophyte and endosperm genome (Jofuku et al., 2005; Ohto et al., 2005, 2009).

The role of the ubiquitin pathway on the grain size determination has been widely investigated over the last years in *Arabidopsis* (Li and Li, 2014). Several members involved in this pathway have been identified. DA1 and DA1-related (DAR) encode for plant-specific ubiquitin receptor protein. DA1 protein might act antagonistically with native DA1 or DAR, and would be negatively regulating cell proliferation in maternal grain tissues (Li et al., 2008). DA2 and enhancer of DA1 (EOD1) encode protein with E3 ubiquitin ligase activity and are also negative regulators of grain size (Xia et al., 2013). In rice GW2 (RING-type E3 ubiquitin ligase) functions as a negative regulator of grain width and weight, the loss of function of GW2 leads to increased cell number, a widers pikelet hull and an accelerated grain milk-filling rate, which increases grain width, grain weight and yield (Song et al., 2007). In recent years, extensive studies of GW2 have been carried out in wheat (Su et al., 2011; Yang et al., 2012; Zhang et al., 2013; Qin et al., 2014; Simmonds et al., 2014, 2016; Hong et al., 2014). Most of them reported that GW2 is a negative regulator of grain size and weight, except the study accomplished by Bednarek et al. (2012), where the authors reported the positive effect of GW2.

In the last decades, many key regulators of grain size have been identified; however, it is still limited the knowledge on genetic control of grain size and weight in crops, and this knowledge is virtually lacking in sunflower. The present research was aimed at evaluating the effect of pre-anthesis shading (source-sink ratio reduction) on flower and grain growth as well as the expression of genes associated with grain size between R3 and physiological maturity in sunflower the dynamics of grain dry matter accumulation and grain dimensions were analyzed in parallel with the expression of AP2 and GW2 genes.

MATERIALS AND METHODS

Field site description, treatments and experimental conditions

A field experiment was conducted at the Agricultural Experimental Station (EEAA) of Universidad Austral de Chile in Valdivia (39°47´S, 73°14´W, 19 m ASL), Chile in the 2014-2015 growing season. Two genotypes contrasting in grain weight and with similar phenology were arranged in a split plot design with three replicates, where the source-sink manipulation treatment was assigned to main plots and genotypes to subplots. Plant density was 6 plants m^{-2} and the dimension of plots (experimental units) was 9 rows at 0,70 m apart. 20 plants were sown per row. The treatments were the outcome of combining (i) two genotypes and (ii) two source-sink rates (control without shading and shading treatment by nets intercepting 80% of incident radiation, imposed during the pre-anthesis period, from R3 to R5). Genotypes were Alybro from Panam Seeds of small size grain (oilseed) and RHA-280 of big size grain (confectionery), from NCRPIS, USDA-ARS.

Sampling and grain measurements

Phenology was recorded by using the scale proposed by Schneiter and Miller (1981). Flowers and grains (the last separated in pericarp and embryo) were sampled from R3 to R9. Samples were individually removed, and were immediately frozen in liquid nitrogen. Frozen samples were stored at -80 °C until analysis. Dry matter, water content and dimensions

(length, width, and height) of flowers and grains were evaluated every three days. Final grain weight, dimensions, and the timing of physiological maturity were estimated using a linear model as in Calderini et al. (1999). The model was fitted using the iterative optimization technique of TableCurve 2D V5.01 (Systat Software Inc., Chicago, IL, USA).

Search and isolation of candidate gene sequences

To find sequences of genes AP2 and GW2 in sunflower we conducted a search in the sunflower transcriptome database Heliagene (https://www.heliagene.org/) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/), based on orthologous genes from *Arabidopsis* and rice. Using the "ExpressionPatterns" tool available in Heliagene, we chose candidate gene sequences, which had greater expression in grain tissue. Sequences were analyzed by blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Putative proteins were predicted by ExPASy (http://web.expasy.org/translate/). A protein analysis was conducted by InterPro (https://www.ebi.ac.uk/interpro/) and Conserved Domain Database (http://www.ncbi.nlm.nih.gov/cdd) to verify the structure of the protein, domains and conserved sites and ensure that the sequences corresponding to the putative genes.

Real-time (qPCR) expression analysis

Total RNA was extracted from flower ovaries, pericarps and embryos from control and shaded plants using the kit NucleoSpin® RNA Plant (Macherey-Nagel). It was treated with DNase I (Invitrogen). First strand cDNA synthesis was performed using an Affinity Script cDNA Synthesis Kit Reverse Transcription System (Agilent Technologies) following the manufacturer's instructions. Three biological replicates for each sampling date were used. Specific 5′-3′ primers for AP2, GW2 and β-tubulin (as internal control) genes from Heliagene database, were designed using Primique (Fredslund and Lange, 2007) (http://cgi-www.daimi.au.dk/cgi-chili/primique/front.py), with high stringency to avoid amplification of non-specific PCR products. All primers were synthesized by Macrogen Inc. (South Korea) (http://www.macrogen.com/). Primer pair sequences were: AP2: AGGATGGGCCAATTTTTAGG (forward), ATGGCAGCCTTATCATACGC (reverse), GW2: GAAGCCATCTGGTTGTCGTT (forward), TGGATGCTAAGAGGCGAACT (reverse), and β-tubulin: GGCGTCTACCTTCATTGGT (forward), TCCATCTCATCCATTCCTTC (reverse) (Meimoun et al., 2014). The amplicon sizes were 92 bp for the AP2 gene, and 115 bp for GW2 gene.

The amplification reactions were performed using Brilliant II SYBR® Green QPCR Master Mix (Agilent Technologies) according to the manufacturer's instructions in an AriaMx real-time PCR system (Agilent Technologies Inc., Santa Clara, CA, USA). PCR conditions were: 95°C for 10 min; 35 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 15 s. No template control (NTC) and no reverse transcriptase control (no-RT) were included for detecting gDNA contamination. A dilution series was built to estimate the amplification efficiency using a cDNA mix as template prepared from control ovaries samples (-14 to 0 days after anthesis). Each reaction was performed in triplicate, and a negative water control was included in each run. Fluorescence was measured at the end of each annealing step. The amplification efficiency was estimated through a melting curve and amplification products were visualized on agarose gels (1.5%, w/v). The relative expression levels were first normalized against the β-tubulin gene and using non-shading samples from day one as calibrator, with a nominal value of 1. The method described by Livak and Schmittgen (2001) was used to make all calculations.

Statistical analysis

The recorded data were assessed by two-way analysis of variance. LSDs were calculated using STATISTICA v7.0 (StatSoft Inc.) and used for mean separation ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The final grain weight of peripheral grain position measured at physiological maturity stage showed a wide range (58.2 – 148.8 mg) of values (Table 1). Both grain weight and dimensions (length, width and heigh) were significantly affected by genotype ($p \leq 0.001$) and the source-sink ($p \leq 0.001$) treatments (Table 1). Previous studies demonstrated that grain weight and grain number are sensitive to shading during pre-flowering in sunflower (Cantagallo and Hall, 2002; Alkio et al., 2003; Cantagallo et al., 2004; Lindström et al., 2006). Our results confirm the high sensitivity of sunflower under strong source shortage at the immediately pre-anthesis stage. These results support that grain traits are set at pre-anthesis, therefore, the size of the ovary would determine the potential weight of grains (Cantagallo et al., 2004; Rondanini et al., 2009) like in wheat (Hassan et al., 2011). The dynamic of dry weight of peripheral ovaries and grains from R3 to maturity is shown in Fig. 1 a, b.

Peripheral grain weight showed a positive relationship with grain length ($r^2 = 0.92$; $P < 0.05$), grain width ($r^2 = 0.98$; $P < 0.05$) and grain height ($r^2 = 1$; $P < .0.001$). Taking into account the close associations shown by the final grain weight and dimensions, especially the height and width, these would seem to be crucial for grain weight determination. In fact, studies by Lizana et al., 2010; Hasan et al., 2011 in wheat show that length grain is very important in final grain weight determination. Unlike wheat where length grain shows better associaciations with grain weight, in sunflower it seems the height and width would be more important in grain weight determination, probably because they are different species, and different architecture grains.

Table 1. Final grain weight, grain dimensions (length, width and height) of peripheral grain position in Alybro and RHA-280 genotypes measured at in physiological maturity.

Genotype	Treatment	Length	Width	Height	Final grain weight (mg)
RHA-280	Control	13,01 a	9,80 a	6,49 a	148,78 a
	S-S	12,30 b	8,40 b	5,21 b	106,97 b
Alybro	Control	10,96 c	6,78 c	4,35 c	79,20 c
	S-S	9,62 d	5,54 d	3,72 d	58,15d
Genotype		***	***	***	***
Treatment		***	***	***	***
Genotype x treatment		***	ns	***	ns

Different letters indicate LSD test differences ($p < 0.05$). S-S: source-sink treatment. *** Mean significant at 0,001 probability level; ns: non-significant.

The putative sequences of AP2 (accession: HaT13l014337) and GW2 (accession: HaT13l009504) were obtained from Heliagene database (https://www.heliagene.org/). Both two sequences in sunflower were named HaAP2 and HaGW2 respectively. These sequences encoding proteins similar to *Arabidopsis* and grape vine (*Vitis vinifera* L.) respectively. The complete coding sequence of HaAP2 shares 100% identity with APETALA 2 (AP2) of *Arabidopsis* (NCBI accession: NP_195410) (Mayer et al., 1999), and the analyses of the protein sequence predict an AP2-like ethylene-responsive transcription factor, DNA-binding domain and AP2/ERF domain. Regarding HaGW2, the sequence shares 61% identity with RING-type E3 ubiquitin ligase (VvGW2) from grape vine (GenBank accession: AII80417.1). The protein analyses of HaGW2 predict a Zinc finger, RING-type domain. These results

support that the sequences evaluated in this study correspond to the HaAP2 and HaGW2 putative sunflower genes.

To investigate the source-sink manipulation effect on HaAP2 and HaGW2 candidate genes and grain development, we evaluate genes expression at eight times from R3 (14 days before anthesis) to R9. (32 days after anthesis), i.e. three times in pre-anthesis, and five times in post-anthesis (Fig. 1). Our results show a differential expression of the two genes between genotypes and among grain tissues.

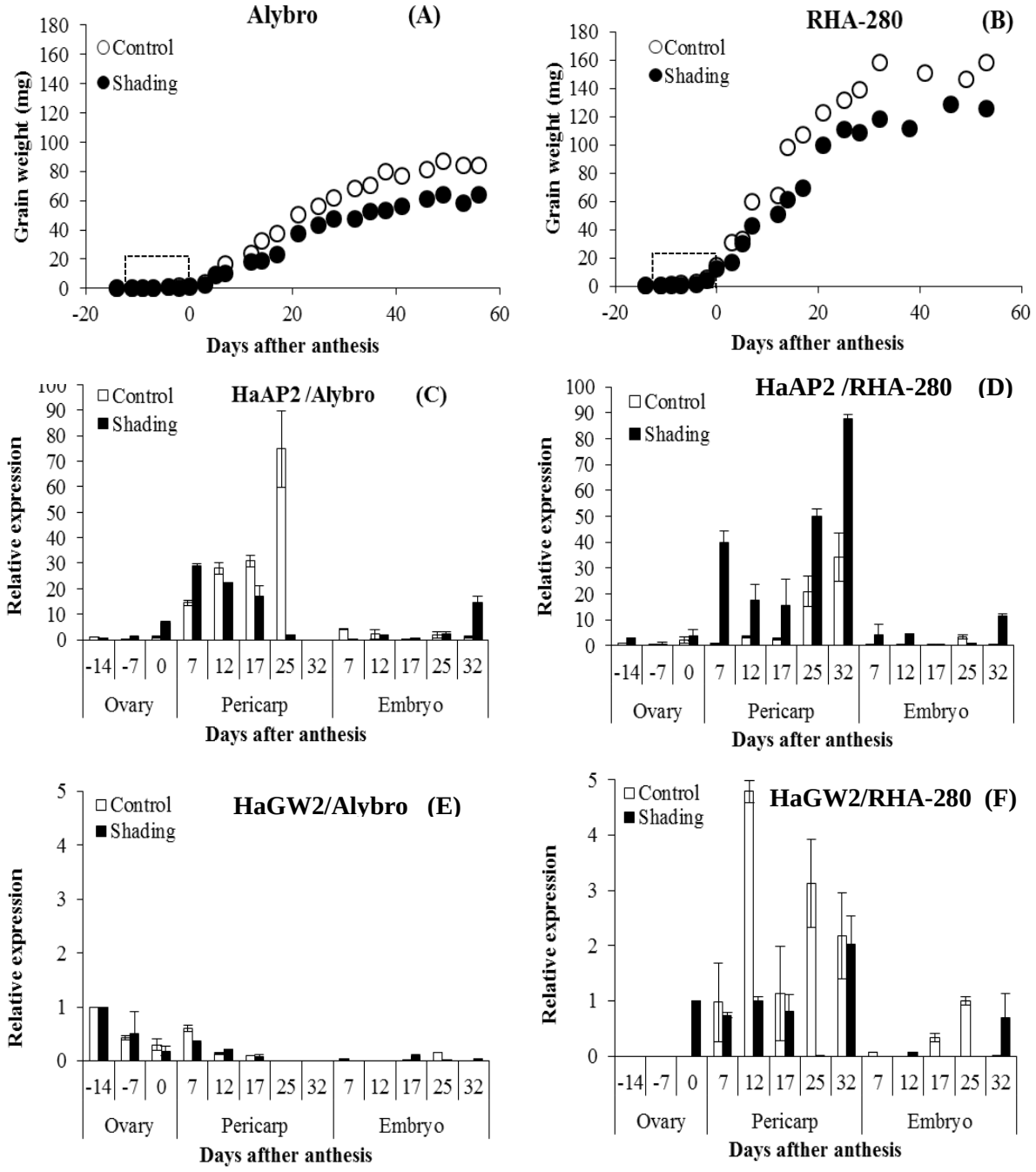

Figure 1. Relative expression of HaAP2 and HaGW2 genes in growing grains of contrasting grains weight genotypes of sunflower before and after anthesis under source-sink manipulation. Time-course of grain dry weight in Alybro (a) (small grains) and

RHA-280 (b) (large grains). Bar dashed line indicates the shading period. Relative expression of HaAP2 in Alybro (c) and RHA-280 (d). Relative expression of HaGW2 in Alybro (e) and RHA-280 (f). Bars on the graphs indicate the standard error. Open symbols indicate the control plants and filled symbols indicate shaded plants.

In control plants the HaAP2 gene showed higher expression in the genotype Alybro (small grain) than in genotype RHA-280 (large grains) (Fig. 1c, d). The expression of HaAP2 in both genotypes was mainly detected in the pericarp tissues, in Alybro from 7 to 25 days after anthesis, while in RHA-280 a longer expression was found (until 32 days after anthesis).

Shaded plants and control plants of Alybro showed similar expression pattern of HaAP2 in pericarp tissues, however, the relative expression in shade plants was lower compared to controls plants between 12 and 25 days after anthesis (Fig. 1c). Surprisingly, higher expression levels in shade plants were found in RHA-280 compared to controls, mainly in the pericarp tissues (Fig. 1d). This higher expression under shading of the large grain genotype parallels with the lower grain weight dynamic and lower final grain weight (Fig. 1d Tabla 1.). These results suggest that this gene may be acting in the pericarp maternal tissues, downregulating the growth of grains. Our results agree with previous studies of AP2, where that the authors suggest that AP2 negatively regulate the size of *Arabidopsis* grains. AP2 may restrict grain growth by limiting cell proliferation and cell expansion in the integuments (Jofuku et al., 2005; Ohto et al., 2005, 2009). In addition, AP2 is required for ovule and seed coat development (Leon-Kloosterziel et al., 1994; Modrusan et al., 1994; Jofuku, 1994).

HaGW2 gene showed low expression in control plants in Alybro genotype (small grain) (Fig. 1e), whereas in RHA-280 genotype (large grain) a higher expression was observed (Fig 1f). The expression of this gene also was mainly detected in the pericarp tissues in RHA-280 and Alybro, although with lower expression in the last genotype, suggesting that the HaGW2 gene might positively affect grain size and weight. The greatest expression of HaGW2 found in control plants of RHA-280 was observed early after anthesis (from 7 to 12 days after anthesis) in agreement with the linear growth phase of the ovule and ovary (Lindström and Hernández, 2015). The expression of GW2 in the pericarp decreased at 12 days after anthesis, when these tissues reached the final size and weight. In shaded plants of Alybro, the HaGW2 gene expression was similar to the controls (Fig. 1e), however, in RHA-280 a lower expression profile was observed in the reduced source-sink treatment compared to the controls (Fig. 1f). Similarly, the expression was tissue-specific in the pericarp. The dry weight of grains under shading was lower than controls during grain filling (Table 1, Fig. 1b), supporting upregulation of this gene in the growth of the pericarp tissue in sunflower.

In plants, the ubiquitin pathway has recently been shown to play important roles in seed size control (Li and Li, 2014), In *Arabidopsis* was reported that DA1, an ubiquitin receptor with two ubiquitin-inter- acting motifs (UIMs), and a single zinc-binding LIM domain (Li et al., 2008), acts as maternal control of seed size. DA1 regulates seed growth by limiting cell proliferation in the maternal integuments of developing ovules and seeds (Li et al., 2008; Xia et al., 2013). In the present study, we found evidence that the HaGW2 gene, a putative RING-type E3 ubiquitin ligase, would be acting in maternal tissues of sunflower, because primarily observed expression in pericarp tissue, but different to most studies that have examined the ubiquitin pathway as a negative regulator of seed size in cereals (Song et al., 2007; Li et al., 2008; Hong et al., 2014; Simmonds et al., 2016), we observe evidence of upregulation. The HaGW2 gene seems to have a positive effect on grain weight and size in sunflower, in wheat, where TaGW2 has been extensively studied in recent years, there are controversial results about the regulation of this gene on grain size (Bednarek et al., 2012). Therefore, it is likely that HaGW2 upregulates the weight and size of sunflower grain.

CONCLUSIONS

A clear association was found in this study between grain growth dynamics and the expression of HaAP2 and HaGW2 putative genes. The expression, tissue-specific pericarp suggests that these genes would be acting by maternal tissues. The present study provides clear evidences on the genetic control of grain growth and could be helpful to improve the knowledge f of grain weight and grain size determination in sunflower.

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PRODUCTIVITY AND QUALITY TRAITS OF SUNFLOWER INBRED LINE COLLECTION OF KAZAKHSTAN

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ABSTRACT

Assessment of 43 restore (R) lines, 36 male – sterile (A) lines and 44 maintainer (B) lines on seeds quality and productivity was done for the searching linkage between economic valuable traits and protein, molecular characteristics. 41 lines were selected as sources for breeding hybrids with high level of oil content, weight of 1000 seeds, total seed number per head and low percent of seeds hull. Screening purity of inbred lines on the base of seeds storage protein electrophoreses revealed 3 heterogeneous lines among restore lines collection (VKU 34RR, VKU 250R, VKU 360R), 3 heterogeneous mail-sterile lines (CMS): VKU 270 A, VKU 116 A, VKU 136 A and 5 heterogeneous maintainer lines : VKU 1B, VKU 183B, VKU 108B, VKU 286B, VKU 110B. According to helianthinin spectra in all sets of inbred lines 4 types of band composition were revealed. Ratio of 1: 2: 3: 4 types in a set of R lines was 79,1%; 4,7%; 11,6% and 9,3%, in a set of A lines 56,4%; 7,7%; 25,6% and 10,3%, in a set of B lines – 61,3%; 4,5%; 25%; 9,0%.

Key words: sunflower, inbred lines, seeds quality, productivity, line purity

THE EFFECT OF SOWING DATE AND DENSITY ON CALLUS INDUCTION AND SHOOT REGENERATION FROM SUNFLOWER ANTHERS

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ABSTRACT

The success of anther culture depends on numerous factors such as genotype, donor plant growing conditions, anther pre-treatment and development stage, as well as incubation conditions. We have investigated the effect of sowing date and sowing density of donor plants on callus induction and shoot regeneration from cultivated sunflower anthers. Anthers were collected from of three commercial sunflower hybrids that were sown in four different sowing dates, and at three different sowing densities. Anthers were surface sterilized and placed on MS-medium based solid regeneration media. The appearance of organogenesis or somatic embryogenesis was observed and obtained data statistically analysed. The experiment was set as completely randomised, with two factors. Callus, somatic embryo, shoot and root regeneration on the anthers of the tested genotypes was observed. Data were analysed by ANOVA. Statistical analysis enabled us to determine effect of sowing date and density on anther culture and shoot regeneration induction. Sowing date had a significant effect on all observed parameters, with earlier sowing dates having significant positive effect on shoot regeneration. Sowing density had no effect on either of observed parameters in all tested genotypes. The obtained results will contribute to the better understanding of the conditions needed for haploid production in sunflower and its introduction in sunflower breeding programs.

Key words: Anther culture, Dihaploid, Donor plant, Regeneration, Sunflower

INTRODUCTION

Anther culture results in sunflower (*Helianthus annuus* L.) have been rather unsatisfactory up to now (Marinković et al., 2003). As in other species, anther culture response of sunflower is strongly affected by numerous factors such as genotype, donor plant growing conditions, anther pre-treatment and development stage, as well as incubation conditions (Gurel et al, 1991; Miladinović et al, 2012). By testing a number of different parameters, that is, donor plant growing conditions and stages, as well as culture media and conditions, appropriate protocol could be worked out for the successful regeneration of shoots - at least for a number of genotypes.

Various environmental factors that the donor plants are exposed to may affect haploid plant production. Light intensity, photoperiod, and temperature have been investigated, and at least for some species, these are found to influence the number of plants produced from anther cultures (Reed, 2005). Seasonal variations have been reported to influence anther response in *Triticum aestivum* (Ouyang et al., 1987) and *Solanum tuberosum* (Tiainen, 1992), while different temperature regimes were found to affect anther response in wheat hybrid plants

(Orshinsky and Sadasivaiah, 1997). Growing season and conditions, as well as donor plant age had an effect on anther culture of *Capsicum annuum* (Ercan et al., 2006; Buyukalaca et al, 2004).

Up to our knowledge, there are no reports on effect of donor plant growing conditions on sunflower anther culture. There are only reports on influence of medium composition variation on the frequency of anther callusing and/or somatic embryogenesis and subsequent plant regeneration (Marinković et al., 2003; Miladinović et al., 2012). Different compositions of media used for establishing anther culture were extensively reviewed by Friedt et al. (1997), and variation of other culture parameters by Nichterlein and Horn (2005).

We have investigated the effect of sowing date and sowing density of donor plants on callus induction and shoot regeneration from cultivated sunflower anthers.

MATERIAL AND METHODS

Anthers were collected from three commercial sunflower hybrids (NS Oskar, NS Fantazija, and Orfej). The hybrids were sown in four different sowing dates and at three different showing densities (30,000; 50,000 and 70,000 plants per ha).

After collection, anthers were surface sterilized and placed on solid regeneration media, supplemented with basic MS macro and micro salts (Murashige and Skoog, 1962), 0.3% gelrite, pH 5.7, while composition of hormones varied (Vasić et al., 2000; Miladinović et al., 2012). Anthers were cultured in the dark at 30^{o}C.

Two experiments were set as completely randomized, with two factors. In the first experiment factors were sowing date and genotype of donor plants, while in the second experiment factors were sowing density and genotype. Callus, somatic embryo, shoot, root and plant regeneration on the anthers of the tested genotypes was observed. The data were transformed by *arc sine* transformation in order to obtain normal distribution of their frequencies, which is required for further statistical analysis. Analysis of variance and Fisher's least significant difference test were performed in statistical program STATISTICA 12.0 (StatSoft Inc., 2013) in order to establish the significance of factor effects and their interaction, and significance of difference among treatments. Based on results of ANOVA, in order to estimate the relative importance of examined sources of variance, expected variances and their contribution to the total variance were calculated.

RESULTS AN DISCUSSION

Sowing date

Regarding contribution of components of variance to the total variance, sowing date had the highest effect on root regeneration since its variance contributed to over 50% of total variance (Figure 1). It also had the strongest effect on callus formation, as well as shoot and plant regeneration, contributing to 45%, 20% and 15% of the total variance, respectively. The effect of sowing date and interaction was similar for embryo formation, as their variances contributed approximately to 25% of total variance, each.

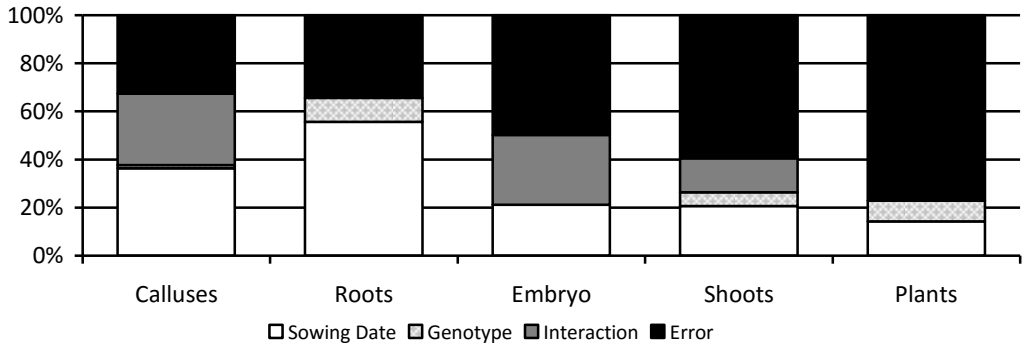

Figure 1. Contribution of expected variances of tested variation sources to the total variance (%)

Although sowing date significantly affected only plant regeneration, LSD test showed that earlier sowing dates had more positive effect on all observed parameters (Table 1). This especially stands for shoot and plant regeneration, as there were no regenerants from anthers collected at later sowing dates. Anthers collected from the plants sown at the earliest planting date had the best androgenic response, as they formed the highest number of calluses and embryos, and had the highest percentage of regeneration of roots, shoots and plants. Genotype had significant effect on shoot and plant regeneration. LSD test indicated that there was no significant difference among tested hybrids for embryo formation.

Table 1. LSD test for percentage of callusogenesis, somatic embryogenesis, root, shoot and plant regeneration at different sowing dates

		Calluses	**Roots**	**Embryos**	**Shoots**	**Plants**
		p	p	p	p	p
Date		0.000	0.000	0.000	0.009	0.071
Genotype		0.027	0.025	0.025	0.606	0.119
Date*Genotype		0.009	0.562	0.562	0.035	0.448
Variant	**Genotype**					
DATE I		86.809a	27.898a	1.612a	0.974a	0.374a
DATE II		83.351a	32.995a	0.784a	0.244ab	0.042ab
DATE III		48.322b	8.441b	1.008a	0.000b	0.000b
DATE IV		52.103b	2.950b	0.014b	0.000b	0.000b
	OSKAR	79.006a	24.313a	0.747a	0.000b	0.000b
	FANTAZIJA	70.243ab	10.526b	0.868a	0.457a	0.211a
	ORFEJ	56.924b	13.811b	0.442a	0.189ab	0.023ab

The values within the same column marked with different letters differed significantly at $\alpha_{0.05}$

Sowing density

Sowing density did not have any effect on the observed parameters (Figure 2). Genotype had the strongest effect on callus formation, as it contributed to 55% of total variance. Embryo formation was equally influenced by genotype and interaction (25% of total variance, each) while interaction had the highest effect on root regeneration (20% of total variance).

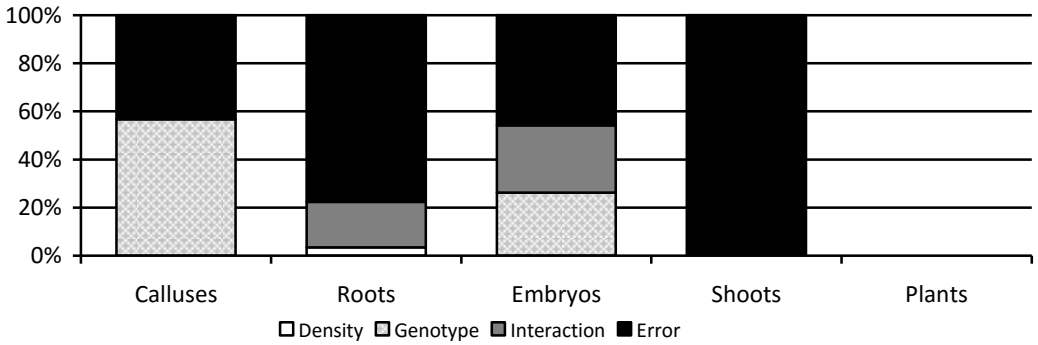

Figure 2. Contribution of expected variances of tested variation sources to the total variance (%)

Analysis of variance showed that sowing density generally had significant effect on tested parameters, but that there were no significant differences among different densities (Table 2). Interaction was significant for all tested traits, while genotype had significant effect on root and shoot formation.

Table 2. LSD test for percentage of callusogenesis, somatic embryogenesis, root, shoot and plant regeneration at different sowing densities

		Calluses	**Roots**	**Embryos**	**Shoots**	**Plants***
		p	p	p	p	p
Density		0.428	0.273	0.863	0.387	-
Genotype		0.000	0.816	0.003	0.387	-
Density*Genotype		0.961	0.186	0.055	0.433	-
Variant	**Genotype**					
30,000		78.057a	19.743a	0.510a	0.000a	-
50,000		70.928a	17.376a	0.694a	0.000a	-
70,000		78.131a	22.920a	0.420a	0.042a	-
	OSKAR	70.408b	19.419a	0.355b	0.000a	-
	FANTAZIJA	63.339b	21.206a	2.182a	0.000a	-
	ORFEJ	90.220a	19.291a	0.014b	0.042a	-

The values within the same column marked with different letters differed significantly at $\alpha_{0.05}$
*This parameter did not vary in some variants, so it was not possible to do variance analysis.

In our study, we have found that sowing date had an effect on establishment and plant regeneration from sunflower anther culture. Higher regeneration frequencies were obtained with plants from earlier sowing dates. The prevailing temperature during the growth of donor plants is reported to play a crucial role in microspore embryogenesis in Crucifers (Pratap et al., 2009). A high frequency of embryogenesis was consistently obtained in donor plants grown at low temperatures (Keller et al., 1987; Dunwell et al., 1985). This could be the reason for better regeneration frequencies in earlier sowing dates in our experiment, as low temperature is thought to increase the number of microspores suitable for embryogenesis due to slow pollen development, and it also prolongs the duration for which suitable microspores are available in a crop (Pratap et al., 2009). The opposite results were observed in wheat anther culture where embryo regeneration was usually greater when anthers were obtained from plants grown at high temperatures than plants grown at lower temperatures (Orshinsky and Sadasivaiah, 1997).

The lack of effect of sowing density on the observed parameters indicates that in our experiment irradiation and the temperature within the canopy were not important for androgenic response in tested hybrids, and that sowing date and temperature conditions during the plant growth have greater effect on this trait.

The results obtained in our study indicate that although genotype plays an important role in sunflower anther culture, the regeneration frequency could be improved by taking care of growing conditions of donor plant. Future studies should be focused on further optimisation of donor plants growing conditions in order to minimize genotype effect and sunflower androgenic potential and enable creation of the environment that will favour haploid plant regeneration.

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DEVELOPMENT OF SUNFLOWER NECROSIS VIRUS (SNV) DISEASE IN SOUTH INDIA

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ABSTRACT

Sunflower is the fourth important oilseed crop in India, with the major area concentrated in its southern states viz. Karnataka, Andhra Pradesh, Maharashtra & Tamilnadu. The crop is largely cultivated under rainfed conditions in these states. Besides abiotic stress, the biotic factors *viz.* viral and fungal diseases directly impact the yield. Sunflower Necrosis Virus Disease (SNV) is reported as the most devastating disease in south India. It was observed for the first time in year 1997 at village Bagepally, in Kolar District, Karnataka State. The disease is caused by Tobacco Streak Virus (TSV) belonging to Genus – *Ilarvirus*, Family - Bromoviridae. Natural infection of the virus on peanut, cotton, green gram, okra, soybean and marigold crop has been reported in India. The virus is naturally transmitted through pollen of weed host *viz. Parthenium hysterophorus* with the aid of *Thrips sp.* Temperature between 25-35 ^{o}C and moderate relative humidity is favourable for spread of *Thrips* vector and TSV. Disease incidence is high in wet and winter cultivation. Key field symptoms are distortion and necrosis of leaf, stem and head. Early infected plants remain stunted and develop malformed heads with chaffy or no seeds. Late infected crop has poor seed setting. Yield losses ranging from 30 to 100% have been reported due to SNV disease in South India. Considering the availability of alternate host crops and *Parthenium* weed prevalence throughout the country, there is a potential threat of spread of SNV in central and north India.

Key words: Sunflower, SNV, TSV, *Parthenium*, Thrips

INTRODUCTION

Sunflower is an important oilseed crop in India, besides groundnut, mustard & soybean. It is cultivated on 6.7 million hectares area (Annon. 2014) in the country with majority of cultivation in the Southern states viz., Karnataka & Andhra Pradesh followed by Maharashtra and Tamilnadu. In North India Sunflower is cultivated in state of Punjab and some parts of North Uttar Prasesh in spring season.

The productivity of sunflower is impacted by both abiotic and biotic stresses. Fungal diseases *Alternaria* blight and Powdery mildew occur in wet and winter season respectively. Among the viral diseases, Sunflower Necrosis Virus Disease is one of the most devastating diseases in South India and is observed in both wet and winter season. The disease was observed for the first time in 1997 in a seed production field near the village of Bagepally, Kolar District, Karanataka State, India (Singh et al., 1977). In subsequent years, outbreaks of this disease in major sunflower-growing states of India, especially Andhra, Karanataka and

Maharashtra, have virtually threatened the sunflower cultivation and yield losses ranging from 30 to 100% have been reported (Chander Rao et al., 2000).

EPIDEMIOLOGY & SYMPTOMS

The Sunflower Necrosis disease is caused by Tobacco Streak Virus (TSV) belonging to the Genus – Ilarvirus, Family - Bromoviridae.(Ravi.et.al., 2001). The virus is reported to naturally infect peanut, cotton, green gram, okra, soybean and marigold crop. Weed sp. *Parthenium hysterophorus* is the common alternate host for the virus. The major method of transmission of TSV is by infected pollen, which can spread by wind or carried by insect vector Thrips sp. (Harvir Singh 2005). Transmission of TSV to plants depends on entry of virus from the infected pollen in plant cells through the feeding injury caused by thrips. Temperature between 25-35 ^{o}C and moderate relative humidity is favourable for spread of Thrips vector and TSV. In Southern India, the SNV disease occurs in Wet and Winter season only. The summers being hot (38-45 ^{o}C) does not support effective multiplication of vector and dissemination of virus.

The SNV disease infects the sunflower crop at all growth stages. This results in severe economic loss. Early infected crop at seedling stage, results in necrosis and death of plant. Crop infected in vegetative stage show symptoms like distortion of apical shoot and necrosis of leaf & stem. Such plants remain stunted and develop malformed heads. The infection during flowering stage affects the seed setting and results in chaffy seeds or no seed set. In susceptible hybrids mosaic and yellowing of leaves is also observed.

CURRENT SCENARIO

Sunflower necrosis disease incidence has a variable trend in the southern states. The disease spread was on rise in Karnataka and Andhra Pradesh states, for a decade after its first appearance in 1997. Subsequently it spread to other states in south. With erratic monsoon pattern there has been rise and fall in area of sunflower crop in wet and early winter cultivations. It also impacts cultivation of other alternate hosts of TSV viz. peanut & cotton. Hence variability in disease incidence is noted across years. Also the availability of disease tolerant hybrids in these states has limited the disease spread and severity.

According to disease survey data in the annual report of All India Co-ordinated Research Project on Sunflower- 2014, the sunflower necrosis disease incidence was variable in different sunflower growing districts of southern states. In eastern dry zone of Karnataka *viz*. Chitradurg district the SNV incidence in wet season at vegetative stage was (20-30%) and at flowering stage (40-45%). In northern districts of the state *viz*. Raichur the disease incidence was (5-10%) at vegetative and (20-45%) at full blom stage, in Bagalkote (8-10%) in vegetative and (30-35%) at flowering and in Koppal (1-2%) at vegetative and (15-20%) at full bloom stage. In Tamil Nadu state, among the sunflower growing districts viz. Erode, Tirrupur, Tiruchi, Dindigul, Tirunelveli and Dharampuri, the SNV incidence in Wet season ranged from (1-4%). In Andhra Pradesh, in the major sunflower cultivation district Kurnool; very low disease incidence (0-4%) was recorded at 15 locations surveyed. In Maharashtra, the SNV disease incidence was recorded to be very low (4-8%) at Akola and Latur districts.

FUTURE CHALLENGES

The sunflower crop is cultivated along with other cash crops like peanut and cotton in south and north part of India. These crops are known to harbour Tobacco Streak Virus, which

is causal pathogen of Sunflower Necrosis disease. Weed host *viz. Parthenium hysterophorus* is the reservoir for TSV and acts as inoculum source. The spread and adaptability of this weed host across different geographies is of serious concern. Simultaneous availability of alternate crops and weed hosts with favourable weather conditions for *Thrips* vector can be a vulnerable combination; favouring dissemination and establishment of the virus, in other sunflower growing areas in India. Indeed there is a need to speed up resistant hybrid development program to counter the challenge of Sunflower Necrosis Virus disease in coming years.

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GENOME WIDE ASSOCIATION STUDIES ON SUNRISE GWA POPULATION

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ABSTRACT

The world oilseed production will face an increasing demand in the next years, for edible oil, biofuel or green chemistry. In the same time, the climate change will cause a water deficit: yield losses of 10 to 30 % have been predicted at 2030 horizon in Europe for sunflower crop. The SUNRISE project aims to improve the oil production from culture of sunflower hybrids in condition of water deficit, thanks to a genomic prediction approach. On the basis of genotypic and phenotypic data, GWAS (Genome Wide Association Study) allows to look for chromosomical regions linked to agronomical traits of interest. The GWA hybrid population was developped to produce these data. This population was an incomplete factorial design of 36 males and 36 females crossed to generate 452 hybrids. Parents were sequenced and SNP calling led to about 2,000,000 SNPs. An association study was performed on phenotype adjusted for field effect, using a mixed model having male, female and their interaction random effects. Results for a multi-locus association analysis will be presented on traits of interest, as flowering time.

Key Words : GWAS, mixed model, parent effects

SCREENING FOR RESISTANCE TO HIGHLY VIRULENT RACES OF SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA*)

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ABSTRACT

Sunflower broomrape (*Orobanche cumana* Wallr.) is a major constraint for sunflower production in most production areas around the world, particularly in areas of Eastern and Southern Europe and the Middle East. The yield losses caused by this parasitic weed can reach 100% for the susceptible cultivars which are heavily infested. The development of resistant cultivars as well as optimized managing strategies is a high priority in sunflower breeding programs all over the world.

Since the problem of resistance to broomrape race E is fully resolved, almost all commercial sunflower hybrids have incorporated resistance gene *Or5*, the main goal in sunflower breeding program in the terms of resistance to broomrape is to find new sources of resistance genes to the new broomrape races which are present in Europe. The objective of this research was to screen newly developed inbred lines in order to find resistance to highly virulent races overcoming race F of broomrape present in Southern and Eastern Europe

Screening procedure of testing for resistance to broomrape was combination of greenhouse and field trials during the several years. First field trials were performed in broomrape infested area in Northern Serbia in two locations where the race present was race E. The greenhouse testing was performed in the winter period under artificial inoculation using broomrape seeds collected from broomrapes attacking sunflower hybrids with incorporated gene of resistance Or5.Only the genotypes with complete resistance to broomrape are further tested in Spain, Turkey and Romania. In this way, the inbred lines resistant to virulent races of broomrape, overcoming race F were detected in NS breeding material. These lines are from the different gene pools and they were fully resistant to races which overcame race F in all tested areas in 2014 and 2015. Preliminary results of studies of the mode of inheritance of resistance to the broomrape races higher than F indicate that this trait is controlled by dominant gene(s).

Key Words : Sunflower, *Orobanche cumana*, resistance, high virulence

PREVALENCE OF SUNFLOWER DOWNY MILDEW AND PATHOGEN VIRULENCE IN THE UNITED STATES NORTH CENTRAL GREAT PLAINS

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ABSTRACT

Genetic resistance is one of the most important management tools of sunflower [*Helianthus annuus* L.] downy mildew caused by *Plasmopara halstedii* [(Farl.) Berl. and de Toni]. However, many resistance genes have been overcome by the pathogen and the incorporation of additional resistance genes into commercial hybrids is needed. Assessment of pathogen virulence is critical for determining what resistance genes should be incorporated into hybrids. The objectives of this study were to determine the prevalence of downy mildew and determine the virulence of *P. halstedii* isolates collected from United States (U.S.) north-central Great Plains states. In 2014 and 2015, 105 and 76 fields, respectively, were surveyed in North Dakota and South Dakota by visually assessing 40 plants at five locations for signs and symptoms of downy mildew. In 2014, 65% of those fields had downy mildew and ten fields (10%) had field-wide incidence levels higher than 5%. In 2015, 78% of fields had downy mildew and sixteen (21%) had field-wide incidence levels higher than 5%. To determine the virulence phenotypes of *P. halstedii*, 185 pathogen samples were evaluated on the international standard nine *P. halstedii* differentials and up to 13 supplemental lines were evaluated as additional differential candidates containing additional resistance genes. Virulence was observed on all nine differential lines and some candidate differential lines. Downy mildew remains common in the north-central U.S., and pathogen virulence on all current differential lines and additional resistance genes indicate that the inclusion of additional differentials is needed.

Key words: Downy mildew, *Plasmopara halstedii*, Races, Resistance genes, Sunflower, Virulence phenotype

INTRODUCTION

Downy mildew caused by the biotrophic, Oomycete pathogen, *P. halstedii* (Farl.) Berl. and de Toni, is an economically significant seedling disease of cultivated sunflower, *Helianthus annuus* L., grown in temperate regions. Cool temperatures around 11°C and wet soil conditions between germination and emergence favor infection of sunflower radicles by *P. halstedii* zoospores (Baldini et al. 2008). If seedlings do not damp-off, cotyledons and the first true leaves become thickened, puckered and chlorotic (Gulya et al. 1997). Later, leaves show chlorosis along the veins and across the leaves while mycelia and zoosporangia appear on the underside of the leaves below the chlorotic areas. Plants that survive are severely dwarfed with shortened internodes and horizontal heads. Yield losses due to downy mildew are dependent on the number of systemically infected plants and their distribution within the field (Friskop et al. 2009).

Qualitative genetic resistance is one of the most important management tools for sunflower downy mildew; however, many previously deployed, single, dominant resistance genes (denoted *Pl*) have been overcome by the pathogen (Tourvieille de Labrouhe et al. 2008). Therefore, the incorporation of additional resistance genes into commercial hybrids as well as the use of fungicidal seed treatments has been and continues to be necessary. A field survey was conducted in order to determine the effectiveness of sunflower downy mildew control in the main production area of the U.S., the north-central Great Plains. Assessment of pathogen virulence is critical for evaluating effectiveness of resistance genes which have been incorporated into hybrids; therefore, a set of nine internationally recognized differential lines became standard in 2000 to identify virulence phenotypes or races of sunflower downy mildew (Tourvieille de Labrouhe et al. 2000). In 2012, Institut National de la Recherche Agronomique (INRA) proposed two additional sets of three differentials to update the race nomenclature bringing the total number of digits in the virulence phenotype code to five (Tourvieille de Labrouhe et al. 2012). These differentials and up to seven supplemental lines lines containing additional sources of resistance were evaluated to determine their effectiveness as additional differential candidates containing additional resistance genes.

MATERIALS AND METHODS

From June 30 to July 10, 2014 and July 8 to 24, 2015, 105 and 76 fields, respectively, were surveyed in the states of North Dakota and South Dakota. To determine field incidence, a visual inspection was made for downy mildew symptoms of 40 plants at five points in an inverted W-shaped pattern for a total of 200 plants. Prevalence was determined based on whether the disease was present or absent in the field. Pathogen isolates were collected from each field for a total of 436 isolates. An additional 125 viable isolates from North Dakota, South Dakota, Minnesota and Nebraska were collected and sent in by personnel from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), state Extension services, and seed companies.

In order to increase *P. halstedii* samples collected, susceptible sunflower seedlings were inoculated in a zoosporangia suspension prepared from symptomatic leaves for three to six hours using methods described by Gulya (1996). Inoculated seedlings were planted in a sand-perlite mixture and grown in the greenhouse for eight to ten days. Then, seedlings were placed in a glass chamber in a cool (16-18^{o}C) room and sprayed with a fine mist of water to achieve 100% relative humidity for 16 to 48 hours to induce sporulatation. The cotyledons covered with zoosporangia were harvested, desiccated, and stored in cryotubes at -80^{o}C.

After inoculum increases were completed, one isolate from each field or research plot was arbitrarily selected for virulence phenotyping. In total, 185 isolates were evaluated on the nine international standard *P. halstedii* differential lines and up to thirteen supplemental lines containing additional resistance genes. Differential seedlings were inoculated and planted using the previously described method. After 11 to 14 days, when true leaves were easily visible, sporulation was induced. Plants were allowed to air dry before susceptibility and resistance of plants was evaluated.

The following new rating system proposed by INRA for susceptible and resistant plants was used: RI = resistant, no sporulation; RII = weak sporulation on cotyledons; SI = susceptible, sporulation on cotyledons and true leaves and SII = abundant sporulation on cotyledons only (Tourvieille de Labrouhe et al. 2012). Moderate, easily visible sporulation on the cotyledons was considered to be a RII reaction. To determine race in the triplet code system, each set of three differential lines is given a numerical value. The first three lines correspond to the first digit (Table 1), the second three lines correspond to the second digit and the third three lines correspond to the third digit. If a line is resistant, it is given a value

of 0. Otherwise, the first line is given a 1, the second line a 2 and the third line a 4. The values for all three lines in each of the three sets are then added. Each digit ranges from 0 if all three lines were resistant to 7 if all three lines were susceptible. The proposed five-digit code adds two additional sets of three differential lines.

Table 1. Standard and Proposed International Differentials.

	Digit	Differential Line	Sunflower Line	Genes
Standard	1	1	Susceptible (MYC 270)	None
		2	RHA 265	Pl_1
		3	RHA 274	Pl_2/Pl_{21}
	2	4	DM-2	Pl_5
		5	PM 17	?
		6	803	?
	3	7	HA-R4	Pl_{16}
		8	HA-R5	Pl_{13}
		9	HA 335	Pl_6
Proposed	4	10	Y7Q	Pl_{6-}
		11	PSC8	Pl_2
		12	XA	Pl_4
	5	13	PSS2RM	Pl_6/Pl_{21}
		14	VAQ	Pl_5
		15	RHA 419	Pl_{Arg}

RESULTS AND DISCUSSION

In 2014, 65% of fields surveyed had sunflower downy mildew and 10% of fields had incidence levels greater than 5% (Table 2). In 2015, 78% of fields had downy mildew and 21% of fields had incidence levels higher than 5%. These fields did not appear to be concentrated in any particular region. Prevalence was high, but yield impacting incidence was low. Yield losses start to occur between 5 and 15% depending on the distribution of systemically infected plants within a field; therefore, if scattered infection occurs, incidence below 15% should result in minimal yield loss (Bradley et al. 2007). In 2015, most infected plants appeared to be scattered throughout the fields, so other plants should have compensated in this incidence range. Over the two years of the survey, six of 181 fields would be expected to have significant yield loss due to sunflower downy mildew.

Table 2. Prevalence and Incidence of Sunflower Downy Mildew for 2014 and 2015.

	2014	2015
Prevalence	65% (68/105)	78% (56/76)
Incidence		
0	65%	55%
0.5 - 4.5%	25%	24%
5 - 14.5%	9%	14%
≥ 15	1%	7%

Virulence was observed on all nine differential lines and some supplemental differential lines (Table 3). Minimal virulence was found on lines HA-R4 and HA-R5, which were released in 1984, containing *Pl16* (1%) and *Pl13* (1%) genes, respectively, (Liu et al. 2012; Mulpuri et al. 2009; Vear et al. 2008). In 1986, the USDA released six downy mildew resistant lines: *Pl6* in HA 335 and HA336 from wild *H. annuus*, *Pl7* in HA337, HA 338 and HA 339 from *H. praecox* and *Pl8* in RHA 340 from *H. argophyllus* (Miller and Gulya 1991). *Pl6* and *Pl7* were found to be similar (Miller and Gulya 1991). Between 2009 and 2013 nine races overcame the *Pl6* gene in the United States (Gulya et al. 2014). Isolates virulent on the *Pl6* gene have been between 38 and 60% since 2011 with an average of 51% (Gulya et al. 2014). Virulence on the *Pl6* gene was found on 47% of the isolates from 2014 and 2015. Seven isolates were found over the two years in North Dakota that were virulent on RHA 340, which contains the *Pl8* gene. No isolates were virulent on both the *Pl6* and the *Pl8* genes. Resistance genes in the supplemental lines evaluated include *PlArg* in RHA 419 and RHA 420 which was released in 1999 from *H. argophyllus*, *Pl17* in HA458 released in 2006 from wild *H. annuus*, an unknown gene in RHA 468 released in 2006, *Pl18* in HA DM 1 released in 2015 from *H. argophyllus* and *Pl15* in RNID a proprietary inbred line from NIDERA in Argentina (DuBle et al. 2004; Paniego et al. 2012; Qi et al. 2015, 2016; Vear et al. 2008). No virulence was found on six supplemental lines containing *PlArg*, *Pl15*, *Pl17*, *Pl18* and two other lines with unknown resistance genes.

Table 3. Results for Standard and Supplemental Sunflower Downy Mildew Differential Lines for 2014 and 2015.

Differential Line		Sunflower Lines	Genes	2014 Isolates Virulent / Isolates Screened	2015 Isolates Virulent / Isolates Screened	Total Isolates Virulent /Isolates Screened	Percent
Standard	1	Susceptible (MYC 270)	None	105/105	80/80	185/185	100%
	2	RHA 265	*Pl1*	105/105	80/80	185/185	100%
	3	RHA 274	*Pl2/Pl21*	101/105	70/80	171/185	92%
	4	DM-2	*Pl5*	83/105	56/80	139/185	75%
	5	PM 17	?	10/105	4/80	14/185	8%
	6	803	?	9/105	3/80	12/185	6%
	7	HA-R4	*Pl16*	1/105	1/80	2/185	1%
	8	HA-R5	*Pl13*	1/105	1/80	2/185	1%
	9	HA 335	*Pl6*	53/105	34/80	87/185	47%
Supplemental		RHA 340	*Pl8*	2/105	5/80	7/185	4%
		RHA 419	*PlArg*	0/105	0/80	0/185	0%
		HA 458	*Pl17*	0/61	0/80	0/141	0%
		HA DM 1	*Pl18*	0/87	0/80	0/167	0%
		RHA 468	?	0/66	0/80	0/146	0%
		TX 16R	?	0/84	0/80	0/164	0%
		RHA 428	?	15/66	0/0	15/66	23%
		RNID	*Pl15*	0/66	0/80	0/146	0%

Based on the current standard nine *P. halstedii* differentials, twelve races were found in 2014 and 2015 in isolates from North Dakota, South Dakota, Minnesota and Nebraska (Table 4). In both years, the most common downy mildew races were 714, 710 and 700,

comprising 77% of the total. Race 774 was the 4th most frequent race in 2014, while race 314 was the 4th most frequent race in 2015. Three races, 304, 707 and 717, have been identified in France, but are new to the U.S. (Virányi et al. 2015). Seven isolates that were virulent on the *Pl8* gene are currently differentiated by the addition of a "+" following the current race type since RHA 340 has not been proposed as a differential. 58% of the 26 fields with incidence greater than 5% had races that were not virulent on the *Pl6* or the *Pl8* genes.

Table 4. ABSTRACT of Sunflower Downy Mildew Races for 2014 and 2015.

Race	2014	2015	Total
304	1	0	1
314	3	10	13
700	18	18	36
700+	1	1	2
704	1	4	5
707	1	0	1
710	32	21	53
710+	1	4	5
714	37	17	54
717	0	1	1
730	0	1	1
734	1	0	1
770	0	1	1
774	9	2	11

+ = Virulent on the *Pl8* gene

A selection of the 185 isolates collected in 2014 and 2015 were virulence phenotyped using the INRA proposed differential lines to determine how races in the main sunflower production area of the U.S. would compare to the 17 races used in the proposal (Table 5) (Gascuel et al. 2015; Tourvieille de Labrouhe et al. 2012).

Table 5. ABSTRACT of Proposed Downy Mildew Races for 2014 and 2015.

Race	2014 Isolates	Proposed Race	2015 Isolates	Proposed Race
304	1/1	30430	---	---
314	0/3	---	9/10	31430
700	7/18	70060	0/18	---
700+	1/1	70060+	1/1	70060+
704	1/1	70471	2/4	70471
707	1/1	70771	---	---
710	14/33	71060	2/20	71060
710+	1/1	71060+	3/3	71060+
710+	---	---	1/1	71070+
714	6/37	71471	2/17	71471
717	---	---	1/1	71771
730	---	---	1/1	73060
734	1/1	73471	---	---
770	---	---	1/1	77062
774	1/9	77473	2/2	77473

+ = Virulent on the *Pl8* gene

Races already evaluated by INRA were the same virulence phenotypes in the U.S., but this is the first time U.S. races 700+, 710+, 734, and 770 have been evaluated with these proposed differentials. One of the 710+ isolates from 2015 conferred virulence on multiple seed batches of Y7Q which has the postulated Pl_{6-} gene, but not on HA 335 with the Pl_6 gene.

CONCLUSIONS

Virulence was observed on all nine differential lines and some supplemental differential lines. Downy mildew remains common in the north-central U.S., and pathogen virulence on all current differential lines and additional resistance genes indicate that inclusion of additional differentials is needed. Use of resistant hybrids in combination with fungicidal seed treatments and crop rotation is currently limiting field incidence based on surveyed fields.

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OILSEED AND CONFECTIONARY (SUNFLOWER (*HELIANTHUS ANNUUS* L.) RESEARCHES IN AEGEAN AGRICULTURAL RESEARCH INSTITUTE (AARI)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops in Turkey. To increase production, sunflower with high yield capacity can be grown at both first and second crop production seasons in the Aegean Region of Turkey. The mission of the "Sunflower Research Project for Aegean Region" at Aegean Agricultural Research Institute (AARI) is to improve well adapted and high yielding varieties, to develop knowledge and technology for sunflower industry in Turkey. A number of different approaches are utilized to achieve the goal. Research is conducted to develop; a diverse germplasm base that leads to enhanced yield potential and quality characteristics; transfer useful traits from wild species, landraces and other sources into cultivated sunflower. To improve sunflower varieties with desired characters, genetic investigations and germplasm development of sunflower with improved yield, oil quality, resistance to disease [Sunflower rust (*Puccinia helianthi* Schw.) and downy mildew (*Plasmopara helianthi* Farl de Toni)], *Orobanche* sp., and adverse conditions. Oilseed and confectionary type of sunflower germplasm (A, B and Rf lines) including improved populations, hybrid and open pollinated varieties have been developed by conventional breeding techniques and biotechnological methods. High oleic types and resistance to herbicide (IMI groups) sunflower varieties are undergoing. The research program is leading to develop oilseed and confectionary type of sunflowers for both first and second crop production seasons. Sunflower landraces were characterized to utilization of development of new varieties. Sunflower germplasm have been developing from sources such as cultivars, populations created through breeding methods and tested for general and specific combining ability were undertaken with line x tester analysis. In the breeding program, lines, candidate and commercial varieties evaluated in preliminary and yield trials as regional basis under first and second crop production season at Aegean Region and other parts in Turkey since 1983. Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance, approximately 500-550 kg da^{-1} at both first and second crop production seasons in Aegean Region of Turkey. Oilseed type of open pollinated variety Ege-2001 was developed by using S 0:1 generation testing (Recurrent selection) method and hybrid variety Turay and parental lines has been registered. In addition effect of plant population, planting time, fertilizing, irrigation, honeybee pollination, on seed yield, oil percentage and other plant characteristics, and silage quality of sunflower (*Helianthus annuus* L.) were determined. Sunflower rust (*Puccinia helianthi* Schw.) race identification under field conditions was determined.

Keywords: Sunflower, *Helianthus annuus* L, *Helianthus* spp., Breeding, Genetics, Germplasm, Hybrid variety, Open polinated variety, Agronomy, Adaptation, CMS line, Restorer line, technology, Sunflower diseases, insects and weeds, Yield components.

INTRODUCTION

Vegetable oils and fats are vital component of human diet because they are an important source of energy. Sunflower is one of the major oilseed crops in Turkey. According to production data, sunflower was grown 657458 ha area with 1637900 metric ton seed production, and average seed yield of 2690 kg ha^{-1} in Turkey in 2014 (Anonymous, 2015). Because of gap for vegetable oil production in Turkey, sunflower is one of the alternative and leading oilseed crops to increase vegetable oil production. Growing sunflower as a first and second crop in Aegean Region is one of the possibility to increase the production. The Aegean Region has suitable ecological conditions for first and second crop sunflower production (Tan, 2007; Tan, 2010; Tan, 2011; Tan, 2014).

Sunflower research activities has been conducted since 1979 and breeding program initiated in 1984 at Aegean Agricultural Research Institute (AARI) in Menemen, Izmir, Turkey. The mission of the Sunflower Research Project is to develop improved germplasm by conventional and biotechnical breeding techniques for both first and second crop production areas in Turkey. New germplasm, breeding lines, hybrid varieties have been developed. To improve oilseed and confectionary sunflower varieties with desired characters, genetic investigations, and germplasm development of sunflower with improved yield, oil quality, resistance to diseaseases such as *Plasmopara helianthi* (Farl.) Berl de Toni., *Puccinia helianthi* Schw., and *Orobanche cumana* Walr. Adverse conditions are also under consideration. This studies are also incorporated with agronomic and other related researches.

RESEARCH FINDINGS

Breeding and Genetics

In the breeding programme a number of different approaches are utilized. Improved germplasm (populations and germplasm), new germplasm for hybrid development and breeding lines of oilseed and confectionary type of sunflower germplasm [A (CMS), B (maintainer) and Rf (restorer) lines] including hybrid and open pollinated varieties have been developed by conventional breeding techniques.

Sunflower germplasm have been developing from sources such as cultivars, populations created through breeding methods or crosses with wild germplasm, and tested for general and specific combining ability, oil percentage, and resistance to prevalent disease and adverse conditions to construct improved variety. In the breeding program, hybrids parental lines have been evaluated and developed according to their General (GCA) and specific combining ability (SCA).

Combining ability studies in oilseed and confectionary sunflower breeding program were undertaken with a set of line x tester including parents for the characters; seed yield, 1000-seed weight, days to flowering, days to physiologic maturity, plant height, head diameter, stem diameter, oil content, fatty acid content (oleic, linoleic, palmitic, and stearic acids), protein content, seed length, seed width, and hull percentage. General (GCA) and specific combining ability (SCA) of inbreed lines and their hybrids were estimated in a line x tester analysis at first and second crop production seasons in 1991. The variances due to GCA and SCA were highly significant for most of the characters under both environments. Based on GCA effects under first and second crop production seasons, the inbreeds (CMS and Rf lines) exhibited desirable GCA effects, and were found to be good general combiners for most of the traits; thus they can be used for developing superior genotypes and hybrids in sunflower (Tan, 1993a; Tan, 2005a, 2005b; Tan, *et. al.*, 2013a; 2015a).

More than 300 oilseed and confectionary type of CMS and restorer (Rf) lines with higher GCA and SCA effect developed since 1983 (Tan, *et. al.*, 2013a; 2015a).

Genetic and inheritance studies in sunflower to understand genetic make-up of the traits and genetic inheritance including disease resistance [Such as *Puccinia helianthi* Schw.; *Plasmopara halstedii* (Farl.) Berl de Toni.] in sunflower, and to improve drought tolerant varieties etc. in the breeding programs under consideration.

In the sunflower breeding program, Parental lines, candidate variety and commercial variety have been evaluated under first and second crop production seasons. Oilseed and confectionary type of sunflower germplasms including hybrid and open pollinated variety have been developed. Oilseed open pollinated variety EGE-2001, and hybrids TURAY, and SUN 2235 ant their parental lines have been registered (Tan, *et. al.*, 2013a; Tan, 2014; Tan, *et. al.*, 2015a).

Sunflower Genetic Resources

Evaluation and characterization of sunflower genetic resources to improve oilseed and confectionary type of sunflower varieties are under consideration. Therefore; sunflower landraces, collected, conserved, regenerated, characterized and evaluated for source of breeding for broaden the genetic base. This studies are allow us to develop new and desired sunflower varieties, breeding lines, and populations (Tan and Tan, 2010; Tan and Tan, 2011; Tan and Tan, 2012; Tan *et. al*., 2013a; Tan *et. al*., 2013b; Tan *et. al*., 2015b).

Orobanche cumana Wallr. Studies

In the breeding program, all test hybrids are tested for genetic resistance and or tolerance of *Orobanche cumana* Wallr. Imidazolinones (IMI) herbicides provide excellent broad-spectrum weed and broomrapes (*Orobanche cernua* and *O. cumana*) in sunflower. some oilseed and confectionary varieties have also been improved for imidazidol herbicide resistance (Tan, *et. al.*, 2013a; 2015a).

On-farm study of *Orobanche cumana* Wallr.

Resistance and susceptibility of sunflower (*Helianthus annuus* L.) to *Orobanche cumana* Wallr. was determined under natural infections. In this on-farm study, sunflower hybrids an control variety of Vniimk-8931 were evaluated for their rates of resistance to *O. cumana*, and evaluated for their agronomic performance. The hybrid varieties found to be consistently resistant, and Vniimk 8931 was susceptible to *Orobanche cumana* Wallr., and had very low yield losses under natural infection. (Tan and Karacaoglu, 1991b).

Rust race identification

Sunflower rust incited by *Puccinia helianthi* Schw. is considered one of the important foliar diseases of sunflower and is present wherever sunflower is grown in the world. The objective of this study was to identify the races of sunflower rust in the main sunflower production areas in Turkey. Experiments were conducted in six provinces (Aydın, Balıkesir, Bursa, Denizli, Izmir, and Edirne) of Turkey in 1992. Race identification of *P. helianthi* was determined in the field conditions. Eighteen differential genotypes were used to identify races of *P. helianthi*. Sunflower rust reaction of the differential genotypes were scored on a scale of 0 to 4, where 0 to 2 = resistant, 3 and 4 = susceptible. Race 1 of *P. helianthi*, causal agent of sunflower rust, was identified at Menemen-Izmir, Susurluk-Balıkesir, Koçarlı-Aydın, Çivril-Denizli. However, Races 1 and 3 of *P. helianthi* were found in Bursa and Edirne (Tan, 1993b; Tan, 1994a; b).

In the breeding program, all lines are selected for *Puccinia helianthi* Schw. genetic resistance under field conditions.

Yield trials

Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance (500-550 kg da^{-1}) at both first and second crop production seasons in Aegean Region of Turkey (Tan, 2010; Tan, *et. al.*, 2013a; Tan, 2014; Tan, *et. al.*, 2015a).

On-farm research and adaptation studies (genotype x environment interaction) to find out higher yielding varieties for different geographical regions and ecological conditions in both first and second crop production seasons. More than 2000 lines, candidate variety and commercial variety have been evaluated in preliminary and yield trials under first and second crop production seasons since 1979 (Tan, *et. al.*, 2013a; Tan, 2014; Tan, *et. al.*, 2015a).

Agronomic techniques have been applied for higher yield and to develop culturing techniques in sunflower.

Effect of plant population on seed yield, oil percentage and other plant characteristics in sunflower

In this study, the highest yield values, 2190 and 1920 kg ha^{-1} were obtained from the plant populations, 40820 and 47620 plants ha^{-1}, respectively. The experiment design was RCBD and plots were consisted of four row 7.70 m in length, spaced 0.7 m apart. Plants spaced 15, 20, 25, 30, 35 and 40 cm on each row of experiments plots. In this study, the most important observation was that degree of lodging effected harvestable yield greatly. Lodging is associated with plant population size, stalk diameter, plant height and wind speed. The number of lodged plants increased when plant population size increased. The maximum wind speed were 9.0 and 12.0 m s^{-1} in 1986 and 1987, respectively. The highest percent of lodged plants were obtained at 95240 plants ha^{-1} (15x70 cm plant spacing) as 69% and 19% in 1986 and 1987, respectively (Tan and Karacaoglu, 1991a).

Effect of planting date on plant characteristics in sunflower

Effect of planting date on seed yield, oil content, fatty acid composition and other plant characteristics in sunflower (*Helianthus annuus* L.) were determined at seven planting date including both first and second crop production times. The highest yields were obtained with early planting in the first and second crop planting times which are at the beginning of April and July, respectively. In general, oil content was not affected by planting times. However, the fatty acid composition was changed significantly by planting dates depending upon temperature. Oleic acid composition was 45% in the early planting time and as planting time was delayed it decreased to 25% in the late planting time. The lineloic acid proportion increased from about 45% to 65% with the delaying plantings from first to second crop planting time. The changes in fatty acid composition can be associated with temperature during seed development of the crop. The plant height, head diameter, and 1000 seed weight were also negatively affected as planting date was delayed (Tan, 1991).

The effects of honeybee pollination on some economic characters of oil type sunflower varieties

The objectives of this research were to determine the effect of honeybee pollination on sunflower varieties (Super-25, AS-503, and ETAE-Y1) in 1997 and 1998, at AARI in Menemen, Izmir. Data were collected on sunflower yield (kg ha^{-1}), 1000-seed weight (g), plant height (cm), head diameter (cm), days to flowering, days to physiological maturity, oil percentage (%), protein percentage (%), hull percentage (%), and seed sizes. The experimental design was randomized block with split plot arrangement with three replications. The main plots were assigned to the pollination treatments and the sub-plots for sunflower varieties. Plot size was 16.17 m^2 and the plant density was 4081,6 plants ha^{-1}. The plots of the treatments were 1) plots with honeybee pollination, 2) plots caged to exclude honeybee and other insect pollination, and 3) and open pollinated plots.

The results of statistical analysis indicated a significant difference among the treatments. Seed yield of honeybee pollinated plants of plots, an average, were 4440 and 4130 kg ha^{-1} in 1997 and 1998 respectively. All cultivars shoved a significant increase in yield in the presence of bees. Seed yield on honeybee pollinated plants of 3 different sunflower varieties were, on average, 95% higher than on plots caged to exclude bees in 1997 and were 124% in 1998. Yields were higher on plots pollinated by honeybees than on those caged to exclude bees and open pollinated plants; but the difference varied between cultivars, and between years. It is concluded that the use of honeybees for pollination were also effective on the seed size, 1000 seed weight, oil and protein content (Tan, 2000; Tan, *et. al*., 2002.).

The effect of irrigation at various growth stages on some economic charecters of first crop sunflower

The objectives of this research were to determine number of and optimum and more economic irrigation during specific growth stages oil type sunflower varieties (Super-25 and Trakya-129) conducted in 1996 and 1997, at AARI in Menemen, Izmir. The experimental design was randomized block with split plot arrangement with three replications. The main plots were assigned to the irrigation treatments and the sub-plots for sunflower varieties. Treatments were: 1. Non-irrigated (Control); 2. One irrigation at the begining of heading stage; 3. One irrigation at the begining of flowering (blooming) stage; 4. One irrigation at the begining of milk stage; 5. Two irrigation at the begining of heading and blooming stages; 6. Two irrigation at the begining of heading and milk stages; 7. Two irrigation at the begining of blooming and milk stages; and 8. Three irrigation at the begining of heading, flowering and milk stages. Data were collected on sunflower yield (kg da^{-1}), 1000-seed weight (g), plant height (cm), head diameter (cm), days to flowering, days to physiological maturity, oil percentage (%), protein percentage (%), hull percentage (%), seed length (mm), seed width (mm), and stem diameter (cm). The results of statistical analysis showed a significant difference among the irrigation treatments. According to the results the best yield obtained from three times irrigation with 427 kg da^{-1} and 373 kg da^{-1} in 1996 and 1997 respectively. Whereas, 347 kg da^{-1} and 265 kg da^{-1} yields were obtained from the control (non-irrigated) plots in 1996 and 1997 respectively.

Marginal analysis method was used for economic analysis. Generally, both registered varieties showed positive response to the applications in question. The 8th application seems as if it is the most profitable treatment in terms of having the highest gross revenues. According to results, three irrigation (Treatments-8) can be applied for high yield; but, one irrigation at the development of head stage is recommended because of satisfactory yield and the maximum marginal revenue (Tan *et. al.* 2000).

Silage quality of sunflowers

Sunflower (*Helianthus annuus* L.) is known as one of the drought tolerant crop. Because of this property, it can be used as an alternative silage crop at both first and second crop production seasons when irrigation is limiting factor. This study was conducted to determine the most suitable harvest stage of sunflowers for silage and evaluate silage quality of sunflowers harvested at different vegetation stage. In this study confectionary material ETAE-14 was planted by machine at populations of 40816 plants ha^{-1} in 1993 at Menemen, Izmir. Plants were harvested at 5 different growth stages (R3, R5.1, R5.5-5.9, R6, R9), and cut about 0.8 - 1.0 cm in length by silage machine then stored in plastic barrels for silage. In the study, the material was evaluated for forage yield, flieg score, sensory quality test, dry matter, crude protein, crude oil, crude fiber, N-free extract, ash, Ca, P, and pH. Research results indicated that harvesting sunflower for silage during R6 stage (complete flowering stage) was found as the most suitable stage for silage (Tan and Tumer, 1996).

Training Programs

Technical and applied training programs organized in each year for both agricultural engineers and farmers.

CONCLUSIONS

In Aegean Sunflower Research Project, desired oilseed and confectionary type of germplasm, populations, lines (CMS and Rf), test hybrids, and varieties developed in the breeding program. Agronomic studies achieved to increase sunflower yield under first and second crop production times in Turkey.

Research findings have been supported that instead of increasing total acreage in sunflower production second crop sunflower production to increase sunflower production in Turkey. Therefore, first and second crop sunflower production should be considered in Aegean Region in order to decrease vegetable oil gap in Turkey.

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PERFORMANCE OF SOME OILSEED SUNFLOWER (*HELIANTHUS ANNUUS* L.) VARIETIES IN AEGEAN REGION OF TURKEY

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ABSTRACT

Sunflower is one of the major and leading oilseed crops in Turkey. It is grown mainly Thrace Region of Turkey. Oilseed sunflower was grown 568995 ha area with 1500000 metric ton seed production, and average seed yield of 2640 kg ha^{-1} in Turkey in 2014. The main objectives of this study were to determine performance of oilseed hybrid varieties which in Aegean region. The experiments were conducted at first crop growing seasons in 2013 on the experiment field of Aegean Agricultural Research Institute in Menemen, Izmir in Aegean Region; Edirne and Luleburgaz in Thrace. The experiments were established in randomized complete block design with four replications. As a material, sunflower oilseed candidate varieties and commercial hybrids were used in this study. Adaptation study were undertaken for the characters seed yield, seed oil content (%), 1000 seed weight, plant height, head diameter, seed length, seed width, hull percentage (%), days to flowering and days to physiological maturity. The results indicated that statistically significant differences were found among the sunflower varieties for the characters in question. The highest seed yield 516 kg da^{-1} and the lowest 347 kg da^{-1} was obtained from the varieties 08 TR003 and TE-TM-2012-2 respectively in Menemen. However, in the rain fed condition, the highest seed yields were 209 and 204 kg da^{-1} were obtained from 08 TR003 in Edirne and LG 5550 in Luleburgaz locations respectively. The results indicated that TE-TM-2012-6 and TE-TM-2012-1 found to be promising candidate hybrids with the high yields over the locations. The results of this study indicated that the production for oilseed sunflower in this region ha the great potentiality. Because of gap for vegetable oil production in Turkey, Aegean Region is one of the possibilities to increase the vegetable oil production.

Keywords: Sunflower, *Helianthus annuus* L, hybrid variety, open pollinated variety, adaptation, yield, yield components.

INTRODUCTION

Because of an increasing world population it is difficult to deal with human feeding in the world. Vegetable oils are an important source of energy. To reduce oilseed production gap in Turkey, it is possible to grow sunflowers with high yield, oil percentage and oil quality; consequently, increasing oilseed production will result in increasing vegetable oil and decreasing import of vegetable oil (Gobbelen et al., 1989; Schneiter, 1997; Tan, 2007).

Turkey is one of the leading countries for sunflower production. According to production data oilseed sunflower was grown on 568995 ha area with 1500000 metric ton seed

production, and average seed yield of 2640 kg ha^{-1} in Turkey in 2014 (Anonim, 2015). However, amount of oilseed production including sunflower is not sufficient for the consumption; therefore, amount of the production should be increased. There are some other potential sunflower production areas such as Aegean Region and South East Anatolia besides main production area of Thrace of Turkey (Fırat and Tan, 1992; Tan, 2007; Tan, 2010a; b; Tan, 2014).

In Sunflower research project at AARI, oilseed and confectionary type of sunflower germplasms including hybrid and open pollinated variety have been developed, and candidate varieties are evaluated in yield trials under first and second crop production season. Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance (500-550 kg da^{-1}) at both first and second crop production seasons in Aegean Region of Turkey (Tan, 2007; Tan, 2010a: b; Tan, *et. al*., 2013a; Tan, 2014; Tan *et. al*., 2015). The Aegean Region that has suitable ecological conditions for first and second crop sunflower production should be considered for sunflower production to decrease vegetable oil gap in Turkey.

The main objectives of this study were: (1) to determine newly developed oilseed hybrids varieties which could be grown with satisfactory yield performance in Aegean region.

MATERIAL AND METHODS

This study was conducted to determine performance of oilseed hybrid for Menemen, Izmir conditions. The experiments including oilseed hybrid cultivars were conducted separately at first crop growing seasons in 2013 on the experiment field of Aegean Agricultural Research Institute (AARI) in Menemen, Izmir. They were also tested in Lüleburgaz and Edirne locations as well. Adaptation study were undertaken for the characters seed yield (kg da^{-1}), seed oil content (%), 1000 seed weight (g), plant height (cm), head diameter (cm), seed length (mm), seed width (mm), hull percentage (%), seed color (white, black, and intermediate), days to flowering and days to physiological maturity.

In this study, as a material, 6 oilseed hybrids sunflower candidate varieties developed at AARI sunflower breeding program were used in the experiments. Restorer lines of these hybrids were developed by sunflower breeding program of AARI and CMS lines of these hybrids were developed by sunflower breeding program of Trakya Agricultural Research Institute (TARI). The newly developed oilseed hybrids used in this study were; TE-TM-2012-1; TE -TM-2012-2; TE -TM-2012-3; TE -TM-2012-4; TE -TM-2012-5; TE -TM-2012-6. Oilseed commercial hybris, LG-5550 (St-4); TURAY (ST-1); LG-5580 (St-5); P64G46 (St-2); 08 TR 003 (St-3) were used as control varieties.

The oilseed hybrid and OP confectionary variety variety experiments were conducted a randomized complete block design with four replications. The experiment plots were consisted of four row 7.70 m in length, spaced 0.7 m apart. There were 22 plants, spaced 0.35 (in Menemen) and 0.30 (in Luleburgaz and Edirne) cm on each row of experiments plots. Recommended agronomic crop production practices were followed. In Menemen and Edirne experiments 50 kg da^{-1} ($N_{10}P_{10}K_0$) and 25 kg da^{-1} ($N_{10}P_{10}K_0$) composed fertilizer applied respectively during the soil preparation. Three irrigation were applied in 2013 (27/06/2013, 12/07/2013, 31.07.2013) in Menemen experiment, however, irrigation were not applied in Edirne and Luleburgaz experiments. In all experiments weed control were routinely applied. The experiments were planted in 10, 20, and 21 May 2013 in Edirne, Luleburgaz and Menemöen locations respectively.

Data were obtained on;

Seed yield (kg da^{-1}): The yield was obtained from each of the two middle rows of the four row plots in the experiment. At the harvest, 1st and 4th rows and first and last plants of the middle row are removed as edge effect in the confectionary variety experiment. The first and last plants of the rows are removed as edge effect in the experiment for evaluation. Heads were hand harvested, threshed, and evaluated at 0% moisture.

Days to physiological maturity: days from planting to R9 stage (Schneiter, and Miller, 1981).

Days to flowering maturity: days from emergence to 75% of the flowering.

1000 seed weight (g): Weight of 1000 seed (g) determined from dried seed (0% moisture) sample.

Oil content (%): Sample from harvested seed was dried to 0% moisture and percent oil was determined by nuclear magnetic resonance (NMR).

Plant height (cm): Height of ten plants were measured at R9 (Schneiter, and Miller, 1981) from ground level to the base of the head (cm).

Head diameter (cm): Head diameter of ten plants were measured at R9 (Schneiter, and Miller, 1981).

Seed size (mm): The length and width of a sample of 10 seeds were measured in mm for only confectionary yield trial.

Hull percentage (%): Sample from harvested seed was dried to 0% moisture and the husk of seed was removed and weighted.

Uniformity: At the 75% of the flowering stage plants were observed whether they were uniform or not.

Statistical analyses were performed to determine the differences among the varieties (Steel, and Torrie, 1980).

RESULTS AND DISCUSSION

Results showed that statistically significant differences were found among the sunflower varieties for the characters in question. In the experiments; the highest seed yields (516, 483, and 443 kg da^{-1}) were obtained from the varieties 08 TR003, TE -TM-2012-6, and TE -TM-2012-1 respectively and the lowest seed yield of 347 kg da^{-1} was obtained from TE -TM-2012-2 in Menemen.

While, the highest seed yields (516, 483, and 443 kg da^{-1}) were obtained from the varieties 08 TR003 (239 kg da^{-1}), TE-TM-2012-6 (237 kg da^{-1}), and TE -TM-2012-1 (221 kg da^{-1}) in Edirne location. In Luleburgaz location, the highest yields; 204 kg da^{-1}, 199 kg da^{-1}, and 190 kg da^{-1} were obtained from the varieties 08 TR003, TE -TM-2012-6 and TE -TM-2012-1 respectively (Tan, *et. al*., 2013).

The lowest flowering days (45 days) observed from ETAE TE-TM-2012-1 and the highest flowering day (50 days) observed LG-5580 (St-5). The lowest physiological maturity days (97 days) observed from 08 TR003 (St-3) and highest flowering day (104 days) observed from P64G46 (St-2). The highest plant height (201 cm) was obtained from ETAE-TM-4 and the lowest plant height (155,50 cm) was obtained from P64G46 (St-2). The highest head diameter (19,6 cm) were obtained from LG-5550 (St-4) and the lowest head diameter (15.5) was obtained from TE-TM-2012-5. The highest 1000-seed weight (82.97 g) was obtained from 08 TR003 (St-3) and the lowest 1000-seed weight (53.54 g) was obtained from TE -TM-2012-2. The highest oil content (45.88 %) was obtained from TANAY, and the lowest oil content (34.55 %) was obtained from TE-TM-2012-3. The highest hull percentage (27.27 %)

was obtained from TE-TM-2012-2, and the lowest hull percentage (20.29 %) was obtained from 08 TR 003 (St-3).

Hybrids varieties had suitable physiological maturity days for both first and second crop production seasons in Aegean and Thrace Regions. These hybrids and especially TE-TM-2012-6 and TE-TM-2012-1 showed satisfactory results of other plant characters in Izmir, Edirne, and Luleburgaz experiments (Tan, *et. al*., 2013a; b).

According to *Orobanche cumana* tests, P64G46 (K), TE-TM 2012-1, TE-TM 2012-2, TE-TM 2012-3, TE-TM 2012-5,TE-TM 2012-6 were found to be tolerant to *Orobanche cumana* (Tan, *et. al*., 2013). The oilseed hybrid variety TE-TM-2012-6 was one of the best candidate hybrid variety for registration because of high yield capacity and high tolerance to Orobanche (*Orobanche cumana* Wallr.) in the yield performance trials in Turkey (Sezgin and Yasar, 2015).

CONCLUSIONS

In the oilseed variety experiments; the highest seed yield (516 kg da^{-1}) and the lowest seed yield (347 kg da^{-1}) were obtained from the varieties. These oilseed hybrids varieties with short physiological maturity days that is suitable for both first and second crop production seasons in Aegean and Thrace Regions.

Research results indicated that Oilseed candidate hybrid TE-TM-2012-6 showed the highest yield and quality performance in Menemen, Edirne and Luleburgaz locations, and found to be highly tolerant to existing races of *Orabanche Cumana* Wallr. (Tan, et. al., 2013a; b; Sezgin and Yasar, 2015), and TE-TM-2012-6 was registered as SUN 2235 in April, 2016 (Sezgin and Yasar, 2016).

Increase in sunflower production could be possible by the expansion of acreage, giving importance to the high-yielding varieties need to be planted. Planting high yielding hybrids in Aegean Region that has suitable ecological conditions for first and second crop sunflower production may play an important role to decrease vegetable oil gap in Turkey.

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Table 1. Oilseed sunflowers yield trials yield, (kg/da), oil content and oil yield, 1000 seed weight (g), and husk percentage (%) values of the varieties. AARI, Menemen-Izmir (2013).

Variety Name	Seed yield (kg da^{-1})*	Yield groups (α=0.01)	Seed yield over St. mean (%)	Seed yield order	Oil content (%)	Oil yield (kg da^{-1}) *	Oil yield over St. mean (%)	Oil yield Order	1000 Seed weight (g)**	Husk Percentage (%)
LG-5550 (St-4)	400	CDEF	90	10	36.83	147	77	12	76.16	25.05
TE-TM-2012-1	**443**	**ABCDE**	**100**	**6**	**43.51**	**193**	**102**	**7**	**55.17**	**22.25**
TE -TM-2012-2	347	F	78	14	36.82	128	67	14	53.54	27.27
TE -TM-2012-3	382	DEF	86	11	34.55	132	70	13	56.42	25.07
TE -TM-2012-4	374	EF	84	12	41.46	155	82	11	58.74	24.10
TURAY (ST-1)	440	A BCDE	99	7	45.30	199	105	6	54.12	25.92
TE -TM-2012-5	372	EF	83	13	42.57	158	83	10	56.33	26.70
TE -TM-2012-6	**483**	**AB**	**108**	**2**	**43.58**	**210**	**111**	**3**	**57.10**	**24.35**
TANAY	478	ABC	107	3	45.88	219	115	2	56.57	20.85
ETAE-TM-4	456	ABCD	102	5	45.16	206	109	4	55.07	21.35
LG-5580 (St-5)	471	ABC	106	4	42.81	202	106	5	68.97	22.18

P64G46 (St-2)	402	CDEF	90	9	41.66	167	88	9	60.54	23.34
08 TR003 (St-3)	516	A	116	1	45.33	234	123	1	82.97	20.29
EGE 2001	422	BCDEF	95	8	44.05	186	98	8	68.09	22.97
CV (%)	9.83								9.87	5.34
LSD (0.05)	60.13								8.67	1.08
LSD (0.01)	80.50								11.60	2.42

* 10% seed moisture; ** 0% seed moisture.

Table 2. Phenological Data of Firs Crop Oilseed Sunflowers Yield Trials. AARI, Menemen-Izmir (2013).

Variety Name	Days to flowering (day)	Pphysiological maturity (day)	Plant height (cm)	Head diameter (cm)	Uniformity (1-5)**
LG-5550 (St-4)	47	103	168.5	19.6	1.5
TE-TM-2012-1	**45**	**100**	**159.1**	**17.7**	**1.0**
TE -TM-2012-2	46	101	174.9	17.3	4.0
TE -TM-2012-3	46	98	165.3	18.4	1.8
TE -TM-2012-4	45	99	165.1	18.2	1.6
TURAY (ST-1)	47	100	187.4	17.9	1.1
TE -TM-2012-5	47	103	183.6	15.5	4.0
TE -TM-2012-6	**47**	**99**	**189.5**	**18.0**	**1.4**
TANAY	47	100	195.4	19.1	3.8
ETAE-TM-4	49	100	201.0	18.5	1.1
LG-5580 (St-5)	50	102	192.4	18.6	1.8
P64G46 (St-2)	48	104	155.5	19.1	1.4
08 TR003 (St-3)	46	97	165.4	18.6	1.4
EGE 2001	47	101	174.9	19.2	2.4
CV (%)	1.22	0.54	3.72	5.56	
LSD (0.05)	0.81	0.77	9.41	1.45	
LSD (0.01)	1.09	1.04	12.60	1.94	

*: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

Table 3. Oilseed sunflowers yield trials yield, (kg/da), oil content and oil yield, and 1000 seed weight (g), values of the varieties. TARI, Edirne (2013).

Variety Name	Seed Yield (kg da^{-1})	Yield Groups (α=0.01)		Seed Yield over St. mean (%)	Seed Yield Order	Oil Content (%)	Oil Yield (kg da^{-1})	Oil Yield Groups (α=0.01)		OilYield Order	1000 Seed weight (g)*
08 TR 003 (K)	239	A		113.1	1	48.0	115	A		1	59.28
TE-TM 2012-6	237	A	B	112.4	2	46.3	110	A	B	2	40.24
TE-TM 2012-1	221	A	C	104.7	3	48.9	108	A	B	3	43.56
EGE 473 A x TT 119 R	217	A	C	102.8	4	46.3	100	B	C	5	50.40
P64G46 (K)	215	B	C	102.0	5	47.2	102	B	C	4	48.24
LG 5550 (K)	206	C	D	97.6	6	42.6	88	D	E	7	55.04
TE-TM 2012-4	204	C	D	96.6	7	46.1	94	C	D	6	40.32
TE-TM 2012-3	200	C	D	95.0	8	43.4	87	D	E	8	44.48
EGE 436 A x TT 119 R	185	D	E	87.6	9	43.4	80		E	10	39.12
TE-TM 2012-2	185	D	E	87.6	10	43.7	81		E	9	40.64
LG 5580 (K)	184	D	E	87.0	11	42.7	78		E	12	39.64
TE-TM 2012-5	169		E	80.0	12	47.6	80		E	11	41.28
CV (%)	7,65						7,60				
LSD (0.05)	22,57						10,24				

* 10% seed moisture.

Table 4. Phenological Data of Firs Crop Oilseed Sunflowers Yield Trials. TARI, Edirne (2013).

Variety Name	Days to flowering (day)	Pphysiological maturity (day)	Plant height cm)	Head diameter (cm)
P64G46 (K)	58	102	133	15
LG 5580 (K)	60	102	147	18
LG 5550 (K)	58	101	137	16
08 TR 003 (K)	57	99	145	16
EGE 436 A x TT 119 R	59	103	158	14
EGE 473 A x TT 119 R	60	107	133	19
TE-TM 2012-1	56	105	124	20
TE-TM 2012-2	58	106	145	16
TE-TM 2012-3	57	105	142	14
TE-TM 2012-4	57	105	147	16
TE-TM 2012-5	58	106	158	15
TE-TM 2012-6	58	105	169	20

Table 5. Oilseed sunflowers yield trials yield, (kg/da), oil content and oil yield, and 1000 seed weight (g), values of the varieties. TARI, Luleburgaz, Edirne (2013).

Variety Name	Seed yield (kg da^{-1})	Yield groups (α=0.01)		Seed yield over St. mean (%)	Seed yield Order	Oil content (%)	Oil yield (kg da^{-1})	(α=0.01) Oil yield groups		Oil yield Order	1000 Seed weight (g)
LG 5550 (K)	204	A		109.5	1	44.7	91	A	C	3	54.66
TE-TM 2012-6	199	A	B	106.7	2	46.9	93	A	B	2	48.12
TE-TM 2012-1	190	A	C	102.1	3	51.1	97	A		1	42.64
EGE 473 A x TT 119 R	187	A	C	100.3	4	48.2	90	A	C	5	43.80
08 TR 003 (K)	185	B	D	99.6	5	49.0	91	A	C	4	50.32
P64G46 (K)	180	C	D	96.4	6	47.3	85	B	D	6	49.48
EGE 436 A x TT 119 R	179	C	D	96.2	7	46.1	83	C	E	8	39.96
LG 5580 (K)	176	C	D	94.3	8	43.0	76		E	11	43.32
TE-TM 2012-5	174	C	D	93.7	9	48.0	84	C	E	7	41.16
TE-TM 2012-3	172	C	D	92.5	10	44.3	76		E	12	41.52
TE-TM 2012-2	168		D	90.3	11	45.7	77	D	E	10	39.56
TE-TM 2012-4	168		D	90.2	12	46.4	78	D	E	9	45.48
CV (%)	7,00						7,01				
LSD (0.05)	18,32						8,58				

* 10% seed moisture.

Table 6. Phenological Data of Firs Crop Oilseed Sunflowers Yield Trials. TARI, Luleburgaz, Edirne (2013).

Variety Name	Days to flowering (day)	Pphysiological maturity (day)	Plant height (cm)	Head diameter (cm)
P64G46 (K)	59	103	137	11
LG 5580 (K)	61	102	146	9
LG 5550 (K)	59	101	144	10
08 TR 003 (K)	57	100	139	11
EGE 436 A x TT 119 R	59	104	164	10
EGE 473 A x TT 119 R	61	106	171	12
TE-TM 2012-1	57	106	144	11
TE-TM 2012-2	59	107	148	9
TE-TM 2012-3	59	105	154	10
TE-TM 2012-4	58	106	159	9
TE-TM 2012-5	59	106	168	9
TE-TM 2012-6	59	104	160	12

Table 7. Oilseed sunflowers yield trials combined data of Edirne and Luleburgaz on yield, (kg/da), oil content and oil yield, and 1000 seed weight (g), values of the varieties.

TARI, Luleburgaz and Edirne (2013).

Variety Name	Seed yield (kg da^{-1})*	Yield groups (α=0.01)		Seed yield over St. mean (%)	Seed yield Order	Oil content (%)	Oil yield (kg da^{-1})	(α=0.01) Oil yield groups		Oil yield order
08 TR 003 (K)	212	A	B	106.8	2	48.5	102,7	A		1
TE-TM 2012-1	206	A	C	103.6	3	50.0	102,6	A		2
TE-TM 2012-6	218	A		109.8	1	46.6	101,5	A	B	3
EGE 473 A x TT 119 R	202	B	C	101.7	5	47.3	95,2	B	C	4
P64G46 (K)	197	C	D	99.4	6	47.3	93,3		C	5
LG 5550 (K)	205	A	C	103.2	4	43.7	89,4	C	D	6
TE-TM 2012-4	186	D	E	93.7	8	46.3	86,0	D	E	7
TE-TM 2012-5	172		F	86.5	12	47.8	82,1	E	F	8
TE-TM 2012-3	186	D	E	93.9	7	43.9	81,6	E	F	9
EGE 436 A x TT 119 R	182	E	F	91.7	9	44.8	81,4	E	F	10
TE-TM 2012-2	177	E	F	88.9	11	44.7	78,8		F	11
LG 5580 (K)	180	E	F	90.5	10	42.9	77,0		F	12
CV (%)	7,38						7.35			
LSD (0.05)	14,26						6.55			

* 10% seed moisture.

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PERFORMANCE OF SOME CONFECTIONARY SUNFLOWER (*HELIANTHUS ANNUUS* L.) VARIETIES IN AEGEAN REGION OF TURKEY

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ABSTRACT

Confectionary sunflower was grown 116322 ha area and 180700 metric ton seed was harvested with the average yield of 1550 kg ha^{-1} in Turkey in 2015. Aegean Region region produce a significant number of approximately 36% of confectionary seed in Denizli, İzmir, Kütahya, Usa, Afyon, Balıkesir, and Manisa provinces of Turkey. However, farmers generally use low yielding landraces for confectionary sunflowers production. The main objectives of this study were to determine confectionary varieties which could be grown with satisfactory yield performance in Aegean Region. The experiments were conducted at the experiment field of Aegean Agricultural Research Institute in Menemen, Izmir in 2009 to 2015. The experiments were established in randomized complete block design with four replications. As a material, open pollinated and hybrid confectionary sunflower candidate varieties were used in the experiments. This study were undertaken for the characters of seed yield, seed oil content (%), 1000 seed weight, plant height, head diameter, seed length, seed width, hull percentage (%), seed color, days to flowering and days to physiological maturity. Results showed that statistically significant differences were found among the varieties for the characters in question. In the experiments; the highest seed yield as 546 kg da^{-1} was obtained from the candidate open pollinated variety ETAE-D1-1-B2 in 2009 and 619 kg da^{-1} was obtained from the candidate hybrid variety ETAE-C-TM-10-2010 in 2011. The results indicated that OP and hybrid varieties could grow with their high yield performance in Aegean Region; thus, in order to increase production landraces or old cultivars need to be replaced with modern varieties.

Keywords: Sunflower, *Helianthus annuus* L, confectionary variety, adaptation, yield, yield components.

INTRODUCTION

Turkey is one of the leading countries for sunflower production. According to production data confectionary sunflower was grown on 116322 ha area and 180700 metric ton seed was harvested and the mean seed yield was 1550 kg ha^{-1} in Turkey in 2015. The Aegean Region has suitable ecological conditions for first and second crop sunflower production. Aegean Region region produce a significant number of approximately 36% of confectionary seed in Denizli, Afyon, Manisa, Uşak, Kütahya, and Izmir provinces (Anonim, 2015).

There is not enough certified seed production with desired quality. Consequently, land races are generally used for Confectionary sunflower production in Turkey.

The land races or local varieties are not suitable for combine harvest because of their ununiformity of the plant development in the field (Tan, 2010b; Tan and Tan, 2010; Tan, *et. al*., 2013b). In Sunflower research project at AARI, oilseed and confectionary type of

sunflower germplasms, open pollinated and hybrid varieties have been developed, and candidate varieties are evaluated in yield trials under first and second crop production season. Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance (500-550 kg da^{-1}) at both first and second crop production seasons in Aegean Region of Turkey (Tan, 2007; Tan, 2011; Tan, 2010a; b; Tan, *et. al.*, 2013a; Tan, *et. al.*, 2015). The main objectives of this study were; to determine open pollinated confectionary varieties which could be grown with satisfactory yield performance for Aegean region.

MATERIAL AND METHODS

The experiments conducted separately at first crop growing seasons in 2013 to 2015 on the experiment field of Aegean Agricultural Research Institute (AARI) in Menemen, Izmir.

The study were undertaken for the characters seed yield (kg da^{-1}), seed oil content (%), 1000 seed weight (g), plant height (cm), head diameter (cm), seed length (mm), seed width (mm), hull percentage (%), seed color, days to flowering and days to physiological maturity.

In this study, open pollinated (OP) confectionary sunflower candidate varieties developed at AARI sunflower breeding program were used in the experiments. The OP confectionary type of varieties, developed by using sunflower collections conserved National Gene Bank at AARI, Menemen, Izmir.

The varieties used in this study.

Varieties	Seed coat color
ETAE-D1-1-B1	White with a light gray stripe
ETAE-D1-1-B2	White with a light gray stripe
ETAE-D1-1-B3	White with a light gray stripe
ETAE-D1-1-B6	White with a gray stripe
D-2012-1-1	White with a light gray stripe
D-2012-1-2	White with a light gray stripe
ETAE-NGL	White
ETAE-ALA (D2)	Dark brown with a light gray stripe
ETAE-Ç-P-1-2	Black
ETAE-Ç-P-11-1	Black
ETAE-K-1	White with a few pale gray stripe
Cigdem (Check variety)	White with gray stripe
Palancı-1 (Check variety)	Gray with a white stripe

Planting times of the experiments were 21.05.2013, 02.07.2014, and 15.04.2015. The experiments were conducted a randomized block design with four replications. The

confectionary variety test experiment plots were consisted of four rows, 8.80 m in length, spaced 0.7 m apart. There were 22 plants, spaced 0.40 cm on each row.

The experiments were conducted on sandy loam soil. Recommended agronomic crop production practices were followed. Fifty kg da^{-1} ($N_{10}P_{10}K_0$) composed fertilizer applied during the soil preparation. Weed control were routinely applied. Three irrigation were applied in 2013 (27/06/2013, 12/07/2013, 31.07.2013) and 2014 (25.07.2014, 22.08.2014, 09.09.2014), and only one irrigation was applied in 2015 (10.07.2015).

Data were obtained on;

Seed yield (kg da^{-1}): The yield was obtained from each of the two middle rows of the four row plots in the experiment. At the harvest, 1st and 4th rows and first and last plants of the middle row are removed as edge effect in the experiment. Heads were hand harvested, threshed, and evaluated at 0% moisture.

Days to physiological maturity: Days from planting to R9 stage (Schneiter, and Miller, 1981).

Days to flowering maturity: Days from emergence to 75% of the flowering.

Plant height (cm): Height of ten plants were measured at R9 (Schneiter, and Miller, 1981) from ground level to the base of the head (cm).

Head diameter (cm): Head diameter of ten plants were measured at R9 (Schneiter, and Miller, 1981).

Seed size (mm): The length and width of a sample of 10 seeds were measured in mm.

Hull percentage (%): Sample from harvested seed was dried to 0% moisture and the husk of seed was removed and weighted.

1000 seed weight (g): Weight of 1000 seed (g) determined from dried seed (0% moisture) sample.

Hectoliter (g): Sample of each parcel hectoliter weight (g).

Oil content (%): Sample from harvested seed was dried to 0 g kg^{-1} moisture and percent oil was determined by nuclear magnetic resonance (NMR).

Uniformity: At the 75% of the flowering stage plants were observed whether they were uniform or not.

Statistical analysis was performed to determine the differences among the varieties (Steel, and Torrie, 1980).

RESULTS AND DISCUSSION

According to three-years results of this research in Menemen conditions, among the confectionary varieties, statistically significant (α: 0.05 and 0.01) differences were found on seed yield, flowering date, physiological maturity date, plant height, head diameter, 1000-seed weight, oil percentage (%), Seed length (mm), Seed width (mm), and hull percentage (%) (Table 1, 2, and 3).

In the experiments; the highest seed yield 537 kg da^{-1} and the lowest 216 kg da^{-1} were obtained from the varieties ETAE-D-2012-1-2 and ETAE-D1-1-B6 respectively in 2013; the highest seed yield 431 kg da^{-1} and the lowest 171 kg da^{-1} were obtained from the varieties ETAE-D-2012-1-1 and ETAE-C-P-1-2 respectively in 2014; and the highest seed yield 326 kg da^{-1} and the lowest 167 kg da^{-1} were obtained from the varieties ETAE-K1 and ETAE-C-P-1-2 respectively in 2015.

The lowest flowering days were recorded from the variety D-2012-1-2 as 50, 47, and 56 days in 2013, 2014 and 2015 growing seasons respectively. However; the highest flowering days (64 days) observed from ETAE-Ç-S-1-2 in 2015.

The lowest physiological maturity days were recorded 104 days (D1-1-B6), 95 days (ETAE-K1 and Ç-P-1-2), and 100 days (Palancı-1) in 2013, 2014 and 2015 growing seasons respectively. However; the highest physiological maturity days (109 days) observed from D-2012-1-2 in 2015. The highest plant height (228.20 cm) was obtained from D-2012-1-1 and the lowest plant height (166.40 cm) was obtained from Ç-P-1-2 in 2013 growing season.

The highest head diameter (27.83 cm) was obtained from D-2012-1-1 in 2015 growing season, and the lowest head diameter (19,40 cm) was obtained from (ETAE-D1-1-B6 and ETAE-Ç-P-11-1) in 2013 growing season. The highest 1000-seed weight 184.60 g (Palancı-1) and 182.90 g (D-2012-1-1) were obtained in 2015 growing season, however; the lowest 1000-seed weight (86,96 g) was obtained from ETAE-Ç-P-11-1 in 2013 growing season.

The highest seed length (25.41 mm) was obtained from ETAE-NGL in 2015 growing season, and the lowest seed length (18.11 mm) was obtained from ETAE-Ç-P-1-2 in 2009 growing season. The highest seed width (8.77 mm) was obtained from ETAE-NGL in 2015 growing season, and the lowest seed width (5.77 mm) was obtained from ETAE-Ç-P-1-2 in 2014 growing season. The highest hull percentage (53.62%) was obtained from ETAE-K1 in 2013, and the lowest hull percentage (35.95%) was obtained from Palancı-1 in 2015 growing season.

CONCLUSIONS

Results showed that statistically significant differences were found among the sunflower varieties for the characters in question.

In the experiments; the highest seed yield for (563 kg da^{-1}) and the lowest seed yield (202 kg da^{-1}) were obtained from the varieties ETAE-D1-2-B2 and ETAE-Ç-P-1-2 in 2009 growing season respectively, in confectionary variety experiments.

Research results indicated that both oilseed hybrids and OP confectionary varieties had suitable physiological maturity days for both first and second crop production seasons in the region.

Research results indicated that the yield performance of the oilseed varieties ranges 171 to 537 kg da^{-1}, and this results shows similarity of the long term adaptation results in Aegean Region (Tan *et. al*., 2013a; 2015).

Planting high yielding varieties in Aegean Region that has suitable ecological conditions for first and second crop sunflower production may play an important role to decrease good quality production gap in Turkey. Research results were also indicated that candidate confectionary varieties with their desired seed characters and quality will aid to decrease seed demands of the farmers.

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Table 1. First Crop confectionary sunflower variety yield trial. AARI, Menemen, Izmir (2013).

Variety name	Days to flowering (days)	Days to physiological maturity (days)	Yield* (kg da^{-1})	Yield Groups (α=0.01)	Plant heigth (cm)	Head diameter (cm)	1000 seed weight (g)	Seed length (mm)	Seed width (mm)	Oil percentage (%)	Hull percentage (%)	Uniformity (1-5) **
D1-1-B1	53	107	372	C	185.6	22.6	118.6	19.86	7.21	25.26	44.31	3.0
D1-1-B2	52	106	411	BC	203.9	21.1	113.2	19.78	7.26	28.60	43.25	2.8
D1-1-B3	52	107	462	AB	196.9	22.6	124.9	19.17	7.47	25.58	44.68	2.5
D-2012-1-1	51	109	485	AB	228.2	21.8	132.7	22.43	7.34	28.89	44.49	2.5
D-2012-1-2	50	108	537	A	218.9	22.3	143.3	22.96	7.75	28.24	44.51	2.5
D1-1-B6	52	104	216	D	171.1	19.4	116.2	21.42	6.11	26.32	41.33	2.5
ETAE-ALA (D2)	52	104	443	BC	202.3	20.2	119.0	21.64	7.55	29.16	45.53	2.3
Ç-P-1-2	58	105	238	D	166.4	21.5	100.2	18.79	5.93	21.83	45.45	2.5
Ç-P-11-1	61	105	280	D	169.6	19.4	86.96	19.79	6.12	24.30	45.90	2.8
ETAE-K-1	53	105	487	AB	189.4	21.1	135.8	19.88	7.48	20.11	53.62	2.3
CV (%)	1.58	0.47	11.59		3.78	5.28	6.42	2.57	4.85	5.16	3.39	
LSD (0.05)	1.22	0.73	66.09		10.59	1.63	11.09	0.77	0.49	1.94	2.23	
LSD (0.01)	1.65	0.98	89.25		14.30	2.19	14.97	1.04	0.67	2.61	3.01	

* Seed yield and 1000 seed weight were adjusted to 0% moisture.

**: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

Table 2. Second crop confectionary sunflower variety yield trial. AARI, Menemen, Izmir (2014).

Variety name	Yield* (kg da^{-1})	Yield Groups (α=0.01)	Days to flowering (days)	Days to physiological maturity (days)	1000 seed weigth (g)	Hull percentage (%)	Seed length (mm)	Seed width (mm)	Uniformity (1-5) **
D-2012-1-1	431	A	48	97	129.29	42.07	23.31	7.76	2.1
D-2012-1-2	353	AB	47	99	150.95	43.86	23.87	8.24	2.6
ETAE-ALA (D2)	385	A	50	93	130.35	42.05	20.55	8.23	2.0
ETAE-K-1	263	BC	49	95	132.45	49.51	20.67	7.80	1.9
ETAE-Ç-P-1-2	171	C	50	95	89.40	42.06	17.91	5.77	1.3
CV (%)	15.53		0.84	0.48	6.95	2.47	2.69	5.68	
LSD (0,05)	76.72		0.63	0.70	13.54	1.67	0.87	0.66	
LSD (0,05)	107.60		0.88	0.99	18.89	2.34	1.22	0.93	

* Seed yield and 1000 seed weight were adjusted to 0% moisture.

**: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

Table 3. First crop confectionary sunflower variety yield trial. AARI, Menemen, Izmir (2015).

Variety name	Days to flowering (days)	Days to physiological maturity (days)	Yield* (kg da^{-1})	Yield Groups (α=0.01)	Plant heigth (cm)	Head diameter (cm)	1000 seed weigth (g)	Seed length (mm)	Seed width (mm)	Hectoliter (g)	Hull percentage (%)	Oil percentage (%)	Uniformity (1-5) **
D-2012-1-1	56	104	279	A	193.9	27.83	164.61	25.05	8.35	206	43.57	24.05	2.3
ETAE-ALA (D2)	57	104	257	A	191.8	26.02	149.58	22.08	8.27	262	42.97	24.38	2.3
ETAE-K-1	59	103	293	A	181.7	25.30	159.66	21.17	7.79	306	47.83	26.58	2.1
ETAE-NGL	60	105	283	A	205.4	28.60	172.35	25.41	8.77	252	44.62	21.10	2.5
ETAE-Ç-S-1-2	64	105	150	B	187.9	22.70	108.27	18.11	5.81	314	39.36	24.20	1.6
Cigdem (St-2)	58	106	286	A	195.4	27.30	142.47	20.16	7.71	300	39.66	26.55	2.3
Palanci-1 (St-3)	56	100	271	A	186.3	24.13	166.14	18.98	8.43	323	35.95	31.90	1.5
CV (%)	1.50	1.37	12.87		3.77	3.67	3.91	2.62	2.29	2.95	3.05	4.52	
LSD (0,05)	1.30	2.12	55.22		10.75	1.42	9.79	0.84	0.27	13.99	1.91	1.71	
LSD (0,05)	1.78	2.90	75.65		14.73	1.94	13.41	1.15	0.36	19.16	2.61	2.35	

* Seed yield, 1000 seed weight, and hectoliter weight (g) were adjusted to 0% moisture.

**: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

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OILSEED AND CONFECTIONARY SUNFLOWER (HELIANTHUS ANNUUS L.) LANDRACES OF TURKEY

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ABSTRACT

Turkey is one of the significant countries for the plant crop diversity. Turkey is also center of origin for many crop species. Flora of Turkey consists of high endemism about 3000 out of the 9500 plant species. Turkey is described as microcenters for some crops which are originated in different part of the world. The sunflower (*Helianthus annuus* L.) and its wild relatives were originated and domesticated in North America, providing an important genetic diversity for crop improvement. It is one of the important oilseed crops and their landraces have significant diversity in Turkey. The extend diversity of sunflower landraces or primitive commercial varieties are also very important source of genetic variability because they have adapted to local environments as a result of natural selection over centuries. Within the framework of National Industrial Plant Genetic Resources Project, sunflower landraces have been collected and conserved ex situ at the National Gene Bank in Izmir, Turkey. There are 389 oilseed and confectionary sunflower accessions conserved and maintained at the National Gene Bank of Turkey. In this study; eco-geographical distribution of sunflower landraces and the characterization result of agro-morphological variation of National sunflower collection will be presented. IPGRI / UPOV characters were evaluated to analyze the similarity and dissimilarity. Principle Component Analysis (PCA) was performed for diversity determination of sunflower accessions. The distribution areas of sunflower samples showed great diversity and very variable for morphological characters. The results of analysis exhibited broad morphological variation model of sunflower land races. The diversity among and between the landraces is result of adaptation of different ecologies and the farmers' selection in their pLITERATURE. The informal seed exchange mechanism among the farmers effect the some degree of similarity of the some accessions collected from different localities of different provinces.

Keywords: Sunflower, *Helianthus annuus* L., landraces, conservation, diversity, agro-morphological variation, eco-geographical variation, characterization, Multivariate analysis.

INTRODUCTION

Turkey is one of the distinctive countries for the plant diversity as being center of origin and/or center of diversity or microgene center for many crop species (Harlan; 1951; Tan, 2010a; Tan, 2010b; Karagoz *et. al*., 2010). Two of the Center of Origin is overlapped in Anatolia. Turkey is also described as microcentres for some crop species that are not originated in Turkey but they are divers in many characteristics. Turkey is the meeting place of three phytogeographical regions; Euro-Siberian, Mediterranean, and lrano-Turanian. Turkey's wealth in plants is apparent in the fact that about 3,700 out of the 11,707 plant taxa are endemic to the area (Güner *et. al*.2012).

Tanksley and McCouch (1997) emphasized that narrowing of the genetic base occurred firstly when changing the wild species into a domesticated species and secondly when landraces were

replaced by modern cultivars. Therefore the landraces, before the replacement with modern varieties should be collected, conserved and evaluated for source of breeding for broaden the genetic base. Highly organized National Plant Genetic Resources Program (NPGRP) of Turkey conducts survey, collection, conservation both *ex situ* and *in situ* (including on farm conservation of landraces), characterization and evaluation of Turkish genetic resources and genetic diversity since 1960s (Tan, 2000; Tan, 2010b).

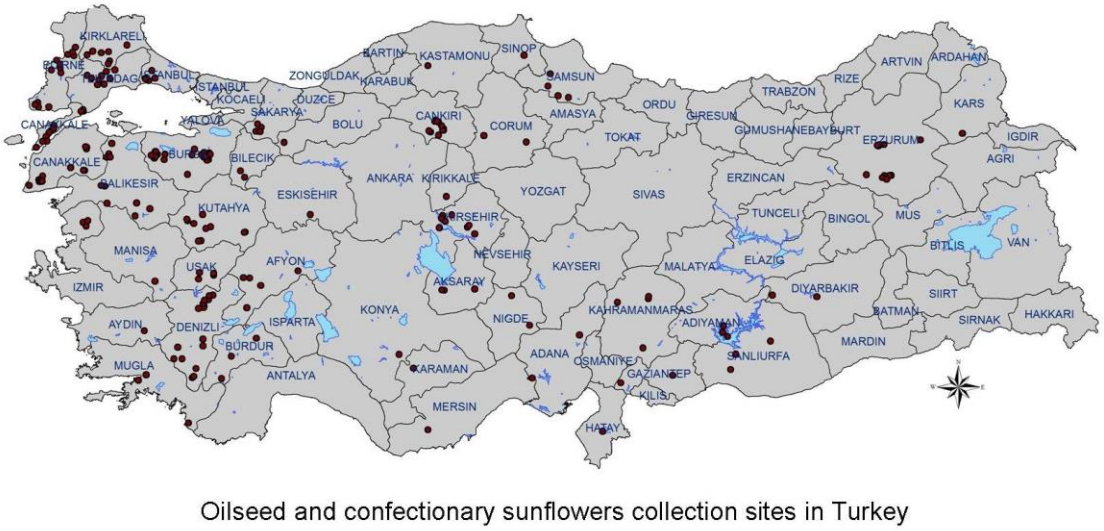
The Industrial Crops Genetic Resources Program of NPGRP is responsible for survey, collection, conservation, regeneration, evaluation, and characterization of industrial crops species (landraces and wild species (Tan and Tan, 2010; Tan and Tan, 2011; Tan and Tan, 2012; Tan et al., 2013). Environmental factors affecting the lost of wild species, the threats on landraces/local varieties are mainly the result of the replacement of landraces with modern varieties and changing the agricultural farming system. So, Industrial Crops Genetic Resources Program has yearly survey and collection program for long-term conservation of the collection at National Gene bank at Aegean Agricultural Research Institute (AARI).

Extremely variable domesticated crops as well as landraces with unique characteristics are still grown by farmers in Turkey. Fragmentation of lands lets farmers run several fields and to keep local landraces with application of traditional farming. Marginal agronomic conditions, especially steep slopes and heterogeneous soils of mountain agriculture make local landraces competitive with improved cultivars, at least in part of farming system. Economic isolation creates market limitation and minimizes to competitive advantages of improved cultivars. Local traditions and preference of diversity lead farmers to keep local landraces are the factors affect the farmers, even modern farmers, to keep their landraces or traditional crops (Tan, 2009).

From different provinces and different sources, like fields, farmer storage, threshing place and local markets of the villages, about 390 accessions confectionary and oilseed type sunflower land races were collected and stored and maintained long-term at National Gene Bank, so far. The collection and passport data, storage and characterization data are stored in National Plant Genetic Resources Data Base (Tan et al. 2015). Figure 1 shows the collection sites of sunflower land races. The collection, passport and characterization data are stored in National Plant Genetic Resources Data Base (Tan and Tan, 1998a; Tan and Tan, 1998b; Tan, 2010a; Tan, 2010b).

The Sunflower and its wild relatives were originated in North America (Heiser ve ark., 1969; Heiser, 1978; Putt, 1978; Zeven and deWet, 1982; Miller, 1987) and providing and important genetic diversity for crop improvement. Landraces are also important source of genetic variability because they have adapted to local environments as a result of natural selections over centuries. Thus, the characterization of existing collection is essential for the breeders. Characterization of genetic resources collections of confectionary and oilseed sunflower is significant to assess collection diversity for increased utilization (Tan and Tan, 2010; Tan and Tan, 2011; Tan and Tan, 2012; Tan et al., 2013; Tan et al., 2015b).

The main objectives of the this study were to analyze the degree of similarity or differences among sunflower landraces and to determine the extent of genetic diversity in sunflower landraces based on agro-morphological traits to provide information and utilization in plant breeding program.



Oilseed and confectionary sunflowers collection sites in Turkey

Figure 1. Sunflower land races collection sites in Turkey (Tan et al., 2015).

MATERIALS AND METHODS

Fifty four confectionary sunflower accessions collected from West and East Turkey, and 36 oilseed accessions collected from West Turkey maintained at National Seed Gene Bank, were characterized for assessing sustainable utilization.

The accessions were grown in two rows and fifty plants. Twenty randomly selected plants were observed from each accession. IPGRI (Anonymous, 1985) and UPOV (Anonymous, 2000) Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Sunflower" were used to observe the thirty two morphological characters of plant, head/flower and seed characteristics (Table 1). The agronomic characters, days to flowering and days to physiological maturity were also recorded (Anonymous, 1985; Anonymous, 2000).

Statistical analysis and Multivariate Analysis (Principal Component Analysis-PCA) were applied to conclude the variation among the accessions (Sneath and Sokal, 1973; Clifford and Stephenson, 1975; Tan, 1983). The statistical values of quantitative characters were calculated (Steel and Torrie, 1980).

Table 1. The observed morphological characters (Anonymous, 1985; Anonymous, 2000).

Plant characteristics: Plant height (cm), Stem width (cm), Branching, Leaf shape of cross section, Leaf shape, Leaf auricules, Leaf wings, Leaf pubescence, Leaf blistering, Leaf serration, Leaf width (cm) Leaf length (cm), Leaf distribution on stem, Stem hairiness, Stem diameter (cm), Leaf angle of lowest lateral veins, Leaf: height of the tip of the blade, compared to insertion of petiole.
Head/flower characteristics: Head diameter (cm), Head attitude, Head shape, Disk flower color, Disk flower anthocyanin coloration, Pollen fertility.
Seed characteristics: 1000 seed weight (g), Seed length (mm), Seed width (mm), Seed main color Seed type (Oilseed/confectionary), Seed hairiness, Seed stripes, Seed shape.

RESULTS

I. Characterization of Confectionary Sunflower Genetic Resources of Turkey

The morphological variation on the observed characters was found highly variable for most of the characters. All accessions have released the fertile pollen, and alternate leaf arrangements, hairy stem, absent branching, short hairy leaves, triangular leaf shape, confectionary type of kernel, dark yellow head flower. No anthocyanin coloration on the disk flower was observed. Plants were mostly vigor.

Almost all leaf characters showed variation. Leaf blistering was mainly strong and medium; Leaf serration coarse and medium; Leaf shape of cross section flat and weakly convex; Leaf auricules medium and large; Leaf wings none or very weakly expressed and weakly expressed; Leaf angle of lowest lateral veins acute and right angle or nearly right angle; Leaf height of the tip of the blade compared to insertion of petiole low and medium. Seed shape presented mainly as elongated, narrow ovoid and broad ovoid. Seed main color was white and whitish grey; Seed Stripes was observed with all types as none or very weakly expressed, weakly expressed and strongly expressed. Head attitude was variable at maturity; mainly half-turned down with straight stem and turned down with slightly curved stem were observed. Head shapes were presented as concave, flat, convex. In case of days to physiological maturity, they exhibited high range (97-104 days) and some of the accessions from Erzurum had shorter maturity period, *i.e.* 97 days, representing earliness. Similar pattern were observed in 1000 seed weight (80.60-183.50 g). The variations on quantitative characters were shown in Table 2.

Table 2. The statistical values of the agromorphological characters (Tan *et. al*., 2013).

Statistical value	Days to flowering	Days to physiological maturity	Plant height (cm)	Head diameter (cm)	1000 seed weight (g)
Mean	50.87	100.69	206.69	21.47	152.44
Min.	46.00	97.00	162.90	16.70	80.60
Max.	60.00	104.00	226.30	25.70	183.50
S^2 (Variance)	6.61	3.43	173.22	3.19	385.82
S (Standard error)	2.57	1.85	13.16	1.79	19.64
SE $\bar{x}$ (Standard error of the mean)	0.35	0.25	1.79	0.24	2.67
CV (%)	5.05	1.84	6.37	8.32	12.89

Statistical value	Seed width (mm)	Seed length (mm)	Leaf width (cm)	Leaf length (cm)	Stem diameter (cm)
Mean	7.56	21.85	12.90	28.24	17.90
Min.	5.60	16.68	2.57	24.00	2.83
Max.	9.16	26.18	33.20	33.30	31.50
S^2 (Variance)	0.47	3.94	161.28	6.17	141.90
S (Standard error)	0.68	1.99	12.70	2.48	11.91
SE $\bar{x}$ (Standard error of the mean)	0.09	0.27	1.73	0.34	1.62
CV (%)	9.05	9.09	98.43	8.79	66.56

Principal Component Analysis showed that the first eight principal components (PRINs) was accounted for 73.721 % of the total variation. The detailed result of PCA with latent roots (Eigen values), percentage variance and cumulative variance values were given in Table 3. First two Principal Components (PRIN1 and PRIN2) accounted with 33.272 % of total variance. Head diameter, leaf shape of cross section, leaf distribution on stem were effective variables on PRIN1 while seed length, 1000 seed weight, head shape, and seed width were effective variables on PRIN2 to form the groups and the scattering the accessions. Highly variable one group was formed and some of the Erzurum accessions and Denizli accessions were separated from the outside of this group (Figure 2).

Table 3. Result of Principal Component Analysis (Tan *et. al*., 2013).

PRINs	Latent Roots (Eigen values)	Percentage variance	Cumulative variance
PRIN 1	4.212	19.147	19.147
PRIN 2	3.107	14.124	33.272
PRIN 3	2.325	10.569	43.841
PRIN 4	1.970	8.956	52.797
PRIN 5	1.398	6.352	59.149
PRIN 6	1.222	5.554	64.703
PRIN 7	1.110	5.047	69.750
PRIN 8	0.874	3.971	73.721

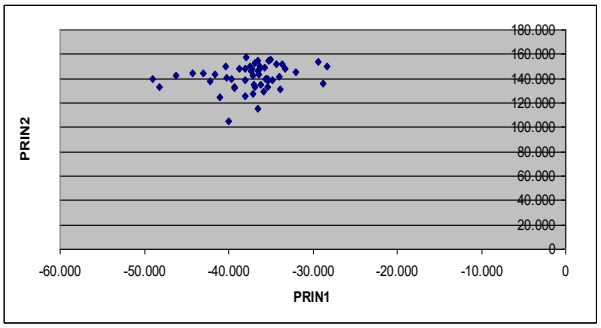


Figure 2. Distributions and grouping of the samples on PRIN1 and PRIN2 (Tan *et. al*., 2013).

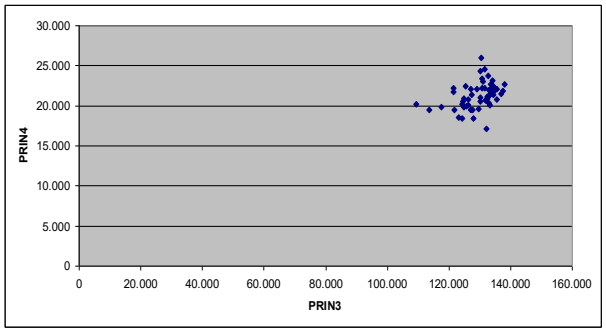


Figure 3. Distributions and grouping of the samples on PRIN3 and PRIN4 (Tan *et. al*., 2013).

Second pairs of Principal Components (PRIN3 and PRIN4) accounted with 52.797% of total variance. Leaf shape of cross section, leaf width, physiological maturity and plant height were effective character on PRIN3 whereas leaf width, leaf length, and leaf blistering were effective

characters on PRIN4. In this scatter one compact group was formed and some accessions of Erzurum province with tall plant heights and with long vegetation period were split out this group (Figure 3). The third pairs of Principal Components (PRIN5 and PRIN6) accounted with 64.703% of total variance were formed by the influence of effective variables leaf blistering on PRIN5 and head attitude on PRIN6. In this scatters, one group were observed as in the other principal component pairs. Pattern was almost same with other scatters and some accessions were scattered outside of the group (Figure 4) (Tan *et. al*., 2013).

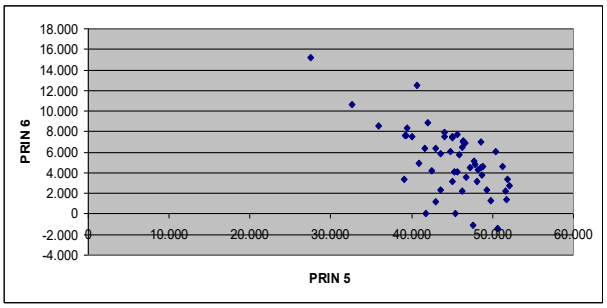


Figure 4. Distributions and grouping of the samples on PRIN5 and PRIN6 (Tan *et. al*., 2013).

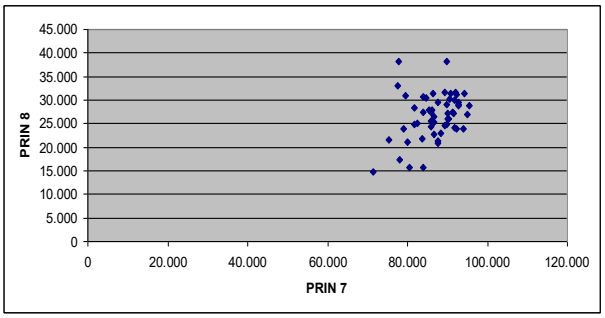


Figure 5. Distributions and grouping of the samples on PRIN7 and PRIN8 (Tan *et. al*., 2013).

The accessions in this group were very distinct from each other mainly with their very variable characteristics of head attitude. Some of the Erzurum and Denizli accessions were scattered from the group in different directions.

The fourth pairs of Principal Components (PRIN7 and PRIN8) accounted with 73.721% of total variance were formed by the influence of effective variables, leaf distribution on stem, plant height, and seed stripes on PRIN7 and leaf distribution on stem, leaf blistering, seed shape and plant height on PRIN8. In this scatters one group were observed (Figure 5). The distribution pattern was very diverse (Tan *et. al*., 2013).

II. Characterization of Oilseed Sunflower Genetic Resources of Turkey

The morphological variation on the observed characters was found highly variable for most of the characters. There was no variation on pollen fertility, type of phyllotaxis, external petal color, number of head, Seed hairiness. All accessions have released the fertile pollen, with hairless seeds, dark yellow ray flower, and alternate leaf arrangements. Plants were mostly vigor. Stems were mostly pubescence. Leaf shape was observed mostly as triangular, but cordate and rounded leaves were also observed and recorded. Head angle was very variable at maturity, and all types were observed (0°, 45°, 90°, 135°, 180° and 225°). Head shapes were also presented as concave, flat, convex and misshapen. Type of branching was another diverse character, but mostly basal branching and top branching were observed. The fully branched with central head were also observed in some plants of some accessions. The variation on quantitative characters was shown in Table 4. In case of plant height, they exhibited high range (157.0-273.5) of variation. Similar pattern were observed in the 1000 seed weight (78.4-142.25 g).

Table 4. Statistical values of the quantitative characters (Tan and Tan, 2012).

Statistical values	Days to flowering	Days to physiological maturity	Plant height (cm)	Head diameter (cm)	1000 seed weight (g)	Husk percentage (%)
Mean	56.00	110.97	185.43	20.61	96.26	28.69
Min.	52.00	108.00	157.00	16.40	78.40	20.95
Max.	71.00	121.00	273.50	27.00	142.25	50.79
S^2 (Variance)	24.74	8.54	878.36	5.01	168.34	57.48
S (Standard error)	4.97	2.92	29.64	2.24	12.97	7.58
$SE_{\bar{x}}$ (Standard error of the mean)	0.83	0.49	4.94	0.37	2.16	1.26
CV (%)	8.88	2.63	15.98	10.86	13.48	26.42

Statistical values	Stem width (cm)	Seed length (mm)	Seed width (mm)	Leaf width (cm)	Leaf length (cm)	Number of leaf
Mean	12.19	6.21	30.69	23.41	22.46	2.38
Min.	10.66	4.92	24.10	16.60	18.00	1.70
Max.	16.70	8.00	45.40	33.00	30.90	3.40
S^2 (Variance)	2.22	0.59	24.44	18.72	15.39	0.17
S (Standard error)	1.49	0.77	4.94	4.33	3.92	0.41
$SE_{\bar{x}}$ (Standard error of the mean)	0.25	0.13	0.82	0.72	0.65	0.07
CV (%)	12.22	12.38	16.11	18.48	17.47	17.39

Table 5. Result of Principal Component Analysis (Tan and Tan, 2012).

PRINs	Latent Roots (Eigen values)	Percentage variance	Cumulative variance
PRIN 1	8.766	35.062	35.062
PRIN 2	3.507	14.029	49.091
PRIN 3	2.194	8.776	57.867
PRIN 4	1.675	6.700	64.567
PRIN 5	1.451	5.803	70.370

Principal component analysis (PCA) showed that the first five principal components (PRINs) accounted for 70.370 % of the total variation. The detailed result of principal component analysis with Latent Roots (Eigen values), Percentage Variance and Cumulative Variance values is given in Table 5. First two Principal Components (PRIN1 and PRIN2) accounted with 49.091 % of total variance. Plant height, leaf length, leaf width, seed length, Stem width and husk percentage were effective variables on PRIN1, and head size, pubescence on leaf and plant vigority were effective variables on PRIN2 to form the groups and the scattering the accessions. Only one group was formed which consist of oil types and confectionary types were separated from this groups (Figure 6). Second pairs of Principal Components (PRIN3 and PRIN4) accounted with 64.567% of total variance. Leaf shape is effective character on PRIN3 and seed length, head flower color, leaf edge and leaf shapes are effective characters on PRIN4. In this scatter one group was formed which consists of oil types and all confectionary types and some oil types with large seed were outside of this group (Figure 7). The third pairs of Principal Components (PRIN4 and PRIN5) accounted with 70.370 % of total variance were formed by the influence of effective variables seed length, head flower color, leaf edge and leaf shape on PRIN4 and type of branching, head flower color and Pubescence at stem on PRIN5. In this scatters one group were observed as in the other principal component pairs. Pattern was almost same the confectionary types were outside of the group (Figure 8) (Tan and Tan, 2012).

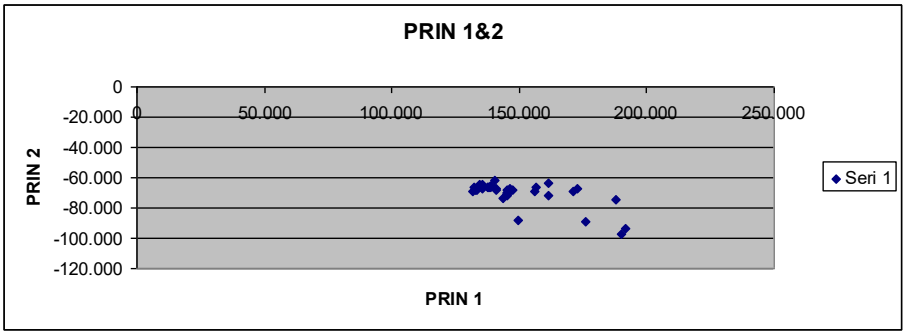


Figure 6. Distributions and grouping of the samples on PRIN1 and PRIN2 (Tan and Tan, 2012).

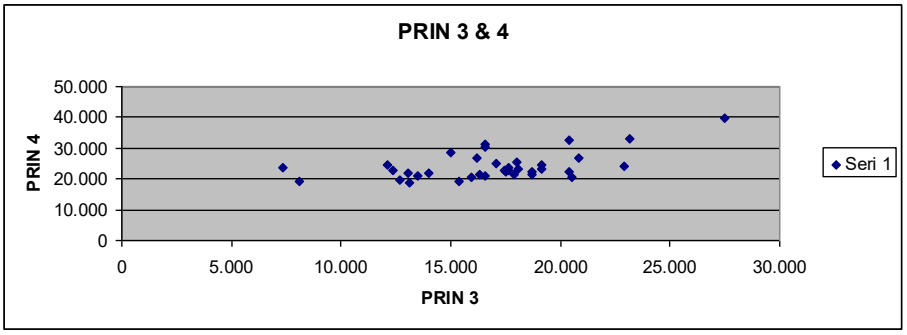


Figure 7. Distributions and grouping of the samples on PRIN3 and PRIN4 (Tan and Tan, 2012).

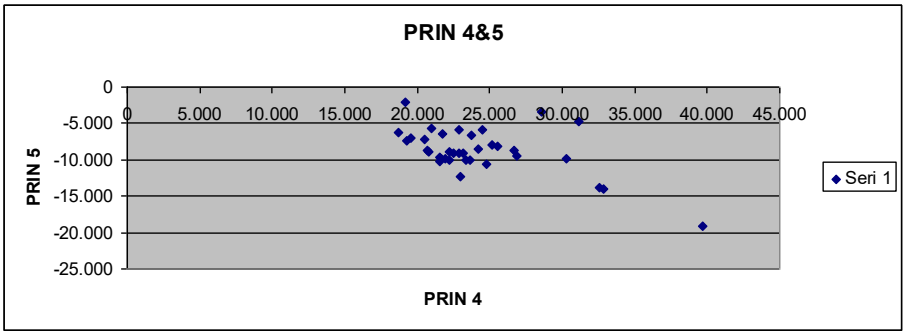


Figure 8. Distributions and grouping of the samples on PRIN4 and PRIN5 (Tan and Tan, 2012).

CONCLUCION

Sunflower land races, especially the confectionary types were very variable for morphological characters. The distinct separation on the morphology of accessions mostly depended on the types of accessions whether oilseed or confectionary types. The variation was also observed not only among accessions but also within the accessions.

Although locality separation by germplasm origin was observed in the accessions, but in general, the origin was not corresponded closely with the grouping pattern. The variation of the land races among and within the provinces and even in the villages on some characters brings up the consideration of the adaptation to different ecological conditions and also the different pLITERATURE of the farmers selection. The some degree of similarity of the some accessions collected from different localities of different provinces may result of the informal seed exchange mechanism among the farmers (Tan and Tan, 2010; Tan and Tan, 2012; Tan *et. al*., 2013).

Landraces show varying degrees of morphological and genetic integrity and may change with time, but they are recognized by farmers on the basis of a number of morphological and agronomic criteria. However, scientists and breeders may look to preserve particular genetic resources of crops, as a means of ensuring that the maximum possible range of genetic variability is available for today and future. Therefore the landraces, before the replacement with modern varieties should be collected, conserved and evaluated for source of breeding. For this purposes the existing sunflower land races still growing by farmers are collected and characterized and used in the sunflower breeding programs.

The genetic diversity plays an important role in plant breeding. Hybrids of parental lines with diverse origin, generally display a greater heterosis than those between closely related parents (Tan, 1993; Tan, 2005). The characterization of existing sunflower collection is essential for the breeders. Thus, the existing confectionary and oilseed sunflower genetic resources collections are started to characterize and evaluate for utilization at the breeding program at AARI.

Sunflower genetic resources have also been using in sunflower breeding program to develop new varieties. Improved germplasm, and breeding lines (A, B and Rf lines) of oilseed and confectionary type of sunflower germplasm, hybrids (TURAY, SUN 2235), open pollinated variety (EGE 2001), hybrids parental lines have been developed by conventional breeding techniques. New oilseed and confectionary type of sunflower hybrids improved for registration (Tan, 2010c; Tan *et. al*., 2015a) for the direct benefit of the countries agricultural sector.

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ABSTRACT

Part of the "Genomic & Genetic of Sunflower" research team in the Laboratory of Plant-Microbe Interactions (INRA, Toulouse, France), the French *Helianthus* Biological Resources Center (BRC) is dedicated to the conservation, multiplication and distribution of seeds of *Helianthus annuus* and its wild or relative species. The germplasm collection is composed of patrimonial and scientific resources, collected or created by INRA since the early 1960's. The patrimonial part of the collection is represented by open pollinated varieties, R-line and B-line varieties and wild ecotypes, for a total of 2250 accessions. The preservation and the availability of this diversity are important for the breeding of new cultivated hybrids and the improvement of the oilseed crop. The French *Helianthus* BRC is also involved in the development, maintenance and diffusion of genetic material dedicated to scientific studies, such as mapping populations, EMS-mutant population or interspecific lines, which represents a total of 2860 entries. Finally, the *Helianthus* BRC is also implicated in the molecular characterization of the patrimonial germplasm by high-throughput SNP genotyping. This helps us to evaluate the genetic diversity and to improve the management of these biological resources.

Key Words : Sunflower; Helianthus; plant genetic resources; ex situ conservation; genetic diversity

19th International Sunflower Conference, Edirne, Turkey, 2016

EVALUATION OF VARIATION ON SUNFLOWER SINGLE CROSSES

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ABSTRACT

Sunflower is the main crop in the rotation system in Bulgaria. Sunflower yield is strongly dependent on the cultivar. A two-year experiment with 3 repeats was carried out to study the effects of climatically difference on yield and 1000 seeds weight. 24 genotypes: 4 *A* lines, 4 *R* lines and 16 hybrids combinations were compared over two years (2013 - drought and 2014 - wet conditions). Variation was highly significant. Two-year experiment for five characteristics (as yield and 1000 seeds weight) hybrids show good adaptation of test hybrids. The *boxplot*-diagram indicated that the hybrid *A3* x *R3* was the best.

Key words: 1000 seeds weight, adaptation, genotypes, hybrid, sunflower, yield.

INTRODUCTION

Agriculture is linked with the development of heterosis in sunflower breeding and the production of a new variety resistant and adapted to the dynamically changing climatic conditions. The creation of hybrids depends on the choice of starting material. The choice of parental forms based on their high combining ability and a complex of economically valuable signs that output components possess. Between plants and environmental conditions there are complex relationships. Under the influence of environmental factors is observed modification variability within a given rate of reaction. These changes characterize the adaptive capacity of the individual genotype to conditions during their development.

Sunflower is economically important oil plant in Bulgaria. Sowing of this trench culture helps the proper crop rotation. In recent years the area planted with sunflower increases and reaches 800,000 ha with an average yield of 2.3 t/ha ("Agrarian Report" by years of MZH). Economic significance is determined by the fact that international trade in sunflower seed is very successful. Important role in seed production of hybrid sunflower varieties separated maternal line with cytoplasmic male sterility (*CMS*), i.e. creation of sterile parent used to avoid castrated stamens.

The existing genetic variability of the cultivated sunflower makes it possible to develop hybrids with a genetic potential for seed yield of over 6 t/ha and seed oil content of over 55 %. However, most often sunflower yields obtained in large-scale commercial production are in the range of 1.5-3.0 t/ha. There are multiple limiting factors preventing the realization of the high genetic potential of this crop. Their removal will enable commercial sunflower yields to stabilize at level of 4 t/ha and above (Skoric, 2012). Competitive characteristic of plants plays an important role in yield formation. They claim that if in agrophytocenosis a potential genetic productivity of individual plants is realized only 10-20 % and 50 % of general phenotypic variance is caused by genetic and ecological differences in the competitive ability, it is difficult to identify the genotype according to its phenotype which leads to the decrease in the efficiency of selection. In the practical selection which is a part of the production of hybrids with a high potential of production, as well as high adaptive potential, a strong influence belongs to the autonomy of epigenetic systems of adaptive reactions to the ecological environment they are located in, or to the dynamics of changes that happen in the environment on the location of cultivation (Kirichenko, 2005).

The aim of the study is to evaluate the genetic potential, using statistical methods, of economically valuable lines in a complex of 5 characteristics and effect of environmental factors on the results obtained in 16 hybrid combinations.

MATERIAL AND METHODS

Four sterile analogues of *B* lines (*A* lines, with *CMS Pet-1*), four *R* lines and 16 hybrids combinations (table 1) were compared over two years. The *B* and *R* lines are received by selection of varieties (*B2* and *B4*) and intraspecific (*B1*) and interspecific (*B3* and all *R*) hybridization (Hristova-Cherbadzhi, 2012, 2009; Hristova-Cherbadzi et al., 2007).

Table 1. Origin of plant material.

I. Lines:	
B1 - *H.annuus* line HA89B x H.annuus line 2607B	*R1* - *H.annuus* line HA89A x *H.neglectus*
B2 - *H.annuus* variety Peredovik	*R2* - *H.annuus* line 2607A x *H.neglectus*
B3 - *H.annuus* line HA89B x *H.nuttallii* ssp. *rydbergii*	*R3* - *H.annuus* line HA89A x *H.nuttallii* ssp. *rydbergii*
B4 - *H.annuus* variety Birimirec	*R4* - *H.annuus* line HA89A x *H.pauciflorus* ssp. *subrhomboideus*

II. Hybrids:			
A1 x *R1*	*A1* x *R2*	*A1* x *R3*	*A1* x *R4*
A2 x R1	*A2* x *R2*	*A2* x *R3*	*A2* x *R4*
A3 x *R1*	*A3* x *R2*	*A3* x *R3*	*A3* x *R4*
A4 x *R1*	*A4* x *R2*	*A4* x *R3*	*A4* x *R4*

Experiences are displayed on the black-earth without fertilization and one hoeing. Experimental hybrids are included in the competitive variety trials ordered in the scheme by the principle of randomized block method, in triplicate, with reporting land area - 20 m^2. The results are represented by boxplot-diagrams in R multiplier for five quantitative traits - yield (kg/dka), 1000 seeds weight (*g*), seed oil (%), diameter head (*cm*) and plant height (*cm*). A two-year experiment with 3 repeats was carried out to study the effects of climatically difference on yield and 1000-seeds weight. *Correlation* and *cluster analysis* were realized. *Correlation analysis* (R Core Team, 2014) is to calculate the correlation coefficient between the values of characteristics yield and weight of 1000 seeds for two consecutive years (2013 - drought and 2014 - wet conditions) and subsequent verification of its statistical significance to evaluate the influence of environmental factors on the values in hybrids and parents. *Bootstrap*-intervals are represented by a histogram, which is designated the primary value of the correlation coefficient as a vertical line, QQ-diagram (Canty and Ripley, 2015; Davison and Hinkley, 1997), visualizing the location of the correlation coefficient and limits of 95 % confidence interval. For *cluster analysis* (R Core Team, 2014) was used the *hclust* function.

RESULTS AND DISCUSSION

***Correlation analysis*.** Zero hypothesis H_0 was that the correlation coefficient is zero, i.e. the value of characteristics were not correlated. The results from correlation analysis are presented on table 2.

For five characteristics hybrids show *very good adaptation* to changing environmental conditions and correlations are high significant- p <0.001, i.e. different climatic conditions in both years (drought and wet conditions) no significantly impact on the appearance of traits. A statistically significant correlation coefficient indicates that the values change synchronously over the years and hybrids. The exception is the trait "diameter head" which also exists statistically proven strong correlation between its values, but it is with hybrids evaluation 0.01 <p <0.05.

This is an evaluation of the total tolerance of the hybrids by traits, i.e. combinations are tolerant to environmental changes. Similar findings were reported for the parental lines, which is the result of their good uniformity. The change in climatic conditions affected most of the "diameter head" in hybrids.

Table 2. *Correlation analysis* of repeatability in years for five quantitative traits.

Quantitative traits	Estimates of correlation coefficient	t-distribution	Degrees of freedom	Lower limit	Upper limit	p-value
yield (hybrids)	0.9683	26.297	46	0.944	0.982	< 2.2e-16
yield (parents)	0.983	13.119	6	0.906	0.997	1.21e-05
1000 seeds weight (hybrids)	0.966	25.27	46	0.94	0.98	< 2.2e-16
1000 seeds weight (parents)	0.998	50.4	6	0.993	0.999	4.096e-09
Seed oil (hybrids)	0.998	66.74	14	0.995	0.999	< 2.2e-16
Seed oil (parents)	0.962	8.73	6	0.803	0.993	0.0001
Diameter head (hybrids)	0.53	2.34	14	0.047	0.812	0.034
Diameter head (parents)	0.979	11.61	6	0.882	0.996	2.449e-05
Plant height (hybrids)	0.834	5.66	14	0.577	0.94	5.883e-05
Plant height (parents)	0.978	11.61	6	0.881	0.995	2.453e-05

***Bootstrap*-intervals for correlation between observations of two traits in years**

Confidence interval on 0.9525 - 0.9855 at hybrids and 0.9288 - 1 at parents for characteristics yield (Fig.1, A and B), and on 0.9485 - 0.9826 at hybrids and 0.9907 - 1 at parents for characteristics 1000 seeds weight (Fig.1, C and D) is observed.

In figure 1A thick vertical line corresponds to the experimental value of 0.968 correlation coefficient between the values for 2013 and 2014 for hybrids for trait 'yield' and in figure 1B value

0.983 of the correlation of parental lines. Since the QQ-diagram (Fig. 1B) can be seen that the distribution of the correlation coefficient is significantly different from normal.

In figure 1C thick vertical line corresponds to the experimental value of 0.966 correlation coefficient between the values for two years for hybrids for trait '1000 seeds weight' and in figure 1D value 0.998 of the correlation of parental lines. Since the QQ-diagram (Fig. 1D) can be seen that the distribution of the correlation coefficient is significantly different from normal.

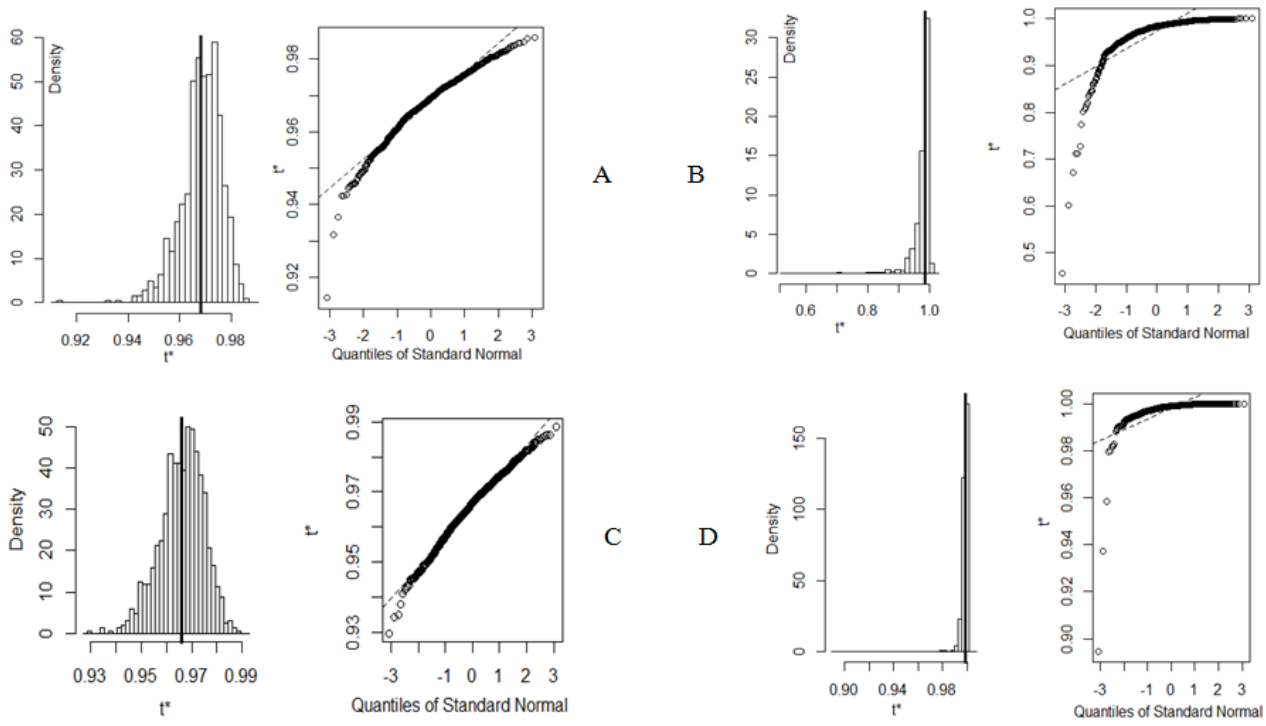

Fig.1. Histogram of the distribution of the bootstrap-values of the correlation coefficient for the characteristic 'yield' in hybrids (A) and in parental lines (B) and the characteristic '1000 seeds weight' in hybrids (C) and in parental lines (D) and their corresponding QQ-diagram.

Relationship does not have significantly change over the years, i.e. studied materials are tolerant to environmental changes with very good adaptability of hybrids, resulting in the statistically significant correlation coefficient. The results obtained from the displayed field experience vary depending on the year and the hybrid combination:

- The average *yield* of hybrids (from 347 kg/dka - in 2013 and 316 kg/dka - 2014 for cross *A2* x *R1* to 503 kg/dka - in 2013 and 509 kg/dka - 2014 for cross *A3* x *R3*);

- For the average *weight of 1000 seeds* in hybrids (from 70.1 g - 2013 and 70.3 g - 2014 for cross *A2* x *R2* to 88.9 g - 2013 and 90.3 g - 2014 for cross *A3* x *R3*).

Selection aim is to create new highly productive hybrids to realize their potential in different agro-ecological conditions. To achieve this, both parent lines of hybrid combinations must have high combining ability. The increase seed yield of new varieties remains one of the most important directions in the selection of the sunflower.

Seed yield is determined by the number and weight of fertile seeds per head (Robinson, 1983). The sunflower head has the ability to compensate for seed damage by increasing the weight of the individual seed Seiler (1997). According Charlet and Miller (1993) 10 % reduction in the number of flowers meet the 20 % reduction in the number of fertile seeds, 18 % of the total weight

of the seed per head to 22 % of the total volume of the seed. Seed yield is a complex character that results from the influence of a large number of traits, which can exert effects individually as well as jointly. The genetic basis of this character is polygenic in nature. Sunflower seed yield is a product of interactions between the genotype and environmental factors that take place throughout the growing season (Skoric, 2012). 1000-seed weight varies depending on genotype and environmental factors, too. The prevailing mode of inheritance of 1000 seed weight is partial dominance, although complete dominance and positive heterosis occur frequently as well.

Yan (2002) declared that typically environment (E) explains most (up to 80 % or higher) of total yield variations, and genotypes (G) and GE are usually smaller. Partitioning of variances revealed the significance of the environmental variance, compared to the genotype and GE interaction variances. It shows that in spite of the same location over the years there were big differences between environments due to different precipitation, temperature and 1-time irrigation in different years (Pourdad and Moghaddam, 2013).

Study the effects of abiotic conditions on plant growth and development, and yield of sunflower is an important prerequisite for the creation of many high-yield crops. Interaction of genotype and environment is important moment to the realization of the genetic potential of many crops, and stability in their production. The potential of sunflower is very high but average yield of sunflower is low and depending on weather conditions. One of reasons is inappropriate selection of varietal composition - parents.

Boxplot-diagram

Boxplot-diagrams for hybrids visually represent variations of maternal (*cms* sterile) and father (fertile) lines regarding on the relevant traits. On the graphs (Fig.2), due to high significant correlation coefficient for the two years, shows the variation of the characteristics. For each trait is evaluated and visualized the genetic potential of parental components. The main range of the relevant parent is defined and different colored rectangle. The solid line within the rectangle is the median, and it is perpendicular to the variation range for the each trait.

The obtained results indicate variation of the five traits in individual lines in different degrees. On the graph shows that the largest potential for trait "yield" means the lines *A1*, *R3* and *R4* for "weight" - *A1*, *A4*, *R2* and *R3*, for "oil" - a *A2* and *R2*, a "diameter" - a *A4* and *R1* and "height" - a A3 and R3. The highest values are around that range maternal *A3* and paternal *R3* line. Combining them to produce hybrid (*A3* x *R3*) is successful and leads to the highest expression of the trait "yield" - 506 kg/dka, due to the overlapping of their main interval of variation (IQR). This is subject to verification by the following analyzes. The lowest values ranging around maternal *A2* and paternal *R1*. Combining these two lines of hybrid (*A2* × *R1*) can be the lowest value for the same trait. In the case in hybrid yield is 332 kg/dka, the lowest in comparison with other results for the remaining hybrid combinations. Some lines for trait "oil", "diameter" and "height" have values close to the median. These lines have very low genetic potential for the trait. For example, the variation of the trait "oil" at *A3* and *A4* is highly asymmetric, which can lead to narrowing of the variation, but this feature may not be an indication of heterosis. Similar asymmetry was observed in the indicators "diameter" of the lines *A1* and *R4*, and "height" of line *A2*.

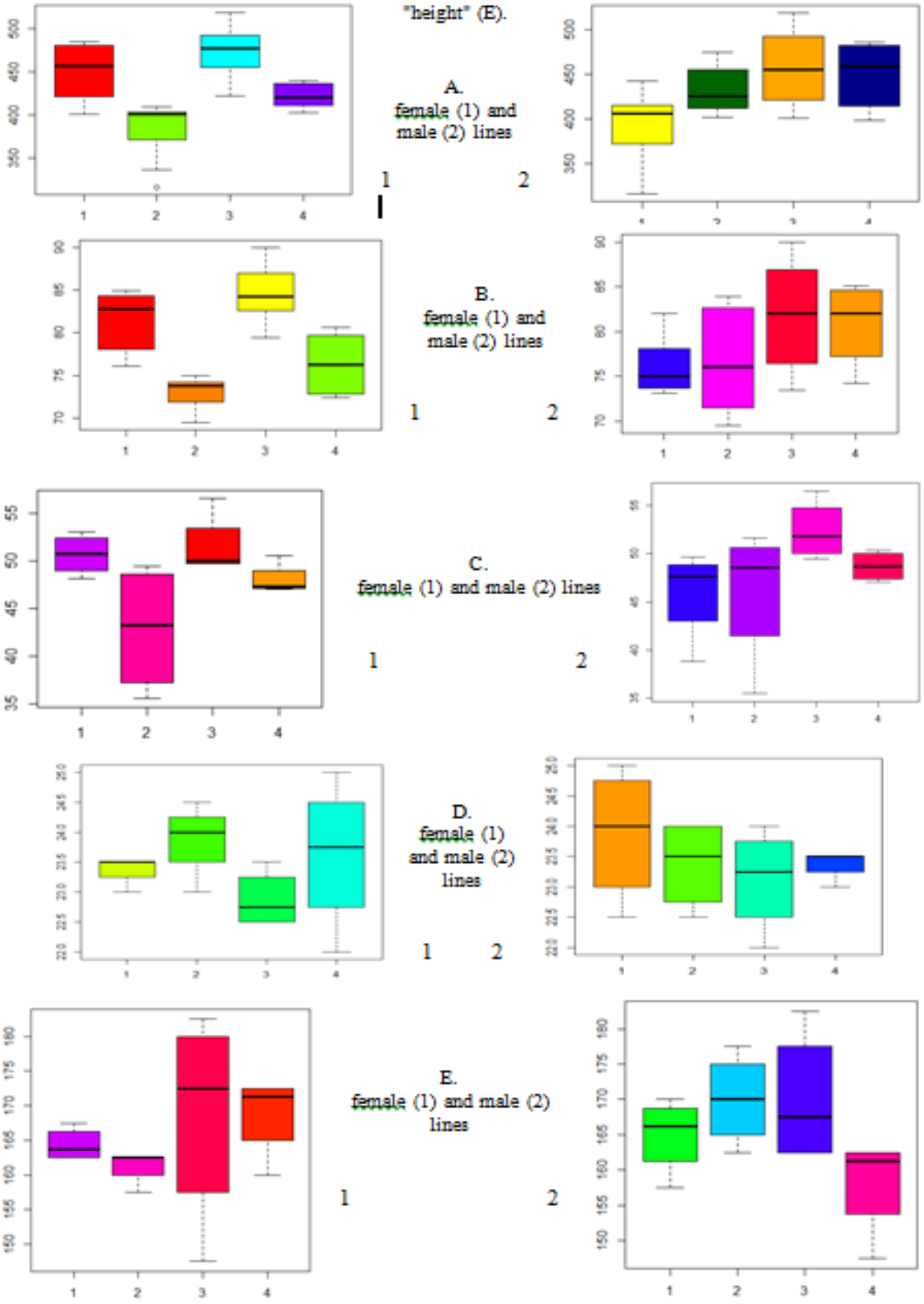

Fig.2. Variation of the characteristics “yield” (A), "1000-seeds weight" (B), "oil" (C), "diameter"

Combining statistical results facilitates breeder in his choice of starting materials in the creation of new hybrids. For example the trait "diameter" in lines *A3* and *R3* values around that range are the lowest, i.e. median or middle observation in the corresponding variation order is the smallest. These same two lines are the highest values for the "oil" and "weight" in hybrid combination (*A3* x *R3*) is the highest manifestation of these traits, i.e. hybrid with the smallest diameter of the heel is the highest yield and the weight of 1000 seeds.

Hybrid combining ***A3*** × ***R3*** is the highest manifestation of traits "oil" and "weight" even the smallest "diameter" and is the most promise, combining the most important economic business qualities.

Cluster analysis

Interspecific hybridization or detection of desirable genes in wild species of the genus *Helianthus* and their insertion into cultivated sunflower genotypes occupies a special place in sunflower production. Heterosis in the sunflower hybrids is highly linked to the genetic distance between the parental lines. With great economic importance for the sunflower is cytoplasmic male sterility - with the including of *CMS* in sunflower (Leclercq, 1969) *Rf* and the identification of genes (Enns et al, 1970; Kinman, 1970) and the creation of lines *R*. Carrying these genes it is possible to use a heterosis breeding for increasing the yield of hybrid seed. Of the hybrid seeds obtained after crossing the two parental forms - *A* (sterile analogues of sterile lines) and *R* (fertility restorer) lines are obtained 100 % fertile F_1 hybrid plants (Putt, 1997). *CMS* system for the production of sunflower hybrid seeds, for first time was used in 1972 (Fick and Miller, 1997).

The *B* and *R* lines, using in this research are received by selection of varieties (*B2* and *B4*) and intraspecific (*B1*) and interspecific (*B3* and all *R*) hybridization (Hristova-Cherbadzhi, 2012, 2009, 2007; Hristova-Cherbadzi and Christov, 2008; Hristova-Cherbadzi et al., 2007). The sterile analogues of *B* lines (*A* lines) were with *CMS Pet-1*.

The results from cluster analysis are presented on figures 3 and 4. Each cluster dendrogram shows the diversity (remoteness) of the materials by grouping parents (Fig.3A) or hybrids (Fig.4A) for 5 traits (Fig.3B, 4B).

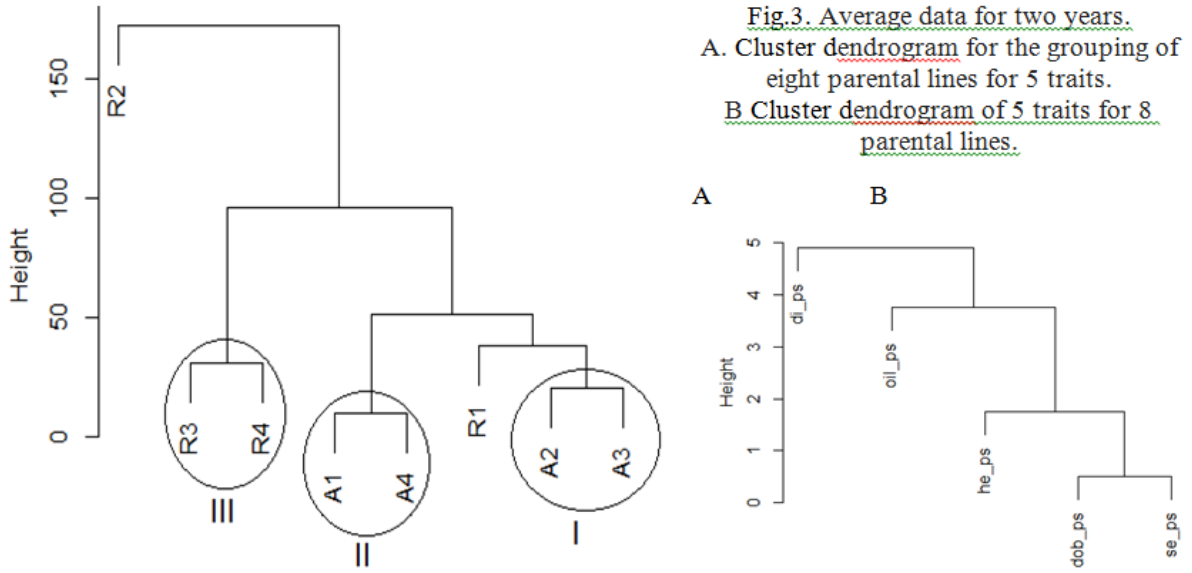

Fig.3. Average data for two years.
A. Cluster dendrogram for the grouping of eight parental lines for 5 traits.
B Cluster dendrogram of 5 traits for 8 parental lines.

Figure 3A shows a design space on the 5 characteristics, where lines *A2* and *A3* are the closest (group I). Next to them is a line *R1*. The lines *A1* and *A4* are in the next near group (II). Group III includes lines *R3* and *R4*. Line *R2* is most remote from all other lines.

Line *B2* is selected from variety Peredovik and line *B3* - after interspecific hybridization *H.annuus* line HA89B x *H.nuttallii* ssp. *rydbergii*. Many years ago line HA89B is selected from variety Peredovik (from J. Miller, Fargo, ND, USA), too.

The result is very interesting because the lines *A3* and *R3* are genetically distant, but at the same time received hybrid combination had strong positive heterosis for traits "1000 seeds weight" and "yield". These two lines (*A3* and *R3*) are received by interspecific hybridization. They are unique in that, that they are obtained after selection of the initial cross *H. annuus* (line HA89*B/A*) x *H. nuttallii* ssp. *rydbergii*. Until 2007 (Hristova-Cherbadzi and Christov, 2008) successful hybridization with this wild perennial deploid subspecies had not yet been reported. The subspecies *subrhomboideus* of the perennial hexaploid species *Helianthus pauciflorus* (*rigidus*), that was crossed with the cultivated sunflower, was studied less, too. Genes that controlled such characters as resistance to *Plasmopara helianthi*, *Phomopsis helianthi*, *Phoma macdonaldii* and *Orobanche cumana*, *Rf* gene for *CMS Pet-1*, suitable type of branching for *R* lines, high oil content (53.43 % for line *R3*) and high combining ability (line *A3*) were transferred.

Differences in expression of the characteristics “yield” and "1000-seeds weight” are the small (Fig.3B). To them next is a "height". In remove of these is the "oil", and finally "diameter".

Figure 4A shows a design space on the 5 characteristics for hybrid combinations. The 16 hybrids can group in three near close groups, too. Only one hybrid stay single, remove from other.

The closeness between the characteristics at hybrids keeps like this at the lines, but it has one difference - places of "oil" and "height" are exchanged. Here the “oil” is closer to the characteristics "yield" and "1000-seeds weight.

Maintaining the relationship between the closeness of the characteristics in parental lines and hybrids is confirming the genetic factor.

The use of statistical analysis in evaluation of the genetic properties of genotypes of *CMS* system together with the use of the selection method - remote hybridization, is practicable and can be further developed.

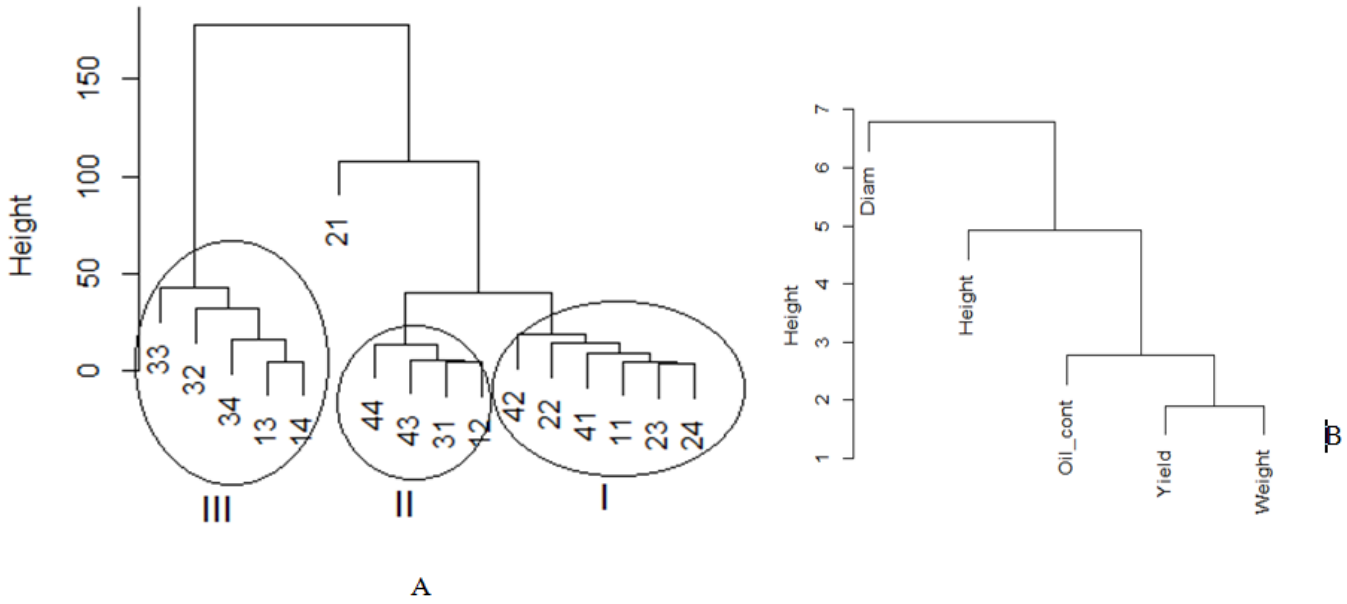

Fig.4. Average data for two years.
A. Cluster dendrogram for the grouping of 16 hybrids for 5 traits.
B. Cluster dendrogram of 5 traits for 16 hybrids.

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HYBRIDIZATION BETWEEN SUNFLOWERS (*HELIANTHUS ANNUUS* L.) AND LESS STEM ROSETTE (*CARLINA ACANTHIFOLIA* ALL.). CHARACTERIZATION OF RECEIVED INTERGENERIC FORMS

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ABSTRACT

Hybridization between sunflower (*Helianthus annuus* L.) and less stem rosette (*Carlina acanthifolia* All.) were made. These were two plants with different habitat in northern Bulgaria. Intergeneric hybrid plants and seeds were obtained in both directions of crossing. There were some outstanding differences between the two intergeneric hybrid groups. The first is related with the pollination and the viability of hybrid plants. Another interesting difference was in the seeds. Seeds of second group plants were larger, with 2-3 times longer size than those from first group, though the maternal parent form was the same. Line had small seeds. New intergeneric hybrids were carriers of *Rf* genes for CMS Pet-1 transferred from *Carlina acanthifolia* All. In crosses with sterile sunflower lines showed 100 % restoration ability.

Key words: *Carlina acanthifolia*, *Helianthus annuus*, intergeneric hybrid, sunflower

INTRODUCTION

Application of intergeneric hybridization in sunflower is hardly realizable work. Under certain conditions and well-chosen parental components enables the creation of rich source material for selection of sunflower (Christov and Panajotov, 1991; Christov et al., 1994, 2004, 2009; Christov and Vassilevska-Ivanova, 1999; Hristova-Cherbadzi, 2007, 2012; Christov, 2013 and etc.). Of particular interest are the plants used in folk medicine. One of these plants is less stem rosette (*Carlina acanthifolia* All.).

MATERIAL AND METHODS

The investigation was carried out at the Vrachantsi, Dobrich, Bulgaria, during the period 2013 - 2015.

For maternal parent form used sterile analogue of line HA-821 and as pollinator *Carlina acanthifolia*: accession №1, found near Vrachantsi, Dobrich (Fig.1) and accession №2 by region Balgarevo, Kavarna.

Fig.1. *Carlina acanthifolia*.

Method of intergeneric hybridization (crossing between cultivated sunflower line HA-821A and *Carlina acanthifolia*), crossing between A lines and intergeneric hybrids, selection,

self-pollination and sib-pollination were used. Morphological characteristics were based on phenotypic observation and biometric measurements during the vegetation period and on laboratory studies of whole plants and seeds. *Rf* genes, in crosses of sterile sunflower lines x *Carlina acanthifolia* and sterile sunflower lines x intergeneric hybrid - F_1 and F_2, were searched. The taste of the kernel is tested in wax and full maturity and after short roasting seeds.

RESULTS AND DISCUSSION

As a result of hybridization between sunflower (*H. annuus*) - line HA-821A and *Carlina acanthifolia* - accessions №1 and №2 total 31 seeds and from them 7 F_1 plants were obtained (Table 1). The number of received seeds for separate inflorescences was from 1 to 8.

Table 1. Crossability of cultivated sunflower *H. annuus* and *Carlina acanthifolia*.

Crosses	Pollinated inflorescences			Total number seeds	Hybrid plants	
	total number	with seed				
		number	%		number	%
H. annuus x *C. acanthifolia* №2 - "first group"	23	5	21,74	12	2	16,67
H. annuus x *C. acanthifolia* №1 - "second group"	23	3	13,04	19	5	26,32

In early March 2014 the seeds were sown in separate containers in a special room with enough light and warm (21-25^{0}C). Then they were seedlings in 30 cm distance from place, where was the *C. acanthifolia* accessions №1. The plants developed normally until flowering. Their height was from 30 to 55 cm. The disk flowers of all plants digressed pollen. One plant from the "second group" (*H. annuus* x *C. acanthifolia* №1) died 3 days after full of blooms. Another plant from the "first group" (*H. annuus* x *C. acanthifolia* №2) died 21 days after blooming. After self-pollination of the plant from the "first group" and two plants from the "second group" are received in total 11 (4 + 7) seeds. Two plants from the "second group" were pollinated with one another. After that 17 seeds were obtained. With pollen from the last two plants was pollinated one sterile plant from the line 92A. After this bekkros number of seeds was 21. In 2014, a second generation plants (2 and 1) were received also. From each group are left spare seeds. Results of the seeds' viability from the first hybrid generation and the viability of hybrid plants from the next generation are presented in Table 2.

Table 2. Viability of seeds and hybrid plants from the second generation, 2014.

Type of pollination	Sown seeds, n	Received plants, n	Vital plants	
			number	%
Self-pollination				
- "first group"	3	2	2	100,00
- "second group"	5	4	4	100,00
Sisterly pollination				
- III group	12	11	10	90,91
Bekkros				
- IV group	15	15	15	100,00

From sown total 8 seeds after self-pollination of F_1 plants, were received 6 F_2 plants. The disk flowers of all plants digressed pollen. After self-pollination of the two plants from "first group" 32 seeds were obtained. Their dimensions were closed to these of line HA-821A. Some of the seeds had gray-black color of the peel and the others were light- motley colored. After self-pollination of 4 F_2 plants from the "second group", 89 seeds were received. The seeds are characterized by large size in length (12-13 mm) and light- motley colored. From III group (since the sisterly pollination of the F_1 plants) 12 seeds were sown. Eleven F_2 plants were obtained. At the beginning of flowering one plant dies from them. The other 10 F_2 plants were fertile. Seven from them were self-pollinated and 201 seeds were obtained. The remaining three plants were left open pollinate. Since all plants the seeds were obtained, but at about 1/3 of the disc florets on each head had not seeds. The best result was obtained at BC_1 plants from the IV group.

From 15 sown seeds, 15 vital plants were received. One from them was male sterile and the other 14 plants - fertile. Eight plants were self-pollinated and 7 (6 + 1) plants were left open pollinate. From all 15 plants, the seeds were obtained. By two inflorescences of lines HA-821A and 92A were pollinated with pollen from F_2 plants (cross *H. annuus* x *C. acanthifolia* №1, "second group"). Seeds were obtained from the 4 pollinated heads. Twenty one seeds from all the numbers of different groups' intergeneric hybrids were sown on April 2015 to obtain plants from third generation. Some results are presented in Table 3.

Table 3. Characteristics of F_2, BC_1, F_3, F_1, BC_1 and F_4 hybrids.

Groups	Sown seeds, n	Received plants, n	Vital plants, n	Inflorescences with seeds, n	Male sterility plants, n
I group	21	17	17	9	-
II group	21	19	19	19	-
III group	21	16	16	16	1
IV group	21	20	20	20	1
V group:					
1 head - HA-821A x II group	21	20	20	20	3
2 head - HA-821A x II group	21	19	19	19	2
1 head 92A x II group	21	21	21	21	2
2 head - 92A x II group	21	20	20	20	1

Six plants from all numbers (groups) were self-pollinated, to receive next generation.

Some of the F_3 plants originating from a cross HA-821A x *C. acanthifolia* №2 had not seeds, although the plants were fertile. Seven of the 8 plants that have not received seeds were open pollinated. This may mean that the work with accession №2 will be difficult. Probably the two accessions №1 and №2 were different. The seeds from all plants II group are distinguished with their length (12-13 mm) and light-motley colored. The seeds from more of plants III group were similar to those of the II group. From other plants, seeds larger than the line HA-821A were received. All plants from the IV group have large seeds, because line 92A was with large seeds. Some of the seeds are colored gray-brown. The seeds of the first two numbers from the V group were larger than those of the line HA-821A, but were smaller than those of the plants from the II group. Seeds from the other two numbers were long and similar to the seeds of plants from the II group. The received fertile plants from all groups showed that in both accessions were established *Rf* genes for CMS Pet-1.

The taste of the kernel from the F_3 plants II group is tested in wax and full maturity and after short roasting seeds. As standards are used variety Favorit and hybrid XL-4337. Taste of nut plants from II group compared with this from nuts on both standard was more similar to hybrid XL-4337, but different in wax maturity.

CONCLUSION

Hybridization between sunflower (*H. annuus*) and two accessions of *C. acanthifolia* was successful. The received interspecies hybrids with the both accessions were differ in some characteristics, such as size of the seeds, in the plant growth and the next generation multiply, carriers of *Rf* genes for CMS Pet-1 and others. Many of the characteristics of the received material to be studied for the future.

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SUNFLOWER VERTICILLIUM WILT: BEHAVIOUR OF COMMERCIAL HYBRIDS IN QUICK TESTS PERFORMED AT CONTROLLED CONDITIONS.

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Introduction and objective. *Verticillium dahliae* is a major pathogen of many important crops. Verticillium sunflower wilt is an important disease in Argentina. The symptoms tend to be rather unreliable for assessing resistance under field condition, since the interaction with environment is often present. There is poor information about the behaviour of Argentinean sunflower hybrids in quick tests performed at controlled conditions. The objective of this work was to quantify the sunflower wilt incidence and severity of hybrids inoculated with two variants of *V. dahliae*.

Materials and methods. Eighteen commercial hybrids from Argentina were tested, including a susceptible and resistant control. Fourteen days old sunflower seedlings were inoculated by root immersion in a conidial suspension of *V. dahliae* (two isolates representative of Argentinean V. dahliae variants inoculated separately). Plants were planted in pots with a mixture of pasteurized soil and perlite and incubated in a growth chamber (12 h, photoperiod, 25 ± 2°C). Forty-five days from the inoculation, the severity and incidence of Verticillium wilt was recorded. The variance of data was analysed and means were compared by turkey test.

Results. The susceptible control had significantly higher *Verticillium wilt* severity and incidence than the resistant one ($P<0.0001$). The two isolates caused differential levels of disease severity and incidence to sunflower hybrids ($P<0.0001$). There was differential behaviour between hybrids in severity and incidence of Verticillium wilt.

Conclusion. The commercial sunflower hybrids show variability in their resistant level against *Verticillium wilt*. One hybrid show a good resistance level to both isolates

Discussion. This information adds knowledge to the breeding for resistance to sunflower *Verticillium wilt*.

Key Words : Sunflower; Verticillium Wilt; Commercial hybrids; Quick tests

ARGENTINEAN AND EUROPEAN SUNFLOWER HYBRID PERFORMANCE IN A *VERTICILLIUM* INFECTARIUM

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Introduction and objective. *Verticillium dahliae* is a major pathogen of a number of economically important crop species. In Sunflower is an important disease in Argentina and increase the importance in the rest of the sunflower world area (special Europe). The expansion to non-traditional areas of disease exposure hybrids to this pathogen. The objective of this work is to compare the resistance level of sunflowers from Argentina (verticillium endemic) and Europa (non *Verticillium)* in monoculture high field pressure (infectarium).

Materials and methods. Fourteen commercial hybrid from Argentina and Europa were tested five years, (including a susceptible and resistant control) in a verticillium infectarium with more than 15 years of continuous sunflower history in Miramar (38°11'14.41"S, 57°55'15.97"W, Province of Buenos Aires, Argentina). The experimental unit were two row of 5m long, the inferctarium have additional irrigation. From R6 to R8 growth stages, severity of Verticillium was quantified. Mix model analysis were used to analyzed the results.

Results. The susceptible control had significantly higher verticillium severity than the resistant one ($P<0.0001$). None of the European materials show similar resistance level of the control (local hybrid). Most of the European materials were highly susceptible. The Argentinean hybrids show a contrasting level of resistance

Conclusion. The European and Argentinean materials show different level of resistance to Verticillium. A higher resistance level were shown by Argentinian hybrids. Some European hybrids had a good level of resistance to Verticillium.

Discussion. This information add knowledge to the breeding for resistance to Verticillium.

Key Words : Sunflower hybrid; Verticillium infectarium

CHARACTERIZATION OF HELIANTHUS TUBEROSUS L. ACCESSIONS FROM VIR COLLECTION

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ABSTRACT

The Jerusalem artichoke *Helianthus tuberosus* L. is distributed throughout the European part of Russia up to about 630 N in gardens and orchards. The population uses its tubers as food (for diabetes prevention), for fodder and as an ornamental plant. Varieties of grown material are not known for sure. Seven accessions of *H. tuberosus* collected in Pskov, Leningrad regions and St. Petersburg were collected and planted in the nursery of perennial wild species in Kuban experimental station of VIR in the Krasnodar region (KES). In the same nursery five accessions of *H. tuberosus* obtained in different years from various habitats in the USA are maintained. We had an opportunity to compare the development of Jerusalem artichoke plants from different places of origin in 2014 and 2015. Spring growth of plants originating from the North-Western of Russia started a little later (April, 15-25) compared with accessions from the USA (April, 4-10), but they flowered earlier: on June, 15 - 26 in 2014 and on June, 20 – July, 14 in 2015. In the maternal populations of these accessions in Leningrad and Pskov regions flowering started on August, 5-10. Accessions of Jerusalem artichoke, grown in KES started flowering on September, 4-20. All the accessions had normally formed flowers. Cytological study showed that anthers produced more than 70% of normal pollen both in Krasnodar region and in the North-West of Russia. For their size the pollen grains were divided into three groups, which may be a consequence of their different ploidy. Study of ovules was not carried out. The seeds were formed only in the conditions of Krasnodar region. In the North-West of Russia plants of Jerusalem artichoke are able to reproduce only vegetatively by tubers.

Key Words : Helianthus tuberosus, Krasnodar region, North-West of Russia, flowering, cytological study

GENETIC RESOURCES FOR THE BREEDING OF LARGE FRUIT SUNFLOWER

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ABSTRACT

On the bases of many years maintenance and several re-sowings among 2230 accessions from VIR collection we have selected 34 genotypes which have not lost the character of a large fruit. Very long fruit accessions from China also were included in this group. These accessions were evaluated in the field at the Kuban experimental station of VIR in Krasnodar region for 3 years for the weight of 1000 seeds. The implementations of achenes was estimated using the microfocus x-ray. The radiation load on the seeds during the investigation, is extremely low and has no mutagenic effect. Three-year observations selected 15 sunflower accessions with weight of 1000 seeds more than 100 grams. Among them modern confectionery varieties bread in all-Russia Institute of Oil crops (VNIIMK) can be marked: Donskoy Krupnoplodniy k-3510, Konditerskiy k-3426, Lakomka k-3526 and also Zaporozhskiy konditerskiy k-3516. High rates of the character show landrace varieties, such as Stadion k-2642 from Bulgaria, Ger-Ger k-1589 from Armenia and the local accessions collected during the expeditions in Primorye kk-2817, 2818, 2835, 2836, 2843 and Argentina k-3583. The highest weight of 1000 seeds is typical for 2 accessions from China k- 3633, k-3586 and line VIR 846 k-3683. The last ones are characterized by light coloration of the achene with gray stripes. Accessions of oil and confectionery use, differing in origin, size of achenes, and the degree of implementation were analyzed with the use of x-ray (Fig). Mathematical analyses of the obtained radiographs were made using the specialized computer program. The largest achenes (fetus' area more than 4,00 mm2) are typical for accessions kk-3586, 3516, 3619, but their implementation is less than 50 %. Accessions with the largest implementation (over 50%) are: kk-1693, 1960, 1961, 2051, 3315, 3351, 3447, 3455, 3553, 3621. Therefore, the largest achenes are typical for one groupe of accessions, and the most implemented – for the other. The weight of 1000 seeds is not always corresponded to the size of the seed and kernel. But accession from China, k-3586 has achenes larger and heavier than all other analyzed accessions. Thus, it is shown a new opportunity for individual selection of genotypes during the creation of the initial material for breeding varieties and hybrids of large-fruited and confectionary sunflower. The method of lifetime estimation of implementation of sunflower achenes using microfocus radiography and specialized computer program for mathematical analyses of x-rays pictures.

Key Words : large fruit sunflower,the implementations of achenes, the microfocus x-ray

CAN GENOTYPE X ENVIRONMENT MANAGEMENT INTERACTIONS (GEMI) BE PREDICTED IN SUNFLOWER MULTI-ENVIRONMENT TRIAL?

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ABSTRACT

Climate change and input reduction in agriculture lead to a diversification of cropping environments with a higher expression of biotic and abiotic stresses. In this context, adapting the choice of cultivars according to their cropping environment is of special importance to increase sunflower productivity. Crop cultivar assessment programs aim at evaluating the performance of new cultivars in multi-environment trials (MET). These are a series of field trials conducted across a range of geographic locations and sometimes over several years. However, choosing a cultivar according to its global performance can be risky because of GEMI, which induce significant variations in the relative performance of cultivars when they are assessed in different environments and submitted to various crop management practices. The analysis of GEMIs could enrich the current information on commercial cultivars, and therefore improve the recommendations on cultivar according to the farmers cropping environment. This study aimed at evaluating the predictive value of statistical methods that model GEMI on cultivar MET. Those methods use environmental covariates quantifying major abiotic stresses. Two approaches were evaluated: the model is performed either directly on the yield variable or on the interaction terms first estimated by a mixed model. For both approaches, several methods are evaluated: factorial regression, PLS regressions, Random Forest and Lasso regression. These models are assessed on a "virtual dataset" generated by SUNFLO, a dynamic model simulating genotype-specific performance of a sunflower crop in contrasted environments. The predictive quality of the statistic models was assessed by cross-validation and their predictive values were compared to the one of an additive model in which GEMI is not taken into account. Then a diagnosis of error of prediction was performed to identify which kind of environment is more difficult to predict. The results obtained showed that the best predictive approach is to model directly GEMIs with the Random forest statistical method. However, compared to an additive model, the improvement of the predictive value achieved by modeling GEMI's remains limited. This improvement is all the worst that the stresses generating GEMI are early in the cropping season. This study shows clearly the inadequacy of the classic statistic methods to model the GEMI in the MET even in an optimistic context (data generated without error on the yield and the environmental covariates).

Key Words : Genotype-environment-management interactions (GEMIs), multi-environment trials (MET), Sunflo

SUNRISE PHENOTYPING DATABASE : A TOOL FOR THE SUNFLOWER COMMUNITY TO SHARE AGRONOMIC, PHYSIOLOGICAL AND MOLECULAR DATA

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ABSTRACT

In the SUNRISE project, a combination of several approaches is developed including the establishment of appropriate and high-throughput phenotyping strategies to characterize the molecular, physiological and agronomical responses of sunflower to variation of the abiotic environment. For instance, large-scale experiments are conducted in multi-environment by the different partners resulting in the acquisition of millions of molecular, physiological, agronomical data points, all associated e.g. through genotypes, and environments. In this context, our community needs to develop a resource to integrate, maintain, store, manage, connect and visualize these valuable data. Our team constituted of agronomists, physiologist, molecular biologist and bioinformaticians developed an information system composed of a database, a query system and a user interface allowing comprehensive data integration and fast interpretation of results. As a first step, the SUNRISE archive was created for collection, storage and long-term accessibility of raw data. From this step on, the collection protocol, description of the environment and data description (metadata) are bound to phenotypic data. Data archiving is made through a secure web portal with user access to identify responsible persons and property. Then, a second step generates from this archive a database for data exploitation. The database schema was built in collaboration between bioinformatics and biologists to structure different type of data and their properties (genotypic information, statistical design, environmental factors, geographical informations, partners,…). A web interface accessible at sunrise.toulouse.inra.fr/phenoDB allows querying the data, viewing them and exporting them. Data access is restricted to authorized users on a file-based system and is therefore very flexible. Importantly, the generic architecture of the archive and the database allows its potential expansion to other types of phenotypic data on sunflower and other species and its use at a larger scale for other public or private projects.

Key Words : Database ; Transcriptomics ; metabolomics ; proteomics ; agronomics ; phenomics

NEW TECHNICAL AND METHODOLOGICAL DEVELOPMENTS FOR SUNFLOWER FIELD PHENOTYPING

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ABSTRACT

INRA is co-developping a set of tools for sunflower phenotyping from leaf to plot level using sensors and imagery based techniques. At the plant level, Mobi-Leaf is a smartphone based application co-developed with Terres Inovia and designed to estimate individual leaf area using the built-in imaging capabilities of the device while collecting the relevant metadata. At plot level, PIETON® is a system designed to measure light transmission using LED based sensors mounted on a stick for simultaneous acquisition of light over and under the canopy to estimate LAI. At the field level, the PHENOME project aims to develop a UAV system for crop phenotyping. The vector is a hexacopter with a payload of 800g. Two sensors combination are available, 1) a high resolution RGB camera, ii) a specially developed 6 band VIS-NIR multispectral camera that can be combined with a thermal infra-red FLIR Tau camera. On sunflower, the first development targeted plant and flower counting and we will now focus on LAI and water stress tolerance using the multispectral camera and thermal IR camera. To overcome the size and weight limitations of UAV, the Phenomobile is an automatic ground vector under development with a 10 m long arm designed to carry a wider range of sensors such as Lidar and spectroradiometers over the canopy. This system, which has active lighting capabilities, will be able to collect data for detailed organ level structure and optical properties and thus complement the canopy approach of the UAV system.

Key Words : high throughput phenotyping, sensor, UAV, image analysis

DIVERSIFICATION OF SUNFLOWER GERMPLASM FOR DIFFERENT IMPORTANT CHARACTERISTICS

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ABSTRACT

Sunflower is a very important crop in the world agriculture. Taking into consideration the high seed yield and oil yield, thanks to the extension of sunflower cultivated varieties and hybrids having a high oil content, this crop has a good place in the hierarchy of dominant crops over the world. Sunflower wild species are the most rich and varied source of favorable genes for the important characteristics of cultivated species. Sunflower interspecific hybrids are very important in breeding, thanks to a very good genetic variability. As the result of our research work, we have obtained many interspecific populations, after crossing sunflower wild species with*Helianthus annuus* cultivated varieties. There have been studied different characteristics, in two years of experiments for the wild species, for the cultivated varieties and for the interspecific populations. Observations regarding flowering duration and vegetation period were recorded. There have been analyzed different morphological characteristics (plant height, number of leaves, petiole length, head diameter, seed wide, seed length, and number of branch) as well as other characteristics, including the seed oil content. Testing resistance to the pathogen *Plasmopara halstedii* and the parasite *Orobanche cumana,* we found that, after 5 generations of selfpollination, some hybrid populations have a good resistance to the pathogen and to the parasite. The obtained data has shown that in the most cases, the differences referring to the cultivated sunflower are statistically significant. Similar results were obtained with the hybrid populations for all analyzed characteristics.

Key Words : sunflower, genetic resources, wild species, analyzed characteristics

CURRENT STATUS OF SUNFLOWER CROP MANAGEMENT IN MOLDOVA

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) is one of the main risk factors in sunflower production, causing significant quality and quantity crop damages. Extension of sunflowers land and its irrational exploitation contributes to the increasing of frequency and intensity of pathogen attack. This work presents the results of integrative study of sunflower farm fields from different geographical areas of Moldova, with the focus on cultivated hybrids of *Helianthus annuus* L., crop rotation and the frequency and intensity of the broomrape attack in natural conditions. Observation and sociological survey of sunflower growers were used as study methods. The investigations were conducted in the period of July-August, 2014, in 80 locations from center, south and north of Moldova. It was found that *Orobanche cumana* Wallr. is preferentially widespread in the central and southern part of country, frequency and intensity of the broomrapes attack, also, being higher in these regions. Around eleven hybrids, especially belonging from Pioneer Seed, Saaten Union and Syngenta companies, were cultivated on analysed fields. One from these hybrids, was found to be resistant (ARENA PR), three tolerant (SY SUBTYL, PARAISO 102 CL and P63 LE10) and others were susceptible and high susceptible to broomrape infection. It was established that efficient and programmed culture of sunflower in a well-organized rotation (using maize and wheat as a proceeding crops, with a return of sunflower to the same field at least after a period of 4 years) decreases the number of plants affected by *Orobanche*.

Key Words : Helianthus annuus L., broomrape, Orobanche cumana Wallr., crop rotation, hybrids

EFFECT OF GIBBERELLIC ACID ON POLLEN DEVELOPMENT IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Differentiation of meiotic cells requires temporally coordinated interactions with anther wall layers and any their defects result in aborted microgametogenesis. Light and electron microscopy investigations highlighted the crucial role of the tapetum in the pollen development. In sunflower degeneration of the tapetal cells is considered to be the result of programmed cell death and aborted microspores may be cause of poor nutrition or defects in pollen coatings secreted by these cells. However, there are many aspects such as signal triggers of cell death, checkpoint factors, signal transduction ways that has not been described in detail and which may provide a clearer picture of tapetum function. More recently, an intensive progress of meiosis and pollen viability studies are associated with use of interspecific hybridization in sunflower breeding. The divergence and heterogeneity of the genus cause difficulties, such as cross incompatibility, high percentage of meiotic abnormalities, resulting in sterility or reduced fertility of interspecific hybrids. These particularities have advantage in gametocides identifying for male sterility induction to achieve hybrid seeds (F1) or model systems for fundamental aspects of pollen development research. The most effective for induction of male sterility is considered to be GA3, but, is known that various genotypes respond differently to the GA3 treatments. Obtained date demonstrated alteration of cellular organization during microspore development in GA3 induced male sterility. Such investigations furnish useful information about the tissues most sensitive to gametocide and contribute as complimentary approaches in highlighting the role of gibberellins in transcriptional regulatory network for anther development.

Key Words : gibberellic acid, Helianthus annuus L., microgametogenesis, GA3 induced male sterility

GENETIC VARIABILITY OF BROOMRAPE POPULATIONS FROM REPUBLIC OF MOLDOVA

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ABSTRACT

Sunflower broomrape (*Orobanche cumana* Wallr.) is one of the most devastating sunflower pathogens in Eastern Europe and Mediterranean region, which annually causes significant yield losses and negative influence oil quality. Virulence of pathogen directly depends on physiological race. Actually, eight races (A-H) of broomrape are described. Knowledge regarding pathogen race could help farmers and breeders to make correct choice for cultivation and breeding of sunflower varieties. Thus, the aim of this study was determination of genetic variability, race and geographical distribution of different broomrape populations from Republic of Moldova. Forty one broomrape populations from Republic of Moldova and one of each from Romania, Ukraine and Spain were investigated. For evaluation of genetic variability were screened 15 SSR primers (Ocum-52, -59, -70, -74, -75, -81, -87, -108, -122, -141, -160, -174, -196, -197 and -206), which are reported previously as the most polymorphic. PCR results were visualized in 8 % polyacrylamide gels. SSR analysis demonstrated different level of polymorphism for investigated populations. The highest polymorphism level was detected for three primer pairs: Ocum-197, -160 and -59. Correlation of obtained data with information about the racial status of populations and their geographical distribution will contribute to identification of specific markers for physiological races of pathogen and will reveal phylogenetic position of populations and their spread.

Key Words : broomrape, physiological race, geografical distribution, genetic variability

MICROSPORE CULTURE RESPONSE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVARS

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ABSTRACT

Androgenic methods are used for the production of haploid plants such as anther and microspore culture. The microspore culture technique plays an important role for efficient production of haploid plants and genetic potential of cultivars is very important for being successful in this method. In this research, different hybrid sunflower cultivars obtained from Trakya Agricultural Research Institute have been selected and their responses to isolated microspore culture were evaluated. Meanwhile the effects of different plant growth regulators and media on androgenic microspore culture were studied in selected cultivars. Effects of microspore developmental stages and sterilization methods on isolation of uninucleate, pure and viable microspores and their culture were examined with the aim of optimizing culture conditions. Capitulum containing anthers were collected when the microspores were at the late uninucleate stage from the field. The florets containing uninucleate microspore were detached from the sterilized capitulum were blended with a blender in 30 ml of cold microspore isolation solution containing 13% sucrose at pH 6 and transfer to modified NLN- medium. After the last centrifugation the microspore density in the pellet was determined. Further studies with isolation method, different media compositions and culture conditions will be necessary in order to develop an efficient microspore isolation and culture technique in sunflower. This research has been supported by TUBITAK KBAG (Project No: 214O274).

Key Words : Sunflower, microspore culture, haploid.

GENOTOXIC EFFECTS OF IN VITRO TISSUE CULTURE CONDITIONS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

In vitro culture of plant cells is useful tool to study the adaptive mechanisms of plants living in adverse environments. In view of these facts, the objective of this work was to understand the genotoxic effects of plant growth regulators and the other parameters in sunflower callus tissues. Hybrid *Helianthus annuus* L. genotypes were grown at field and used as plant material in this study. The capitulums were collected when the microspores were at uninucleate stage for anther culture. Plating density was around 20 anthers per control and media supplemented with plant growth regulators petri dishes. Plated anthers were pretreated with cold for 24, 48 or 72 hours at 4°C and heat for 0, 2, 4, 8 or 12 days at 35 0C in the dark. Obtained calli from the control and supplemented with different plant growth regulators media (0,5 mg/l IAA+0,5 mg/l BAP; 0,5 mg/l NAA+0,5 mg/l BAP; 0,5 mg/l 2,4D+ 0,5 mg/l BAP) were used as a material to detect the DNA damage levels by Comet assay. In our preliminary studies, the highest frequency of calli induction (95%) was obtained at 350C for 2 days on the MS medium supplemented with 0, 5 mg/l 2,4D and 0, 5 mg/l BAP. Examples of nuclei with different levels of DNA damage in the comet assay were evaluated. Preliminary screening suggest that callus tissues of sunflower could be useful for studying the genotoxic damage due to different pretreatment applications. This research has been supported by TUBITAK KBAG (Project No: 214O274).

Key Words : Sunflower, callus, DNA damage, plant growth regulators.

NEW RACE OF BROOMRAPE IN SOUTH REGION OF UKRAINE

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ABSTRACT

Nearly all plants of well-known hybrid Brio (Syngenta) which were sown in 2013 in the fields of Izmail district of Odessa region were infected with broomrape. There were 10 to 15 sprouts of parasite on each plant of hybrid. We collected seeds from this broomrape plants and infected, in controlled conditions (greenhouse) the differential lines of sunflower LC-1093 (F) and RO-1-1(G). Susceptibility of these lines was 92% and 36% with the intensity of the defeat 5.7 and 0.6 respectively. So there was appearing the new virulent race H of broomrape. The results of this research show that there is a big risk to grow hybrids of sunflower with the stability to F race of broomrape in South region of Ukraine.

Key Words : broomrape, virulent race H

TISSUE CULTURE STUDIES IN SUNFLOWER

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ABSTRACT

Unfortunately, tissue culture studies on sunflower in Turkey are not sufficient when we compare them with similar studies on Earth. Tissue culture techniques offer important approach about sunflower breeding and germplasm conservation. This report was written for unroll the scientific knowledge on this subject in our country and encourage the researchers to study on tissue culture of sunflower. According to the literature, a study which was made in the first half of the 90s on anther culture of sunflower was the first investigations in Turkey. At the same time, hypocotyl and cotyledon explants of sunflower were cultured in another research. In the second half of the 90s, developing a regeneration procedure from different type of explants and determining transformation protocols studies were made. Following these studies in the early 2000s, micropropagation protocol via direct somatic organogenesis from hypocotyl and cotyledon explants of sunflower was carried out. In the same period, shoot and then whole plant regeneration was formed from mature and immature somatic embryos. Gene transfer studies with *Agrobacterium tumefaciens* were also seen at the same period. Finally in 2010s, anther culture technique and germplasm conservation by slow growth technique were used in sunflower tissue culture. Although these researches established an important scientific knowledge about sunflower tissue culture in Turkey, this is not sufficient yet. Therefore, there is an urgent need to make more *in vitro* studies on sunflower which is an important agricultural plant for Turkey. Also, it is essential in terms of sectoral that the information obtained in this studies should be transferred to agricultural practice.

Key Words : in vitro, *Helianthus annuus*, Breeding, Germplasm Conservation

WIDE (INTERSPECIFIC AND INTERGENERIC) HYBRIDIZATION IN SUNFLOWER (*HELIANTHUS ANNUUS* L.): A TOOL FOR CREATION OF GENETIC VARIABILITY AND SELECTION OF DESIRED TRAITS

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ABSTRACT

Hybridization is a reunion between differentiated genetic materials. The domesticated sunflower (*Helianthus annuus* L.) is one of the most important oil species with a comparatively narrow genetic base. Most of the important agronomic characters of a sunflower cultivar such as yield, oil content, oil quality, response to abiotic stress, and also many of pathogen –resistance traits would benefit from wild *Helianthus* species. The strategy using wide hybridization between *H. annuus* and its wild relatives (annual and perennial sunflowers), and also some species from related genera of Compositae (Asteraceae) has proved to be successful approach for development of introgressed plants with a wide range of variability. Herewith, we describe several advanced sunflower lines developed after wide hybridization *H. annuus* x *H. mollis*, *H. annuus* x *Verbesina encelioides*, *H. annuus* x *Verbesina helianthoides*, *H. annuus* x *Tithonia rotundifolia*, and *H. annuus* x *Echinacea purpurea*. Analyses of morphological and agronomic traits that characterized the phenotype of these lines as well as some biochemical parameters are presented.

Key Words : wild species, *Helianthus*, productivity, inheritance, Compositae, cultivated sunflower, wide hybridization

AGRO-MORPHOLOGICAL DIVERSITY OF TUNISIAN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is a summer crop in Tunisia. All Tunisian sunflower seed production is sold for snack food and marketed as roasted dry fruits (glibettes) in the kiosks. The morphological variation and the systematical status of 30 accessions of sunflower collected from different geographical areas of Tunisia were assessed based on 19 qualitative and 8 quantitative traits. Turkich sunflower was used as a reference variety. The data underwent an analysis of variance and a discriminant analysis. Significant differences ($p<0.05$ and $p<0.001$) among accessions within and between origins were revealed for the majority of the qualitative and quantitative traits. The genetic diversity within accessions (Shannon and Weaver's index) was high (H'= 502) and varied according sites of collection. The major proportion of the variation was attributable to individual differences within accessions. The Accessions A22 from the Beja locality were more polymorphic and exhibited the highest genetic diversity (= 0,787). The discriminant UPGMA dendrogram performed on all measured traits showed that most accessions clustered independently to their geographical origin and the variation within the same sites was extremely important. The geographic location did not seem to be the main factor structuring the variability of the studied accessions. There proved to be high phenotypic variability in the Tunisian material for the vigor descriptors.

Key Words : *Helianthus annuus*, accessions, genetic variation, morphological traits, UPGMA

MOLECULAR STUDIES OF RESISTANCE MECHANISMS IN SUNFLOWER AGAINST *OROBANCHE CUMANA* WALLR.

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ABSTRACT

Sunflower is one of the most important annual oilseed crops in the world. Sunflower broomrape *(Orobanche cumana* Wallr.) is a holoparasitic plant and infects sunflower roots. This parasitic infection is a crucial problem in all countries, but it is mainly observed in south Europe and Turkey. In order to decrease the infection problem, chemical and biological treatments were used in the past. However, herbicide utilization is not economical and causes health problems in humans whereas biological control treatments do not give trustable results. Therefore, molecular studies are necessary to understand the resistance mechanism of sunflower against *O. cumana* for a long-term solution to this parasitic plant problem. *Orobanche* spp. (broomrapes) cause significant damage by depriving organic nutrients, minerals and water from the host through a special organ called haustorium. Some monogenically and dominantly inherited resistance genes are identified as *Or1, Or2, Or3, Or4* and *Or5* that are controlled by a root-specific promoter as expected. In order to hinder the parasitic infestation, at least two independent resistance components were suggested to be activated simultaneously in sunflower roots. Here, we present recent molecular studies of resistance mechanisms in sunflower against *Orobanche cumana* genotypes.

Keywords: *Orobanche cumana*, resistance, sunflower, molecular studies

SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA* WALLR.)

Orobanche (*Phelipanche*) spp. (broomrapes) are pathogenic agents attacking sunflower with parasitic angiosperms, and they are a significant threat to sunflower crops worldwide (Parker, 2013). Diploid (2n) *O. cumana* species have chromosome number of 38 and an estimated genome size of 1.42 Gb (Piednoël et al., 2012). Unfortunately, the complete transcriptome and genome sequences are not available for this species (Molinero-Ruiz 2015). In the Parasitic Plant Genome Project website, only Expressed Sequence Tags (ESTs) from *Phelipanche aegyptiaca* are publicly accessible (PPGP, 2015).

There are several species of *Orobanche* that cause major economic losses in the world; e.g. *P. aegyptiaca* (Pers.) Pomel, *O. crenata* Forsk, *P. ramosa* (L.) Pomel, *O. minor* Sm., *O. cernua* L. (Parker, 2013). *O. cumana* limits the range of sunflower species in the wild and sunflower is parasitized by *O. cumana* crop. These species are found in a wide range in the wild, and they damage sunflower species economically (Kreutz, 1995, Parker, 2013). While the resistance of host range in other parasitic systems is non-complete, horizontal and regulated by many genes, sunflower resistance to broomrape is vertical, race specific and regulated by a few

single dominant genes (Pérez-Vich et al., 2013). Vertical resistance means the genes are mainly controlled by the gene-for-gene interactions between the host resistance genes and related parasite avirulance genes (Flor, 1971). Generally, these interactions are defined by physical characters of parasite and dominant avirulance genes that are regulated by dominant resistance genes (Fernández-Martínez et al., 2015). Kaya (2014) reported a total of eight races (namely, A to H) of *O. cumana*. However, terminology of race identification is confusing. Because, the same race classification is used without corresponding other studies in different geographical areas. Velasco et al. (2016) reported that there were some marked differences between the classified populations of F from Romania and Spain about virulence against in a set of differential lines. Sunflower resistance against broomrape is reported as a trait controlled by a few single dominant genes. This resistance was reported against the races of E (Sukno et al., 1999; Vranceanu et al., 1980; Lu et al., 2000; Ish-Shalom-Gordon et al., 1993; PérezVich et al., 2004), G (Velasco et al., 2012) and F (Pacureanu-Joita et al., 2004; Pérez-Vich et al., 2002).

In the parasitizing process, while parasite cells move towards host vessels, a specialized endophytic organ called haustorium is formed in the development of vascular connections (Hibberd&Jeschke, 2001). The haustorium is used for water or nutrient (nitrogen compounds and carbohyrates) uptake. While host factors for the haustorium development (haustorium-inducing factors or HIF) are determined in some *Orobanchaceae* species, those factors are not characterized in *Phelipanche* and *Orobanche* species (Yang et al., 2015). Studies on developing molecular markers related with avirulence genes in *O. cumana* plants are advancing. To provide accurate information about race classification of *O. cumana* populations and individual tolerant plants, markers developed from avirulence genes will be used as extremely impressive tools even though there are difficulties in studying the gene-for-gene interactions between sunflower host genes and parasite genes of *O. cumana*. Molecular marker studies concerning avirulance genes involve the development of new lines, crossing between them and selfing segregated broomrape populations (Velasco et al., 2016). Further studies on genetic diversity of *O. cumana* and its interaction with different sunflower populations will give breakthrough for the understanding of resistance mechanism of *O. cumana* in sunflower diversity (Pineda-Martos et al., 2013). The gene-for-gene interaction of *O. cumana*-sunflower was reported by Pineda-Martos et al. (2013). They showed that *O. cumana* race E avirulence in the presence of resistance gene *Or5* was inherited as a single dominant gene. Additionally, in gene-for-gene relationships between dominant resistance genes for *Or5* gene in sunflower, resistance to broomrape E but not to race F, avirulance is determined in presence of presence of the dominant *Or5* gene by avirulence gene Avr_{Or5}.

Unfortunately, genetic diversity of *O. cumana* has not been studied on global scale. In a recent study conducted by Pineda-Martos et al. (2013), two separate gene pools of *O. cumana* were identified in either south or central Spain according to their distribution as weedy forms in sunflower crops instead of distribution in the wild. The two gene pools included very low internal variability, and they were genetically very distant from each other. This phenomenon is known as the "founder effect", where two genetically distant gene pools are introduced simultaneously in two separate emergence events. However, this interpretation depends on low diversity (Pineda-Martos et al., 2013). Gene transfer was found to continue between *O. cumana* populations parasitizing sunflower populations in the wild as well as in the agricultural fields (Pineda-Martos et al., 2014). In any case, gene transfer from parasitizing wild plants to weedy populations may suggest additional mechanisms. That may create genetic diversity for latter populations. A large genetic diversity in *O. cumana* populations may contribute to the mechanisms that cope with the resistance barriers. Gene transfer in the opposite direction (from weedy populations to parasitizing wild plants) shows ecological significance because the

potential movement of new alleles of wild *O. cumana* virulence may be seen under selection pressure of agricultural fields (Velasco et al., 2016).

Inheritance of resistance to broomrape race F in sunflower line K-96 was studied and quantitative trait loci (QTL) of potential value for marker assisted pyramiding of resistance genes were characterized (Akhtouch, 2016). To search inheritance of broomrape resistance, susceptible line P-21 and the line P-96 were analyzed with oligogenic recessive resistance. K-96 and P-96 were found to have a minor effect on broomrape resistance. Therefore, they could be suitable donors in marker-assisted sunflower breeding programs against broomrape resistance. Broad transgressive segregation is observed in crosses with P-96 for susceptibility in F2 generation. Molecular analyses of both lines have identified different resistance alleles in each line. Crosses with P-21 proposed that the resistance trait(s) could be controlled by dominant-recessive epistasis at two loci. Five QTLs of linkage groups of 2, 3, 4, 5 and 6 control the broomrape resistance traits in susceptible line P-21 (Akhtouch, 2016). Additionally, it is found that the linkage groups (LG) of 4 and 5 control the plant height. This suggests a pleiotropic effect of plant height in broomrape resistance (Akhtouch, 2016).

STRATEGIES FOR SUSTAINABLE RESISTANCE

The use of reduced number of sunflower sources of resistance to broomrape, which are dominant and monogenic, increasingly contribute to the continuous development of resistance. Huge efforts are devoted by companies to create commercial hybrids with new resistance genes that are identified so far. Unfortunately, broomrape is distributed to new areas that were not infected by the virus before, such as Tunisia (Amri et al., 2012), France (Jestin et al., 2014), and northern Spain (Pineda-Martos et al., 2013). Very virulent populations of the races G and H are prevalent in several areas (Antonova, 2014; Pacureanu, 2014). Their use in strategy of broomrape control for genetic resistance will support the development of new races even though most of the broomrape populations are controlled as vertical resistance sources. In sustainable genetic control of broomrape, major genes controlling different resistance mechanisms are needed to take into consideration. Molecular markers can be developed with major genes to sustain the effects of resistance.

Vrânceanu et al. (1980) in Romania identified a set of differential lines having resistance to five successive broomrape races A, B, C, D, and E. They are controlled by the dominant genes of *Or1*, *Or2*, *Or3*, *Or4* and *Or5*, respectively. Molecular studies carried on resistance to race E indicated that its resistance is controlled by the major dominant *Or5* gene. *Or5* gene is probably localized in the telomeric region. and is related with linkage group 3 (LG3) of the sunflower genetic map (Lu et al., 1999; Tang et al., 2003; Pérez-Vich et al., 2004; Márquez-Lema et al., 2008). Regarding to this knowledge, four QTLs associated with the number of broomrape shoots per plant were determined (Pérez-Vich et al., 2004). Imerovski et al. (2013) indicated that resistance genes *Or2*, *Or4* and *Or6* are strongly associated with simple sequence repeat (SSR) markers of LG3. Regardless of *O. cumana* race populations, it is possible to develop resistant sunflower genotypes (Škorić & Pacureanu, 2010). A mutation of *ACETOLACTATE SYNTHASE* (*AHAS*, EC 4.1.3.18,) gene in maize (*Zea mays*, var Black Mexican Sweet) (Shaner et al., 1984) and pea (*Pisum sativum* L. var Alaska) (Ray, 1984) was shown to be used in the control of broomrape infestation. Therefore, similar strategies may also work in sunflower. Based on the introduction of different *AHAS* alleles to obtain resistance in sunflower, three different herbicidal technologies are available (Sala et al., 2012). They are Clearfield® technology based on Imisun sunflowers, Clearfield Plus® technology based on CLPlus sunflowers and and Sures sunflowers (Sala et al., 2012). The first commercial herbicide tolerance (HT) in sunflower is called as

'Imisun'. Crossing of imidazolinones-tolerant plants with cultivated sunflower lines results in IMI-tolerant populations (Al-Khatib et al., 1998). The partially dominant allele *Ahasl1-1* and the other a modifier or enhancer factor controls the inheritance of Imisun (Miller and Al-Khatib, 2002; Bruniard and Miller, 2001). The second IMI tolerance trait in sunflowers as CLPlus is regulated by partially dominant nuclear allele Ahasl1-3. Its expression is developed by seed mutagenesis and selection with imazapyr (Sala et al., 2008). The third one is Sures sunflowers. They are developed from wild sunflower populations discovered in USA (Al-Khatib et al., 1999). Forward crossing and selection with the herbicide tribenuron accesses the tolerance allele *Ahasl1-2* and result in the trait known as Sures (Miller and AlKhatib, 2004).

RPG01 is one of the sunflower lines that carry *Or5* gene conferring resistance to race E of *O. cumana*. Identification of sequence characterized amplified region (SCAR) markers regarding to broomrape resistance gene *Or5* could be a significant tool in breeding resistance against broomrape race E (Lu, 2000). Lu et al. (2000) found five SCAR markers significantly linking to the *Or5* locus. They are RTS05, which is at 5.6 cM from the *Or5* locus, RTS28, RTS40 and RTS29 that are in distance interval of about 20 cM from the resistance gene, and UBC120_660 that is the only one in opposite side of the *Or5* locus. Identification of *Or5*-linked SCAR markers in broomrape resistance studies will help selection of new resistant lines in molecular genetics of broomrape resistance genes in sunflower (Lu, 2000).

MECHANISMS OF RESISTANCE AGAINST *OROBANCHE CUMANA* WALLR.

Conducting physiology-based breeding and pyramiding resistance genes underlying different resistance mechanisms will support to obtain information on the physiological basis of the different resistance sources on broomrape resistance (Pérez-Vich et al., 2013). Importance of the pH in root system for broomrape resistance is indicated in early studies of sunflower. Rihter (1924) mentioned that susceptibility to this parasite is promoted by low pH values in the roots of the host plant. On the other hand, Lozano-Cabello et al. (1999) notified that basic soils with pH 8.14 gave lower number of broomrape stems per sunflower plant compared to acid soils with pH 6.17 in Spain.

Plant defensins are basic peptides around 5-10 kDa and show antifungal activity (De-Zelicourt et al. 2007). After attachment and before necrosis of *O. cumana*, it is shown that roots of resistant sunflower genotype (LR1; *Helianthus annuus* x *Helianthus debilis debilis*) encodes a putative defensin by the gene *HaDef1* (Letousey et al. 2007). It supports that defensins could play significant roles in the resistance of sunflower genotype LR1 by leading to parasite death (De-Zelicourt et al. 2007). De-Zelicourt et al. (2007) confirmed that *HaDef1* activates cell death in *Orobanche* parasitic plants.

CONCLUSION

In summary, the parasitic broomrape (*Orobanche* spp.) is a major global issue that acts as a huge risk in sunflower production in Southern and Eastern European countries every year, causing 50% losses in the yield (Molinero-Ruiz 2015). In broomrape resistance of sunflower, next generation sequencing technologies, metabolomics and their applications on parasitic weeds will be necessary to exploit. Application of different bioinformatics approaches with known *O. cumana* target genes obtained from model plants of sunflower will lead to a rapid and effective characterization of broomrape (*Orobanche* spp.) resistance mechanisms in sunflower. Although there are several sunflower genes characterized in resistance against broomrape (*Orobanche* spp.), molecular basis of resistance has not been completely comprehended. Broomrape resistance in sunflower can be managed by the use of molecular techniques such as marker-

assisted selection, QTL identification and associating mapping. Additionally, the application of different molecular methods such as transcriptomics will help the identification of new sunflower broomrape resistance genes. Therefore, the use of molecular markers, RFLP, SSR, QTL, RAPD, TRAOP, and physiological markers will be the most reliable and the most easily applied methods to screen sunflower breeding materials for broomrape resistance.

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THE RESISTANCE OF ADVANCED HIGH OLEIC RESTORER LINES AND THE EVALUATION OF THEIR HYBRDS' YIELD TRAITS

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ABSTRACT

Downy mildew is the most serious disease in sunflower production in Turkey. To determine the adaptation and combining abilities of inbred lines is key issue in sunflower hybrid breeding. F8 and F9 stage restorer lines having high oleic acid content crossed with 9661-A (CMS) female line used as female tester for general combination ability were used in the study. These restorer lines developed in National Sunflower Hybrid Breeding Project conducted by Trakya Agricultural Research Institute (TARI) were selected as resistant to the broomrape parasite *(Orobanche cumana)* which is the major problem in most of sunflower growing areas in Turkey among other inbred lines in TARI. Mildew tests were conducted in artificial inoculation collected from infected plants as a mixture at various locations across Thrace region in 2014. In downy mildew tests, plant samples having a blend of all the mildew races were dried under shadow as 24-48 hours then they were preserved in the cooler at -80 Cº. Based on downy mildew test results, the hybrids with F8-R SN: 1, 2, 7, 8, 17 and F9-R SN: 9, 10, 12, 13 restorer lines were found fully resistant and others were found mid resistant. To determine their performances of these 18 hybrids, yield trial was conducted as randomized complete blocks design with 3 reps in 4 rows and with three commercial controls at Edirne location in 2015. For seed yield, the number of 5, 15 and 12 inbred line hybrids exhibited higher performances than commercial checks. While the numbered of 4, 5, 12, 15 and 16 line hybrids were found as resistant to downy mildew whereas other hybrids were found as mid resistant. As a result, high oleic, resistant to downy mildew and broomrape restorer lines and their hybrids and also exhibited higher yield and quality performance ones were determined. If they keeps higher performances in this year, they will send registration trials and the same testers will be used for developing sunflower hybrids for downy mildew and high oleic types.

Key Words : Sunflower, High Oleic, Downy Mildew Resistant, Tester, Restorer Line

MOLECULAR GENETICS

PROTEOMIC RESPONSE OF SUNFLOWER TO DROUGHT STRESS

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ABSTRACT

Proteomics technique was used to identify of proteins involved in drought tolerance and sensitivity of sunflower inbred lines. Based on two years field evaluation under drought stress in flowering stage among 16 sunflower lines RGK 21 and BGK 329 were identified as more drought sensitive and tolerant lines respectively. In comparison of proteomic pattern using 2DE, 21 of 347 and 27 of 363 protein spots were affected significantly by drought stress in sensitive and tolerant lines respectively, among them 18 proteins were identified in sensitive and 24 in tolerant line by nano-LC MS/MS mass spectrometry. In sensitive and tolerant lines 81 and 52% of identified proteins were down-regulated respectively. Cytoplasm-chloroplast /metabolism-energy related proteins constituted the major group of identified proteins. The results indicated that preservation of relevant water statues by morpho-physiological changes, supporting natural cellular metabolism, and changes in energy and antioxidant defense related proteins were the main factors for adaptation and drought tolerance of sunflower.
Keywords; Sunflower, Proteomics, Drought, Root

INTRODUCTION

Sunflower as a main oilseed crop is a drought tolerant crop however its productivity is greatly affected by drought stress (Chimenti et al., 2002). There are different; morphological, physiological or molecular mechanisms at different stages for drought tolerance in plants (Farooq *et al*., 2009). At the molecular level drought tolerance is related to differential expression of some stress induced proteins as late embryogenesis abundant, aquaporins and heat shock proteins (Bartels and Sunkar, 2005). Generally it is believed that under drought stress photosynthesis related proteins are reduced as a result of stomata closure while energy and defense related proteins are increased to meet energy demands and to protection of sub-cellular structures (Dinakar *et al.* 2012).

Nowadays proteomics is an essential methodology for large-scale analysis of proteins in various fields of plant biology (Komatsu *et al.* 2007) which has been used for analysis of proteome changes against drought stress in different plants There are limited studies about response of sunflower to drought stress. Castillejo *et al.* (2008) and Fulda et al (2011) used this approach to identification of proteins involved in sunflower tolerance to drought stress. Linking proteome changes to physiological changes providing protein biomarkers for selection programs enable plant breeders to develop new varieties with enhanced drought tolerance. Considering the limited information about sunflower response to drought stress in protein level proteomics technique was used to compare changes of proteins induced by drought stress in roots of sensitive and tolerant sunflower inbred lines.

MATERIALS AND METHODS

Protein extraction from roots of sunflower was adapted from oervious work (Ghaffari et al., 2013) by some modifications. Protein contents were determined using the Bradford (1976) method with bovine serum albumin as the standard. After 2-DE electrophoresis the gels were

stained with coomassie brilliant blue for 1 h, and then destained with 35% methanol and 10% acetic acid for 12 h. 2-DE images were obtained using a GS-800 calibrated densitometer scanner and the position of individual proteins on gels was evaluated with PDQuest software. The isoelectric point and molecular mass of each protein was determined using 2-DE standard marker. Comparative analysis, were performed using PDQuest software. Student's t-test was used to assess the statistical significant of differences in protein abundance between control and drought treatment. The protein spots were excised from 2-DE gels and subjected to reduction and rehydration process using DigestPro. The desalted peptide solution was analyzed by nano-liquid column (LC)-MS/MS.

A nanospray LTQ XL Orbitrap MS was operated in data-dependent acquisition mode with the installed XCalibur software were used to obtaining MS/MS spectra. Full-scan mass spectra were acquired in the Orbitrap over a mass range of 150 - 2,000 m/z with a resolution of 15,000. The 3 most intense ions above an intensity threshold of 1,000 units were selected for collision-induced fragmentation in the linear ion trap at normalized collision energy of 35% after accumulation to a target value of 1,000 intensity units. The resulting peptide sequence data were used to search the NCBInr protein database using the MASCOT search engine. A homology search of the amino acid sequences of identified proteins was performed against the NCBI non-redundant sequence database using BLASTP to assign protein identities.

RESULTS AND DISCUSSIONS

Comparison of 2-DE gels revealed 347 reproducible protein spots in drought sensitive (RGK 21) and 363 in tolerant (BGK 329) line, among them 18 were affected by drought stress in sensitive and 24 in tolerant line significantly. Drought stress made lower reduction in abundance of identified proteins in BGK 329 which implies the more flexibility of the tolerant line to endurance of drought damages. This is also indicated as a general rule by Rossignol et al. (2006). Of the affected protein spots in RGK 21 the relative abundances of 4 were increased and 14 decreased. Of the protein spots in BGK 329, relative abundances of 10 were increased, 11 decreased, 2 protein spots turned up as new spots and one spot disappeared. According to the direction of changes all identified proteins categorized in 6 groups.

Classification of all differentially changed protein spots under drought stress based on cellular function revealed more impressibility of metabolism followed by energy related proteins in both sensitive and tolerant lines. Relative abundance of all metabolism related proteins were decreased in sensitive line but 36% of these proteins were increased in tolerant line. Relative abundance of disease/defense related proteins were decreased in sensitive while increased in tolerant line. According to the sub-cellular localization, cytoplasmic followed by chloroplastic proteins were primary drought responsive proteins in both lines. There was more reduction while more increase in relative abundance of cytoplasmic proteins in sensitive and tolerant lines respectively, however chloroplastic proteins were down expressed considerably in both lines.

Among the identified proteins, 15 protein spots were commonly affected by drought stress in both lines; relative abundance of 4 of them were increased and 6 decreased . Relative abundance of the 5 remaining protein spots i.e. sucrose synthase, bifunctional polymyxin resistance protein ArnA-like, ATP synthase subunit beta, glyceraldehyde-3-phosphate dehydrogenase, putative cytosolic NADP-malic enzyme (protein spots 1, 9, 14, 32 and 33) were expressed differentially; decreased in sensitive while increased in tolerant line. There were 3 proteins specific to RGK 21 and 9 to BGK 329. The abundances of RGK 21 specific proteins were decreased while 3 of BGK 329 specific proteins; carbonic anhydrase, Cu/Zn superoxide dismutase, methionine synthase (spots 7, 13, 29) were increased.

Two groups of proteins had same changes in two lines. Oxygen-evolving enhancer (OEE) and ferredoxin-NADP reductase (FNR) as major photosynthetic proteins were suppressed in both lines**.** Impairment to the OEE as a result of drought stress has been reported by Vander Willigen et al. (2003). OEE has a key role in providing of reducing power to the electron transport chain (ETC) in chloroplast by splitting of H_2O. It seems that OEE act as a primary drought sensor and changes of its abundance can shift normal ETC mode to stress tolerance mode. Involvement of FNR in ROS scavenging and NADPH/NADP+ homeostasis (Rodriguez et al. 2007) expresses this protein as a common intermediate in response to drought stress. **I**nduction of tubulin alpha-3 chain in both lines as a cytoskeleton-related protein which is also involves in cell division can enhance cellular structure to repel drought injuries.

Reduction of enolase (spot 16) assumed as a sign of metabolic impairment to the glycolytic pathway in sensitive line. Down turning of enolase can limit malate supply for mitochondria which is an explanation for reduction of ATP synthase and energetic depletion in sensitive line. Drought induced accumulation of carbonic anhydrase (spot 7), Cu/Zn superoxide dismutase and methionine synthase was assumed as a BGK 329 specific response to endurance of drought injuries. The same results reported in other plants previously (Merewitz et al. 2011). Carbonic anhydrase has a key role in CO_2 enrichment before Calvin cycle. The energetic cost of this adaptation is more ATP which is provided by increased abundance of ATP synthase in the tolerant line.

Enhancement of ROS scavenging capacity by induction of Cu/Zn-SOD constituted one of the major aspects of drought tolerance in BGK 329. Induction of this protein by drought was also reported by Salekdeh et al. (2002). Increased demand for more methyl groups for lignification justifies accumulation of methionine synthase in tolerant line too. Involvement of this protein in osmotic adjustment (Yan et al. 2005), imply critical role of osmo-protection and lignification in drought tolerance of BGK 329.

Differential changes in abundances of 5 proteins (spots 1, 9, 14, 32 and 33) can be a cause for different response of the lines to drought stress. Drought induced accumulation of sucrose synthase has been also reported in Fern-Ally (Wang et al. 2010). We suggest that drought induced accumulation of sucrose synthase in tolerant line was an adaptive mechanism to energy conservation and maintaining cell structure integrity by invigoration of osmotic adjustment.

Induction of mitochondrial ATP synthase (spot 14) in tolerant line assumed as a compensatory mechanism to meet energy demand and to alleviation of drought effects within mitochondrion. Kotapalli et al. (2009) also observed similar results with peanut. Regarding the role of NADP-malic enzyme in breaking down of malate which results in reduction of turgor in guard cells and so on in stomata closure (Laporte et al, 2002) induction of this protein in tolerant line assumed as an adaptive mechanism for water conservation under drought condition. The results of this study indicated that sunflower genotypes shut down photosynthesis by down expression of ETC related proteins as a protective mechanism to avoid generation of ROS during drought condition. Proteomic changes related to ROS scavenging, energy conservation, cell structure integrity and water conservation constituted the major aspects of drought tolerance in sunflower.

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APPROACHES FOR IMPROVEMENT OF RESISTANCE TO POWDERY MILDEW IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Powdery mildew disease caused by *Golovinomyces cichoracearum* (DC) V.P. Heluta var. *Cichoracearum* has become a serious problem in sunflower cultivation in India since the last decade. Initially, the disease was confined to *rabi* crop (October-March) at flowering and post-flowering stages but in the recent past, the pathogen attack is witnessed during all seasons and all stages of crop growth necessitating resistance deployment strategies. A screening method and scoring scale were developed for reliable identification of genotypes resistant to the disease. Screening of germplasm, breeding lines and wild *Helianthus* species resulted in identification of two interspecific derivatives namely HIR-1734-2 (EC-633077) and RES-834-3 (EC-633089), two exotic lines namely PI 642072 (EC-595333) and USDA-25 (EC-537925) and ten *Helianthus* species namely *H. argophyllus, H. agrestis, H. debilis, H. praecox, H. angustifolius, H. atrorubens, H. rigidus, H. salicifolius, H. pauciflorus* and *H. resinosus* tolerant to the disease. Based on consistent reaction in different accessions and across seasons, four accessions namely RES-834-3, PI 642072, *H. debilis* and *H. praecox* along with the highly susceptible line PS 2023 were studied extensively for host-pathogen interactions, biochemical profiling of defense related enzymes and transcript profiling in control and post-infected samples which indicated different mechanisms of tolerance. Development of mapping populations (RILs, BC_1F_1) involving the resistant donors are in various stages towards mapping of genes conferring resistance to powdery mildew in sunflower.

Keywords: Sunflower, powdery mildew, *Golovinomyces cichoracearum*, differential transcripts, host-pathogen relationships

INTRODUCTION

In India, powdery mildew disease was sporadically observed before 2006, but during the year 2006-07 it was reported in high intensity (80%) on *rabi* crop (October-March) in some areas around Bengaluru and Raichur which increased over the years (Anonymous, 2007). Polycyclic nature and short life cycle of the pathogen under conditions of high humidity resulted in rapid spread of the disease to all the sunflower growing states (South, Central and North India) and seasons (rainy, spring and summer) in India (Sujatha et al., 2015). The disease begins during the post-flowering stage as minute discoloured specks on leaves from which powdery mass radiates on all the sides. All the aerial parts of the host are covered with white powdery mass containing mycelia and conidia of the fungus. At present, the disease is seen regularly in all sunflower growing areas of the country in moderate to severe form. A field experiment on yield loss assessment of powdery mildew in sunflower was conducted and the results revealed that, at 30% and 64% of disease severity levels the seed yields were reduced by 20.5% and 52.6%, respectively (Anonymous, 2014) necessitating research for development of appropriate

management strategies. Yield reduction is mainly due to the reduced photosynthetic activity, physiological changes and increased rate of senescence.

SCREENING AND IDENTIFICATION OF RELIABLE SOURCES OF RESISTANCE TO POWDERY MILDEW

It is reported that three genera namely *Golovinomyces cichoracearum* f.sp. *helianthi* (syn *Erysiphe cichoracearum* DC ex Meret; *Oidium asteris punicei* Peck), *Leveillula taurica* (=*Leveillula compositarum*) and *Podosphaera xanthii* Castagne Braun & Shishkoff (=*Sphaerotheca fuliginea* auct p.p.) are the causative agents of powdery mildew in sunflower; of which, *G. cichoracearum* is of the most common occurrence in all the continents (Saliman et al., 1982; Gulya et al., 1991; Chen et al., 2008). Classical identification methods based on microscopical analysis and spore trapping are labour intensive and require considerable experience in differentiating the morphologies of the powdery mildews (Grote et al., 2002). Hence, morphological characteristics supported by molecular analysis of the powdery mildew isolates collected from different geographical locations in India using the powdery mildew specific ITS universal primer pair (Bardin et al., 1999) and also primers that are specific to the ITS regions of *P. xanthii*, *G. cichoracearum* and *L. taurica* indicated that disease infection is caused by *G. cichoracearum* (Prathap Reddy et al., 2013). Reliable sources of resistance are not available in the released cultivars and the parental lines of hybrids. Hence, wild *Helianthus* species, backcross inbred lines, interspecific derivatives, core germplasm set, inbred lines and few exotic accessions were screened under field conditions by simulating the conditions followed by rescreening under artificial inoculation conditions (Fig. 1). Sources of resistance were identified in five annual wild species namely *H. argophyllus, H. agrestis, H. debilis, H. niveus, H. praecox* and six perennials namely *H. angustifolius, H. atrorubens, H. rigidus, H. salcifolius, H. pauciflorus* and *H. resinosus*, two interspecific derivatives (HIR-1734-2/EC-633077, RES-834-3/ EC-633089) and two exotic lines accessions (PI 642072/EC-595333/TX16R, USDA-25/EC-537925). The species, *H. strumosus* was highly susceptible and harboured the pathogen throughout the year. Seven different methods described earlier (Karuna, 2010) were tested; of which, dusting of spores from infected leaves on to the healthy leaves of the test plants proved to be the most convenient and effective method of infection. Artificial screening showed low infestation of powdery mildew on ID-25 (RES-834-3) and other accessions (TX16R, EC-537925) with negligible conidial count (2500 conidia/cm^2) when compared to 1,30,000 conidia/cm^2 in the control (Prathap Reddy et al., 2013). Based on the differential response of the accessions derived from diverse genetic backgrounds, a 0-9 scale for obtaining reliable estimate of the disease has been devised based on the percentage of leaf area as well as the spread of the disease on the plant on different leaves (Prathap Reddy et al., 2013; Sujatha et al., 2015). Crosses were effected with the resistant and susceptible lines and plant-pathogen interaction studies in lines with contrasting reaction were done to understand the mechanism of resistance in different sources.

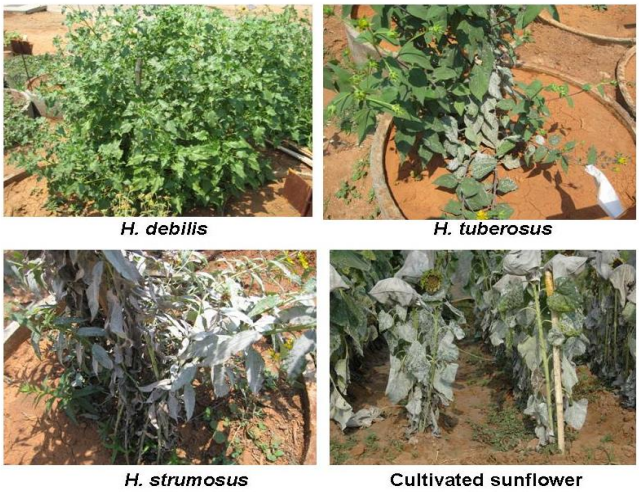

Fig. 1. Reaction of wild sunflowers to powdery mildew

HOST-PATHOGEN INTERACTIONS AND DIFFERENTIAL EXPRESSION OF GENES

The infection process of *G. cichoracearum* was studied in sunflower which included immune/resistant (*H. debilis, H. praecox*), tolerant (RES-834-3, TX16R) and susceptible (Morden, PS 2023A) genotypes both in controlled environment and field conditions. Inoculation was done by dusting the conidia on leaf blades of plants using camel hair brush. At 8, 12, 16, 20, 24, 36, 48, 72 and 96 hours following inoculation, leaves were sampled, cleared and stained. Powdery mildew infection in susceptible (2023B) line was within 8 hrs while spread and infection was slow in TX16R. There was no conidial germination and hyphal growth even after 4 days in *H. debilis* and *H. praecox.* Biochemical analysis of the accumulation of reactive oxygen intermediates (ROIs) such as superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) was done using ROI-specific dyes such as nitroblue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB). This study provided a distinct accumulation pattern during host-pathogen interaction and it was observed that the level accumulation of ROIs was higher in resistant than susceptible genotypes. It is presumed that a higher inducible level of ROIs during infection in resistant lines is responsible for the arrest of the pathogen.

Following preliminary light microscopic and biochemical analysis, the host-pathogen interactions were studied by transcriptome profiling. Leaves of the resistant species- *H. debilis*, *H. praecox*, *H. niveus*, tolerant genotypes- TX16R, USDA-25 and the susceptible genotype - PS 2023B were dusted with the powdery mildew conidia from infected leaves of the susceptible accession (PS 2023B). Infected leaves were fixed at 0 (no infection), 24, 48 and 72 hours post infection (hpi) and subjected to transcriptome profiling. Libraries were prepared using TruSeq RNA library prep kit (Illumina) and were sequenced (PE-2x100) on HiSeq to obtain 80 million reads per sample. Following filtration of organelle genome and non-coding RNA sequences, the cleaned reads were aligned to the reference genome of *H. annuus* cv. Ha-412-HO with a gene model downloaded from Genomics of Sunflower database using Tophat2 tool. Results showed that in each of the donors, the mechanism of resistance varied as evident for the upregulation and downregulation of genes following infection. Maximum number of genes upregulated in response to the pathogen infection was observed in TX16R and *H. praecox* (Table 1).

Table 1: Total up and down regulated genes in transcript level [P value <= 0.01 and FPKM >= 1] found using Cuffdiff analysis

Samples	Up Regulated	Down Regulated
2023_B_Control vs 2023_B_Pool (24,48,72 hpi)	779	335
TX16R_Control vs TX16R_Pool (24,48,72 hpi)	4,464	211
ID25_Control vs ID25_Pool (24,48,72 hpi)	441	723
*H. niveus*_1452_Control vs *H. niveus*_1452_Pool (24,48,72 hpi)	909	263
*H. praecox*_1823_Control vs *H. praecox*_1823_Pool (24,48,72 hpi)	3,818	186
*H. debilis*_Control vs *H. debilis*_Pool (24,48,72 hpi)	308	468
2023_B_Control vs TX16R_Pool (24,48,72 hpi)	677	803
2023_B_Control vs ID25_Pool (24,48,72 hpi)	892	435
2023_B_Control vs *H. niveus*_1452_Pool (24,48,72 hpi)	1,252	797
2023_B_Control vs *H. praecox*_1823_Pool (24,48,72 hpi)	3,824	204
2023_B_Control vs *H. debilis*_Pool (24,48,72 hpi)	677	803

Analysis was done to check the genes which are commonly upregulated and downregulated in the susceptible versus resistant donors, among the resistant lines, and the tolerant lines, which are presented in Fig. 2 and 3, respectively. Only two genes were commonly upregulated in the susceptible and resistant genotypes while no genes were commonly downregulated between the two groups. The tolerant genotypes (TX16R and ID-25) had 14 and 19 genes in common that were upregulated and downregulated, respectively. Venn diagrams showed more common genes between *H. praecox* and *H. niveus* than those between *H. debilis* and *H. niveus.*

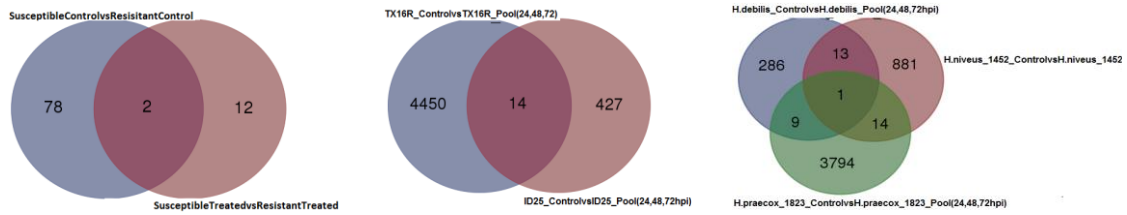

Fig. 2 Venn diagram showing commonly upregulated genes in different groups

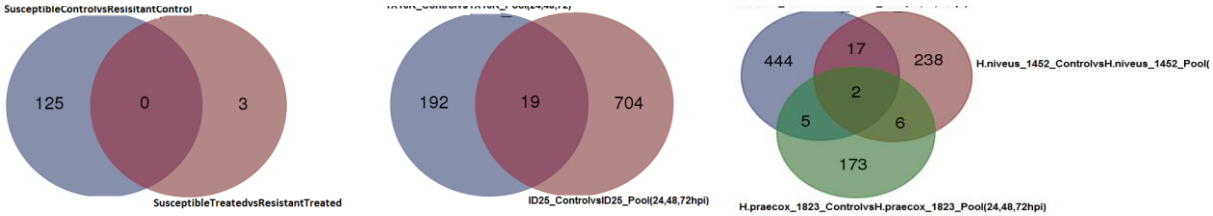

Fig. 3 Venn diagram showing commonly downregulated genes in different groups

Pathway enrichment was performed using Reactome database (Fig. 4). Pathway analysis indicated that the MAPK/MAPK6/MAPK4 signaling cascades are involved in *H. praecox;* Vesicle-mediated transport and membrane trafficking, regulation of HSF1-mediated heat shock response in *H. debilis*, mRNA splicing in TX16R, purine catabolism and detoxification of ROS in the susceptible genotype (PS 2023B).

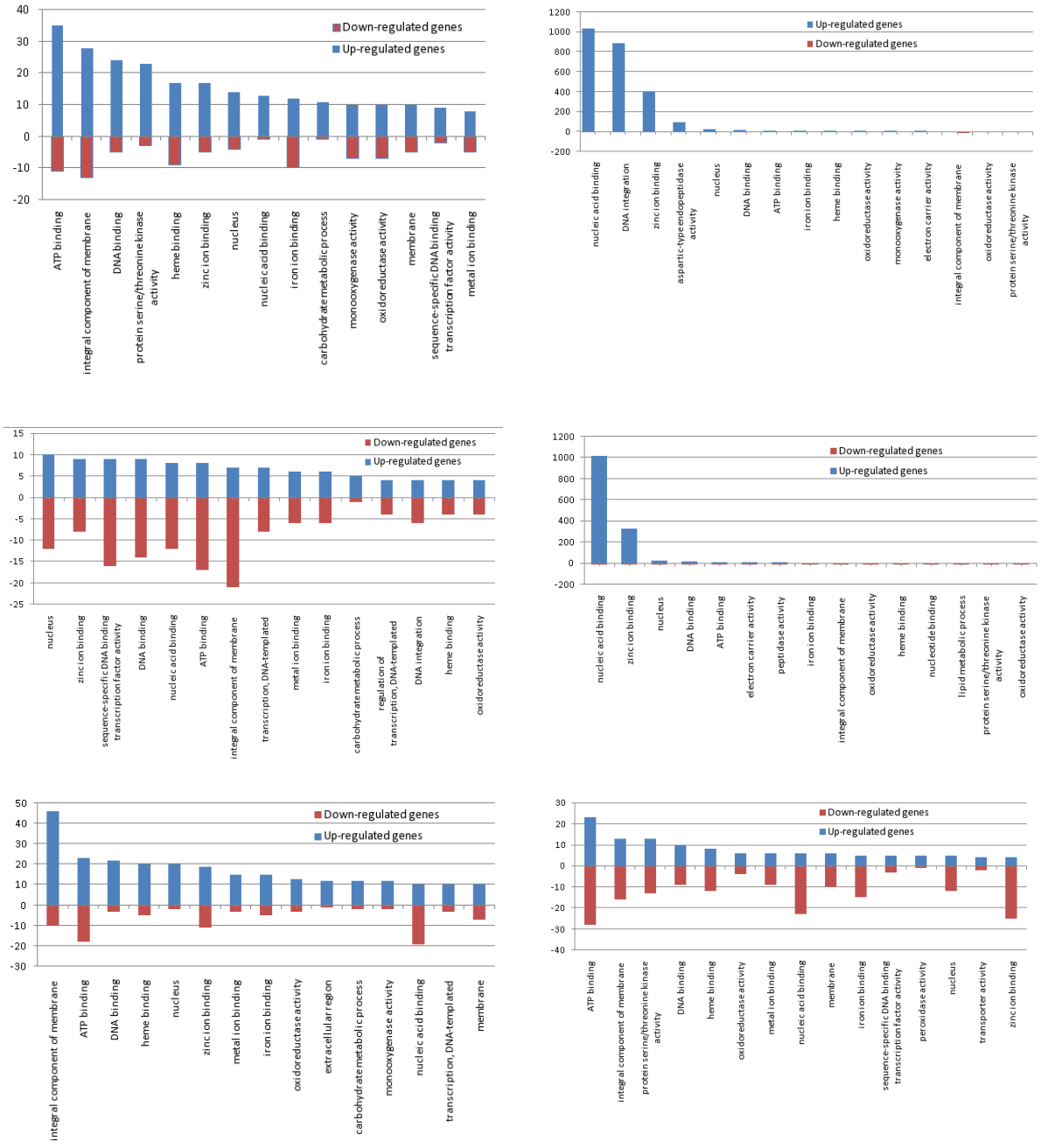

Fig. 4 Ontological analysis of differential expressed genes in PS 2023B, *H. praecox*, *H. debilis*, TX16R, *H. niveus* and ID-25 (in control vs infected)

The transcriptome data was explored for WRKY, Kinases and MAPK in the up and down regulated genes across all the pair-wise combinations. In ABSTRACT, there were 412 genes related to Kinases, 3 MAPK genes and 19 WRKY related genes from both up and down regulation. Work on validation of the key genes for their role in conferring resistance to powdery mildew in sunflower is underway.

TOWARDS MAPPING GENE(S) FOR RESISTANCE TO POWDERY MILDEW

Among the identified sources of resistance to powdery mildew, PI 642072 (TX16R) was selected as resistance source for mapping gene(s) that confer resistance to powdery mildew in sunflower. The F_1s were made by crossing PS 2023A (highly susceptible) and TX16R (resistant to powdery mildew) and further selfed to develop F_2 population. The F_1s showed resistance reaction to powdery mildew infection suggesting dominance nature. Variation for resistance to powdery mildew in F_2 population appeared to be quantitative (did not fit into Mendelian ratios) (Fig. 5) The F_2 population was advanced further by single seed descent method in order to develop recombinant inbred line (F_7-RIL) population for use in mapping of powdery mildew resistance in TX16R.

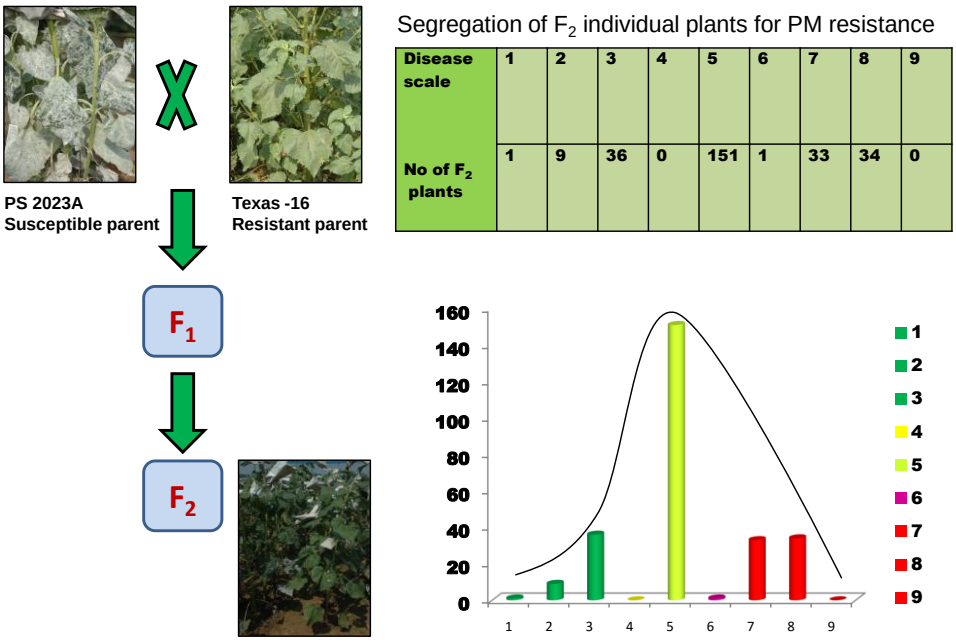

Fig. 5 Inheritance of resistance to powdery mildew in F_2 population produced from the cross: PS2033A x TX16R

Furthermore, interspecific crosses of cultivated sunflower with annual diploid species (*H. argophyllus*, *H. debilis* and *H. praecox*) were also made. The F_1s were confirmed for hybridity using SSR markers (ORS925, ORS505 and ORS898) and characterized for their reaction to powdery mildew. The F_1s involving *H. debilis* and *H. praecox* were highly resistant suggesting the dominance nature of resistance to powdery mildew in these sources. Development of backcross inbred line (BIL) populations is in progress towards mapping of powdery mildew resistance from wild sources. Till date, about 2100 sunflower specific SSR markers are available in public domain (Tang et al., 2002). The transcriptome data generated for the six genotypes has been mined for SSRs and SNPs and the additional markers would be used for trait mapping.

Thus, based on the importance and severity of the disease which is increasing over years and across seasons, the future line of research priorities would include determination of genetics

resistance to powdery mildew in different resistant donors including wild *Helianthus* species, introgression of resistance from the identified resistant donors into promising parental lines and molecular mapping of genes, which would enable marker-assisted selection (MAS) for resistance to powdery mildew in sunflower breeding.

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COMPARISON OF CYTOPLASMIC MALE STERILITY BASED ON PET1 AND PET2 CYTOPLASM IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Commercial sunflower hybrid breeding is exclusively based on the PET1 cytoplasm. However, diversity on the level of the cytoplasm is desired to reduce the susceptibility to potential pathogens. The PET2 cytoplasm represents a new CMS source with high potential for hybrid breeding. As the PET1 cytoplasm, CMS PET2 originates from an interspecific cross of *H. petiolaris* with *H. annuus*. However, rearrangements observed in the PET2 cytoplasm are totally different from the PET1 cytoplasm. The PET1 cytoplasm is characterized by the co-expression of *atpA* and *orfH522* and the presence of the CMS-specific 16-kDa-protein, whereas in the PET2 cytoplasm due to a duplication of the *atp9* gene, followed by an insertion of 271 bp of unknown sequences, two new open reading frames *orf288* and *orf231* are created. Both *orf*s, which share homology to the *atp9* gene, are co-transcribed and a clear reduction of this co-transcript can be observed in the anthers of fertility-restored hybrids. The *orf*s encode proteins of 11.1 kDa and 7.9 kDa, respectively. New markers linked to the *Rf1* gene and to the *Rf_PET2* restorer gene have been identified by AFLP analyses and have been developed from BAC-end sequences. Comparative mapping using SSR-markers demonstrated that both restorer genes are located on linkage group 13 close to each other, which facilitates cloning of both restorer genes.
Keywords: Cytoplasmic male sterility, fertility restoration, CMS PET1, CMS PET2, marker

INTRODUCTION

Cytoplasmic male sterility (CMS) is a maternally inherited trait in higher plants in which these fail to produce or shed viable pollen (Horn 2006). In most cases mutations in the mitochondrial DNA lead to new open reading frames (ORFs) that encode CMS-specific proteins that interfere with the pollen development, a process that has a high demand on energy supply (Horn et al. 2014). For hybrid breeding, CMS is of special interest because it allows directed crosses when using CMS lines as mother lines. In addition, CMS can be restored by dominant nuclear genes, so called restorer of fertility (*Rf*) genes. This allows the restoration of fertility in F_1 hybrids. Improvements in yield and yield stability of sunflower hybrids require the development of new lines, which are resistant to diseases, e.g. fungal pathogens, or which have improved oil quality. Marker-assisted breeding can accelerate the back-cross programs and is especially useful if the trait like fertility restoration can only be assessed as late as the flowering stage, a rather advanced period in the plant development (Neuhaus & Horn 2004). Therefore the development of co-dominant markers closely linked to the fertility restorer gene is of great importance for sunflower breeding programs. In addition, the isolation of the fertility restorer gene is of general interest to the research community to understand the molecular mechanism behind fertility restoration (Horn 2006).

The development of co-dominant markers closely linked to the locus of the fertility restorer gene *Rf1* would present a great improvement for marker-assisted breeding in sunflower hybrid production. The selection for the presence of the restorer gene *Rf1* (homozygous or heterozygous) could be performed at a very early stage of plant development.
Worldwide, only one CMS source, the so called PET1 cytoplasm, has been used for commercial sunflower hybrid production, so far (Nichterlein & Horn 2005). This CMS source is the result of an interspecific cross between *Helianthus petiolaris* and *H. annuus* (Leclerq 1969). The PET1 cytoplasm is characterized by the *orfH522* that encodes for a 16-kDa-protein (Horn et al. 1991, Köhler et al. 1991, Laver et al. 1991). However, more than 70 CMS sources have been described for sunflower (Serieys 2005), but only about half of them have been analyzed for the molecular mechanisms leading to CMS (de la Canal 2001, Horn 2002, Horn & Friedt 1999, Horn et al. 2002). However, diversity on the cytoplasm side is desired to avoid the pathogen specialization as the one observed in the maize T-cytoplasm (Miller & Koeppe 1971), exclusively used up to then in maize hybrid production. In sunflower, the PET2 cytoplasm, which was also derived from an interspecific cross of *Helianthus petiolaris* and *H. annuus* (Whelan & Dedio 1980), might be an interesting alternative (Horn & Friedt 1997). However, molecular characterization of the PET2 cytoplasm as well as markers for the restorer gene *Rf_PET2* would be required.
Markers linked to the restorer gene *Rf1*, responsible for fertility restoration of hybrids based on the PET1 cytoplasm, have been identified (Horn et al. 2003, Kusterer et al. 2005). The restorer gene has been placed on linkage group 13 of the sunflower reference map using SSR-markers (Kusterer et al. 2005). In order to isolate the restorer gene *Rf1* by a map-based cloning approach (Kusterer et al. 2004 a, b) a bacterial artificial chromosome (BAC) library has been constructed for the restorer line RHA325 (Özdemir et al. 2002, 2004). Markers were hybridised against high density BAC filters of two BAC libraries (RHA325 and HA383) to identify positive BAC clones. Using BAC fingerprinting, cloning and sequencing of BAC ends, the BAC clones were organized into contigs around the restorer gene *Rf1* (Hamrit et al. 2008, Hamrit 2009).
All markers linked to the restorer gene *Rf1* described up to now are dominant markers, which do not allow distinguishing the homozygous from the heterozygous fertile plants. Here we present the development of a co-dominant CAPS-marker linked to the *Rf1* gene. In addition, the molecular mechanism behind the PET2 cytoplasm will be elucidated as well as the close location of the restorer genes *Rf1* and *Rf_PET2* on linkage 13 by comparative mapping.

MATERIALS AND METHODS

Plant material

HA89 (maintainer line of CMS PET1 and CMS PET2) was used to study the male fertile cytoplasm, CMS line PET2 (Whelan and Dedio, 1980) maintained by RHA265, which is a restorer line of CMS PET1, and CMS line PET1 (Leclercq, 1969), maintained with HA89 and the fertility-restored hybrid PET2 (RHA265) x IH-51 were used for comparing CMS PET1 and CMS PET2 source. Mapping of the *Rf_PET2* was performed in the F2 population RHA265(PET2) x IH-51.
The investigations on the restorer gene *Rf1* were performed using the fertility restorer line RHA325, homozygous for the dominant *Rf1* allele, the fertility maintainer line HA342, homozygous for the recessive allele *rf1*, and bulks of the F2 population of the cross HA342 x RHA325. Each of the F2 bulks consisted of 10 individuals from the F2 population, which were either homozygous for the recessive allele of the fertility restorer gene (S1 and S2) or for the dominant allele (R1 and R2). Total genomic DNA was extracted from leaves according to the protocol of Doyle & Doyle (1987).

Cloning and sequencing mitochondrial DNA

Mitochondrial DNA was isolated using the procedure of Köhler et al. (1991). The *HindIII* digested mtDNA was blotted on Hybond N+ membrane (GE Healthcare) after separation on a 0.8 % agarose gel. Hybridizations with *atp9* as probe were performed according to the manufacturer's instructions using ECL Direct™ Nucleic Acid Labeling and Detection System (GE Healthcare) for the detection of restriction polymorphisms. The *Hin*dIII digested mtDNA fragments were cloned into pUC18 vector and the resulting recombinant plasmids were used to prepare a mitochondrial DNA library. Positive clones were selected by *Hin*dIII hybridization pattern with *atp9* as probe and sequenced.

Analysis and use of BAC-end sequences

Eleven positive bacterial clones (BAC-end sequences) were included in the investigations, which had been identified by hybridizations with markers linked to the restorer gene *Rf1* (Hamrit 2009) on the basis of the two available BAC-libraries in sunflower (Özdemir et al. 2004; Clemson University Genomics Institute-http://www.genome.clemson.edu). The BAC ends had been sequenced using SP6 and T7 primers. The BAC-end sequences of 100L22, 94F15, 147A3, 67N4, 261F19, 126N19, 447N6, 59J13, 450J13, 450B06, 480G04 and 139A17 were included in this study. The software BioEdit 7 was used for processing and analysis of the BAC-end sequences with the aim to develop STS (Sequence-Tagged-Site)-markers for back-mapping of these BAC clones in the population. The design of STS-primers was carried out by the program Web primer (http://www.yeastgenome.org/cgi-bin/web-primer). The design of CAPS (Cleaved Amplified Polymorphic Sequence) markers was realized with the help of the program NEB cutter V2.0 (http://tools.neb.com/NEBcutter2/).

PCR amplification of STS- and CAPS-markers

PCR amplification with STS-primers was performed with 15 ng DNA in the PCR cycler 2700 under the following conditions: 3 min denaturation at 94°C, followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing using different temperatures depending on the primers (range between 55°C and 64°C), 30 sec polymerization at 72°C of polymerization; followed by a final 7 min period of elongation at 72°C. For CAPS-marker 480G04_BsrGI, the PCR products were directly digested with the restriction endonuclease BsrGI. The amplified products were separated by 2 % agarose gel electrophoresis at 100 V for 35 minutes. The PCR-products were visualized with ethidium bromide solution.

Cloning and sequencing of PCR products

PCR products were cloned using the pGEM®-T Easy vector (Promega). After minipreparation, plasmids were sequenced using T7 and SP6 primers.

AFLP and SSR analyses

Amplified fragment length polymorphism (AFLP) analyses were done as described by Vos et al. (1995). For the *Rf1* gene, three new preamplifications were used: (1) E02 and M01, (2) E02 and M02 and (3) E02 and M04. For the selective amplifications, 16 *Eco*RI primers were combined with 48 *MseI* primers. For the *Rf_PET2* gene, AFLP analyses were performed based on the preamplification of E01 and M02 as primers. For the selective amplification, 16 *Eco*RI primers (E31 to E46) and 16 *Mse*I primers (M47 to M62) were combined.

SSR analyses were performed as described in Sajer et al. (2013). Using the SSR primer combinations for ORS317, ORS630 and ORS1030 of linkage group 13 (Tang et al. 2005) and

the M13tailing procedure (Oetting et al. 1995) PCR products were labelled with IRD800 and separated on the DNA Analyzer 4300 (LI-COR, Biosciences).

RESULTS AND DISCUSSION

Molecular characterization of the PET2 cytoplasm

Comparing the Southern hybridization pattern of mitochondrial DNA (*HindIII* digested) from the PET2 cytoplasm and the male fertile cytoplasm a restriction polymorphism was detected using the *atp9* gene as probe. One fragment of 3.4 kb was identical in the male fertile cytoplasm and CMS PET2, but an additional fragment of 4.1 kb was only present in CMS PET2. Cloning and sequencing of the *HindIII* fragments showed that the 3.4-kb-fragment contained the regular copy of the *atp9* gene, whereas the PET2-specific 4.1-kb-fragment contained a split second copy of *atp9*, which resulted in two new open reading frames of 228 bp and 231 bp (Figure 1). Both *orf*s show partial homology to *atp9*. The insertion of 271 bp splitting the *atp9* represents sequences of unknown origin. The new *orf*s encode proteins of 11.1 kDa and 7.9 kDa, respectively. RT-PCR analyses showed that the two PET2-specific *orfs* are co-transcribed and that the co-transcript is specifically reduced in the anthers of fertility-restored hybrids. Mitochondrial genes of the F1F0 ATP synthase are the most frequent genes involved in creating cytoplasmic male sterility (Horn et al. 2014). In sunflower, one other CMS source, the PEF1 cytoplasm, which originates from an interspecific cross of *H. petiolaris* ssp. *fallax* and *H. annuus* (Serieys & Vincourt 1987), also showed changes in the *atp9* gene, here a 0.5-kb-insertion in the 3'-UTR, associated with the male sterility phenotype (de la Canal et al. 2001).

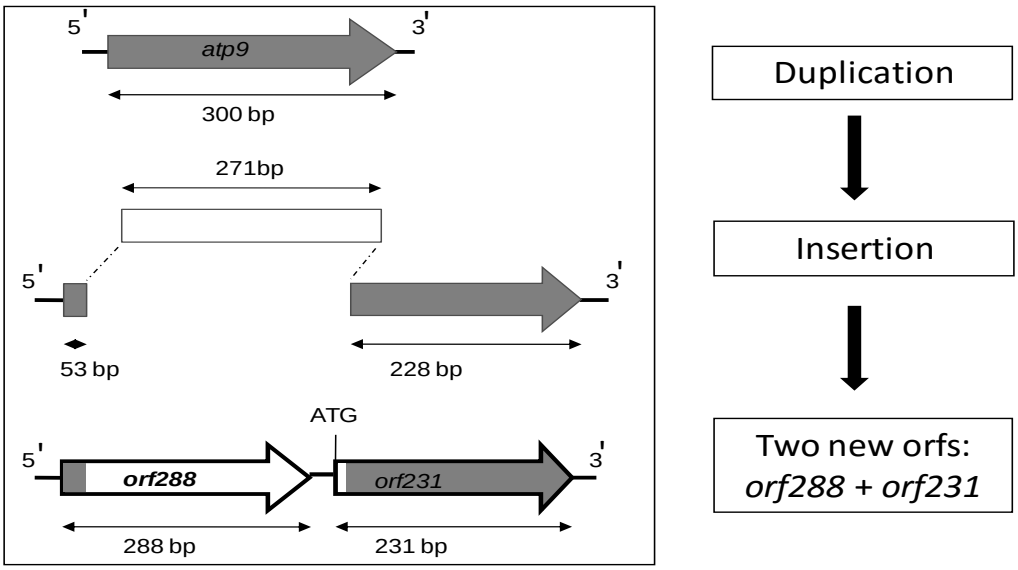


Figure 1: Model of the recombination events

Even though both CMS cytoplasm PET1 and PET2 were derived from an interspecific cross between *H. petiolaris* and *H. annuus*, the mechanism behind the male sterility of CMS PET2 is totally different than in the CMS PET1 cytoplasm, which makes it interesting to use the CMS PET2 cytoplasm for commercial sunflower hybrids. Now that the mechanism is known primers specifically differentiating the two CMS sources can be developed.

First round of developing of STS-markers for *Rf1* from BAC-end sequences

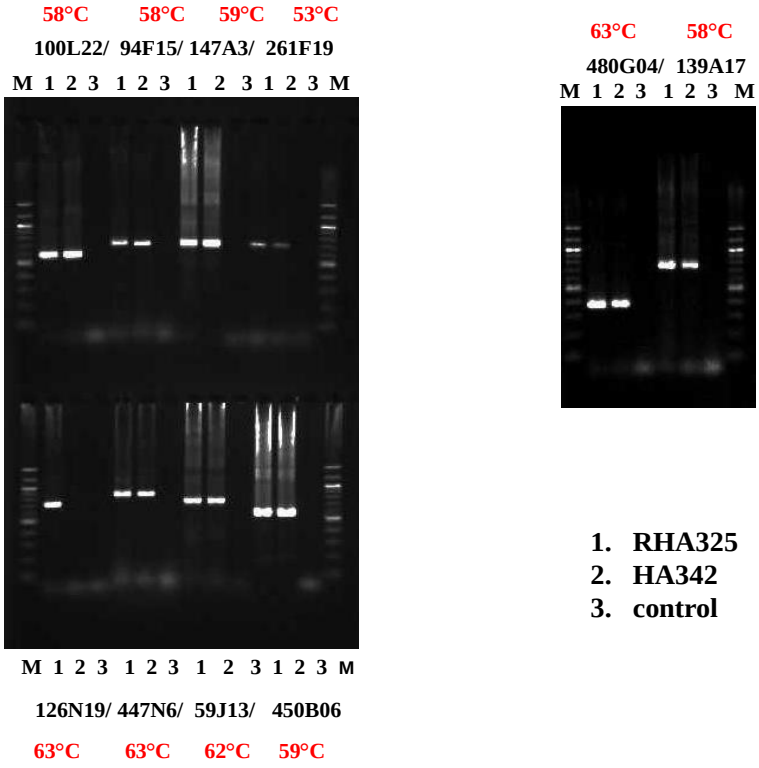
Based on the positive BAC-clones identified during the process of preliminary chromosome walking at the *Rf1* locus (Hamrit 2009), 11 STS-primer combinations were derived from the BAC-end sequences (Table 1). With the aim to improve the quality of the amplified products with the respective primers, a series of PCR-reactions with different annealing temperatures were tested. The optimal parameters for PCR-amplification for each of the investigated primers are given in Table 1.

Table 1: Design of STS-primers from the BAC-end sequences for back mapping the identified positive BACs

BAC-end	Primer name	Primer sequences 5' - 3'	T_A	Expected size of PCR product	Observed size of PCR product	Mono-/ Polymorph
100L22	100L22_for	GAACTTGCTAAATGTTAACGAG	58	573 bp	578 bp	monomorph
	100L22_rev	ATGCAAAAACCGCCTAAG				
94F15	94F15_for	TTAGTCGCCATGTGTACCGAT	58	712 bp	716 bp	monomorph
	94F15_rev	CCACTTTCGATGATGGAGTTG				
147A3	147A3_for	GTTATGCCCGATATCGTAAT	59	702 bp	709 bp	monomorph
	147A3_rev	ACCATTTTAAGTCCCGTAAG				
67N4	67N4_for	TTTCTTGTGTTTTACGATGCC	52-55	714 bp	-	-
	67N4_rev	TGTAACCGTCCGGAACAAAA				
261F19	261F19_for	ACCAAAAGGATCTAGAACTG	53	708 bp	717 bp	monomorph
	261F19_rev	CATTTTAAGGTCATATGGGC				
126N19	126N19_for	ACGCTGTGGCAATAAGACACA	63	701 bp	707 bp	monomorph
	126N19_rev	ACTTTGCAATTGTCACCAAAA				
447N6	447N6_for	TTCATGCTTTTAGCTGCCTGT	63	879 bp	884 bp	monomorph
	447N6_rev	TGCAGTTTAACTGCCCAAGA				
59J13	59J13_for	GCTTCTTGTGCTTCTTTTAAC	62	726 bp	726 bp	monomorph
	59J13_rev	TATCATGACGCTATCGGTTG				
450B06	450B06_for	AGCAGATTGTCAATCGGACAG	59	560 bp	564 bp	monomorph
	450B06_rev	GCTGAAAGATGAGCATCCAA				
480G04	480G04_for	GGTTCACATGGTGTGGATAA	63	361 bp	365 bp	monomorph
	480G04_rev	CTTCAATCAGACATCTATAGAGA				
139A17	139A17_for	GTAACGACTAGCAGGCAATAACA	58	717 bp	720 bp	monomorph
	139A17_rev	TGCGGACGTGAAATAGG				

The primers were tested with the parental lines RHA325 and HA342 to detect polymorphisms that would allow using them directly as markers. The PCR products showed the expected sizes (Table 1), but all showed monomorphic patterns between RHA325 and HA342 (Figure 2). The primers derived from 67N4 and 126N19 did not result in good PCR amplification products.

Figure 2: PCR products using the STS-primers derived from the BAC-end sequences for amplification from the lines RHA325 and HA342



Development of STS- and CAPS-markers for *Rf1* from cloned PCR-products

As the PCR amplification products with the derived STS-primers were monomorph for the parental lines (RHA325 and HA342) the PCR-products were cloned into the pGEM-T vector and sequenced to detect single nucleotide polymorphism (SNP) between the two lines that can be used to develop markers. Positive clones were obtained from the PCR products of the primers derived from the BAC-end sequences of 100L22, 147A3, 480G04, 450B06, 139A17, 59J13, 94F15. On the basis of the sequences analyses, variations in the nucleotide sequence of the investigated DNA fragments from RHA325 and HA342 were found (data not shown); these will be used to design specific polymorphic STS-markers and CAPS-markers. First results are shown for 480G04 (Table 2).

For the BAC clone 480G04, both types of markers could be designed. Based on variations in the 480G04 sequences of the two parental lines RHA325 and HA342, the specific STS-primers 480G04_RH325 and 480G04_HA342 were developed, which combined with the 480G04_rev primer specifically amplified a PCR product from RHA325 or HA342, respectively. Testing the primers in the bulks confirmed the association of the polymorphisms with fertility restoration (data not shown). However, these markers are again dominant.

More interesting was the development of a co-dominant marker based on a SNP in the restriction site of *BsrGI* between RHA325 and HA342. Whereas the PCR product of 361 bp of RHA325 (480G04_for/480G04_rev primer combination) is cut into a 138-bp-fragment and a 223-bp-fragment, the PCR product of HA342 remains uncut (Figure 3). The use of this CAPS-marker in the bulks showed its linkage to the fertility restorer gene. However, this marker still needs to be mapped to the restorer gene *Rf1* in the F2-population. This co-dominant marker will be very helpful in distinguishing plants, heterozygous or homozygous for the restorer gene *Rf1*.

Table 2: Development of STS- and CAPS-markers from cloned sequences

BAC-clone	STS-marker				
	Primer name	Forward primer sequences 5'-3' (reverse as before)	Expected size (Line)	Observed size (Line)	Monomorph/ Polymorph
480G04	480G04_ HA342	TTTGTGGGCTTCGTTTAATCGG	278 bp (HA342)	278 bp (HA342)	Polymorph (dominant)
480G04	480G04_ RHA325	TTTGTGGGCTTCGTTTAATAAC	277 bp (RHA325)	277 bp (RHA325)	Polymorph (dominant)
BAC-clone	CAPS-marker				
	Restriction enzyme	Cutting sequence	Expected size (Line)	Observed size (Line)	Monomorph/ Polymorph
480G04	BsrGI	5′…T˘GTACA…3′ 3′…ACATG˘T…5′	138 bp/223 bp (RHA 325) 361 bp (HA342)	138 bp/223 bp (RHA 325) 361 bp (HA342)	Polymorph (co-dominant)

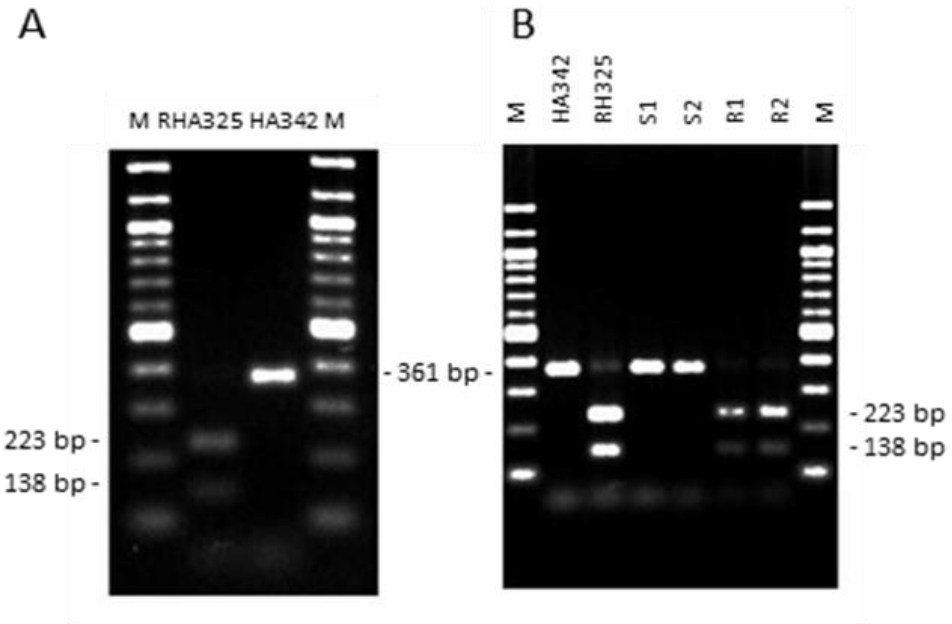


Figure 3: CAPS-marker 480G04_BsrGI. DNA was amplified using the primer combination 480G04_for/480G04_rev and digested with the restriction endonuclease *BsrGI*. A. parental lines (RHA325 and HA342) and B. RHA352 and HA342 as well as bulks (S1, S2, R1, R2). M: 100 bp marker

Comparative mapping of the *Rf1* and *Rf_PET2* gene

New AFLP-markers linked to the restorer gene *Rf1* have been identified as well as first AFLP-markers closely mapping to the *Rf_PET2* gene. Some of these have been cloned and sequenced.

The restorer gene *Rf1* had been mapped to the linkage group 13 (Kusterer et al. 2005) using SSR-markers of the general genetic map of sunflower (Tang et al. 2003). In order to see if the *Rf_PET2* restorer gene might be also located on the same linkage group, three SSR markers (ORS317, ORS630 and ORS1030) bordering the *Rf1* gene were mapped in the F2 population RHA265(PET2) x IH-51 segregating for the *Rf_PET2* gene. Comparative mapping showed that *Rf_PET2* maps less than 10 cM from the *Rf1* gene. This will facilitate cloning of the two restorer genes. Comparing the mechanism of fertility restoration from *Rf1* and *Rf_PET2* will give a better understanding about the processes behind fertility restoration in sunflower.

Conclusions

The molecular basis of the new CMS source PET2 was revealed, which now allows the development of markers differentiating between CMS PET1, CMS PET2 and the fertile cytoplasm. In combination with the identification of AFLP-markers linked to the restorer gene *Rf_PET2* this new CMS source would be now ready to be used for the development of commercial sunflower hybrids.

In addition, a step forward has been made in chromosome walking at the fertility restorer locus *Rf1*. The BAC-end sequences can be used to obtain new overgo probes for subsequent hybridizations against BAC-filters for identification of new positive BAC-clones. However, BAC clones have still to be back-mapped by markers. Their arrangement in contigs will ultimately result in a complete contig around the *Rf1* gene. The newly identified AFLP-markers for the *Rf1* gene will be helpful for this work as well. All markers described up to now have been dominant markers, which do not allow differentiation between homozygous and heterozygous fertile plants. Therefore, the development of the co-dominant CAPS-marker 480G04_BsrG I for the fertility restorer gene *Rf1* is especially interesting. Both, the two new STS-markers as well as the CAPS-marker will be used to back-map the BAC-clone 480G04 in the F2-population and to confirm its position in the region of gene *Rf1*. Interestingly, the restorer gene *Rf_PET2* seems to be located less than 10 cM from the *Rf1* gene.

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IDENTIFICATION OF *HADELLA, HAGID1* AS WELL AS *HASLEEPY* AND *HASNEEZY* GENES INVOLVED IN GIBBERELLIN SIGNALING IN SUNFLOWER

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ABSTRACT

Mutations in the gibberellin (GA) biosynthesis or signaling pathway lead to significant changes in shoot growth (dwarfism/gigantism) and were responsible for the so-called 'green revolution' in cereals. Knowledge about the GA metabolism offers starting-points for targeted breeding in sunflower. However, so far we know little about the components of the GA signaling pathway in sunflower. Using the sunflower genome sequence database, kindly provided by the Loren Rieseberg group (www.heliagene.org), we screened for similar sequences to known DELLA, GID1 and SLEEPY proteins from other plant species. Next, identified sequences of the HA-412-HO scaffold database were used for primer design and the existence of the genes was verified in the line HA383 using DNA as well as cDNA. Here we describe the identification and characterization of four DELLA (HaDELLA1-2 and HaDELLA-like1-2), five HaGID1A-E and nine F-box genes (HaSLEEPY1A-F and HaSNEEZY1-3). For functional analysis, we studied sunflower SLEEPY and DELLA homologs in Arabidopsis thaliana. Our complementation studies of the Arabidopsis SLEEPY mutant sly1-10 demonstrated that HaSLY1A encodes a functional F-box protein, which is fully able to compensate the sly1-10 mutant phenotype. Overexpression of HaDELLA1 in wild-type Arabidopsis induced dwarfism and late-flowering.

Keywords: Gibberellins, *Helianthus annuus*, plant hormones, signal transduction

INTRODUCTION

Three major regulators are responsible for perception of GAs by the GA signaling pathway: the GA receptor GID1 (GA-insensitive dwarf1), the DELLA protein and SLY1/GID2 (SLEPPY1/GA-insensitive dwarf2) as part of the SCF E3 ubiquitin ligase. GID1 was characterized as soluble receptor for GA by studies of GA-insensitive dwarf rice mutants (Ueguchi-Tanaka et al., 2005). In Arabidopsis, three GID1 encoding genes (AtGID1A, AtGID1B and AtGID1C) were identified (Nakajima et al., 2006). Analyses of the gid1 double and triple mutants as well as interacting studies with DELLA proteins revealed that GID1 orthologs have redundant but also distinct roles in plant development (Iuchi et al., 2007). In contrast, there is very little knowledge available about GID1 encoding genes in sunflower. Recently, Blackman et al. (2011) has published one partial GID1-like sequence (about 37%, HaGID1B), which shows high similarities to GID1B (At3g63010) in Arabidopsis. However, transcriptome analysis with the focus on GA synthesis and signaling genes of Gerbera hybrida indicate that species of the compositae family contain various homologous GID1 genes (Kuang et al., 2013). In Gerbera, nine different GID1 transcripts were detected and in H. annuus three corresponding hits were found by local BLASTN indicating the existence of more than one GID1 gene in sunflower.

DELLA proteins are nuclear transcriptional regulators that repress the GA signaling pathway and belong to the GRAS gene family (Tian et al., 2004). Several plant species like rice (SLR1) and tomato (Pro) contain only a single gene encoding for DELLA (Ikeda et al., 2001; Jasinski et al., 2008). In contrast, other species like A. thaliana have multiple DELLA genes (Tyler et al., 2004). Ramos et al. (2013) have recently reported that the semidominant mutant sunflower allele, Rht1, which maps to linkage group 12 of the sunflower public consensus map, completely cosegregated with a haplotype of HaDELLA1. Phenotypic effects of this allele include shorter height and internode length, insensibility to exogenous gibberellin application, normal skotomorphogenetic response, and reduced seed set under self-pollinating conditions (Ramos et al., 2013). In addition to the known HaDELLA1 sequence similar EST sequences provide initial indications of a second HaDELLA gene (Blackman et al., 2011), but it remains to be determined whether that C-terminal sequence is part of a true DELLA protein or belongs to another member of the large GRAS gene family.

The F-box protein GID2/SLY1 interacts with DELLA proteins, especially in the presence of GA activated GID1, via the C-terminal GGF and LSL domains (Sun, 2008). The N-terminal F-box domain is necessary for the interaction with the SCF complex (Smalle & Vierstra, 2004). However, the molecular mechanisms behind GA signaling have been extensively studied in plants such as Arabidopsis and rice, which have only a single gene encoding SLY1/GID2. In sunflower, just one putative SLY1 sequence (HaSLY1) has been described so far (Blackman et al., 2011), but it seems to be very likely that sunflower, like Gerbera hybrida with six putative GID2/SLY1 transcripts (Kuang et al., 2013), will have more than one SLY1 homolog.

MATERIAL AND METHODS

Screening of the sunflower genome for GID1, DELLA and SLEEPY homologs

The genome database of the sunflower line HA-412-HO provided by Loren Rieseberg (www.heliagene.org) was screened for similar sequences to known GID1, DELLA1 and SLY1 sequences of Arabidopsis thaliana. Therefore the 3.1 GB scaffold fasta file (Celera_14libs_sspace2_ext.final.scaffolds) was converted into a local nucleotide database by using the stand alone blast program Blaststation-Local (www.blaststation.com). We also used published sequences of GA signaling genes of Lactuca sativa (Sawada et al., 2008) due to its close relationship to sunflower as well as previously published EST sequences of putative sunflower genes involved in GA signaling (Blackman et al., 2011).

In silico analyses of sunflower GA signaling homologs

To compare the hits of the scaffold database showing highest E-values with GID1, DELLA or SLY1 proteins we performed phylogenetic analyses. Therefore, we choose GID1, DELLA and SLY1 homologs of sequenced higher land plant genomes, which were described in the literature or detected by BLASTP and TBLASTN tools of the databases NCBI (National Center for Biotechnology Information) and Phytozome (http://www.phytozome.org). We also included amino acid sequences of Selaginella moellendorffii (lycophyte) as well as homologous protein sequences of Physcomitrella patens (moss). Several studies dealt with the subject of evolutionary origin of GA signaling. These studies indicate that the GA signaling evolved after the divergence of bryophytes from land plants (Wang et al., 2015). Furthermore, it was demonstrated that homologs of GID1, DELLA, and SLY1 work similar in S. moellendorffii and in flowering plants, whereas no evidences were found for functional conservation of genes in P. patens (Hirano et al., 2007). For this reason, sequences of P. patens were used as outgroup, whereas S.

moellendorffii sequences served as exclusion limits to eliminate similar but unrelated sequence, like other GRAS or F-box proteins. The "one click" mode of Phylogeny.fr (Dereeper et al., 2008) offered a quick and easy method for handling large volumes of sequences and was used to get a first impression how much of the hits really belonged to the GA signaling members. Next, Neighbor-joining trees were computed using the JTT matrix-based method and bootstrap test of 1000 replicates to confirm the evolutionary distance.

Verification of GA signaling scaffold sequences in HA383

Based on scaffold sequences forward and reverse primers were designed encompassing the respective coding sequence of GID1, DELLA and SLY1 homologs. Genomic DNA from leaves of HA383 was subjected to standard PCR (Taq DNA polymerase NEB). The obtained fragments were cloned in pGEM®-T Easy vector (Promega) and sequenced using SP6 and T7 primers designed for sequencing inserts cloned into this vector. Nucleotide polymorphisms were verified by using a proof reading Phusion DNA polymerase (Thermo Scientific). To get accurate information on the exon-intron structure of the genes, RNA from HA383 was isolated according to the protocol for RNA extraction from different tissues of grapevine and other woody plants (Gambino et al., 2008). All RNA samples have undergone a DNAse treatment (DNAse I; Fermentas) to remove DNA contaminations. Complementary DNA (cDNA) was obtained from total RNA by using RevertAid H minus cDNA synthesis kit (MBI Fermentas) and used for amplification of coding sequences.

Functional analyses of the sunflower SLEEPY and DELLA homologs HaSLY1A and HaDELLA1

PCR amplicons of HaSLY1A and HaDELLA1 coding sequences flanked by restriction sites were ligated into corresponding cloning sites of the 35S cassette. The entire 35S cassettes, now including cds of HaSLY1A or HaDELLA1, were excised and inserted into the vector pGREEN0029 via the EcoRV restriction site (http://www.pgreen.ac.uk). These vectors were used to transform Arabidopsis mutant sly1-10 or Landsberg erecta wild-type plants by using Agrobacterium tumefaciens strain GV3101psoup. After selection with BASTA (phosphinotricine, 0.1%), integration of the 35S:HaSLY1A or 35S:HaDELLA1 transgene was confirmed by PCR using a primer specific for the 35S promoter and reverse primers for the respective coding sequences (cds). In the screen for HaSLY1A complemented plants that were homozygous for sly1-10, we used the specific primer pairs sly1-10f/2-63r and sly1-10f/sly1-10r2 for PCR with genomic DNA to verify the absence of the wild-type Arabidopsis AtSLY1 gene and the presence of the sly1-10 allele, respectively (McGinnis et al., 2003). Primer for the gene At2g09990 encoding the 40S ribosomal protein S16 were used as positive control for DNA content. Arabidopsis plants were grown under a 10-h-light/14-h-dark (22°C/18°C) cycle at 100 to 150 µmol photons m^{-2} s^{-1} in controlled environment chambers, or a 12-h-light/12-h-dark cycle in the case of HaDELLA1-overexpression (OE) lines and the corresponding Arabidopsis wild-type.

RESULTS AND DISCUSSION

Identification of GID1 homologs in the sunflower genome

GID1, the soluble receptor for GA, was first characterized in rice (Ueguchi-Tanaka et al., 2005). A reason for this surely was that no other GID1 isoform was able to compensate phenotypic alterations based on mutations in this single gene. In contrast, in Arabidopsis three GID1 proteins

(AtGID1a, AtGID1b and AtGID1c) with redundant but also distinct roles in plant development were identified (Nakajima et al., 2006). In 2011, one sunflower EST sequence (BU028290) was published, which encodes the C-terminal part of an Arabidopsis AtGID1b similar protein (Blackman et al., 2011). However, our genome databases screenings revealed that the genomes of most eudicots contain at least two different genes encoding GID1 homologous proteins. In addition, Kuang et al. (2013) found three corresponding hits to GID1 homologs in Gerbera hybrida. In H. annuus, local BLASTN indicated several HaGID1 isoforms. By using the sunflower EST BU028290, which is similar to AtGID1b, for BLAST searches in the HA-412-HO scaffold database we detected an ORF of 1005 bp, which was identical to BU028290 in the 3'-end. The resulting amino acid sequence showed more than 90% identities when compared to Lactuca sativa LsGID1A and LsGID1B sequences (Sawada et al., 2012). Nevertheless the start ATG, as well as the next 13 amino acids could not be detected in that scaffold. By using the Lactuca GID1 sequences (LsGID1A and LsGID1B) we found a second scaffold, which was identical to the first scaffold in the 3'-end. In addition, this scaffold contained a start ATG followed by a 39-bp-sequence, which showed high similarities in the amino acid sequence to LsGID1A and LsGID1B, 890 bp upstream from the 1005-bp-ORF. To confirm the exon-inron structure we used RT-PCR with primers encompassing the full-length cds (binding in the 5'- and 3'-UTR region) and amplified the mature HaGID1B cds. We were thus able to guarantee that both scaffolds contained parts of HaGID1B, which consists of two exons and one intron of 890 bp. We utilized the HaGID1B sequence and found four other highly similar sequences (HaGID1A, C, D and E) within the HA-412-HO genome (Figure 1). Like HaGID1B, HaGID1A and HaGID1E consist of two exons separated by an intron of 577 bp and 885 bp, respectively. We anticipate that HaGID1C and HaGID1D have similar structures but the first exon and the following intron are missing due to truncated scaffold sequences. Phylogenetic analyses using the second exons, the main parts of HaGID1C and HaGID1D cds, have clearly shown that the resulting protein sequences belong to GID1 receptors (tree not shown). Consequently, our findings reveal that sunflower contains at least five HaGID1 isoforms and it seems to be very likely that HaGID1s, like Arabidopsis GID1 proteins, mediate a complex network with overlapping but also distinct functions.

Identification of DELLA homologs in the sunflower genome

DELLA proteins belong to a subfamily of the plant specific GRAS gene family. DELLAs contain a conserved C-terminal GRAS domain and a unique N-terminal DELLA domain, which is essential for GA-induced degradation (Sun, 2011). In rice and barley, one single gene codes for the only DELLA protein, called SLENDER1 due to the elongated stem phenotype (Ikeda et al., 2001; Chandler et al., 2002). In contrast, Arabidopsis contains five DELLA proteins with partly overlapping but also distinct functions (Daviere & Achard, 2013). There is some evidence that the genome of sunflower contains at least two different genes (HaDELLA1 and HaDELLA2) coding for DELLA proteins (Blackman et al., 2011). However, functional evidence for the HaDELLA proteins is missing, although Ramos et al. (2013) could show that the reduced height in some dwarf sunflower lines is based on a SNP in the DELLA motif of HaDELLA1, which leads to a single amino acid change from DELLA to DELPA (Ramos et al., 2013). At the beginning of our search for DELLA homologs in sunflower, we first aligned the EST sequences of HaDELLA1 published by Blackman et al. (2011). The ESTs coded for an N-terminal HaDELLA part as well as for a C-terminal GRAS domain, but did not overlap in the middle. For that reason, we tried to amplify the whole cds by using primers binding in the 5'- and 3'-UTRs and were able to fill the observed gap of 41 amino acids between the N- and C-terminal part. The HaDELLA1 amplicons differed in size by about 300 bp, when we used DNA or cDNA of the

line HA383. This is due to the fact that the HaDELLA1 cds is divided into two parts by a 325-bp-intron. In the next step, we wanted to find out whether the putative HaDELLA2 EST (CD850340), lacking the DELLA domain, is part of a true DELLA protein and searched for identical scaffold parts in the HA-412-HO genome. Indeed, CD850340 is part of an 1701-bp-ORF, not interrupted by introns, with a typical DELLA domain. In addition to HaDELLA1 and HaDELLA2, we detected two other coding sequences having putative DELLA domains. These sequences were named HaDELLA-Like1 and HaDELLA-Like2 due to the modified DELLE and DELLF motifs, respectively. Besides these DELLA sequences, we found many other hits of unrelated putative GRAS proteins, which were excluded by phylogenetic analyses. The HaDELLA-Like1 coding sequence consists of 1710 nucleotides. The HaDELLA-Like2 cds still remains fragmentary (1521 bp), lacking about 50-60 amino acid at the end. Despite many attempts to amplify transcripts, we could not detect expression of HaDELLA-like2 in different organs or developing stages of the line HA383, a prerequisite for RACE analyses. Like for HaDELLA-Like2, HaDELLA-Like1 transcripts could not be detected. However, it seems rather unlikely that HaDELLA-Like1 is just a pseudogene because HaDELLA-Like1 (also referred to as RGL2 by Mandel) was identified as a gene of likely agronomic importance in evolutionary analyses of crop-related traits in sunflower (Mandel et al., 2014). The authors speculated that HaDELLA-Like1, which co-localized with a QTL for seed dormancy, might perhaps have something to do with lesser or no dormancy of primitive and improved varieties compared to the strong seed dormancy observed in wild sunflowers. Investigations of Arabidopsis RGL2 revealed that RGL2 transcript levels rise rapidly following seed imbibition and then decline rapidly as germination proceeds (Lee et al., 2002). Further studies are needed to find out whether HaDELLA-Like1 is a functional equivalent of AtRGL2. Hower, it is conceivable that the expression level of HaDELLA-Like1 may be lower in improved varieties without dormancy.

Identification of SLEEPY and SNEEZY homologs in the sunflower genome

The two orthologous F-box genes GID2 and SLY1 of rice and Arabidopsis are needed for GA-stimulated DELLA degradation by the 26S proteasome (Wang & Deng, 2011). A second F-box protein SNE (SNEEZY) in Arabidopsis is only partially able to compensate the sly1 phenotype by overexpression. Extensive studies of AtSLY1 and AtSNE overexpression in the Arabidopsis sly1-10 mutant suggest that one reason, why SNE is unable to fully compensate the mutant phenotype, is that SLY1 regulates a broader spectrum of DELLA proteins than SNE (Ariizumi et al., 2011). However, rice and Arabidopsis have only a single gene for GID2/SLY1 and SNE, respectively, whereas other plant species like Gerbera hybrida have more SLY1 homologs (Kuang et al., 2013). In sunflower, one full-length cds of a putative SLEEPY protein (HaSLY1, now named HaSLY1A) was described so far (Blackman et al., 2011) but initial work of our group revealed the existence of a second HaSLY1 isoform (HaSLY1B).

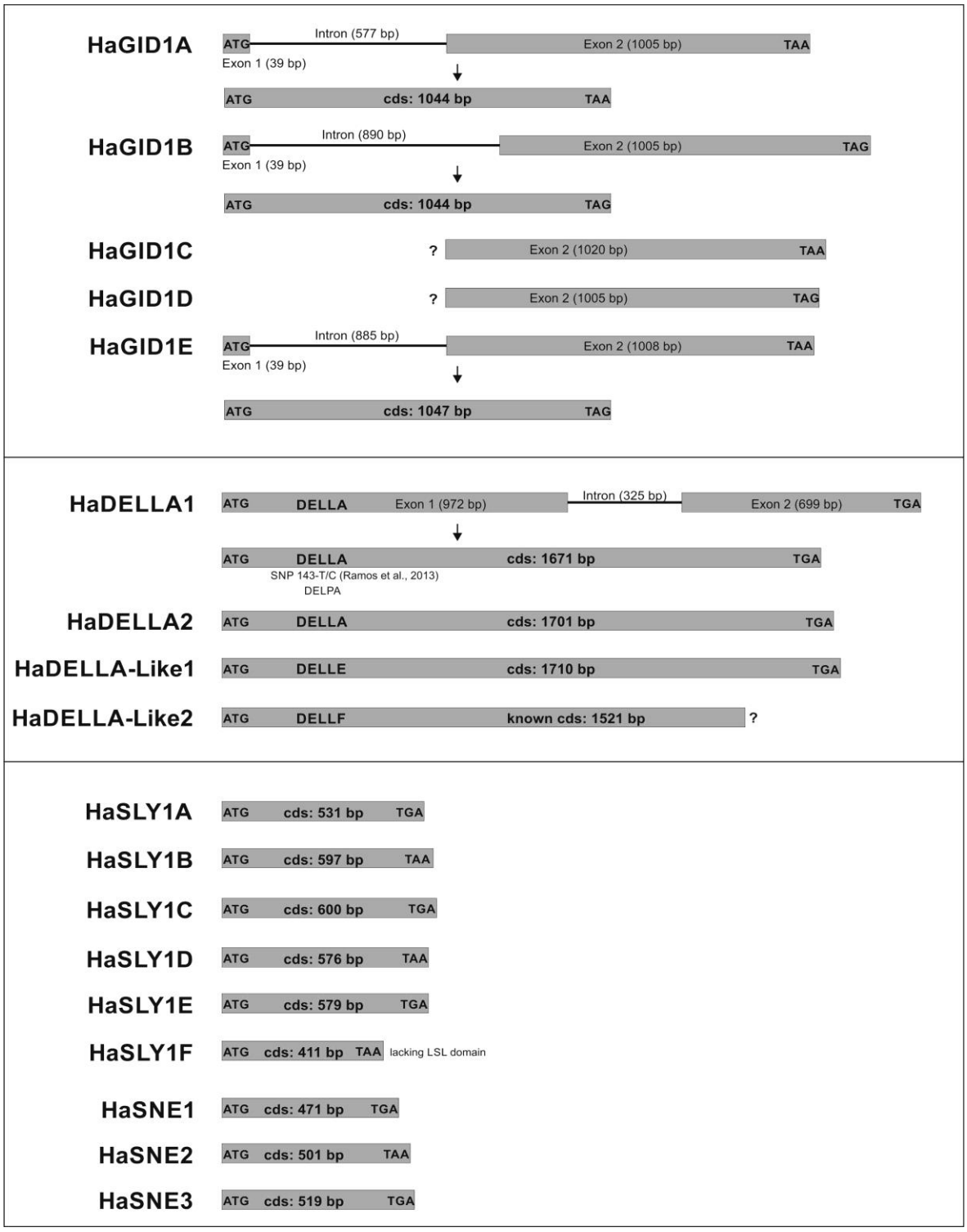

Fig. 16: Schematic overview of genes involved in GA signaling in the sunflower line HA383

Through the access to the genome database we were able to identify all in all nine coding sequences showing similarities to Arabidopsis SLY1, some of them being incomplete. Like HaSLY1A and HaSLY1B, four other coding sequences also showed high identity values to AtSLY1 and were named HaSLY1C, D, E and F. The last three related sequences, on the other hand, had a significantly lower correlation to AtSLY1 and HaSLY1s. We assumed that these

three proteins (now HaSNE1, 2 and 3) could be hitherto undetected sunflower SNEEZY homologs due to amino acid identities of about 50% to AtSNE. The coding sequence of HaSNE3 was incomplete in the scaffold database assembled by the program Celera but we detected the full-length cds in the Ha412Newbler20120907_gene database (www.heliagene.org). The complete cds of HaSLY1E, lacking the 5'-end in the scaffold databases, was obtained by RACE-PCR. Assuming that the HaSLY1F sequence was unusually short due to sequencing or assembly errors, we were surprised to find the same TAA stop codon in the sunflower line HA383 at position 409, which leads to a shortened F-box protein. Attempts to obtain RACE-PCR products as well as RT-PCR amplicons have failed. In contrast to all other HaSLY1s, HaSLY1F seemed to be not expressed. Complementation studies of truncated AtSLY1 forms provided evidence for malfunction of SLEEPY proteins without the LSL domain (Ariizumi et al., 2011). Together with the fact that no HaSLY1F expression was detectable the unusually short form suggests that HaSLY1F may represent a pseudogene.

In summary, our findings of nine F-box proteins in sunflower, as well as the detection of six putative SLY1 transcripts in the transcriptome of Gerbera hybrida ray florets (Kuang et al., 2013) suggest that in the Asteraceae family genomes may contain a large number of duplicated F-box proteins.

Functional analyses of sunflower SLEEPY and DELLA homologs

The very low regeneration rates in sunflower make it almost impossible to generate stable knockout or knockdown mutant lines. Therefore, we chose the model plant Arabidopsis for functional analyses of the sunflower GA signaling homologs. In this work, we describe our general approach for functional analyses using HaSLY1A and HaDELLA1 as examples. The sly1-10 mutant provides a good basis for complementation studies with SLY1 homologs. In Arabidopsis the overexpression of AtSLY1 under the control of the 35S-promoter fully complemented the dwarfism of the loss-of-function mutant sly1-10 (McGinnis et al., 2003). The full-length ORF of HASLY1A gene was cloned into plant transformation vector pGREEN0229 including the 35S-promoter and terminator (Fig. 17 A). The sly1-10 mutant was transformed with these constructs.

The genotyping analyses clearly showed that all lines contained the respective T-DNA and were homozygous for the sly1-10 mutation (Fig. 17 C). The growth of overexpression lines was compared with untransformed sly1-10 plants as well as wild-type (Ler) plants. HaSLY1A-overexpression lines grew significant faster than untransformed sly1-10 plants and resulted in a similar growth type as the wild-type plants (Fig. 17 E). These results show that the HaSLY1A protein was able to rescue the genetic defects of sly1-10.

Accumulation of DELLA proteins results in dwarfism and often in delayed flowering onset. Typical examples of this are GID1 and SLY1/GID2 knockout lines (Sasaki et al., 2003; Strader et al., 2004), as well as mutations in DELLA genes responsible for the so called 'green evolution' in cereals (Boss & Thomas, 2002). Therefore we tested the phenotypic changes of Arabidopsis wild-type plants (ecotype Ler) by overexpressing HaDELLA1 under the control of the 35S-promoter. The HaDELLA1 overexpression lines showed a delayed flowering onset, slightly decreased rosette diameter and shorter stems (Fig. 17 D). We have not yet made statistical evaluations (waiting for the T3-generation), but the phenotypic changes of HaDELLA1-OE line are in line with observation of plants showing DELLA accumulation.

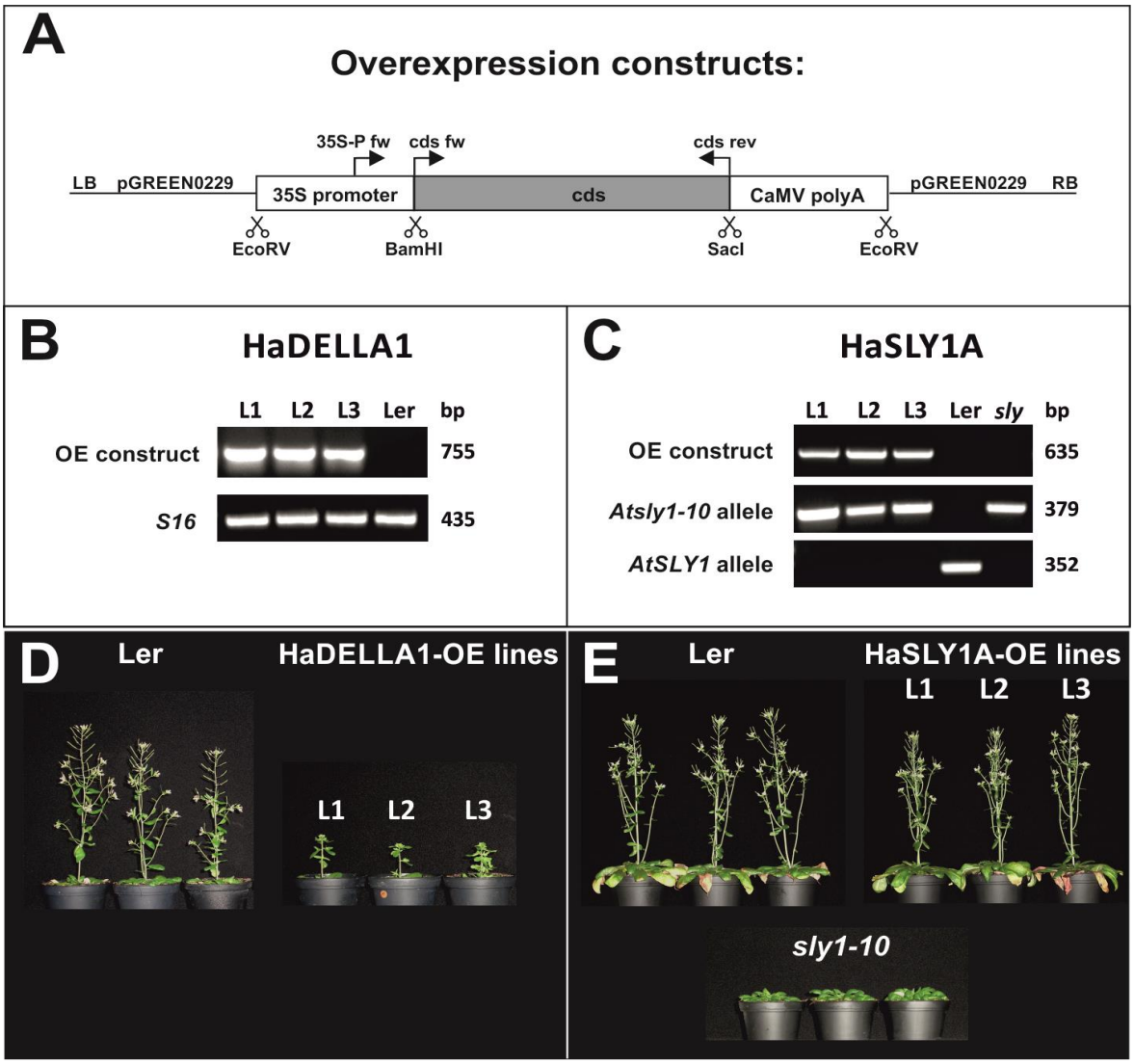

Fig. 17: Overexpression of HaDELLA1 in wild-type Arabidopsis induced dwarfism and late-flowering, whereas overexpression of HaSLY1A rescued sly1-10 dwarf phenotype. A: Schematic overview about cloning strategy of overexpression constructs (see methods for detailed information). B, C: Genotyping of HaDELLA1 (Ler background) and HaSLY1A (sly1-10 background) overexpression lines (L1-L3). D, E: Phenotyping of overexpression lines compared to corresponding Arabidopsis wild-type and sly1-10 mutant plants.

Currently, we analyze these overexpression lines, as well as overexpression lines of the other GA signaling genes detected in sunflower to get more detailed knowledge about their functions.

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QUANTITATIVE DETERMINATION OF SUNFLOWER IN MIXED CONCENTRATE FEEDS BY REAL TIME PCR

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ABSTRACT

Feeding farm animals such as, high-yielding dairy, poultry, swine and beef cattle at certain periods with a balanced and adequate mixed concentrate feed is a must. Concentrate feed is produced from grain feed materials that goes through various processes and mixed with oilseed meals, food industry by-products and feed additives. The aim of this study was to determine proportional amounts of sunflower in mixed concentrate feeds by DNA-based Real Time Polymerase Chain Reaction (RT-PCR) in order to monitor compliances of mixed feed labels, investigate the appropriateness of the required quality criteria, detect and avoid unintended feed materials that are mixed in imported plant raw materials. For this purpose, concentrate feed samples that were prepared at the laboratory by weight to weight and also the samples with known ingredients from a local feed plant were used to test quantity of sunflower. Genomic DNA (gDNA) extracted using commercial kits and evaluated for quality and quantity prior to use in a PCR assay. For the specific detection of sunflower and taxon specific (for the relative quantification) a fragment of the *helianthinin* and *actin* genes were selected respectively using gene specific primers and probes. The calibration curve was established on the basis of five samples. The average value of the slope of the standard curve was within the range of -3.1 to -3.6, and the R2 coefficient was ≥ 0.98. This study, first time showed that sunflower in a mixed concentrate feed can be quantified by DNA-based RT-PCR with a high precision.

Key Words : Mixed Concentrate Feeds,Sunflower, RT-PCR, Quantitative Determination

EVALUATION OF WRKY AND MYB TRANSCRIPTION FACTORS IN SOME DOWNY MILDEW INFECTED SUNFLOWER LINES; MICROARRAY DATA ANALYSIS

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ABSTRACT

Downy mildew, which is caused by *Plasmopara halstedii*, is a major plant disease significantly affecting the sunflower (*Helianthus annuus*) production. Yield losses can reach up to 100% in heavily contaminated fields, even could lead the abandoning of cultivation. When considering the worldwide cultivation of sunflower as oil crop, plant losses have serious impact on the country economy as well as on the global food security. So far, thirty-six*P. halstedii* pathotypes/races were identified all over the world. In this study, expression profiles of *WRKY* and *MYB* transcription factor (TF) genes were analyzed in four downy mildew (race 334 and 710) infected sunflower genotypes such as PSC8 (susceptible), XRQ (resistant), and RIL43 and 88 (cross between XRQ and PSC8). Microarray data, including 60 normal vs disease samples were retrieved from NCBI GEO Data Sets (access. GSE25717). For array analyses, sunflower lines were grown in compost under 70% humidity, 20°C temperature and 16h day/8h night light periods. Germinated seeds (2-3 days) were inoculated with downy mildew or water for control for 3hrs, and then following 6 or 10 days of inoculation, plants were harvested for RNA extraction and array analyses. Data were normalized with GCRMA algorithm in Bioconductor package. Analyses demonstrated the 18 putative *WRKY* and 49 putative *MYB* TFs in downy mildew infected sunflower genotypes. In *WRKY* TFs, *WRKY-b* (Heli023654), *WRKY4* (Heli013712 and Heli000574), *WRKY1* (Heli029273) and *WRKY30*(Heli009222) TFs demonstrated significant downregulation in most downy mildew-sunflower interactions while the rest mainly upregulated in infected lines. Hierarchical clustering with Euclidean distance showed that*WRKY-b* (Heli023654) and *WRKY4* (Heli013712 and Heli000574) TFs in genes, and 10 days PSC8, RIL43 and RIL88 lines infected with race 710 in conditions have similar expression profiles. In addition, without line consideration, 6 or 10 days plants infected with race 334 had similar expression pattern and clustered closely, however, plants infected with 710 race demonstrated divergence in clustering based on plant age (6 or 10 days). In*MYB*, hierarchical clustering of TFs demonstrated three main clusters, including considerably up- and downregulated genes, and the genes with mosaic pattern. In addition, plant age seemed to have determinative effect in gene expression patterns. All these implicate that infection period (plant age) and pathotype (race 334 or 710) play an important role in modulation of expression profiles of *WRKY* and *MYB* TFs. However, further molecular and physiological studies are required to elucidate the relationship between these two TFs as well as with their interactors. Comparative analyses of *WRKY* and *MYB* TFs in susceptible, resistant and cross sunflower lines could significantly provide valuable insights to understand the gene regulatory elements in downy mildew-sunflower interactions as well as could pave the way for their biotechnological manipulation to improve the commercially important sunflower lines.

Key Words : Pathotype, race, array, mildew, inoculation, TF

DE NOVO SEQUENCING OF THE HELIANTHUS ANNUUS AND OROBANCHE CUMANA GENOMES

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ABSTRACT

The sunflower (*Helianthus annuus*) genome is diploid, large (N=17 chromosomes spanning 3.6Gb) and complex. It has been shown to be composed of over 81% of transposable elements (Staton et al., 2012). The strategy using short Roche and Illumina reads from a range of library sizes coud not completely solve the great complexity of this genome. In this context, within the SUNRISE project, we started an ambitious approach to generate a high quality genome sequence of the INRA line XRQ, by exploiting the PacBio RSII sequencing progresses. In the frame of the HeliOr project, PacBio sequencing was also used to sequence the genome of another sunflower INRA line (PSC8) and the genome of the parasitic plant *Orobanche cumana* (the broomrape of sunflower).*Orobanche cumana* is a non-chlorophyll plant that specifically infests sunflower by fixing to its roots. It then uses a part of the mineral nutrients absorbed by sunflower and a part of the assimilates produced by the sunflower. Broomrape can cause yield loses up to 90%. Very few genomic resources are available for this species but its genome is diploid and around 40% of the sunflower genome size (N=19 chromosomes, 1.5Gb). In this paper, we will present our approach, the overall assembly process and a first analysis of the genomes annotation.

Key Words : genome sequencing, PacBio, sunflower, PacBio

IN VITRO POLLEN VIABILITY IN SOME WILD TYPE SUNFLOWER GENOTYPES (HELIANTHUS SPP)

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ABSTRACT

The evaluation of pollen viability is one of the essential criteria for pollinator's characterization. This study was carried out to understand the relationship between *in vitro* pollen viability and pollination and/or seed set in eight genotypes of wild type sunflower [(*Helianthus petiolaris* subsp. *petiolaris,* (1), (*H. petiolaris,* (1), *H. annuus* subsp. *lenticularis*, (1), *H. petiolaris* subsp. *petiolaris*, (1), *H. argophyllus*, (3), *H. maximiliani*, (1)] Two pollen viability tests, [TTC (2,3,5-triphenyl tetrazolium chloride), Asetocarmine] were used to estimate pollen viability. The data taken from three pollen characters (viable, semi viable, death) were analysed statistically by Jump statistical programme. Significant differences among genotypes, dies and interactions between genotypes and dies at 1% probability levels were found at all examined characters. The percentage of pollen viability varied from 48.6 to 99.1% by acetocarmine test and from 25.3 to 68.5% by TTC test. The highest pollen viability was obtained from diffent origin of *H. argophyllus* number 34 (% 79.53*)* and 35 genotypes (% 75.33) while *H. petiolaris* subsp. *petiolaris* had the lowest (%42.2). The acetocarmine had the highest pollen viability (83.3%) followed by TTC (%37.7). When the interactions examined acetocarmine died the wild sunflower pollens at 99.1%, TTC died the lowest rate of *H. petiolaris* pollens (%25.3). Among the wild type sunflowers studied *H. argophyllus* appeared to be suitable pollinators with respect to the criteria investigated.

Key Words : Wild type sunflower genotypes, pollen viability

CHARACTERIZATION OF SUNFLOWER INBRED LINES WITH HIGH OLEIC ACID CONTENT BY DNA MARKERS

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ABSTRACT

Sunflower is one of the most important oilseed crops due to high oxidative stability of its oil with high oleic acid content. Screening high oleic sunflower genotypes by standard methods such as Gas Chromatography (GC) is time consuming and expensive. Using molecular markers associated with high oleic acid trait is a useful tool in order to facilitate sunflower breeding program. For the purpose of genotyping the sunflower lines for high oleic content, two markers were chosen; SSR marker and HO PCR specific fragment. The results showed that high oleic containing hybrids expressed a specific SSR band at 246 bp length, and also HO PCR specific fragment at 870 bp length. The results were confirmed by determining the fatty acid composition. The results of this work allowed to validation of two DNA markers in sunflower inbred lines for high oleic acid traits.

Key words: *Helianthus annuus* L., Marker-assisted selection, SSR, Oleic acid composition

INTRODUCTION

Sunflower is one of the most important oil crops in the world. It is produced in the world generally for human and non-food purposes (cosmetics, paints, etc.) due to the oil and fatty acid composition of the seed being adapted to these uses. Sunflower oil contains high level of unsaturated fatty acids (88%); linoleic acid (48-74%), oleic acid (14-40%) and also saturated fatty acids; palmitic acid (4-9%) and stearic acid (1-7%) (Singchai et al., 2013; Nagarathna et al., 2011). It is desirable for human consumption because of its favorable fatty acid composition (Baydar and Erbas 2005). Oleic sunflower production and consumption started rapidly both for healthy frying oil, and also non-food purposes in recent years. Non-food applications in particular require oleic acid content that is stable and higher than 90% (Vannozzi 2006). Diets containing vegetable oil with high oleic acid content have been reported to be most effective for preventing cardiovascular diseases (Delplanque et al., 1997; Broun et al., 1999). Increase of oleic acid content has become one of the major goals to improve vegetable oil quality (Lacombe et al., 2004). In order to reach this aim, Sunflower lines and hybrids which have high oleic acid content in their seeds have been obtained by selection programs from HO (High oleic) Pervenet mutant by chemical mutagenesis (Soldatov 1976). The mean content of oleic acid of the seeds from Pervenet population is higher than 65% whereas this content in normal LO varieties is about 20% (Berville et al., 2009). Because of the interest in oleic acid and also the agronomic performance of HO varieties carrying the Pervenets mutation compared with the LO varieties, these varieties are widely used in the world (about 1.2 million ha, CETIOM 2002). The phenotypic determination (fatty acid analysis) does not allow rapid and early determination of HO genotypes and also cannot provide differentiation of homozygotes from heterozygotes for

the mutation. The use of molecular markers has become popular tool for the genetic and breeding studies and it is rapid, cheaper and simple when suitable markers were developed (Varshney et al., 2005). Therefore, marker assisted selection (MAS) analysis is necessary at genomic level allowing rapid and earlier determination of homozygous HO genotypes for sunflower breeding studies.

The aims of this study are characterization of sunflower inbred lines with high oleic acid content by DNA markers and evaluate the effectivity of two marker types developed by Berville et al. (2009).

MATERIALS AND METHODS

Plant materials

For the purpose of screening on high oleic acid genotypes, around 300 sunflower F_3 (K2-R-SN-9/13) individuals obtained from a cross between high oleic acid and low oleic acid lines were used. Leaves were collected from the field, labeled with individual number and stored kept at -80ºC until further use.

DNA isolation

Before DNA isolation leave samples were homogenized with Retsch® Model MM300 Mixer Mill. Different manual DNA isolation methods (Dellaporta et al., 1983; Doyle and Doyle 1987; Li et al., 2007; Azmat et al., 2012; Souza et al., 2012; Healey et al., 2014) and also DNA isolation kits (NANObiz Plant Genomic DNA Isolation Kit, Vivantis GF-1 Plant DNA Extraction Kit and i-genomic Plant DNA Extraction Mini Kit) were tested in order to obtain high quality and quantity DNA for PCR analysis. Finally i-genomic Plant DNA Extraction Mini Kit was selected and used for DNA isolation from all samples. Concentration of each DNA was measured with Qubit® 2.0 Fluorometer and the quality of DNA was checked by 1% agarose gel electrophoresis, stained with RedSafe Nucleic Acid Staining Solution and visualized by Gel Imaging System Vilber Lourmat Quantum ST5. Each of the extracted DNA was diluted as 50 ng per µl and was stored at -20 ºC for later uses.

PCR analysis

Genotyping of high oleic (HO) and low oleic (LO) sunflower individuals was performed with two primer pairs; SSR (N1-1F/N1-1R) and HO PCR specific fragment (N1-3F/N2-1R) that were chosen from the patent obtained by Berville et al. (2009) (Table 1). PCR amplification was carried out using 20 µl volume containing 100 ng of template DNA, 2 mM $MgCl_2$, 1X reaction buffer, four dNTPs (each 0.2 mM), 10 pmol of each primer (forward primer WellRed D4 fluorescent dye labeled) and 1.5 U of Taq-polymerase. The PCR profile for SSR (N1-1F/N1-1R) consisted of 5 min denaturing at 94 ºC, followed by 35 cycles of 1 min denaturing at 94 ºC, 1 min annealing at 50 ºC and 1 min extension at 72 ºC, with a final extension of 10 min at 72 ºC. The PCR profile for HO PCR specific fragment (N1-3F/N2-1R) consisted of 5 min denaturing at 94 ºC, followed by 35 cycles of 1 min denaturing at 94 ºC, 1 min annealing at 58 ºC and 1 min extension at 72 ºC, with a final extension of 10 min at 72 ºC. Amplified PCR products were controlled by 2% agarose gel electrophoresis, stained with RedSafe Nucleic Acid Staining Solution and visualized by Gel Imaging System Vilber Lourmat Quantum ST5 (Figure 1 and

Figure 2). SSR (N1-1F/N1-1R) fragments were scored in a Beckman Coulter GenomeLab™ GeXP Genetic Analysis System and fragment sizes were calculated by its Software.

Table 1. Characteristics of markers used to analyze HO and LO sunflower genotypes

No	Primer type	Primer name	Primer sequences (5′-3′)
1	SSR	N1-1F	TTGGAGTTCGGTTTATTTAT
		N1-1R	TTAGTAAACGAGCCTGAAC
2	HO PCR specific fragment	N1-3F	GAGAAGAGGGAGGTGTGAAG
		N2-1R	AGCGGTTATGGTGAGGTCAG

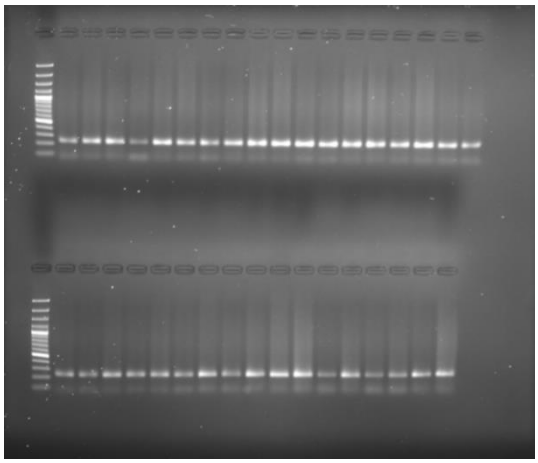

Figure 1. Amplified fragments with SSR (N1-1F/N1-1R) primer for sunflower individuals (First lane is 100 bp DNA Ladder)

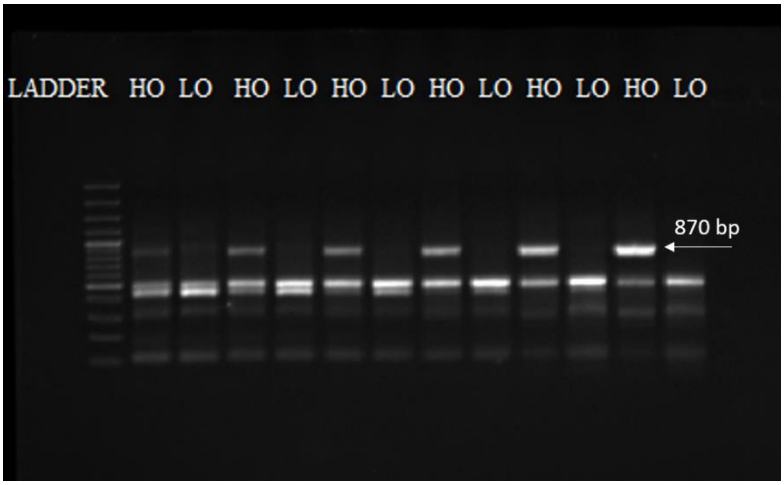

Figure 2. PCR amplification of HO and LO genotypes with HO PCR specific fragment (N1-3F/N2-1R) (First lane is 100 bp DNA Ladder)

PCR amplification of SSR (N1-1F/N1-1R) primer leads to 243/246/249 bp fragments corresponding to 15/16/17 TTA repeats, respectively. The HO genotypes has 16 TTA repeats whereas LO genotypes has 17 TTA repeats for the studied sunflower individuals. DNA sequence analysis with reverse primer was carried out to confirm repeat motifs corresponding to HO genotypes (Figure 3 and 4).

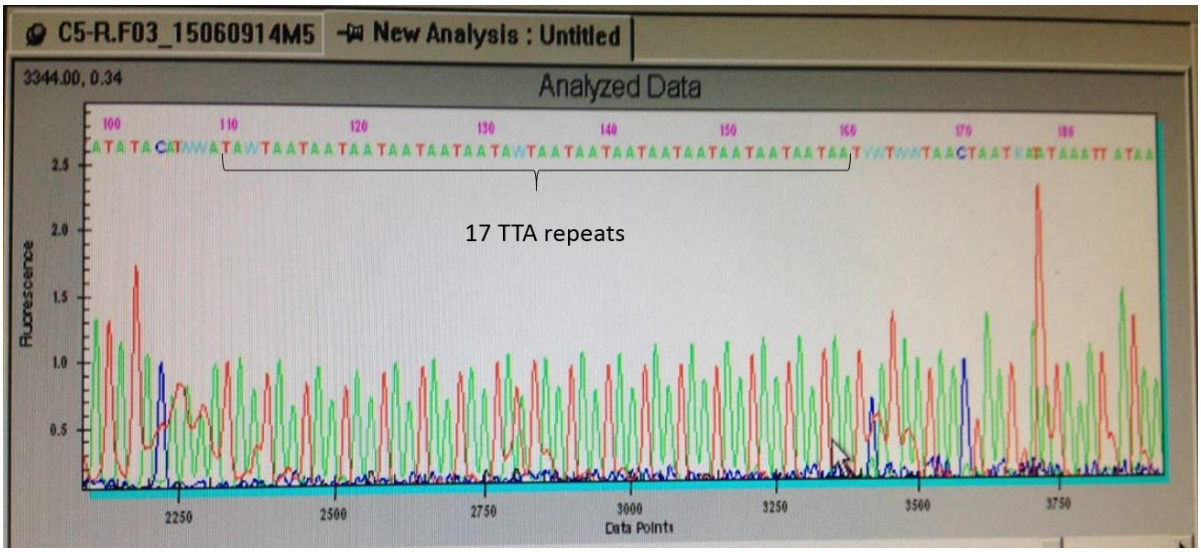

Figure 3. DNA sequence analysis of LO genotypes that have 17 TTA repeats (249 bp)

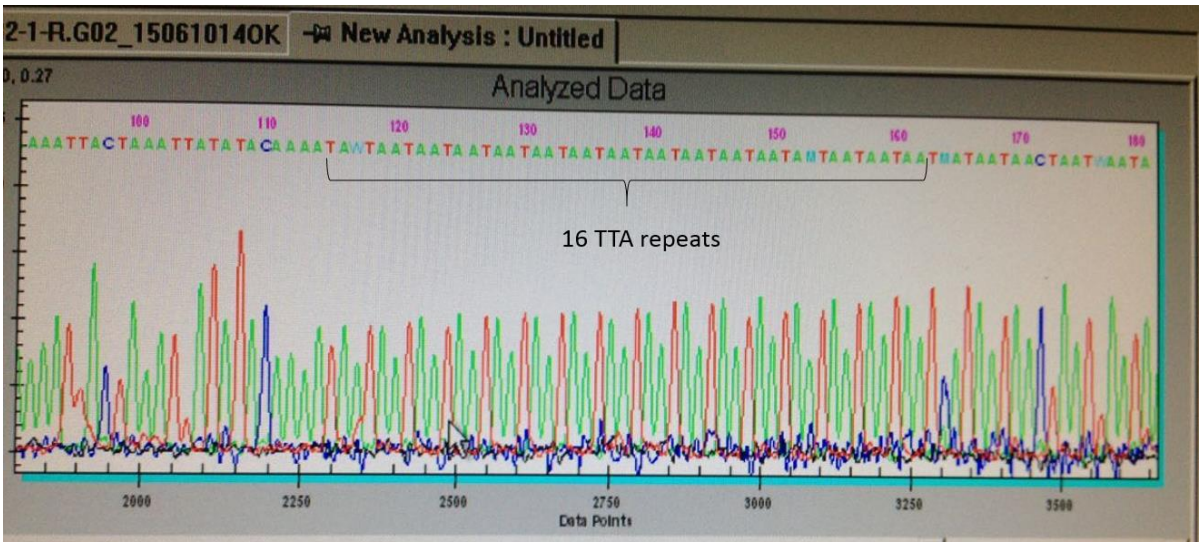

Figure 4. DNA sequence analysis of HO genotypes that have 16 TTA repeats (246 bp)

RESULTS AND DISCUSSION

The Pervenet mutation was labelled by the polymorphism of the SSR locus located on the Δ12-desaturase gene intron (Berville et al., 2009). Alleles and genotypes of studied sunflower individuals were determined for analyzed SSR (N1-1F/N1-1R) locus. According to DNA fragment analysis for SSR locus 246/246 Homozygous, 249/249 Homozygous and 246/249

Heterozygous genotypes were identified (Figure 5). In order to confirm HO sunflower genotypes, all studied individuals were screened with HO PCR specific fragment (N1-3F/N2-1R). The Pervenet mutation was labelled by the 870 bp PCR fragment across the 5' insertion point by HO PCR specific fragment (N1-3F/N2-1R) (Berville et al., 2009). The results showed that high oleic containing sunflower individuals (HO genotypes) showed a specific band at about 870 bp length which was absent in low oleic (LO) genotypes (Figure 2).

After evaluation of all studied sunflower individuals (Totally 300 F_3), 183 of them were HO genotypes, the others were LO genotypes. The results were confirmed by determination of fatty acid composition using gas chromatography in all the studied individuals. According to fatty acid analysis, the oleic acid content was obtained from 60-92% for HO genotypes and below 60% (minimum 22.8%) for LO genotypes.

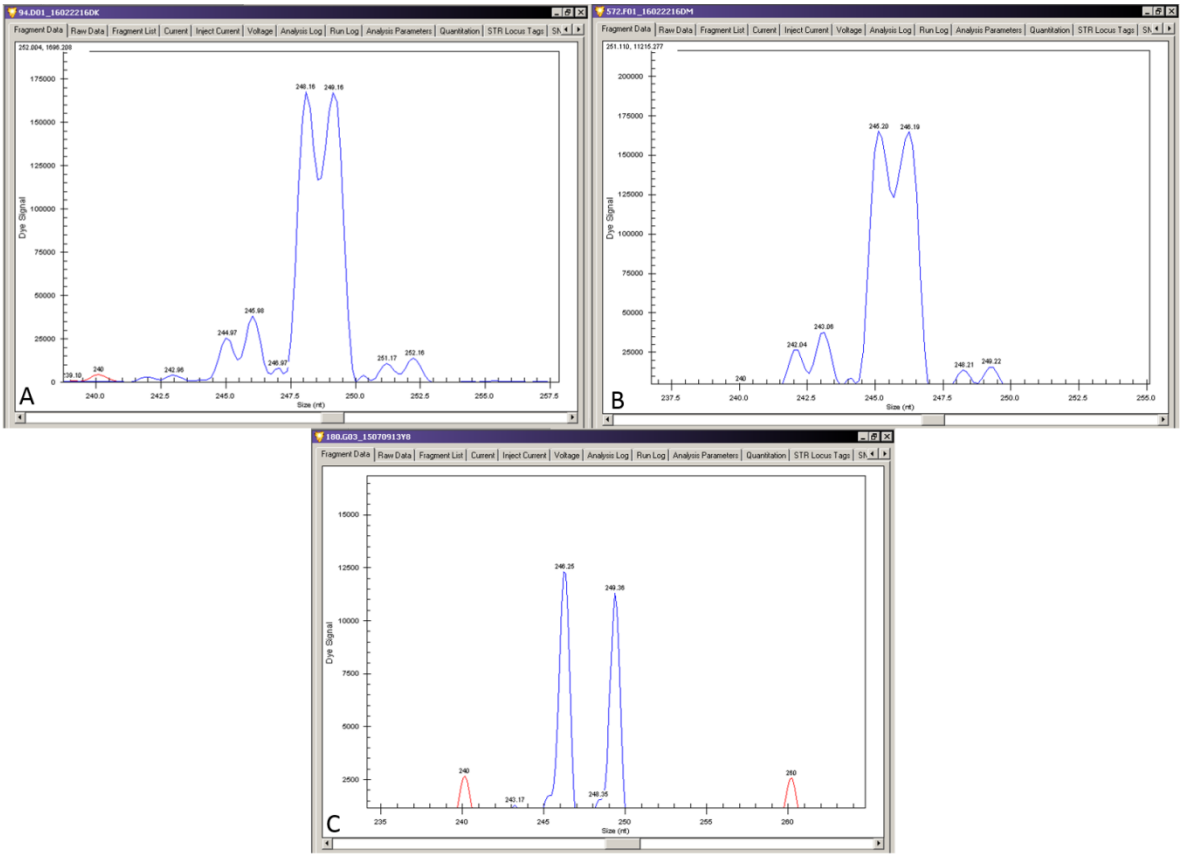

Figure 5. DNA fragment analyses results for (N1-1F/N1-1R) primer A) 249 bp (Homozygous LO genotype), B) 246 bp (Homozygous HO genotype), C) 246 bp/249 bp (Heterozygous HO genotype)

Various sunflower lines and hybrids have been studied to distinguish HO genotypes from LO genotypes by different researchers and molecular marker types such as RAPD or SSR (Dehmer and Friedt 1998; Nagarathna et al., 2011; Grandon et al., 2012; Singchai et al., 2013). Nagarathna et al., (2011) studied around 350 sunflower genotypes including RHA-lines, cms lines, inbreds and germplasm lines to screening on high oleic acid. In Nagarathna et al., (2011) For the purpose of genotyping the sunflower lines for high oleic content, HO PCR specific fragment (N1-3F/N2-1R) were chosen and also the seeds were used for the determination of fatty acids (linoleic acid, oleic acid, palmitic acid and stearic acid) using gas chromatography. They

reported that the genotypes having a specific band (at 800 to 900 bp) showed high oleic content. Singchai et al., (2013) studied the developed lines that used as the representative of low and high oleic acid sunflowers for genotyping. They screened thirty seven SSR primers including 34 primers of ORS set, 2 primers of ha set and N1-3F/N2-1R primer to identify DNA samples from two lines (high and low oleic acid contents). Out of the 37 SSR primers screened for polymorphism, 10 SSR primers including N1-3F/N2-1R generated differentiating bands between the high and low oleic content lines. With the 10 SSR markers they studied, Singchai et al., (2013) reported that it is possible to identify the genetic markers linked to high oleic acid trait which may be useful for further sunflower breeding program.

As a conclusion PCR analysis with selected primers enabling to amplify either the Δ12HOS allele and thus the Pervenet mutations (N1-3F/N2-1R) or the SSR locus (N1-1F/N1-1R), lead to discriminate HO and LO genotypes. Consequently, these primers may be used in selection programs to identify genotypes carrying the Pervenet mutation. However, these markers especially SSR (N1-1F/N1-1R) need to be further validation in different sunflower populations in order to confirm their capability to identify high and low oleic acid contents.

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GENETIC ENGINEERING STUDIES ON SUNFLOWER

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ABSTRACT

Domestication of sunflowers (*Helianthus annuus*) by humans for particular structures that are desirable to humans in a relatively benign environmental conditions and stresses have forced these plants to undergo evolutionary increases in the yield, but at the cost of reduced defense mechanisms against biotic and abiotic stresses and diseases. A multitude of factors such as insects and diseases reduce the sunflower yield, and research to develop pest resistance, herbicide resistance, increasing oil per acre yield of sunflower holds indispensable. Wild species of sunflower contain rich source of useful genes, which needs to be transferred to cultivated ones. Though improved transformed techniques have been reported, more efficient transformation protocol needs to be explored. There are some studies involving transgenic sunflower plants to increase yield, oil content, insect/fungal resistance, stress tolerance and production of biopharmaceutical proteins. Studies involving ecological impact of *Bt* sunflowers with regards to "gene flow" remains controversial. Stable transformation is relatively time-consuming with low regeneration rate and has left sunflower transformation recalcitrant. For this reason, transient expression in sunflowers have gained attention in studies of function of promoters, regulation of gene, subcellular localization of proteins, protein stability, protein-protein interactions and small RNA function. In this presentation, we will attempt to give an overview of the genetic engineering studies in sunflower with main challenges, achievements and future prospects.

Key words: Transgenic sunflower, Transient expression, Stress resistance, Insect resistance, Oil yield, Biopharming.

INTRODUCTION

Cultivated sunflower (*Helianthus annuus* L.) has its origin from North America and is one of the few major food crops in the world (Harter *et al*., 2004; Blackman *et al*., 2011). Domestication of sunflowers by humans for particular structures that are desirable to humans in a relatively benign environmental conditions and stresses have forced these plants to undergo evolutionary increases in the yield, but at the cost of reduced defense mechanisms against biotic and abiotic stresses and diseases (Mayrose *et al*., 2011). Mayrose *et al*. (2011) studied growth traits of sunflower under benign environmental condition which they found to be greater for the domesticated genotype population than that for the wild population, but with a drop in defense response in domesticated sunflowers when exposed to biotic and abiotic stresses. Additionally, it was found that lepidopteran pests preferred domesticated sunflowers more than the wild sunflowers in experimental agriculture fields (Chen and Welter, 2002). It was also revealed that

Botrytis cinerea and drought had more negative consequences on domesticated sunflowers than the native plants (Mayrose *et al*., 2010). A multitude of factors such as insects and diseases reduce the sunflower yield, and molecular biology research with a focus on transgenic sunflowers to develop pest resistance, herbicide resistance, increasing oil per acre yield of sunflower holds indispensable as well as its study on ecological impact is pressing.

VARIOUS TECHNIQUES IN GENETIC ENGINEERING OF SUNFLOWERS

Various approaches in production of transgenic plants have been used, but with low efficiencies in transformation: Polyethylene glycol (PEG)-induced vector uptake of pCAMVNEO into protoplasts isolated from sunflower seedling hypocotyls (Moyne *et al*., 1988), microprojectile bombardment (Knittel *et al*., 1994; Laparra *et al*., 1995; Hunold *et al*., 1995) and electroporation (Kirches *et al*., 1991). PEG-induced vector uptake turned out to be mainly labor intensive and some other protocols with *Agrobacterium*-mediated transformation of sunflower plants have been used (Bidney *et al*., 1992; Laparra *et al*., 1995; Rao *et al*., 1999; Weber *et al*., 2003; Ikeda *et al*., 2005; Mohamed *et al*., 2006).

One possibility as to the reason for less studies with stable transformation experiments with sunflowers could be because of no efficient and reproducible protocol for sunflower transformation (Radonic *et al*., 2008). Selection of transformants, tissue regeneration, long life cycle of sunflower plants, time-consuming homozygous lines generation mainly as compared to the time required to obtain homozygous transgenic *Arabidopsis* plants have possibly made stable transformation of sunflower to be used to elucidate metabolic or signal pathways of sunflowers recalcitrant. This has led to some studies to choose *Arabidopsis* or tobacco heterologous system or transient expression in sunflower leaves to further unravel gene functions in sunflowers (Manavella and Chan, 2009; Cabello *et al*., 2012; Cabello *et al.*, 2016; Tata *et al*., 2016).

THE DEVELOPMENT OF INSECT TOLERANT TRANSGENIC SUNFLOWERS

A polyphagous insect *Helicoverpa armigera* (Noctuidae; Lepidoptera) is reported to cause 20-25% yield losses in sunflowerss and sometimes upto 40-70% in severe conditions (Ranasingh and Mahalik, 2008). Westdal (1975) found that the sunflower beetle *Zygogramma exclamationis* (25 larvae per plant) reduced seed production in sunflower as much as 30%.

Cry1F-transgenic sunflowers were obtained with a *Cry1F* gene ("Bt" gene) isolated from *Bacillus thuringiensis* which conferred resistance against *Spilosoma virginicia* and *Rachiplusia nu*. Compared to the control, increased tolerance of transgenic plants against larvae at the seedling and preflowering stages were found during the feeding assay with transgenic leaf discs. *Cry1AC* gene was used to develop a transgenic line of *Bt* sunflowers by Pioneer Hi-Bred and Dow AgroSciences which produce CrylAc protein that is lethal to *Lepidopteran* (moth) larvae (Snow *et al*., 2003). Snow *et al.* (2003) reported that the transgenic plants yielded considerably more inflorescences with more mature seeds in more inflorescences and higher number of viable seeds per plant as compared to non-transgenic controls. They observed that the transgenic plants in a greenhouse experiment even without the insect pests produced no difference in the seeds or inflorescences. The study concluded that the transgene itself didn't actually cause the benefit in these transgenics, but the protection from lepidopeterian resulted in the gain of fecundity in transgenics. They suggested that the wild sunflowers and weedy populations near to the cultivated transgenic sunflowers would render recurring events of "gene flow" from the transgenics and it could have detrimental effects on the native lepidopteran herbivores and other populations of coleopteran and dipteran herbivores.

THE DEVELOPMENT OF FUNGAL RESISTANT TRANSGENIC SUNFLOWERS

Charcoal rot disease caused by *Macrophomina phaseolina* in sunflower causes losses on more than 500 cultivated and wild plant species (Khan, 2007). *Alternaria* blight caused by *Alternaria helianthi* is reported to reduce seed and oil yield by 27-80% and 17-33% respectively (Reviewed by Mukhtar, 2009). *Sclerotinia* has been reported to cause damage upto 50% in sunflower in UK (Tu, 1989). Fungal pathogen *Plasmopara halstedii* causes Downy mildew and can lead to more than 50% yield loss (Hvarleva *et al*., 2009).

Oxo-transgenic sunflower plants were obtained by introducing wheat germin gf2.8 *OXO* gene to confer resistance against fungal disease *Sclerotinia* head rot (Lane *et al*., 1991; Lu *et al*., 2000; Hu *et al*., 2003). However, it has been of concern if OXO enzyme could be a human allergen (Jensen-Jarolim *et al*., 2002). The probability of transgenic wild plants being a worse weed is scarce as *OXO* transgene will diffuse neutrally on its escape because the transgenic wild plants do not produce ample number of seeds than the wild population (Burke and Rieseberg, 2003). Chapman and Burke (2006) also ruled out the possibility of "gene flow" concluding that the natural selection is the key in spread of favorable transgene alleles. Human lysozyme gene under CaMV 35S promoter and *Nos* terminator in a binary vector containing *NPTII* and *GUS* marker genes was incorporated in sunflowers using hypocotyl explants with *Agrobacterium*-mediated transformation conferred resistance against mold disease causing *Sclerotinia sclerotiorum* (Sawahel and Hagran, 2006). Lectin or proteinase inhibitor genes have been used to engineer sunflower with insect resistance (Schuler *et al.*, 1998).

THE DEVELOPMENT OF ABIOTIC STRESS TOLERANT TRANSGENIC SUNFLOWERS

Yeast metallothionein gene (*CUP1*) from yeast was incorporated into sunflower to evaluate tolerance of transgenic plants to heavy metals at the callus stage and selected heavy metal-tolerant lines of the transgenic sunflower calli. The results showed use of transgenics to obtain abiotic stress tolerance in sunflowers (Watanabe *et al*., 2005). LBA4404 strain harboring T-DNA containing dsRNA-suppressor of proline dehydrogenase gene, produced based on the *ProDH1* gene of Arabidopsis, was integrated into the genome of sunflower plants transformed *in vitro* and *in planta* to increase sunflower tolerance level to water deficiency and salinity (Tishchenko *et al*., 2014**)**.

MOLECULAR PHARMING IN SUNFLOWERS

Plants have the ability to bring about protein stability and bioactivity by glycosylation and posttranslational modification, and plant and animal cell protein synthesis pathway are alike; it is estimated that to synthesize pharmaceutical proteins in plants is highly economical than using fermentation techniques and mammalian cell cultures (Rybicki *et al*., 2010; Ma *et al*., 2003). Guan and Wang (2014) successfully expressed CTB-LK (Cholera toxin B subunit-Lumbrokinase), peeled seeds of which if administered orally to rats and mice had significant antithrombotic effect, in sunflower seeds using *Agrobacterium* mediated transformation. This study also concluded that the CTB-LK expression in sunflower seeds eradicated the requirement for protein downstream processing. Similarly, the use of *Agrobacterium rhizogenes*-mediated transformation of topinambour in sunflower plants, callus and "hairy" root cultures proved sunflower plants to be a good source of recombinant interferon alpha 2b protein. The plasmid vectors with interferon gene fused with *Nicotiana plumbaginifolia* L. calreticulin apoplast

targeting signal driven by *35S* CaMV promoter or root-specific *Mll* promoter to obtain transgenic *H. tuberosus* cultures with high antiviral activity (Maistrenko *et al*., 2015).

THE DEVELOPMENT OF SUNFLOWERS WITH INCREASED FATTY ACID OIL CONTENT BY MUTAGENESIS

Modifying oil quality is crucial as it is one of the edible oils worldwide known for its salubrious quality and lipid peroxidation (Moschen *et al*., 2014). Many sunflower lines have been developed with elevated saturated fatty acid content with greater than 25% of fatty acids compared to 12% in normal sunflower using physical or chemical mutagenesis. Osorio *et al*. (1995) developed CAS-3 and CAS-5 mutants with high amount of stearic acid and palmitic acid contents respectively. Fernández-Martínez *et al*. (1997) reported CAS-12 mutants with high palmitic acid and oleic acid contents. Fernández-Moya *et al*. (2002) developed CAS-14 mutants with upto 37% stearic acid content. Velasco *et al*. (2008) used ethylmethane sulfonate as a chemical mutagen and obtained M2 seeds from a single M1 plant with 5-39% palmitic acid content. 10-30% of palmitic acid was obtained from the progenies of all selected M2 seeds.

THE DEVELOPMENT OF TRANSGENIC SUNFLOWERS WITH INCREASED FATTY ACID OIL CONTENT

The Δ9-stearoyl-(acyl carrier protein) desaturase coding sequence from *Ricinus communis* was transferred in sunflower under the control of seed-specific promoter and terminator sequences of *Hads10*. Seed oil composition analysis showed significant decrease in stearic acid content in the seeds obtained from transgenic plants. Some progenies exhibited saturated fatty acid content below 10% whereas other plants had elevated palmitic acid content with reduced stearic acid content (Rousselin et al., 2002). Hydroxymethylglutaryl-CoA (*Hmgr*-CoA) and *Erwinia uredovora* phytoene desaturase (*Crtl*) genes were introduced into sunflower to obtain potential increase in oil quality (Dagustu *et al*., 2008).

OTHER MOLECULAR STUDIES IN TRANSGENIC SUNFLOWERS

Post-transcriptional gene silencing (PTGS) in transgenic sunflower expressing *glucuronidase* (GUS) activity has been performed using grafting procedure. In two weeks silencing was observed and the study showed that the RNA infiltration in sunflower induces transient silencing and is not transmitted to offspring (Hewezi *et al*., 2005). Shulga *et al*. (2015) reported first transgenic sunflower with alteration in *HAM59* expression to study the function of *HAM59* MADS-box gene in sunflower which is involved in formation of reproductive organs of flower.

The elucidation of role of PLFOR48 sequence in resistance to mildew in sunflower was studied assessing loss of function, by expressing antisense cDNA PLFOR48 construct in RHA 266 sunflower line. Transgenic sunflower lines displayed severe developmental abnormalities. The same antisense expression in transgenic tobacco lines resulted in higher susceptibility to *Phytophthora parasitica*. It was reported that TIR-NBSLRR R genes in sunflower and tobacco have a dual role in plant development and fungal resistance (Hewezi *et al*., 2006).

CURRENT STIUATION AND CHALLENGES

A progress is still being made in efficiently transforming sunflower crops but stable transformation of sunflower plants is just yet time-consuming in generating homozygous lines and in regeneration of tissue. Also, sunflower has a long life cycle and transient expression of genes can be an alternative method in elucidating molecular mechanisms such as function of promoters, regulation of gene, subcellular localization of proteins, protein stability, protein-protein interactions and small RNA function (Manavella and Chan, 2009). Despite of this constraint, several studies by developing transgenic sunflowers are still being conducted.

Wild sunflower species provide greater contribution as a rich source of genes in crop improvement to bring about economic viability in cultivates species as major oilseed global crop (Seiler and Fredrick, 2011). As having a narrow background in domesticated sunflowers with deficient genes, discovery of unique genes from wild sunflower plants is indispensable and is still underway. This could help in developing transgenic sunflowers with desired traits from wild population.

Clearfield and Express Sun technologies saw restriction on growing of genetically modified crops for "not being biotech product" (Reviewed by Kaya, 2015). Genetically modified crops have always been a matter of debate and public acceptance regarding this remains divided with some people being reluctant on the use of biotechnology in crop amelioration.

FUTURE DIRECTIONS IN GENETIC ENGINEERING OF SUNFLOWER

Transgenic technology holds imperative role in sunflower breeding and exerts strong promises to increase yield, oil content, insect/fungal resistance, stress tolerance and production of biopharmaceutical proteins. Albeit having improved transformed techniques in sunflower, more efficient transformation protocol needs to be explored for generating increased success rates in obtaining transgenic sunflowers as well as search for candidate genes with elite traits in developing transgenic crops does remain apparent.

Traits that are being studied in sunflower for environment release is sparse. Sunflower is known to have a high exposure to gene flow ultimately generating continuous variability. Strict environmental monitoring is inevitable to preclude undesired outcomes (Cantamutto and Poverene, 2007).

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MAPPING OF A BROOMRAPE RESISTANCE GENE IN SUNFLOWER LINE LIV-17

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ABSTRACT

Broomrape (*Orobanche cumana* Walr.) is a parasitic plant that causes severe yield loses in Southern and Eastern Europe and in some areas of Asia. While the application of herbicides is limited for environmental and economic reasons, breeding for resistance is regarded as the most effective solution. Closely linked molecular markers can facilitate and accelerate the process of introducing resistance genes into breeding material. Here we report mapping of a new resistance gene in LIV-17, sunflower inbred line which was resistant to broomrape populations from Spain, Romania and Turkey. Bulk segregant analyses was done on F2 population from a cross LIV-17/HA-26-PR using 210 SSR markers. Preliminary results showed that the resistance gene was placed in LG3 of the sunflower genetic map. Identification of closely linked molecular markers which will enable marker-assisted selection is underway.

Key Words : sunflower, broomrape, SSR

SCREENING OF THE PRESENCE OF OL GENE IN NS SUNFLOWER COLLECTION

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ABSTRACT

Providing high quality oil is of great interest for oil companies. When it comes to sunflower oil, there are two types of oil on the market: high linoleic and high oleic. High oleic oil is considered a healthier version of oil, since it rich in omega-9 fatty acids that are oxidative more stable than linoleic fatty acid (omega-6 fatty acid), dominant in common sunflower oil. Development of high oleic sunflower genotypes was enabled by the discovery of Pervenets mutant sunflower population. In the IFVCNS, there is a great collection of sunflower inbred lines with wide range of oleic acid content (OAC). From the collection, we have chosen 62 genotypes for determination of OAC. In addition we used molecular marker reported by Schuppert et al. (2006) to screen for presence of the mutation that led to increase in OAC. The OAC in lines in which the presence of the mutation was detected ranged between 36.48 and 88.61% (mutant lines derived from high oleic line L31 – 36.48 – 56.58 and standard inbred lines 58.25 – 88.61%); while in lines where OAC varied between 14.24 and 34.46% this mutation was not detected. These results will help in choosing the best parental lines in future breeding programs, while the marker used will enable quick detection of the mutation. In addition it showed that the mutation in mutant lines most likely did not affect the analyzed part of the FAD2-1D sequence.
Key words: Oleic acid, Helinathus annuus L., marker assisted selection

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the third most important oilcrop in the world. Sunflower oil is naturally rich in polyunsaturated omega-6 fatty acid, linonelic acid, ranging between 50 and 70%, while the content of monounsaturated omega-9 fatty acid, oleic acid, ranges between 20 and 25% (Kabbaj et al., 1996). The creation of mutant sunflower cultivar Pervenets (Soldatov, 1976), obtained by dimethyl-sulfate (DMS) treatment lead to broadening of sunflower breeding programs and allowed creation of high oleic sunflower lines and hybrids. Today, the end user is dictating what type of oil is in high demand. Therefore, breeders create and conduct their breeding programs in compliance with the market demand.

In today's market, high oleic oil is considered to be healthier than high linoleic, since it rich in omega-9 fatty acids that are oxidative more stable than linoleic fatty acid. This trait is important in food industry since a lot of processing activities include higher temperature treatment or converting some unsaturated fats into saturated fats in order to achieve higher melting point. The conversion is important in margarine production because oil stays solid at room temperature. Bearing in mind benefits that high oleic oil has on human health and in industry due to their temperature stability, introduction of *Ol* gene in breeding programs originating from Pervenets became the basis for sunflower breeding for high oleic lines and hybrids, since Pervenets mutant has oleic acid content (OAC) greater than 65% (Soldatov, 1976; Lacombe and Bervillé, 2001, Lacombe et al., 2004).

There are different reports about the inheritance of OAC. Initially, Urie (1984) reported dominant mode of inheritance, while Fick (1984) reported partially dominant mode of inheritance. Later on, there were reports of existence of a modifier gene (Urie, 1985; Miller et al., 1987; Fernández et al., 1999) or one or more genes that influence OAC (Fernández-Martínez et al., 1989; Pérez-Vich et al., 2002; Velasco et al., 2000). In general, OAC varies depending on the genetic background of the recipient genotype.

The molecular change underlying the increase in OAC in Pervenets is the duplication of the *FAD2-1* allele. FAD2 (oleoyl-phosphatidyl choline desaturase) is an enzyme that catalyses synthesis of linoleic acid from oleic acid (Okuley et al., 1994). Three FAD genes are present in sunflower genome: *FAD2-1*, *FAD2-2*, *FAD2-3* (Hongtrakul et al., 1998; Martínez-Rivas et al., 2001). Of those three, only *FAD2-1* is strongly expressed in developing seeds (Hongtrakul et al., 1998). Partial duplication of this gene led to silencing of the *FAD2-1* gene, thus decreasing the activity of FAD enzyme leading to accumulation of oleic acid (Lacombe et al., 2002). *FAD2-1* was reported to cosegregate with *Ol* gene at LG14 (Lacombe and Bervillé, 2001; Pérez-Vich et al., 2002; Schuppert et al., 2006). Hongtrakul et al. (1998) and Schuppert et al. (2006) reported that duplicated sequence of *FAD2-1* does not differ from the corresponding wild type sequence.

So far, different molecular tools were used for analysis of *FAD2-1* gene (Hongtrakul et al., 1998; Dehmer, and Friedt, 1998; Lacombe and Bervillé, 2001; Lacombe et al., 2000; 2002; 2004; 2009, Schuppert et al., 2006). Some of these reports include identification or development of molecular markers for detection of *FAD2-1*. At the Institute of Field and Vegetable Crops there are 2 registered high oleic sunflower hybrids, however we are expanding our breeding program for creation of greater variety of high oleic hybrids.

In present work sunflower inbred lines were selected from a considerable sunflower collection developed at the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS) and was screened for presence of *Ol* mutation by use of INDEL molecular marker reported by (Schuppert et al., 2006). This marker is developed to detect presence of *Ol* mutation since forward primer corresponds to the intergenic region present in *Ol* mutation and reverse primer is complementary to coding region of *FAD2-1*. To verify molecular results, OAC of chosen lines was analyzed by use of gas chromatography (GC). The main goal of present work was to evaluate the efficiency of the INDEL marker for marker assisted selection in IFVCNS lines and to identify the best high oleic parental lines for future crossings.

MATERIAL AND METHODS

Plant material

Chosen plant material for analysis includes lines that are used in current breeding program at the IFVCNS and vary in OAC (Table 1). Additionally, four mutant lines (M-1, M-2, M-3, M-4) derived from high oleic proprietary line developed at the IFVCNS were analyzed in order to try do detect changes on a molecular level that underlined decrease in OAC.

Plants were grown in growth chamber in Klasmann Deilmann Substrat 1 until reaching two leaf-pair stage when leaves were sampled for DNA extraction. Out of each examined sunflower line a bulk sample of 10 plants was formed and plant leaves were kept at -70^{o}C until DNA extraction.

Oleic acid content

Oil samples were obtained by pressing of 2 grams of seeds in a hydraulic press (Sirio, Mikodental 10 tons stregth, cc 400 bars) to yield approximately 0.5 ml of oil available for GC analysis. In the reaction vial 270 µl of TMSH (transesterification agens) was added to exactly 30 µl of oil, well shaked in the vortex, and kept at room temperature for an hour.

Table 1. Tested sunflower genotypes, their oleic acid content and obtained molecular profiles (presence or absence of a part of the *FAD2-1D* sequence)

Genotype - sunflower line	Oleic acid content in %	Presence of *FAD2-1D* mutation*	Genotype - sunflower line	Oleic acid content in %	Presence of *FAD2-1D* mutation *
L1	88.61	+	L34	79.17	+
L2	87.74	+	L35	78.98	+
L3	87.39	+	L36	78.56	+
L5	87.04	+	L37	77.35	+
L6	86.63	+	L38	77.06	+
L7	86.62	+	L39	76.42	+
L8	86.56	+	L40	75.33	+
L9	86.29	+	L41	74.15	+
L10	85.94	+	L42	70.22	+
L11	85.55	+	L43	69.40	+
L12	85.41	+	L44	69.33	+
L13	85.10	+	L45	68.29	+
L14	84.92	+	L46	68.29	+
L15	84.72	+	L47	63.45	+
L16	84.69	+	L48	62.04	+
L17	84.17	+	L49	58.25	+
L18	84.04	+	M-4	56.58	+
L19	83.63	+	M-3	50.13	+
L20	83.49	+	M-1	49.93	+
L22	83.45	+	M-2	36.48	+
L23	82.91	+	L50	34.46	-
L24	82.88	+	L51	28.30	-
L25	82.31	+	L52	24.52	-
L26	81.64	+	L53	22.03	-
L27	81.39	+	L54	21.53	-
L28	81.29	+	L55	21.47	-
L31	80.57	+	L56	17.31	-
L30	80.37	+	L57	17.03	-
L32	79.51	+	L58	14.24	-
L33	79.47	+			

* presence of amplified band (+), absence of amplified band (-)

The oleic acid was identified using a reference mixture of fatty acids methyl esters (FAME). A multi-standard from Supelco (FAME RM-1, Cat. no. O7006) containing the methyl esters of palmitic, stearic, oleic, linoleic, linolenic and arachidic fatty acids was used to confirm the retention times as well as to confirm that the peak areas reflected actual composition of these mixtures.

Oleic acid content analysis was performed on Agilent 5890 gas chromatograph equipped with flame ionization detector (FID) and split/splitless injector (split ratio of 1:50). The separation was performed on a fused silica capillary column (HP-INNOWAX, 30m×0.25mm i.d., and 0.25µm film thickness). Helium was used as carrier gas at a constant pressure of 53kPa at 50°C min). The temperature program was as follows: initial temperature of 50°C was held for 1 min, increased to 200°C at a rate of 25°C/min, then increased to 230°C at a rate of 3°C/min, and and hold for 18 min. The injector and detector temperatures were set at 250 and 280°C respectively. The sample volume injected was 1 µl. The results were processed using ChemStation software and expressed as the percentage of individual fatty acids in the oil sample.

Molecular analysis

DNA was extracted from leaves by use modified CTAB protocol (Permingeat et al., 1998). For detection of *FAD2-1D* sequence primer pair F4-R1 was used (Schuppert et al., 2006). PCR was performed as described by Schuppert et al. (2006) in mix described by Dimitrijević et al. (2010). Products of PCR amplification were run on 2% agarose gels and visualized with the BIO-Print system (Vilber Lourmat, Marne-La-Vallée, France).

RESULTS AND DISCUSSION

Oleic acid content varied between tested lines, ranging from 14.24 to 88.61% (Table 1, Figure 1). Thirty one sunflower line (L1-L31) had OAC higher that 80% , 22 lines (L32-L49 and M-1, M-2, M-3, M-4), had OAC ranging between 36 and 80% and 9 lines (L50-L58) had less than 36% OAC. Even though mutant lines, (M-1, M-2, M-3, M-4) originate from high oleic line, GC analysis showed significant decrease in OAC (Table 1).

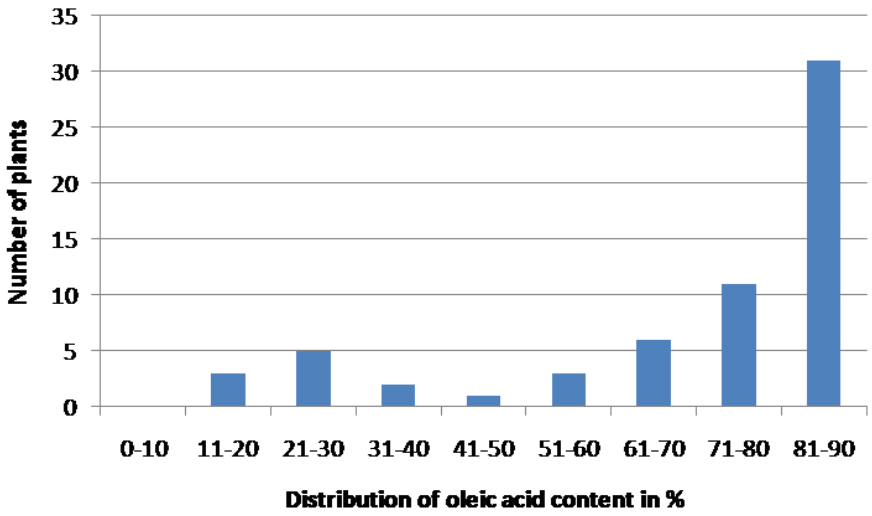

Figure 1. Distribution of oleic acid content (%) in examined sunflower genotypes

In order to examine the presence of *Ol* mutation in tested lines F4-R1 primer was used (Schuppert et al., 2006). Out of 62 chosen genotypes, seeds of three lines did not germinate; consequently they were excluded from the molecular analysis. Molecular marker used amplified a band of expected length (approximately 650 bp) in all sunflower lines, except in lines L50-L58 that had low OAC ranging from 14.24 to 34.46% (Figure 2). Presence of an amplified band in all tested mutant lines showed that there is an *Ol* mutation present in examined lines, consequently

some other changes on a molecular level must have happened and caused significant decrease in OAC. Since EMS was used for treatment of wild-type line, small nuclear changes could have occurred in *FAD2-1D* sequence, as EMS most frequently induces SNPs (G to A and C to T point mutations) (McCallum et al., 2000), as was the case with high oleic mutant lines developed by León et al. (2013). Consequently, there is a possibility that some small changes occurred in amplified sequence which could not be detected by electrophoresis. Alternatively, some changes might have occurred in other parts of *FAD2-1D* sequence or somewhere else in sunflower genome. However, this is unlikely since most of the reports on molecular changes in fatty acid composition occurred in the sequence of encoding enzymes (León et al., 2013).

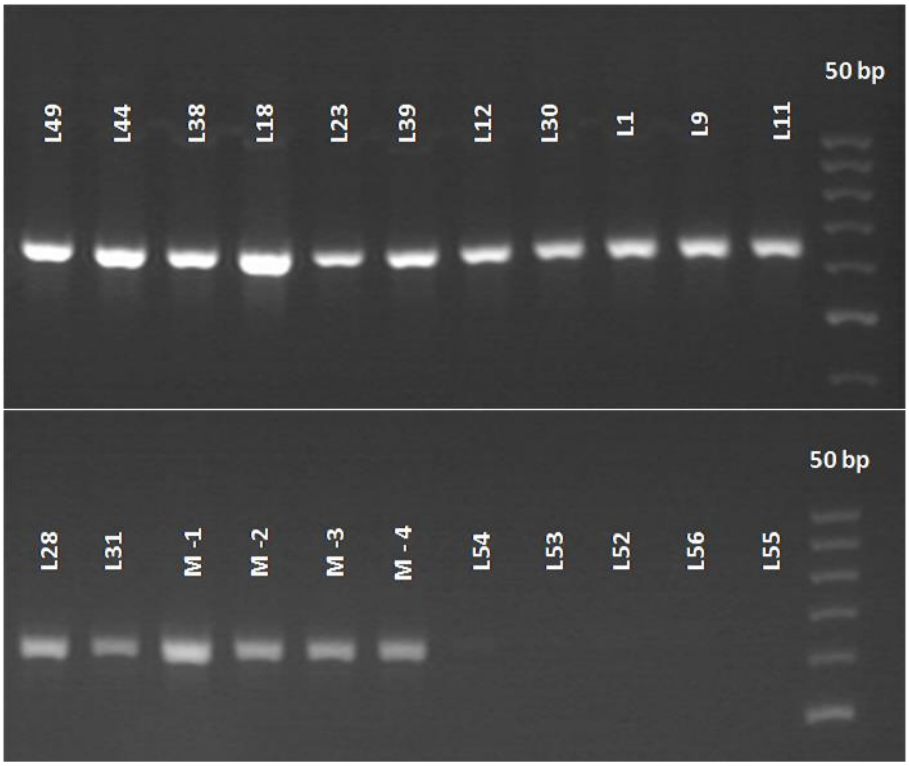

Figure 2. Molecular profiles of high oleic, low oleic and mutant sunflower lines obtained by amplification with F4-R1 (Schuppert et al., 2006) (DNA ladder 50 bp, Thermo Scientific)

In this study, we examined OAC in a set of sunflower lines and established that there is a great variation in OAC in comparison to studies performed by Lacombe et al. (2004), since in this research high oleic lines with OAC ranging from 83 to 91%. and low oleic lines with OAC ranging from 23 to 39% were used for molecular studies. The great variation in OAC could be explained by the fact that OAC is influenced not only by genetic background (Lancombe et al., 2001; Schuppert et al., 2006), but also by the environmental conditions, primarily temperature, but also by sowing date etc. (Triboï-Blondel et al., 2000; Flagella et al., 2002; Izquierdo et al., 2002; Del Gatto et al., 2015).

Molecular marker used in this study successfully identified high oleic genotypes and could therefore be used in marker assisted selection in IFVCNS. However, *Ol* mutation was detected in mutant lines that had lower OAC, as well. This means that molecular breeders should always be aware of the genetic background used in breeding and verify results with GC.

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SEASONAL TIME-COURSE OF EXPANSIN EXPRESSION IN FLOWERS AND GROWING GRAINS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Grain weight is a key component of yield and quality of sunflower. Taking into account that the ovaries of flowers became the pericarp of grains in grasses and dicots such as sunflower, it has been proposed that the maternal tissues impose a physical restriction to growing grains in these crops. The physiological processes supporting the hypothesis that the pericarp controls grain weight (GW) are only starting to be understood. Expansins (Expns.) are proteins that play a key role in plant cell growth by inducing the loosening of cell walls in plants, which determines the cellular growth expansion. The objective of the present study was to assess the seasonal time-course expression of Expn. genes in ovary, pericarp and embryo of flowers and growing grains of sunflower. Two sunflower genotypes contrasting in grain size and weight were sown in a split-plot design with three replicates at the Agricultural Research Station of the Universidad Austral de Chile in Valdivia, Chile. Ovaries and grains (divided in pericarp and embryo) were sampled at pre-anthesis and post-anthesis, respectively. Relative quantification of mRNA levels of Expns. was evaluated by qPCR. Final GW was different between genotypes (80 and 150 mg). Expression analysis by qPCR showed that specifics Expns. (Expn.3, 4 and 5) are associate better with grain growing dynamics in sunflower, and a variation in the expression of Expns. between genotypes contrasting in GW and in flower and grain tissues across the developmental stages.

Keywords: grain weight, expansin expression, yield, sunflower

INTRODUCTION

Expansins (Expns.) are proteins inducing cell wall extension (McQueen-Mason et al. 1992). They have been known as "factors that loosen the cell wall", allowing relaxation of the cell wall during cell expansion, playing a major role in growth. Expns. are involved in different physiological processes as the cell wall disassembly during fruit ripening (Brummel et al. 1999), organogenesis of leaves (Fleming et al., 1997), differentiation of vascular cells (Cho and Kende, 1998) and root system architecture (Ma et al., 2013; Marowa et al., 2016); however, there is little information about the role of Expansins in specific tissues like grains.

A recent study shows the evolutionary divergence between classes of Expns. especially α and β groups in grass and dicots plants, due to the difference in the composition of the cell wall,

highlighting that the β Expns. family has expanded considerably in grasses (Sampedro et al., 2015). This was important to consider when characterize Expns. in reproductive structures of sunflower.

In wheat, it has been estimated that there are at least 30 α and 65 β Expns. Generally, a multigene family suggests that different members may play unique developmental or tissue-specific roles. This may be the situation of the α Expn. group, which has shown organ or development stage specificity in wheat (Liu et al., 2007). The present study hypothesizes that some Expns. will be specifically involved in the pericarp extension, and that their expression could be related to grain growth dynamics in sunflower. Previous experiments found expression of 6 different Exp. cDNA sequences in pericarp tissues of wheat at 14 days after anthesis. Out of the 6, 4 matched known wheat Expn., whereas the other 2 were novel wheat Expns., similar to sequences described for *Festuca pratensis* and *Oryza sativa* (Calderini et al., 2006; Lizana et al., 2010).

A strong relationship between grain size dynamics, water content and Expn. expression was found in wheat grains during grain filling. Water content of grains was consistent with high abundance of Expns. gene transcripts (Lizana et al., 2010). Plant cell expansion is turgor-driven and regulated by cell wall mechanical properties and is related to Expns. proteins (Cosgrove, 2015), a key component in grain enlargement according to Lizana et al. (2010).

From the background outlined above, the expression of TaExpA6 transcript is consistent with grain elongation in wheat (Lizana et al., 2010). Therefore, the following questions arise:

i) Are there Expns. associated with grain growth in sunflower?
ii) Is the elongation of the pericarp and embryo driven by different Expns.?
iii) Is the timing of Expn. expression similar between the pericarp and embryo?

The objectives of this work was to identify the expression of Expns. And their time course in ovary, pericarp and embryo of sunflower during the growth of the reproductive organs.

The study of Expns. at both pre- and post-pollination and their relationship with the dynamics of grain growth will be key to a better understanding of processes controlling GW. It will also make it possible to establish the relationships among dry matter, water content, and Expns. expression in flowers and growing grains of sunflower.

MATERIALS AND METHODS

Plant material and field experiment

Two sunflower genotypes contrasting in GW and adapted to the south of Chile, Alybro (oilseed) and RHA280 (confectionery) were sown under field conditions at the Experimental Station of the Universidad Austral de Chile in Valdivia (39◦47′S, 73◦14′W). The genotypes were laid out in a randomized complete block design with three replicates. Sowing date were 20 of October as in previous evaluations in Valdivia. Plots consisted of seven rows, 0.70 m apart and 5 m long with a plant population density of 6 plants m^{-2}. Plots were fertilized at sowing with N, P and K based on soil analysis, ensuring that the crop was not affected by any nutrient limitation. Weeds, insects and diseases were prevented or controlled, and regular watering was supplied to complement rainfall throughout the experiment to avoid water stress.

Phenology, flower and grain sampling

Phenology of the crop was followed during the growing season according to the scale by Schneiter and Miller (1981). Individual plants were tagged at R3 (Schneiter and Miller, 1981) to evaluate the seasonal time-course of development, dry matter, water content and dimensions of

flowers and grains as in previous studies (Rondanini et al., 2009). Grains were split into pericarp and embryo at each sampling. Two capitula per replicate were harvested at 2 or 3 days intervals, florets and grains were sampled from two places in the capitulum at each sampling date (grains from peripheral position). Harvested flowers and grains were immediately processed (to measure fresh weight and dimensions) or preserved into cryotubes and quickly immersed in liquid nitrogen for molecular analysis. Samples were stored at -80°C until processing.

Grain weight and dimension measurements

Four flowers and grains from the peripheral position were weighed after harvest at each sampling date to determine fresh weight. In the case of grains, they were immediately separated into pericarp and embryo and each component weighed. In early phases of grain development the embryo included the aqueous endosperm (present at the early stages of embryo development and consumed during the embryo growth). Plant material were dried for 72 h at 60°C to determine dry weight.

Absolute water content (mg) of flowers and grains (pericarp and embryo) were calculated as the difference between fresh and dry weight, while water concentration (%WC, on a fresh weight basis) was estimated as the ratio between absolute water content and fresh weight, expressed as a percentage. Flower and grain dimensions (length, width, and height) were recorded quickly after sampling using an electronic caliper; this was measured in four grains.

Molecular analysis: In silico analysis and primer design

The first objective was to identify *in silico* the Expns. sequence genes expressed in grain of sunflower. Public databases of sunflower genome (https://www.heliagene.org/) enabled a search for putative Expns. using the BLAST tool with *Helianthus annuus* trascriptome and Zinnia elegans Exp. 3 mRNA sequence (GenBank: AF230333.1) because this is a related species of sunflower. The sequences obtained from BLAST were evaluated using the bioinformatic tool option "expression patterns" making it possible to consider Expns. putative expression patterns in plant organs. Expns. sequences expressed mainly in grain were chosen.

Selected sequences were aligned using the program Clustal W2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to reveal the number of unique sequences. Sequences were searched against the non-redundant GenBank DNA and protein database using BLASTn and BLASTX (Altschul et al., 1990, 1997) and against the Uni Prot database resources using BLASTX. The best matches were used as the foundation for sequence identity-based annotations. Sequences were used in BLASTx searches to confirm that they correspond to Expn. Transcripts. In addition, nucleotide sequences were translated into protein with the ExPASy bioinformatic tool (http://web.expasy.org/translate/) to mark off the coding region for the design of specific primers. These primers were designed using the "PRIMIQUE" tool to detect different sequences of the gene family (Fredslund and Lange, 2007). Two primer pairs were chosen for the same sequence.

A bibliographic search were conducted for housekeeping genes of sunflower for use as an endogenous control to normalize the data for differences in input RNA and the efficiency of reverse transcription between the various samples. Primers reported by previous studies for elongation factor 1 (EF1), S19 protein, β-tubulin, actin, ubiquitin and 18S of sunflower grains were evaluated (Brunner et al., 2004; Layat et al., 2014; Meimoun et al., 2014; Oracz et al., 2008; Pramod et al., 2012).

RNA extraction and RT-PCR

Total RNAs were isolated with the RNeasy Plant Mini kit (Qiagen) according with the manufacturer's instructions. The kit provides a choice of lysis buffers depending on the amount and type of secondary metabolites in the tissue; therefore, the RNA extraction protocol should be standardized. The quality and concentration of RNA were measured by spectroscopy with Nanodrop (nd-1000, Thermo Fisher Scientific, USA).

The isolated RNA was pretreated with DNasaI. First-strand cDNA was synthesized from 250 ng RNA using the ImProm-II™ Reverse Transcription System. The oligo(dt)16-18 primer/template mix was thermally denatured at 70°C for 5 minutes and chilled on ice. A reverse transcription reaction mix was assembled on ice to contain nuclease-free water, reaction buffer, reverse transcriptase, magnesium chloride, dNTPs and ribonuclease inhibitor. 1u/µl of Recombinant RNasin® Ribonuclease Inhibitor was added. The template-primer combination was added to the reaction mix on ice. Following an initial annealing at 25°C for 5 minutes, the reaction was incubated at 42°C for up to one hour. The synthesized cDNA (20µl) was stored at -20°C. As a negative control, an RNA sample was replaced by water in this procedure.

Quantification of mRNA levels by using real time PCR (qPCR)

The PCR reaction was performed in a final volume of 25µL and containing 12,5µL Brilliant II SYBR Green PCR Master Mix (Stratagene, Agilent technologies), 1 µL 10 µM forward and reverse primers and 8,5 µL of sterile deionized water. After an initial DNA polymerase activation step at 95◦C for 10 min, the samples were subjected to 35 amplification cycles (95◦C for 15 s,60◦C for 15 s, and 72◦C for 15 s). No-template and no-transcriptase controls were included to detect genomic DNA contamination.

A melting curve was generated by incubating the reaction at 95°C for 15 s, 25°C for 1 s, and 70◦C for 15 s and then slowly increasing the temperature to 95 ◦C. The target gene expression was quantified with the method proposed by Livak (2001) by using the Agilent AriaMx software to calculate the transcript abundance relative to the calibrator, which becomes sample 1, and all other quantities are expressed as an n-fold difference relative to the calibrator.

After confirming the amplified specific products, a standard curve of each pair of primers was created with the product from the previous amplification. A dilution of 1: 1000 was prepared and then 7 serial dilutions were prepared by a factor of 10 starting from the 1:1000 dilution of the previously amplified product. This was achieve the efficiency of the primers.

Samples were subjected to service sequencing. Resulting sequencing chromatograms be viewed, evaluated and aligned. Sequence alignments and database searches were carried out using NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and https://www.heliagene.org/.

Statistical analysis

Final grain weight was estimated using a bilinear model as in Calderini et al. (1999). The model was fitted using the iterative optimization technique of Table curve V 3.0 (Jandel, 1991). Data of final grain weight and variables from grain growth dynamics were assessed by ANOVA using the software STATISTICA v. 7.0 (Stat Soft, Inc., 2004). The LSD test (5%) was employed for differences among genotypes.

RESULTS AND DISCUSSION

Grain weight and dimensions were significantly different between genotypes ($P \leq 0.001$) as we expected. Grains of the confectionery genotype (RHA280) were havier in RHA280 (149 mg) than in Alybro (79 mg) as well as grain dimensions (Fig. 1a and b). These differences were evident as early as 3 days after antehsis (DAA) (Fig. 1). Moreover, a linear association ($r^2= 0.77$)

between final grain weight and ovary weight was found in this study (data not shown). These results agree with previous studies where positive relationships between grain weight and the weight/size of carpels at anthesis have been reported in sunflower (Cantagallo et al., 2004), sorghum (Yang et al., 2009) and wheat (Hasan et al., 2011). Therefore, the pre-anthesis period has proven critical to determining GW (Calderini et al., 1999; Ugarte et al., 2007). RHA280 had a mean of 465 filled grains and 184 empty grains per capitulum, and Alybro, 1497 filled grains and 80 empty grains per capitulum (data not shown).

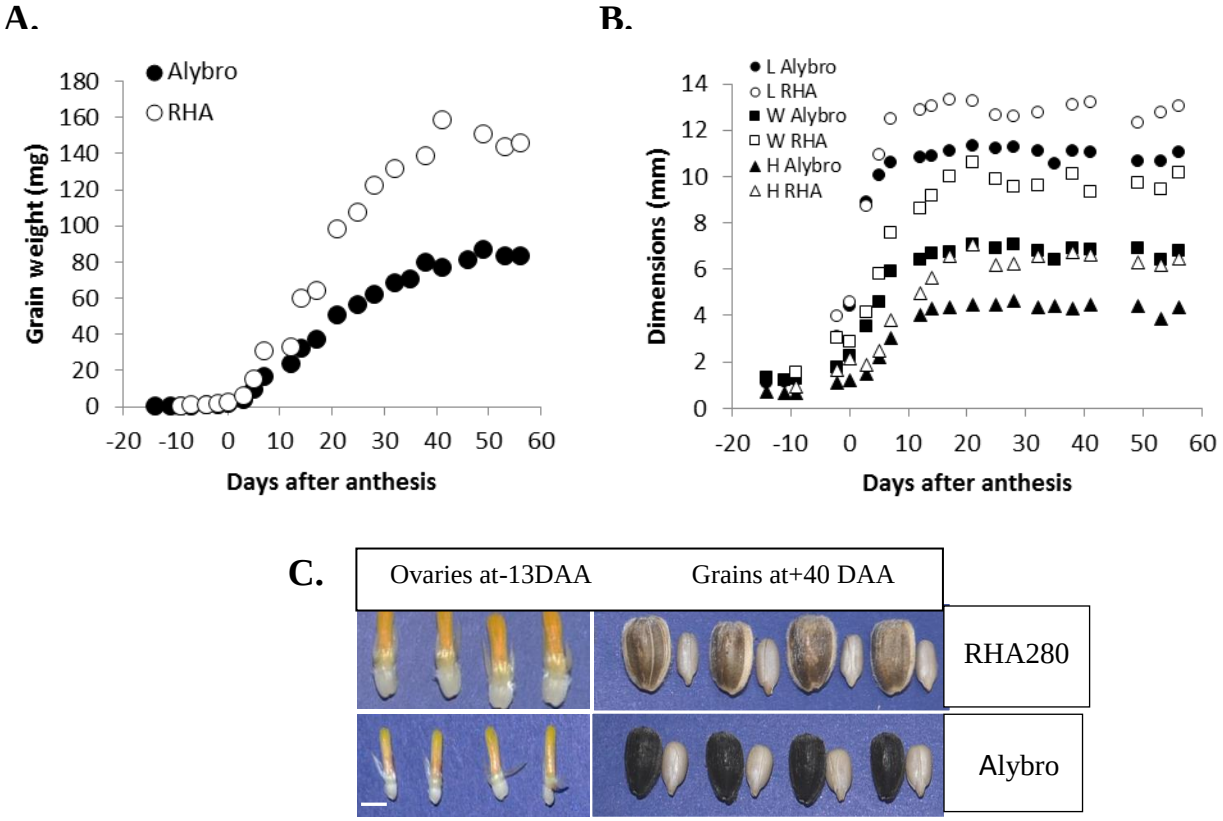

Figure 1. **Time-course of grain weight and grain dimensions of peripheral grains of two sunflowers genotypes (RHA280 and Alybro).** Dynamics of grain dry weight (A) and dimensions (B) of RHA280 (open symbols) and Alybro (closed symbols) are shown. The photos of ovaries (-13 DAA) and grains (+40 DAA) of both genotypes are also shown, Scale bar: 4 mm. (C). Grains were separated into pericarp and embryo. L: Length, W: width, H: high.

The expression of three putative Expns. Genes, named by us Expn. 3, Expn. 4 and Expn. 5 (accession in Heliagene database: Ha412T4l900C0S1, HaT13l007346, HaT13l009552, respectively) according to the name of the similar Expn. sequence with a maximum value of identity in the results of a BLAST, were chosen and evaluated by qPCR along the development of the reproductive organs in both genotypes.

Taking into account the dynamics showed in Figs. 1 and 2, it was found that some Expns. could be specifically involved in the grain tissues extension, suggesting their expression would control grain size of sunflower. For example, Expn. 4 follows a similar time course than the grain growth dynamic of genotypes (Fig. 2). Lizana et al., 2010 showed that the expression of few Expns. is associated with grain elongation when the grain is growing in wheat. The present is the first study showing parallelism between growing grains and Expns. expression in grain tissues of sunflower.

In addition to the time course of expression, specifics Expns. Seem to be controlling the extension of ovary, pericarp and embryo. Expn. 3 and Expn. 4 was found specific to maternal tissues (ovary and pericarp) and Expn. 3 was more abundant in the pericarp. On the other hand, Expn. 5 was found more abundant in the embryo (Fig. 2). The timing of Expn. 3 and Expn.4 in the pericarp showed a higher abundance at +7 DAA when in sunflower, the growth of the pericarp levels off soon after flowering, i.e., 8 days after anthesis at R5.1 (Rondanini et al., 2009; Lindström and Hernández, 2015). Interestingly, the confectionery genotype showed higher abundance of Expns. genes later than the oil genotype Alybro (Fig. 2), suggesting that Expns. isoforms control the growth in flowers and grains of sunflower. Furthermore, it has been hypostatized that the pericarp imposes a physical restriction to growing grains in grasses (Calderini et al., 1999; Ugarte et al., 2007; Yang et al., 2009), which might explain the importance of the pre-flowering phase for GW determination.

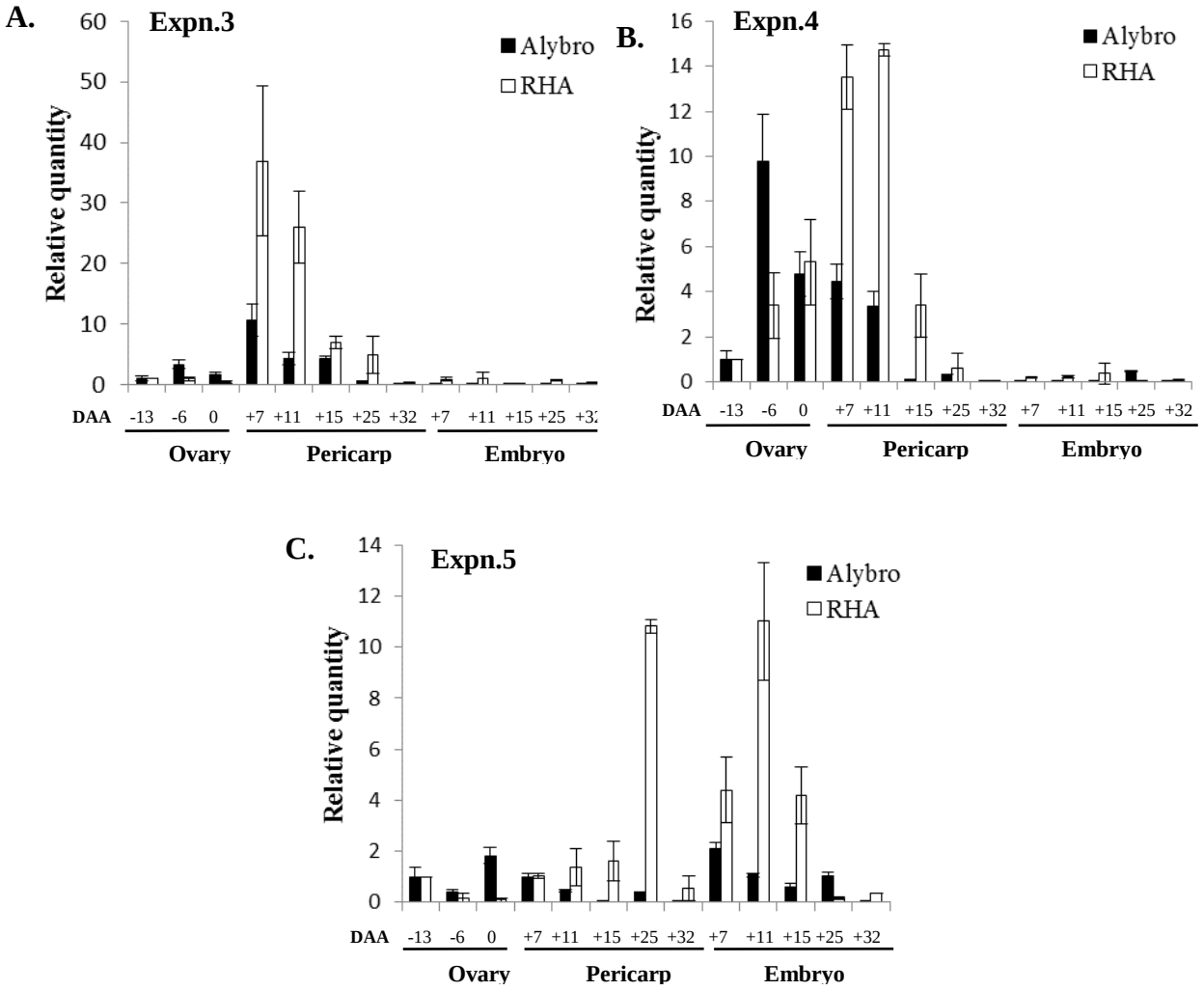

Figure 2. Seasonal time-course of expansin expression in ovary, pericarp and embryo of sunflower. A, B, C. Relative quantity analysis of three expansins genes, this target genes were quantified and normalized to β Tubulina using the Livak method (n=3).

In dicots, such as the model plant *Arabidopsis thaliana*, the control of GW by the growth of the inner integument during ovary and early phases of seed development has been demonstrated,

supporting the hypothesis that potential GW is controlled by the outer seed tissues (Adamski et al., 2009; Fang et al., 2012; Xia et al., 2013; Du et al., 2014). Remarkably, ovary mass at anthesis associate with final pericarp mass across both sunflower genotypes (see Table 2 in Lindström and Hernández, 2015). The present study support the hypothesis that the Expns. play an important role in the extension of grain maternal tissues. The importance of the pericarp on early grain development of wheat was stated many years ago (Rijven and Banbury, 1960), but the physiological processes through which the pericarp controls the final size of the grain is only now beginning to be understood (e.g., Garcia et al., 2005; Léon-Kloosterziel et al., 1994; Schruff et al., 2006; Song et al., 2007).

In sunflower and other crops, such as wheat, sorghum and coffee tree, maternal tissues (ovary/pericarp) have been proposed to control the potential grain size (Calderini and Reynolds, 2000; Yang et al., 2009; Budzinski et al., 2011; Lindström et al., 2006). In these crop species, the maternal tissues undergo rapid cell wall division and expansion, which in turn might determine the physical limit of the endosperm or embryo of the fruit (Budzinski et al., 2011). During the rapid cell wall expansion is where the Expns. proteins are acting.

In a previous study, transcripts of CaExpA2 were detected only in the pericarp of coffea fruit during the later stages of fruit maturation and ripening, highlighting the participation of these isoforms in the regulation of fruit size (Budzinski et al., 2011). Another study addressing fruit elongation showed two Expns. genes (ExpA4-a and ExpA5) inducing fruit length in cucumber, suggesting that the cell-wall related genes are required for fruit elongation in these species (Jiang et al., 2015). Studies by Harrison et al. (2001) found two Expns. in strawberry highly expressed during the process of rapid expansion of immature fruit, while four were expressed mainly during fruit ripening. In agreement, similar results were found in peach (Hayama et al. 2003), pear (Hiwasa et al. 2003) and banana (Asha et al. 2007), showing that high levels of mRNA coding for Expns. were observed in rapidly growing tissues, and multi-gene family of Expns. exhibited differential expression patterns for the different phases of fruit development. In the light of results shown in the present and previous studies, we propose Expn. 4 as a driver of grain growth in sunflower.

CONCLUSIONS

The analysis of results showed in this study supports different expression of Expns. genes between genotypes and across the developmental stages of flower and grain growth. The expression of Expns. variable in ovary, pericap and embryo tissues.

Expns. genes associated with grain dynamics would contribute to a better understanding of mechanisms controlling grain size in sunflower, especially Expn. 4, it is a good candidate for future research.

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CHARACTERISATION AND MAPPING OF A LOCUS CONTROLLING LIGHT-YELLOW RAY FLORETS IN SUNFLOWER

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ABSTRACT

In Sunflower, Ray Florets (RF) color may be yellowish-white, light-yellow, medium-yellow, orange-yellow, orange, purple or reddish brown. However among this high variability of colors, the most widespread encountered in Elite germplasm is medium-yellow. In Syngenta germplasm, some lines display a light-yellow coloration of RF. This light-yellow trait is referenced as a DUS (Distinctiveness, Uniformity, and Stability) trait by official instances for registration. It has been found by Fick (1976) and confirmed by Sharypina *et al*. (2008) that a lemon-yellow RF coloration is inherited according to the principle of recessive epistasis with respect to yellow coloration. Whether or not light yellow and lemon yellow colors are similar is not clearly established. Yue *et al*. (2008) proposed that two recessive genes Yf1 and Yf2 control the occurrence of lemon-yellow RF. The authors were able to map one of the genes controlling the lemon-yellow RF trait Yf1 on linkage group 11. In the present work, an F2 population segregating for light-yellow RF was developed. The observed ratio in our F2 population is in accordance with the hypothesis of one single gene. We were able to establish its position on linkage group 3. A co-dominant SNP molecular marker linked to the gene was developed and is disclosed to the community.

Key Words : Ray Florets color, Genetic Mapping, Single Nucleotide Polymorphism

EXPRESSION PROFILES OF DROUGHT INDUCED WRKY TRANSCRIPTION FACTORS IN SOME SUNFLOWER CULTIVARS; MICROARRAY DATA ANALYSIS

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ABSTRACT

Drought or water scarcity is an unbalanced state between the water availability in soil and its physiological demand by plant. Drought stress significantly inhibits the plant growth and development, and reduces the crop productivity as well as it is predicted to become more severe in years to come. Sunflower is mainly regarded as an oil crop able to grow under water-scarce conditions. However, not all sunflower genotypes are homogeneous in water usage regimes under water available and scarce conditions. Thus, this study aimed to analyze the gene expression profiles of drought induced *WRKY* transcription factors (TFs) in eight sunflower (*Helianthus annuus*) cultivars such as Inedi, Melody, Tekny, SF028, SF107, SF109, SF193 and SF326. Two different drought stress scenarios such as Fixed Duration Stress (FDS; 7days/0-0.57 FTSW) and Fixed Intensity Stress (FIS; ≥7days/0-0.09 FTSW) were implemented to understand the *WRKY*s-drought relationship in sunflower plants. Sunflower Affymetrix microarray with 96 samples, including 32423 probesets were retrieved from NCBI GEO Data Sets (access. GSE25719). For array analyses, sunflower cultivars were grown in pots filled with 15 L of substrate, including 50% clay loam, 40% potting soil and 10% sand in greenhouse at 17oC/night and 20-25oC/daylight temperatures. 96 pots were arranged in six blocks, each containing 16 plants with eight treatment and eight control groups. All pots were daily watered and three times fertilized for 25 days before drought stress treatment. A total of 18 putative *WRKY* TFs were analyzed in eight drought-induced sunflower cultivars. Hierarchical clustering analysis demonstrated that expression profiles of *WRKY4* (Heli013712 and Heli000574) and *WRKY30*(Heli009222) TF genes show more divergence from others. Besides, SF028 (FIS), SF107 (FIS), SF326 (FIS) and Tekny (FDS) cultivars demonstrated more similar clustering pattern. Revealing the gene expression profiles of*WRKY* TFs in various sunflower cultivars under different water regimens will provide valuable insights to elucidate the drought-induced transcriptional regulatory elements or mechanisms in plants, with purposes of enhancing the crop productivity and yield.

Key Words : Water scarcity, FDS, FIS, genotype, WRKY, cultivar

HIGH THROUGHPUT GENOTYPING TOOLS IN SUNFLOWER

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In the frame of the SUNRISE project (http://www.sunrise-project.fr), 72 sunflower lines have been re-sequenced at an average depth of 10X with paired-end HiSeq sequences. The mapping of the reads on a reference genome sequence (XRQ line), identified 6 348 868 SNPs. We sampled 586 985 SNPs located on 14 129 genomic scaffolds. These SNPs were selected to maximize the diversity and to have at least one polymorphic SNP per genomic scaffold for each of 18 recombinant inbred lines populations designed to perform nested association mapping. All SNPs were used to produce an AXIOM® genotyping array. This tool allows to genotype 96 samples simultaneously. From genotyping data obtained with this 600k array, we sampled a subset of 49 449 SNPs located on 14 021 genomic scaffolds. These 49 449 SNPs were selected for the production of another AXIOM® array that allows the simultaneous genotyping of 384 samples. These 2 high throughput genotyping tools will help to better characterize the sunflower genetic resources and will accelerate sunflower breeding.

Key Words : genotyping, sunflower, High-throughput, SNP, polymorphism

MAS SELECTION ON OLEIC TYPE SUNFLOWER BREEDING

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Oleic type sunflower is new trend in sunflower production in the world. Its market is increasing year by year recently. On the other hand, new oleic type hybrids are developing and releasing into the market. High Oleic (HO) trait is controlling a gene calling *Ol* utilized and obtained from mostly to a high oleic mutation of variety Pervenets by Soldatov 1976. Although, there are some other sources for high oleic content up to 90% from Bulgaria and Italy, etc. Pervenets mutations are using worldwide as the donor to develop high oleic content inbred lines and hybrids in sunflower breeding programs. However, as a seed trait, oleic type plants could determine after harvest so it needs to wait until seed tests to select oleic types until seed trashing. However, when applied MAS analysis high oleic plants could be determined as much as early stages so it helps extremely to breeders both reducing costs and time wasting and also accurate selection. Different RAPD, SSR (microsatellite) markers were determined until today in different studies. These studies enabled to detect the genetic behavior of high oleic of QTL and linked markers efficiently then leaded to use of molecular tools practically in sunflower breeding programs. On the other hand, PCR analysis with HO specific fragments enabled to amplify either the Pervenets mutation itself or the polymorphism of the SSR locus (TTA repeat variability) located on the Δ12-desaturase gene intron. These markers lead to discriminate genotypes carrying Pervenets mutation and genotypes without mutation. Consequently, the HO PCR specific fragments or SSR markers may be used in selection programs to identify genotypes carrying the Pervenets mutation. However, these markers need further validations in different genetic sources to classify sunflower genotypes accurately based on their oleic acid contents. For example, the length of the SSR depend on the lines that have been used to convert the LO in HO. Therefore, amplified SSR locus should be sequenced from different progenies, because the SSR size estimation may vary depending of the plants and of the PCR reaction. Furthermore, HO PCR specific fragments could not able to distinguish homozygous HO genotypes from Heterozygous HO genotypes so this type primers may be used first selecting HO genotypes (both homozygous and heterozygous) and then extra selection with SSR markers should be done further. As results, further studies need on MAS selection in oleic acid content in sunflower and not dependable to genetic background, practical and widely used molecular markers determining HO in the breeding programs broadly were not released yet for public interest and uses.

Key Words : Sunflower, MAS, Oleic type, breeding

DNA MARKER DETECTION OF DOWNY MILDEW (PLASMOPARA HALSTEDII) RESISTANCE IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Downy mildew, caused by *Plasmopara halstedii*, is one of the most destructive diseases in cultivated sunflower (*Helianthus annuus* L.) responsible for significant yield loss. The development and testing of DNA markers of important agronomic traits and in particular markers of resistance to downy mildew is considered to be one of the most priority tasks for breeding resistant sunflower to downy mildew. Resistance for *Plasmopara halstedii* is inherited and provided by dominant genes. Some of downy mildew resistance genes were characterized, but only a few of them were associated with molecular markers. The study was carried out on five different sunflower crosses as parents and their F2 individuals developed by Thrace Agricultural Research Center in Edirne. To perform the molecular genetic analysis, genomic DNA was isolated from leaf tissues. Genotyping of these materials has been currently carried out using 117 SSR of 4 *Pl*-loci including *Pl6*, *Pl8*, *Pl13* and *Plarg* associated with the resistance of sunflower to downy mildew. Further studies with genotyping and validation of resistance will be especially promising for the marker-assisted selection of sunflower with respect to resistance to the downy mildew. This research has been supported by TUBITAK TEYDEB 1501 Program (Project No: 3150030).

Key Words : Sunflower, downy mildew, SSR-markers, marker assisted selection

THE MOLECULAR GENETIC DIVERSITY OF THE BROOMRAPE (*OROBANCHE CUMANA* WALLR.) POPULATIONS OF TURKEY

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) which is a holoparasitic plant infecting the sunflower roots is considered as one of the most important constraints for sunflower production in Turkey. The study of natural *Orobanche* populations can help us to understand gene pool dynamics, population size structure and geographical distribution, environmental adaptation and centers of origin. Since the pathogenic composition of broomrape populations has changed over the years, nucleic acid markers provides a clear advantage according to morphological traits due to the stability and the power to identifying new broomrape races. The objective of this research is to study the genetic variation of *Orobanche cumana* Wallr. populations in order to (1) determine the genetic differences between them, (2) infer the inter and intra-population variability and (3) to determine the geographical influence on genetic diversity mainly from Thrace and Adana regions of Turkey by simple sequence repeats. Two field expeditions were conducted along the Thrace region and Adana, where the distribution of *O. cumana* in the wild has been seen, to collect fresh tissue and mature seeds of twenty seven *O. cumana* populations. Orobanche floral buds were used for DNA extraction for SSR analysis. Genetic diversity and population structure are currently being studied with SSR markers in *O. cumana* populations. Obtained results will led to better understand the evolution of parasitic plants and contribute to the establishment of improved crop breeding and management strategies for *O. cumana* control. This research has been supported by TUBITAK BIDEB (Program No: 2216).

Keywords: *Orobanche cumana*, SSR markers, parasitic plants, genetic diversity.

THE DEVELOPMENTAL FEATURES OF THE OVULE AND EMBRYO SAC IN THE HERMAPHRODITE FLOWERS OF *HELIANTHUS ANNUUS* L.

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INTRODUCTION

The inflorescence of *Helianthus annuus* L. has two types of flowers in a single capitulum; central hermaphrodite disc flowers and peripheral pistillate ray flowers (Herman, 2000; Cvejić et al., 2016). In the present study, the developmental features of the ovule and embryo sac were investigated.

MATERIAL AND METHODS

The capitulum at various stages of development were collected from Tekirdağ (Turkey). Firstly, the diameter of capitulum were measured, hermaphrodite flowers were morphologically analysed by stereomicroscope (Olympus 970931). The samples were prepared for light microscope analysis. The material was fixed in acetic-alcohol (1:3, v/v) and embedded in paraffin blocks. The blocks were sectioned at 8-15 μm by Leica RM2235 rotation microtome and sections were stained with hematoxylin. The preparations were photographed with an Evolution LC color camera and an Olympus BH-2 microscope, and the images were analyzed with Image-Pro Express Version 6.0 scientific image processing and analysis software. For SEM analysis, the plant material was fixed in 2.5% glutaraldehyde in 50 mM cacodylate buffer, pH 7.0 (Platt *et al.*, 1983) and then dehydrated with an increasing ethanol gradient: from 70% up to 100%. Then, the material for drying were kept in various percentages of ethanol-HMDS solution at room temperature (Topçuoğlu *et al.*, 2009). Then, coated with 11 nm of gold by using an automated sputter coater and then examined with a SEM (JEOL JMS-59 10LV).

RESULTS

In *Helianthus annuus* L., a hermaphrodite flower contains a pistil with inferior type of ovary which lies below the attachment of other floral parts. The ovule shows basal placentation. It differantiates as small and homogeneous mass on the ovary and consists of the cells with dense cytoplasm and small nuclei. The ovule is unitegmic type, namely have one integument. This situation occurs with the disappearance of one of the integuments or with the merge of the two integuments. Together with the differantiation of the integument and the megaspore mother cell, the funiculi which contacts the ovule to ovary begins to curl. The ovule becomes anatropous at megaspore tetrad stage. The micropyle is long and narrow.

The ovule is *tenuinucellate type. Namely,* megaspore mother cell differantiates just below the nucellar epidermis. The nucellar cells expand and create a loose tissue around the embryo sac. The nucellar tissue can be observe in the mature ovule.

The development of female gametophyte conforms to Polygonum type. Megaspore mother cell produces linear megaspore tetrad by regular meiosis. Functional megaspore which is the the largest one locates at the chalazal part. It undergoes three successive mitosis and forms 2, 4 and 8 nucleated embryo sac, respectively. Two nuclei locate the chalazal part and the other two nuclei locate the micropyle part in 4 nucleated embryo sac. The mature, 8 nucleated embryo sac

contains an egg cell, two polar nuclei, three synergid cells and three antipodal cells. The egg cell and two synergids locate the micropyllar part of the embryo sac and a cell wall is formed around the this cells. This structure is called egg aparture. The egg cell is bigger than the synergids and locate between the synergids. In the egg cell, there is a nucleus in the chalazal part and a big vacuole in the micropyle part of the cell. The cytoplasm is a thin layer and locate only on the periphery. The polarization in the synergid cells is the opposite. Althought the nuclei are found in the base, vacuoles are found in chalazal part. Antipodal cells are smaller than the other cells of the embryo sac. The synergids and the antipodal cells are ephemeral. They become blunt after the fertilization.

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BIOTIC AND ABIOTIC STRESS TOLERANCE

EVALUATION OF SUNFLOWER GENOTYPES TO STEM ROT CAUSED BY SCLEROTINIA SCLEROTIORUM UNDER FIELD CONDITIONS

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ABSTRACT

Stem and root rot caused by *Sclerotinia sclerotiorum* is the most important devastating disease of confectionary sunflowers in West Azarbayjan province of Iran particularly Khoy area. To evaluate reaction of confectionary and oilseed sunflowers against the disease, 76 genotypes including both types were inoculated by the pathogen at grain filing stage. The isolate 302 collected from the infected area (Khoy) and mass produced, was used for the experiment. Seven millimeter mycelial plugs of PDA medium including 3-day-old culture of the pathogen were put on injured site of the individual plants at 40 centimeter height. A small piece of wet cotton and two layers of Parafilm for maintaining moisture and fixing the fungal plug were employed for all treatments. The lesion length of inoculated stems was measured seven and 14 days post inoculation. The results of data analysis demonstrated significant differences of lesion length and single head yield between the genotypes. Line S53 with mean 63 millimeter lesion length and S6B with 13 millimeter lesion length demonstrated the most and least progress and infections, respectively. The local land races of confectionary sunflower including Shamshiri and Badami were more susceptible in comparison with Pestei ones against the disease.

Key Words : confectionary sunflower, *Sclerotinia sclerotiorum*, reaction.

ADVANCES IN HOST PLANT RESISTANCE TO SUNFLOWER INSECT PESTS IN NORTH AMERICA

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ABSTRACT

Resistance of wild (annual or perennial) sunflowers to insect pests is a recurring idea in the scientific literature. Though wild sunflowers may often be refractory to certain insect pests, recent research illustrates that cultivated sunflower germplasm also has considerable variation in susceptibility to insects, and that many opportunities exist to exploit host plant resistance in cultivated sunflowers. For the sunflower moth (*Homoeosoma electellum*), there are very high numbers of capitate glandular trichomes available in inbred lines and progress is being made in mapping the genes that determine trichome number. Though there are differences in the composition of secondary plant compounds in the glandular trichomes of wild and cultivated *H. annuus*, the contents of cultivated sunflowers retain toxicity or repellency to sunflower moth larvae. For the red sunflower seed weevil (*Smicronyx fulvus*), strong resistance in breeding material exists, but it not well understood. Development of germplasm and mapping of the weevil resistance is ongoing. Lastly, there is significant variation in susceptibility of sunflower inbreds to the banded sunflower moth (*Cochylis hospes*). While some public inbreds appear to be as refractory as previously identified cultivars, it is not clear whether resistance of inbred parents will translate into equivalent or better resistance in hybrids. Specific mechanisms of resistance are not well understood in all cases, but the traits found in resistant accessions and inbreds identified for North American insects should be applicable to some pests in other parts of the world.

Key Words : sunflower moth, banded sunflower moth, red sunflower seed weevil, antibiosis, glandular trichomes

DISTRIBUTION OF PLASMOPARA HALSTEDII PATHOTYPES IN HUNGARY

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ABSTRACT

The oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni, the causal agent of sunflower downy mildew, is one of the most damaging diseases in the world. There are several pathotypes (at least 36) of the pathogen which greatly influence both the effectiveness of fungicides and the resistance genes incorporated into new sunflower hybrids to combat this disease. The number of *P. halstedii* pathotypes is increasing rapidly. Recently, new pathotypes, 704 and 714, have been identified in Hungary, while as many as 5 races (100, 330, 700, 710, 730) were distributed before 2010 in the country. Our objectives are to continuously monitor this pathogen and identify pathotypes of *P. halstedii.* Samples were collected at 20 different sites in Hungary between 2012-2014 from sunflower hybrids containing *Pl6* resistance gene.and from volunteer plants. Examination of isolates was carried out using a set of sunflower differential lines based on the internationally standardized method for race identification of *P. halstedii*. Disease assessment was first performed based on the appearance of white sporulation on cotyledons. A second evaluation of true leaves was made on 21-day-old plants. According to our results six different pathotypes of the pathogen were determined. Pathotypes 704, 714 and 700 were the most widespread while 730 and 710 were also common during the examination period. Occurrence of a new pathotype, 734, is also suspected but not proved during the survey. Thus, continuous survey and identification of the virulence phenotype of *P. halstedii* is essential for sustainable sunflower breeding and plant protection.

Key Words : sunflower, downy mildew, races.

THE EFFECTS OF APPLIED HERBICIDES ON YIELD AND OIL QUALITY COMPONENTS OF TWO OLEIC AND TWO LINOLEIC SUNFLOWER (*Helianthus annuus* L.) HYBRIDS

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ABSTRACT

The aim of the present study was to investigate the effect of application at the recommended dose by their manufacturer of five herbicides including different *active* ingredients herbicides on seed yields, some morphological and phenological characters, oil content of seed and oil fatty acid profiles of two oleic and two linoleic sunflower hybrids. One of oleic and linoleic hybrids were tolerant to the *imazamox* herbicide as the Clearfield trait. The experiments were conducted under field conditions during 2014 and 2015 in Lüleburgaz, Turkey. Used five traditional herbicides having different active ingredients as pre-plant, pre-emergence or post-emergence were Bonoflan WG with benfluralin, Stomp® Extra with pendimethalin, Challenge 600 with aclonifen, Targa Super with quizalofop-p-ethyl and Intervix® Pro with imazamox active ingredients. Intervix® Pro traditional herbicide with imazamox active ingredient decreased in plant heights of two Clearfield hybrids (LG 5542 CL and Colombi) about 10% in both years. This herbicide had insignificant negative effects on seed oil content and oleic aid ratio of Clearfield sunflower hybrid "LG 5542 CL" in 2014. Stomp® Extra with pendimenthalin active ingredient applied pre-emergence herbicide decreased the number of days to flowering of linoleic sunflower hybrid "64LL05" and oleic sunflower hybrid "64H34" in 2014 and 2015. In the other hand, Bonoflan WG with benfluralin application to "64H34" cultivar had the highest seed yield in 2015. Herbicide residue in harvested seeds of all applications was not detected upper than limits.

Key words: Fatty acids, herbicide application, sunflower, oil content, yield components

INTRODUCTION

Weed control in crop production is very important. Crop yield decreases from 10% to 40% were observed when the sunflower was weeded during the first four weeks after emergence (Wanikorn, 1991; Delchev and Georgiev, 2015). The most common preferable method by the farmers for weed management is chemical application which is economical in short term and faster result to get rid of weeds. Agrochemicals are looked upon as a vehicle for improved crop production technology though it is a costly input. Pesticide use has become inevitable in modern agriculture, and increased several folds during the last four decades Over use of these chemicals have severe effects on environment that may lead to an immediate and long term effects (Sequtowski and Kortekamp, 2011; Bhandari, 2014)

There are so many study made on effects of pesticides on environment and human healthy. Degradation of these compounds in the environment and extensive or inappropriate use by farmers can lead to the contamination of various ecosystems. Widespread distribution of pesticides is also known to cause problems to the apiculture industry and in surface waters (Centner and Nicholas Eberhart, 2014; Lari et al., 2014). Severe effects of herbicides have also been announced in agency reports of International Organizations (EPA, 1996; FAO, 2013; EFSA, 2014).

Many pesticides are harmful, and cause to death for bee population. There are some pesticides that kill the bees directly. Since bees are the most important pollinators of crops, the use of pesticides can considerably reduce the yield of cross pollinated crops. Bees may be contaminated by pesticide residues during harvesting and contaminants can be transported on bee bodies or with forages to the hive, from where they can be transferred into honey (Ünal et al, 2010; Bargańska et al, 2014).

The persistence of pesticides in soil and their residual effects on sequential crops have been reported by many researchers (Wicks et al, 1969; Demircioğlu and Maden, 2007; Anonymous, 2009; Süzer and Büyük, 2010; Baranski et al, 2014: Serim and Maden, 2014). These chemicals (herbicides) are also caused some damage to crops or non-target plants due to wrong usage with technical implementation (Torun and Uygur, 2011).

The most of part of previous researches have been on the effects of the application of herbicides for weed control in weed-crop competition. Some of them were on crop productivity, and the grain yield and the oil content of sunflower seeds were also measured in order to confirm the importance of successful weed control (Simic et al., 2011; Reddy et. al., 2012; Knezevic et al., 2013.Petcu and Ciontu, 2014; Jursik et al., 2015; Suryavanshi et. al., 2015)

There were vanishingly small number research to determine the effects of herbicides at the normal dose on the yield and quality components of sunflower. The aim of the present study was to investigate the effect of application at the recommended dose by their manufacturer of five herbicides including different *active* ingredients on seed yields, some morphological and phenological characters, oil content of seed and oil fatty acid profiles of two oleic and two linoleic sunflower hybrids.

MATERIALS AND METHODS

The data presented in this paper were collected as a part of a larger study to investigate the effect of application of five herbicides including different *active* ingredients on the some agronomic characters of sunflower in 2014 and 2015. Field experiments were conducted on farmer fields in Karamusul village (41º 24′ N, 27º 21′ E, elevation 46 m) of Lüleburgaz, Kırklareli at one of main sunflower-growing regions of Turkey. Some properties of experimental area soils were given in Table 1. Soil properties in both year were similar. The had clay loam texture. There was no any important problem in soil properties of experimental areas for sunflower production.

Table 1.Some chemical properties of the experimental field soil

Year	SOM (%)	PH	Lime (%)	Salt (%)	N (%)	P_2O_5 ppm	K ppm	Ca ppm	Cu ppm	Fe ppm	Mn ppm	Mg ppm	Zn
2014	1.92	6.7	0.57	0.07	0.08	14.28	212	4225	1.88	12.13	15.16	414	2.23
2015	1.88	6.8	0.68	0.08	0.09	14.36	228	4313	1.79	12.48	15.38	432	2.33

SOM = soil organic matter

Table 2 shows some meteorological data during two growth season. The second year was very dry after R6 growing period although rainfall was fairly well and steady during almost all vegetative and reproductive growth period in 2014.

Table 2.Climatic data during growing periods of Sunflower in 2014 and 2015

Month	Rainfall (mm)		Relative humidity (%)		Temperature (°C)	
	2014	2015	2014	2015	2014	2015
April	47.0	69.8	83.6	75.3	12.5	11.1
May	80.0	5.8	79.9	69.5	16.9	18.8
June	51.4	42.8	76.2	69.2	21.2	21.3
July	131.6	4.8	73.4	65.3	23.8	24.5
August	19.2	2.6	73.8	63.1	24.2	25.3
September	121.4	63.0	81.8	74.2	18.9	21.8

Table 3 shows some properties of cultivars in this study. Two high oleic and two high linoleic sunflower hybrids were used. One of oleic and linoleic hybrids were tolerant to the *imazamox h*erbicide as the Clearfield technology.

Tablo 3. Some properties of sunflower cultivars in this study

	Sunflower cultivar	Seed company	Clearfield/non-Clearfield	Oil fatty acid profile
1	LG 5542 CL	Limagrain	Clearfield	High Linoleic
2	64LL05	Pioneer	non-Clearfield	High Linoleic
3	Colombi	Syngenta	Clearfield	High Oleic
4	64H34	Pioneer	non-Clearfield	High Oleic

Active ingredients, application rates, application times and trade names, manufacturer of herbicides.were given in Table 4. All herbicides were applied by backpack sprayer at the recommended dose from their manufacturer. “Intervix® Pro” was applied to Clearfield cultivars “LG 5542 CL and Colombi”. Other four herbicides “Bonaflan WG, Stomp® Extra, Challenge 600 and Targa Super” were applied to non-Clearfield cultivars “64LL05 and 64H34”. Each cultivar also had a control “untreated” plot for each replication.

Table 4. Active ingredients, application rates, application times and trade names, manufacturer of herbicides.

	Trade name	Manufacturer	Active ingredient	Dose (ml/ha)	Application time
1	Bonaflan WG	Dow AgroSciences	Benfluralin, 60 g/ltr	2500	Pre-Plant
2	Stomp® Extra	BASF	Pendimethalin, 450 g/ltr	3000	Pre-Emergence
3	Challenge 600	Bayer	Aclonifen, 600 g/ltr	1250	Post-Emergence
4	Targa Super	Sumi Agro	Quizalofop-P-Ethyl, 50 g/ltr	1000	Post-Emergence
5	Intervix® Pro	BASF	Imazamox, 40 g/ltr	1250	Post-Emergence

The experiments were laid out in randomized complete block design (RCBD) with split plot arrangement having fourteen sub-plots including five different herbicide applications and untreated control plots on four sunflower cultivar with four replications in 2014 and 2015.

Each plot was set up in planting at 5.0 m × 2.8 m = 14.0 m^2. Planting was done on May 21, 2014 for the first year and on April 27, 2015 for the second year with an intra-row spacing of 30 cm and a row-to-row spacing of 70 cm. The reason of late planting in the first year was heavy rainfall. The experimental field in each year was fertilized as 300 kg ha^{-1} with 20-20-0 (NPK) prior to sowing. In each growing season, observations such as plant height, time to flowering, head diameter, one thousand seeds weight, seed yield, oil and protein contents of seed, and oil fatty acids.

Pesticide (herbicide) residues analysis were done on harvested seeds from all plots belonging to herbicide applications within each block according to TS EN 15662 by private firm. In analyses, GC MS/MS and LC MS/MS instruments were used for benfluralin, and pendimethalin, aclonifen and imazamox, respectively. UPLC MS/MS instrument was used only to analysis of Quizalofop-P-Ethyl.

Statistical analysis was conducted according to Standard procedures for a randomized complete block design with split plot separately for Clearfield and non-Clearfield cultivars. The SAS System was used to generate the analysis of variance (ANOVA) for determining treatment effects on the dependent variables (SAS Institute, 1997). Treatment mean comparisons were based on F-Protected Least Significance Differences (LSD) comparisons at $P \leq 0.05$.

RESULTS AND DISCUSSION

In this research, "Intervix® Pro" was applied to Clearfield cultivars "LG 5542 CL and Colombi". Other four herbicides "Bonaflan WG, Stomp® Extra, Challenge 600 and Targa Super" were applied to non-Clearfield cultivars "64LL05 and 64H34". Each cultivar had a control "untreated" plot within each replication. Thus, analysis of variance were done separately for Clearfield and non-Clearfield cultivars.

Table 5.Analysis of variance (mean square) of some sunflower yield and yield components with herbicide applications as separately for Clearfield and non-Clearfield cultivars in 2014 and 2015

Clearfield cultivars	Plant height	Head diameter	Stem diameter	1000 seed weight	Days to 50% flowering	Test weight	Seed yield
Year	3333.97**	4.22**	0.29^{ns}	0.12^{ns}	11.28*	0.01^{ns}	290.65^{ns}
Cultivar (Cul)	1275.0**	18.06**	2.31**	25.10^{ns}	5.28*	24.67**	407.55^{ns}
Application (App)	1933.33**	0.27^{ns}	4.33**	45.13^{ns}	2.53^{ns}	0.34^{ns}	293.79^{ns}
Year*Cul	223.82**	0.11^{ns}	0.49*	0.17^{ns}	2.53^{ns}	0.03^{ns}	7378.08*
Year*App	0.24^{ns}	0.45^{ns}	0.11^{ns}	0.25^{ns}	0.03^{ns}	9.14^{ns}	50.45^{ns}
Cultivar*App	12.09*	1.74^{ns}	4.13**	4.96^{ns}	1.53^{ns}	0.14^{ns}	13.49^{ns}
Year*Cul*App	16.06*	1.24^{ns}	0.07^{ns}	0.01^{ns}	0.03^{ns}	0.02^{ns}	0.22^{ns}
C.V.	**1.16**	**3.81**	**4.75**	**5.42**	**1.27**	**3.51**	**10.26**
non-Clearfield cultivars							
Year	2472.87**	48.88**	1.62*	0.16^{ns}	56.11**	0.61^{ns}	63429.28**
Cultivar (Cul)	416.42**	177.40**	0.74*	164.37*	66.61**	1.46^{ns}	2921.07^{ns}
Application (App)	468.97**	0.77^{ns}	1.18**	51.67^{ns}	8.89**	4.22^{ns}	1340.64^{ns}
Year*Cul	0.08^{ns}	2.96^{ns}	0.46^{ns}	0.77^{ns}	3.61*	0.20^{ns}	3015.35^{ns}
Year*App	246.23**	0.35^{ns}	0.09^{ns}	0.31^{ns}	0.46^{ns}	0.56^{ns}	563.62^{ns}
Cultivar*App	398.21**	3.66^{ns}	1.92**	193.17**	9.27**	3.18^{ns}	523.25^{ns}
Year*Cul*App	269.81**	1.14^{ns}	0.33^{ns}	0.82^{ns}	1.33*	0.55^{ns}	1052.91^{ns}
C.V.	**2.11**	**9.83**	**7.17**	**7.22**	**1.00**	**3.81**	**14.37**

* and ** : **: Significant differences based on ANOVA are shown at $P < 0.05$ and $P < 0.01$, respectively. ns: non significant

Tablo 5 shows analysis of variance of some sunflower yield and yield components with herbicide applications as separately for Clearfield and non-Clearfield cultivars in 2014 and 2015

According to ANOVA results, Herbicide application affected significantly at $P < 0.01$ on plant height, stem diameter of Clearfield cultivars. For the non-Clearfield cultivars, herbicide application had significant effect on plant height, stem diameter and days to 50% flowering.

Herbicide application to Clearfield cultivars had significant effect on stearic acid of seed oil while the effects of Year*Cultivar*Application interaction on oleic and linoleic acid of seed oil was significant at $P < 0.05$ statistical level (Table 6). For non-Clearfield cultivars, the effects

of Year*Cultivar*Application interaction on oil content and behenic acid (C22:0) were significant at $P < 0.01$ and $P < 0.05$, respectively.

Table 6.Analysis of variance (mean square) of oil content and fatty acid composition with herbicide applications as separately for Clearfield and non-Clearfield cultivars in 2014 and 2015

Clearfield cultivars	Seed oil content	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C22:0 Behenic	C24:0 Lignoceric
Year	5.07 ns	0.17 ns	1.42**	24.68 ns	23.21 ns	0.01 ns	0.01 ns
Cultivar (Cul)	30.69**	14.20**	1.22**	13763.9**	12667.2**	0.01 ns	0.01 ns
Application (App)	0.99 ns	0.03 ns	0.06*	1.04 ns	1.66 ns	0.01 ns	0.01 ns
Year*Cul	2.14 ns	0.27*	0.33**	0.63 ns	0.47 ns	0.05 ns	0.01 ns
Year*App	1.62 ns	0.12 ns	0.01 ns	13.55 ns	7.73 ns	0.02 ns	0.01 ns
Cul*App	0.12 ns	0.14 ns	0.03 ns	0.13 ns	0.34 ns	0.01 ns	0.01 ns
Year*Cul*App	0.01 ns	0.05 ns	0.03 ns	37.37*	41.29*	0.04 ns	0.01 ns
C.V.	**3.39**	**4.61**	**3.45**	**4.69**	**10.18**	**19.34**	**23.77**
non-Clearfield cultivars							
Year	257.22**	0.01 ns	0.01 ns	449.07**	654.25**	0.19*	0.01 ns
Cultivar (Cul)	289.98**	77.36**	13.3 **	42187.6**	38949.1**	0.17*	0.08 ns
Application (App)	5.71 ns	0.13 ns	0.10 ns	16.12 ns	0.84 ns	0.03 ns	0.01 ns
Year*Cul	61.65**	0.57 ns	0.37*	19.52 ns	5.09 ns	0.04 ns	0.03 ns
Year*App	5.87 ns	0.15 ns	0.05 ns	14.24 ns	1.55 ns	0.02 ns	0.01 ns
Cul*App	15.30*	0.35 ns	0.07 ns	20.74 ns	5.96 ns	0.04 ns	0.01 ns
Year*Cul*App	13.93**	0.38 ns	0.04 ns	19.01 ns	7.08 ns	0.07*	0.01 ns
C.V.	**5.22**	**9.33**	**8.61**	**8.54**	**12.77**	**22.10**	**33.16**

* and ** : **: Significant differences based on ANOVA are shown at $P < 0.05$ and $P < 0.01$, respectively. ns: non significant

Variations in yield and yield components by herbicide applications are given in Table 7. Intervix® Pro had negative effect on plant height. Plant height of Clearfield cultivars by Intervix® Pro with Imazamox (40 g/ltr) active ingredient was shortened 10.59% than untreated plots. This herbicide had also significant negative effect on stem diameter. The negative effects of Intervix® Pro on seed yield, test weight and head diameter of Clearfield cultivars were insignificant.

Although ANOVA of herbicide application shows insignificant effect on yield components of non-Clearfield cultivations, it created different $LSD_{0.05}$ groups for plant height, stem diameter, 1000 seed weight, days to 50% flowering, test weight and seed yield.

Targa Super with Quizalofop-P-Ethyl (50 g/ltr) application decreased seed yield per hectare according to other herbicides while it was in the same statistical group with untreated plots. Otherwise, Bonaflan WG application had the highest seed yield in the first group. The lowest plant height was measured in untreated plots. Oppositely to the effects of Intervix® Pro on Clearfield cultivars, other four herbicides application to non-Clearfield cultivars increased plant height according to untreated plots. Stomp® Extra with pendimenthalin active ingredient applied pre-emergence herbicide decreased the number of days to flowering of linoleic sunflower hybrid "64LL05" and oleic sunflower hybrid "64H34". It had positive effect on stem diameter and seed test weight.

Table 7. Variations in yield and yield components by herbicide applications

	Plant height (cm)	Head diameter (cm)	Stem diameter (cm)	1000 seed weight (g.)	Days to % 50 flowering	Test weight (kg/hl)	Seed yield (kg/ha)
Clearfield cultivars							
Untreated	146.80 a	18.61	6.20 a	67.61	75.25	34.99	2437.98
Intervix® Pro	131.26 b	18.42	5.47 b	69.98	75.81	34.79	2377.38
$LSD_{0.05}$	**1.19**		**0.20**				
		0.52		**2.74**	**0.71**	**0.90**	**181.68**
non-Clearfield cultivars							
Untreated	113.96 c	15.82	5.38 c	73.79 ab	72.06 a	34.75 ab	2036.5 ab
Bonaflan WG	122.34 b	15.49	5.54 bc	76.25 a	71.63 a	34.89 a	2251.3 a
Stomp® Extra	126.43 a	15.40	6.10 a	71.78 b	70.13 b	35.25 a	2147.6 ab
Challenge 600	122.25 b	15.25	5.79 b	74.94 ab	71.69 a	33.86 b	2100.7 ab
Targa Super	127.86 a	15.64	5.69 b	72.55 ab	71.56 a	34.74 ab	2028.2 b
$LSD_{0.05}$	**1.83**		**0.29**	**3.77**	**0.51**	**0.94**	**214.95**
		1.08					

*: Within each column for Clearfield and non-Clearfield cultivars, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.

Table 8 shows the variations in seed oil content and fatty acid compositions by herbicide applications. According to results, only stearic acid (C18:0) was affected by herbicide application. Intervix® Pro decreased content of stearic acid in seed oil of Clearfield cultivars.

Positive effects on seed oil content, linoleic acid and behenic acid, and negative effects on oleic acid and palmitic acid of this herbicide were insignificant at $P < 0.05$ statistical level. Insignificant positive effect of Bonaflan WG was observed on seed oil content. It also increased insignificantly palmitic and stearic acids. The highest oleic acid was found in untreated plots.

Table 8. Variations in seed oil content and fatty acid compositions by herbicide applications

Clearfield cultivars	Seed oil content (%)	C16:0 Palmitic (%)	C18:0 Stearic (%)	C18:1 Oleic (%)	C18:2 Linoleic (%)	C22:0 Behenic (%)	C24:0 Lignoceric (%)
Untreated	42.09	5.06	2.77 a	61.76	28.62	0.78	0.34
Intervix® Pro	42.44	5.00	2.68 b	61.40	29.08	0.81	0.35
LSD$_{0.05}$			**0.07**				
	1.05	**0.17**		**2.13**	**2.16**	**0.11**	**0.06**
non-Clearfield cultivars							
Untreated	42.31	4.58	2.90	63.21	27.61	0.74	0.31
Bonaflan WG	42.63	4.78	3.09	60.59	28.08	0.64	0.27
Stomp® Extra	42.32	4.61	3.06	62.47	28.12	0.72	0.28
Challenge 600	41.24	4.65	3.00	62.72	27.87	0.72	0.30
Targa Super	42.76	4.55	2.95	62.61	28.16	0.75	0.33
LSD$_{0.05}$	**1.56**	**0.31**	**0.18**	**3.77**	**2.53**	**0.11**	**0.07**

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P< 0.05$.

Variations in some important yield and oil character according to cultivars, years and applications are given in Table 9. Plant height of LG 5542 CL and Colombi was affected negatively. by Intervix® Pro application in both years. In LG 5542 CL, Intervix® Pro application in 2014 and 2015 decreased plant height 12.28 and 11.44%, respectively. Decreases in Colombi were 7.93 and 11.09% for 2014 and 2015, respectively. Colombi in 2014 was affected negatively more than in 2015.

Table 9. Variations in some important yield and oil character according to cultivars, years and applications

Cultivar	Year	Application	Seed yield (kg/ha)	Days to % 50 flowering	Plant height (cm)	Seed oil content (%)	C18:1 Oleic (%)	C18:2 Linoleic (%)
LG 5542 CL	2014	Untreated	244.52	75.50	149.46a	44.03	41.67	47.73
		Intervix® Pro	254.22	76.50	131.10b	43.78	37.98	51.23
		LSD$_{0.05}$	**45.13**	**3.18**	**7.00**	**2.37**	**9.49**	**8.83**
	2015	Untreated	227.54	73.75b	132.75a	42.23	40.25	49.52
		Intervix® Pro	222.52	74.75a	117.56b	42.95	43.48	46.51
		LSD$_{0.05}$	**27.41**	**0.99**	**2.24**	**4.23**	**4.62**	**4.44**
	2014	Untreated	222.42	76.25	164.73a	41.40	81.40	10.24
		Intervix® Pro	229.86	76.25	151.66b	41.46	81.77	9.62

Colombi		**LSD$_{0.05}$**	**40.54**	**1.30**	**1.30**	**3.51**	**8.26**	**8.99**
		Untreated	261.49	75.50	140.27a	40.71	83.73	7.005
	2015	Intervix® Pro	263.58	75.75	124.71b	41.59	82.38	8.96
		LSD$_{0.05}$	**29.02**	**0.80**	**2.35**	**3.16**	**2.33**	**2.80**
		Untreated	181.01	71.75	117.13d	44.55b	38.16	51.73
		Bonaflan WG	178.31	71.50	122.00c	44.45b	36.46	52.91
	2014	Stomp® Extra	169.12	71.00	123.82c	45.08ab	34.58	54.94
		Challenge 600	168.36	71.50	128.46b	45.21ab	38.40	51.30
64LL05		Targa Super	157.94	72.00	137.68a	46.07a	34.87	54.84
		LSD$_{0.05}$	**29.92**	**1.55**	**2.19**	**1.12**	**4.78**	**4.34**
		Untreated	230.38	69.75b	115.10b	42.70	42.57	46.68
		Bonaflan WG	246.10	68.75c	111.76c	42.87	42.10	47.43
	2015	Stomp® Extra	246.10	68.75c	111.76c	43.50	42.01	46.69
		Challenge 600	233.80	68.75c	110.89c	44.34	41.84	47.07
		Targa Super	241.32	71.25a	124.28a	42.79	42.58	46.72
		LSD$_{0.05}$	**45.14**	**0.69**	**2.36**	**3.43**	**3.30**	**3.66**
		Untreated	174.60	74.25a	121.80c	43.01ab	83.23	8.61
		Bonaflan WG	196.26	73.75a	135.22b	43.61a	83.15	8.98
	2014	Stomp® Extra	210.89	71.00c	135.32b	42.88ab	84.80	7.27
		Challenge 600	206.12	73.50ab	139.28a	43.00ab	82.10	9.59
64H34		Targa Super	188.69	72.25bc	120.57d	42.59b	83.76	8.09
		LSD$_{0.05}$	**46.74**	**1.25**	**1.16**	**0.97**	**9.73**	**9.26**
		Untreated	228.61b	72.50a	101.80d	38.98a	88.87	3.41
		Bonaflan WG	279.84a	72.50a	120.39b	39.58a	80.67	3.00
	2015	Stomp® Extra	232.94b	69.75c	134.83a	37.82ab	88.48	3.57
		Challenge 600	232.01b	73.00a	110.37c	32.42b	88.53	3.51
		Targa Super	223.33b	70.75b	128.91a	39.59a	89.24	2.97
		LSD$_{0.05}$	**44.76**	**0.51**	**7.17**	**5.78**	**12.29**	**1.63**

*: Within each column for each cultivar and year, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.

The second year depend on dry condition especially after R6 growth stage caused to stress on plants. It decreased seed oil contents. Thus, Intervix® Pro application in 2015 affected negatively some yield and quality components more than 2014. Generally, the decreases were insignificant. In seed yield and linoleic acid of LG 5542 CL by the herbicide application were also observed decreases in 2015 although oleic acid content of Colombi affected negatively in 2015. The otherwise, some insignificant increases by Intervix® Pro were determined similar in seed yield of LG 5542 CL in 2014 according to untreated plots.

In 64LL05 cultivar in 2014 and 2015 had the highest plant height by Targa Super application. Engrossingly, plant height of untreated plots was the lowest in 2014. Targa Super herbicide application also resulted the highest days number to flowering of 64LL05 in 2015. In addition, this herbicide application was in the first highest seed oil content group with Challenge

600 and Stomp® Extra in 2014. Although insignificant differences were found in seed yield of 64LL05 cultivar, untreated plots gave the highest seed yield in 2014. In the second year, Bonaflan WG and Stomp® Extra gave the highest seed yield in 64LL05.

In the other hand, Stomp® Extra and Targa Super decreased significantly days to the flowering and seed oil content of 64H34 in 2014. The other applications including untreated plots were in the latest group for flowering in this year. The highest plant height of 64H34 cultivar in 2014 was observed in Challenge 600 application while Targa Super application gave the lowest plant height. The differences among herbicide applications for oleic acid and linoleic acid content of 64H34 in 2014 were not significant at $P < 0.05$ statistical level. However, Stomp® Extra application had the highest seed yield and oleic content of seed oil. Untreated plots gave the lowest seed yield in 2014.

Herbicide application created statistically significant groups for seed yield, number of days to flowering, plant height and seed oil content of 64H34 in 2015. Bonaflan WG application to 64H34 in 2015 gave the highest seed yield. It increased significantly seed yield according to untreated plots and other herbicide applications. Although Targa Super was in the second group with the other applications except Bonaflan WG, it created the lowest seed yield. Stomp® Extra decreased the number of days to flowering 64H34 in 2015 similar to the first year. The highest plant height was also measured in Stomp® Extra with Targa Super application although Targa Super had negative effect on plant height in 2014.

Results of pesticide (herbicide) residues analysis on harvested seeds belonging to herbicide applications are given in Table 10. Analyses were done according to TS EN 15662. In analyses, GC MS/MS and LC MS/MS instruments were used for benfluralin, and pendimethalin, aclonifen and imazamox, respectively. UPLC MS/MS instrument was used only to analysis of Quizalofop-P-Ethyl. Active ingredient residue on harvested seed from herbicide applications belonging to each block and each replication were not detected according to limit.

Table 10. Pesticide (Herbicide) residues analysis on harvested seeds

Trade name	Active ingredient	Limit (LOQ)	Unit	Result	Instrument Analysis method
Bonaflan WG	Benfluralin, 60 g/ltr	0.01	mg/kg	Not Detected	GC MS/MS TS EN 15662
Stomp® Extra	Pendimethalin, 450 g/ltr	0.01	mg/kg	Not Detected	LC MS/MS TS EN 15662
Challenge 600	Aclonifen, 600 g/ltr	0.01	mg/kg	Not Detected	LC MS/MS TS EN 15662
Targa Super	Quizalofop-P-Ethyl, 50 g/ltr	0.01	mg/kg	Not Detected	UPLC MS/MS J.of AOAC Int. Vol. 90. No.2.2017
Intervix® Pro	Imazamox, 40 g/lt	0.01	mg/kg	Not Detected	LC MS/MS TS EN 15662

Delchev and Georgiev (2015) and Suryavanshi et al. (2015) also reported results in the same direction with this study. But they usually emphasized the effective and efficient use of pesticides. Agrochemicals (pesticides and fertilizers) are looked upon as a vehicle for improved crop production technology though it is a costly input. Balance use, optimum doses, correct

method and right time of application of agrochemicals ensures increased crop production. The requirement of fertilizers and pesticides for crops differ according to soil and meteorology. On a large scale, success of pesticide application is depend on farmer knowledge and education (Bhandari, 2014).

The results show that some of herbicides could have hormonal positive effect on some characters with yield, morphological, physiological, seed oil content and fatty acid composition of sunflower while the effects of others are negative or in significant. It is a great result we could not find residue of herbicides in harvested seeds from application plots. The results lead to need more new researches on determination stress or hormonal effects of pesticides under different ecological conditions for evaluating effects of genotype, growth stage and environmental.

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GENETIC CHARACTERIZATION OF THE INTERACTION BETWEEN SUNFLOWER AND OROBANCHE CUMANA.

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ABSTRACT

Orobanche cumana is a major disease in cultivated areas around the black sea and in Spain. The pathogen spread recently to several other countries (France, China …). During the last ten years, several new *O. cumana*races have emerged but very few efficient methods were available to control their development. Genetic resistance was the more efficient and introgression of major resistance loci was successfully used to produce new resistant sunflower varieties. With the recent emergence of new virulent races, novel resistance loci need to be mapped and characterized. A recombinant inbred line population, derived from the cross between the lines HA89 and LR1, was used to map QTLs controlling quantitative resistance to race F. The phenotyping has been conducted on the 107 lines of the population at different stages of the interaction. We evaluated each line for (i) the capacity of their root exudate to induce germination of *O. cumana* seeds, (ii) their ability to induce incompatible attachment, (iii) the number of broomrape tubercles in growth chamber, and (iv) the number of broomrape emergences in the field. Different response profiles were observed at these 4 stages of development, indicating several resistance mechanisms in sunflower. Interestingly, even if the two parental lines showed a close resistant phenotype, we observed a large diversity of the resistance level in the population. Combined with this detailed phenotyping analysis, we performed the genotyping of the sunflower recombinant inbred lines using an AXIOM array of 586 985 SNPs. QTLs will be mapped for the different traits.

Key Words : Orobanche cumana, sunflower, QTL, resistance

ISOLATION AND IDENTIFICATION OF PATHOGEN OF SUNFLOWER *FUSARIUM* WILT

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ABSTRACT

Fusarium spp. is one of the most destructive fungi which could infect a wide range of plants and cause wilt diseases. After infection, the lower leaves of sunflower showed the typical symptoms as discoloration, irregular, mottled and wilt. In the field, the symptom of the Fusarium wilt is very similar with sunflower yellow wilt caused by *Verticillium dahliae*. It is rather difficult to distinguish them. In August of 2014-2015, sunflower wilt samples, showing the above described symptom, were collected from Inner Mongolia, Jilin, Liaoning, Nixia and Xinjiang province of China. The pathogen was isolated from the diseased stem and identified the pathogen with Koch's postulates. The CLA, SNA and PDA medium were used to observe three types of conidias such as macroconidia, microconidia, and chlamydospores. Combined the morphology characteristic and the PCR results (with ITS and EF-1α primes), 31 isolates were identified as seven different species of *Fusarium* spp., including *F. oxysporum* (9 strains), *F. verticilloides* (2 strains), *F. lateritium* (1 strain), *F.acuminatum* (4 strains), *F. redolens* (2 strains), *F. equiseti* (2 strains) and *F. proliferum* (11 strains). Inoculation test was performed with both the stem or root wound inoculation method. All 31 strains could cause the wilt of sunflower seedling under lab condition. In conclusion, 7 species of Fusarium spp. was the causal agent of sunflower fusarium wilt in China and *F. proliferum* and *F. oxysporum* are the dominant species of sunflower fusarium wilt.

Key Words : Sunflower wilt; Koch's postulates ; Fusarium species.

PCR COMBINED WITH GFP TAGGED VERTICILLIUM DAHLIAE CONFIRMED THE SEEDS TRANSMISSION OF SUNFLOWER *VERTICILLIUM* WILT

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ABSTRACT

Verticillium wilt of sunflower (*Helianthus annuus* L.) is a widespread and destructive disease caused by the soil-borne fungal pathogen *Verticillium dahliae* (*V. dahliae*). The quick spreading of Verticillium Wilt in sunflower planting region of China promoted us to consider the possibility of seeds transmission the pathogen. Therefore, knowledge on the contamination of the seeds by *V. dahliae* is critical for understanding the infection cycle of sunflower yellow wilt and also to develop the efficient ways to control the spreading of this disease. In this study, sunflower seedlings were inoculated with conidial suspensions of GFP tagged isolate. Colonization and developing were studied under confocal microscopes. After 12 to 96 hour post-inoculation (hpi), conidia germinated and formed hyphal colonies on the root tips and in the root elongation zones. Hyphae colonized in cortical tissues and vascular elements after 2 weeks inoculation (2wpi). 10 wpi later, the xylem of the upper stem, sunflower disc including the pericarp and seed coat, had been colonized by the pathogen. Moreover, pathogen DNA could also be detected by PCR in the pericarp and seed coat. Additional experiment was performed to detect the transmission rate of seeds of different sunflower cultivars was conducted with PCR. Our result indicated that the transmission rate of sunflower seeds ranged from 10-25% among all tested cultivars. In conclusion, seed transmission is the main way for the long distance transmission of sunflower *V. dahliae* and seed pretreatment should be done to control the infection of sunflower seedling in the future.

Key Words : sunflower (*Helianthus annuus* L.); *Verticillium dahliae* ; seed transmission

RAPID INVITRO SCREENING OF SUNFLOWER GENOTYPES FOR MOISTURE STRESS TOLERANCE USING PEG-6000

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ABSTRACT

Sunflower is an important oilseed crop, cultivated mainly under rainfed situation the productivity is very low and experiences moisture stress during flowering and terminal growth stages. To overcome such situations, there is need to identify the moisture stress tolerant genotypes to develop drought tolerant varieties and hybrids. Present experiment was conducted mainly to screen large number of genotypes for moisture stress tolerance at laboratory level using chemical PEG-6000 (Poly Ethyl Glycol) at MARS, UAS, Raichur. A total of 160 genotypes screened with two replications of each treatment (0, 10, 15, 20, 25 and 30% of PEG-6000) in two factorial CRD fashion. All the treatments, genotypes and G X T interactions were significant for all five characters studied. The treatment mean indicated the germination per cent, shoot length, seedling length and seed vigour reduced drastically with increased concentrations of PEG. Based on the drastic reduction in seed germination and growth at two critical concentrations 20% and 25% PEG were considered for screening the germplasm. The genotype x treatment interactions at 20 and 25% PEG exhibited significant differences for few genotypes studied, hence these concentrations are considered as critical doses to identify/isolate the real moisture tolerant genotypes. Comparative performance indicated that shoot length was most affected one among the characters studied. From this study the moisture stress tolerant B-lines, R-lines and germplasm lines are identified as most tolerant lines to moisture stress induced by PEG-6000 and further suggesting that these inbred lines could be used as parental lines to develop moisture stress tolerant hybrids and varieties in the heterosis breeding programme

Key words: PEG-6000, Germination per cent, Root length

INTRODUCTION

Sunflower (*Helianthus annuus* L.) plant belongs to *Asteracae* or *Compositae* family and it is native to the temperate North America. It is a highly cross pollinated crop which is adaptable to a wide range of agro climatic situations, having high yield potential, suitable for cultivation in all seasons due to its day neutral nature (photo insensitivity) and can fit well in various inter and sequence cropping systems. In India, the major area is under rainfed cultivation. The crop experiences severe moisture stress during flowering and terminal growth stages. In order to overcome moisture stress at such critical stages of crop, there is need identify the moisture stress tolerant genotypes at germination stage itself. Germination stage is an important stage to maintain adequate population for obtaining prominent yield. Hence the present investigation was mainly to identify genotypes which can grow under moisture stress conditions through germination test under laboratory conditions using moisture stress inducing agent PEG-6000 having a molecular weight of 6000. Geetha *et al.,* (2012) proposed the use of PEG-6000 for

screening for drought tolerance under laboratory conditions and may be useful complementary method to field screening to overcome difficulties like uncontrolled climatic conditions, heterogeneity of soil, large amount of plant material to and time and labour consumption making field trials difficult for drought screening of genotypes. Turhan and Baser (2004) suggested that prior to field trial; an *in vitro* approach could be useful in screening and selecting for drought response. Ahmad *et al.* (2009) identified G-101 and 64-A-93 from *in vitro* response of sunflower genotypes to drought stress imposed at germination and seedling growth stages at five water stress levels induced by PEG.

MATERıALS AND METHODS

In vitro screening of genotypes is an alternate and easy method to screen large number of genotypes with limited space and time, a total of 160 genotypes which includes (12 maintainer lines, 12 restorer lines and 136 germplasm lines) were screened by using a water stress inducing chemical i.e. poly ethylene glycol (PEG-6000). Many studies indicated that PEG-6000 is water stress inducing agent and it is best to screen germplasm for drought stress tolerance under laboratory conditions (Turhan and Baser, 2004; Somers *et al.* 1983; Ahmad *et al.* 2009 and Geetha *et al.* 2012).

Totally there are six treatments (0 control) 10, 15, 20, 25 and 30 g of weighed powder of PEG-6000 are dissolved in 100 ml of distilled water separately to prepare 0 (control), -0.6 (10 %), -0.9 (15 %), -1.2 (20 %), -1.5 (25 %) and -1.8 (30 %) M Pa concentrations, respectively and two replications were maintained for each osmotic potential in two factorial-CRD fashion. Twenty seeds of each genotype were rinsed and soaked for ten minutes using distilled water, later kept the seeds on the germinating paper in each petri plate and then treated with different concentrations of PEG-6000 and observations were recorded on germination per cent, root length, shoot length, seedling length and seed vigour seven days after sowing in petriplates.

The germination per cent, seedling length and seed vigour was calculated by using the formulae.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

Seedling length (cm) = Total length of shoot (cm) + Total length of root (cm).

Seed vigour= [Seedling length (cm) × Germination percentage]

RESULTS AND DISCUSSION:

The analysis of variance for five characters on 160 sunflower genotypes under laboratory screening using PEG-6000 is presented in the Table 1. The analysis of variance exhibited that all the treatments, genotypes and genotypes x treatment interactions are found to be highly significant for all the five characters studied.

The mean treatment effects obtained for five characters are presented in Table 2. The significant differences were noticed for all the characters at (0 control), 10 and 15 per cent concentrations of PEG-6000. But 20, 25 and 30 per cent of PEG-6000 concentration none of the character found significant except germination percent. The overall treatment means indicated that germination per cent, shoot length and seedling length are drastically reduced with increasing the concentrations of PEG-6000 (20, 25 & 30%). But indicating that 20% and 25% concentration are the critical doses for screening moisture stress tolerant lines.

The results of the genotype x treatment interaction of 160 sunflower genotypes for five characters under laboratory evaluation are presented in Table 3. The *per se* performance of interaction effects of 160 sunflower genotypes are screened by using PEG-6000 at 0, 10, 15, 20, 25 and 30 per cent concentrations. The results revealed that at 10 and 15 per cent PEG majority of the genotypes showed highly significant mean *per se* performance. At 30 per cent PEG-6000 majority of the genotypes could not survive and could not exhibit significant *per se* performance for the characters studied and 30 per cent PEG acted as lethal dose. At 20 percent PEG, the germination percent ranged from 12.50 to 100 while at 25 per cent PEG it ranged from 5.0 to 95 percent. The root length ranged from 0.52 to 12.65 cm and 0.10 to 6.56 cm at 20 and 25 per cent PEG respectively. Shoot length ranged from 0.25 to 2.50cm and 0.12 to 1.20cm at 20 and 25 per cent PEG respectively. Similarly the seedling length ranged from 0.80 to 14.09 and 0.30 to 7.76cm at 20 and 25 per cent PEG respectively. Hence, the genotypic treatment interaction at 20 and 25 per cent were chosen to compare with control.

The germination per cent showed highly significant differences for majority genotypes at 0 per cent (control). The germination per cent at 20 per cent indicated 94 out of 160 genotypes were showed significant *per se* performance, while, at 25 per cent PEG, 24 out of 160 genotypes were found to be significant in comparison with control. Among CMS lines CMS 857B, among restorer lines R-78, R-12-2 and among germplasm lines GP_6-305, GP_6-2255, GP_6-371, GP_6-424, GP_6-442, GP_6-863, GP_6-11, GP_6-969, GP_6-310, GP_6-118, GP_6-211, GP_6-1576, GP_6-326, GP_6-714, GP_6-967, GP_6-1060, GP_6-366, GP_6-614, GP_6-54, GP_6-1102 and GP_6-1072 exhibited significant *per se* performance. The majority of the genotypes showed decreased the germination percent with increased concentrations of PEG-6000 compared to control. These results accordance with reports of Kaya *et al.* (2006), Iqbal and Asraf (2006), El Midaoui (2003), Ahmad *et al.* (2009) and Saensee *et al.* (2012). However at 15 per cent or its equivalent M pa reduced germination per cent was reported by Geetha *et al.* (2012) and Sheidaie *et al.* (2012).

The root length at 20 per cent PEG indicated 62 out of 160 genotypes were showed significant. While, at 25 per cent concentration five genotypes were found to be significant in comparison with control. Among CMS lines CMS 857B, among restorer lines R-78 and among germplasm lines GP6-1072, GP6-714 and GP6-1254 exhibited significant *per se* performance. The majority of the genotypes exhibited decreased root length at higher concentrations (25 and 30 % PEG) compared to control these results accordance with reports of Kaya *et al.* (2006), however some authors El. Midaoui *et al.* (2003), Geetha *et al.* (2012) and Sheidaie *et al.* (2012) reported restricted root length at 15 per cent PEG-6000 or its equivalent M pa.

The shoot length at 20 per cent concentration indicated only two out of 160 genotypes showed significant *per se* performance, while at 25 per cent concentration none of the genotypes were found to be significant. At higher concentrations of PEG majority of the genotypes showed decreased shoot length compared to control. These results are in accordance with reports of Kaya *et al.* (2006) and Ahmad *et al.* (2009). However some of the authors El Midaoui *et al.* (2003), Geetha *et al.* (2012) and Sheidae *et al.* (2012) reported that drastic reduction in shoot length drastically reduced at 15 per cent of PEG-6000. Fulda *et al.* (2010) reported the shoot growth was more affected than root growth at more than10 per cent of PEG-6000.

The seedling length at 20 per cent PEG indicated 33 out of 160 genotypes showed significant *per se* performance, while, at 25 per cent PEG only one genotype R-78 exhibited significant *per se* performance. The seedling length was arrested at higher concentrations of PEG-6000. These results are in accordance with the reports of Khodarampour (2011) in maize at

20 per cent PEG or its equivalent Mpa. Manuhara *et al.* (2013) reported sunflower callus growth inhibited with increased concentration of PEG-6000.

Seed vigour at 20 per cent PEG indicated 26 out of 160 genotypes showed significant *per se* performance, while at 25 per cent PEG none of the genotypes were found to be significant. The seed vigour decreased with increased concentration of PEG-6000, these results are in accordance with the reports of Barros and Rosseto (2009).

Comparative performance of 160 sunflower genotypes under laboratory conditions using water stress inducing chemical PEG-6000 at 20 and 25 per cent concentrations are presented in Table 4 for five characters. The germination per cent under 20 and 25 per cent concentrations reduced by -25.06 and -58.14 per cent, respectively compared to control (0 %), the root length was also reduced by -25.88 to -68.81 %, the shoot length was reduced by -86.30 to -93.53 %, seedling length was reduced from -56.76 to -81.45 %, seed vigour reduced by -65.14 to -89.50 %. Based on overall mean performance 38 promising genotypes were identified *viz.*, CMS 857B, CMS 104B, CMS 378B, R 78, R-12-2, R 630, GM 71R, GP_6-305, GP_6-371, GP_6-118, GP_6-714, GP_6-1072, GP_6-325, GP_6-2255, GP_6-424, GP_6-442, GP_6-863, GP_6-11, GP_6-969, GP_6-310, GP_6-211, GP_6-1576, GP_6-326, GP_6-967, GP_6-1060, GP_6-366, GP_6-614, GP_6-54, GP_6-1102, GP_6-1254, GP_6-1616, GP_6-420, GP_6-219, GP_6-517, GP_6-912, GP_6-734, GP_6-586 and GP_6-589 found to be moisture tolerant genotypes.

Table 1. Analysis of variance for five different characters in sunflower under laboratory evaluation using PEG-6000

Source	DF	Germination per cent	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seed vigour
Treatment	5	384834.50**	3121.93**	1838.55**	7956.16**	80230000.00**
Genotype	159	1853.89**	30.13**	4.343**	48.714**	474957.23**
Treatment x Genotype	795	344.26**	5.76**	1.62**	9.50**	97651.51**
Error	960	13.25	0.06	0.03	0.11	1740.33

Table 2. Treatment means of six different concentrations of PEG-6000 for five characters in sunflower

Treatment	Germination per cent	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seed vigour
0	96.35**	6.22**	6.50**	12.72**	1228.00**
10 %	91.84**	8.41**	3.22**	11.63**	1074.00**
15 %	86.50**	7.24**	1.85	9.08**	797.99**
20 %	72.20**	4.61	0.89	5.50	428.06**
25 %	40.33	1.94	0.42	2.36	128.63
30 %	8.47	0.37	0.13	0.50	11.60
Mean	65.95	4.80	2.17	6.96	228.10
S E	0.20	0.01	0.01	0.02	2.33
CD 5%	0.56	0.04	0.03	0.05	6.46
CD 1%	0.74	0.05	0.04	0.07	8.51

Table 3: Genotype x Treatment interaction effects for five different characters using PEG 6000 at 0, 20 and 25 % concentrations

Sl. No	Genotypes	Germination per cent			Root length (cm)			Shoot length (cm)			Seedling length (cm)			Seed vigour		
		PEG 6000 concentrations			PEG 6000 concentrations			PEG 6000 concentrations			PEG 6000 concentrations			PEG 6000 concentrations		
		0 %	20 %	25 %	0 %	20 %	25 %	0 %	20 %	25 %	0 %	20 %	25 %	0 %	20 %	25 %
1	CMS-17B	72.50	35.00	0.00	3.02	1.70	0.00	3.04**	0.43	0.00	6.06	2.13	0.00	440.05	75.50	0.00
2	CMS-103B	90.00**	50.00	35.00	3.50	2.67	1.95	3.52**	0.90	0.65	7.02	3.57	2.60	631.80	179.10	91.00
3	CMS-104B	97.50**	72.50	32.50	2.00	5.92**	2.80	2.84**	0.74	0.37	4.84	6.66	3.17	471.15	482.40	103.20
4	CMS-148B	100.00**	100.00**	70.00	1.60	2.81	0.95	3.50**	0.59	0.33	5.10	3.40	1.28	510.00	340.00	89.60
5	CMS-335B	100.00**	90.00**	65.00	3.47	2.11	1.53	3.70**	0.62	0.35	7.17	2.73	1.88	717.00*	245.70	122.20
6	CMS-338B	100.00**	90.00**	45.00	3.37	1.91	0.68	3.80**	1.00	0.35	7.17	2.91	1.03	717.00*	262.45	46.50
7	CMS-351B	100.00**	85.00**	40.00	5.31*	3.85	0.12	3.00**	0.62	0.20	8.31**	4.47	0.32	831.00**	379.20	12.60
8	CMS-378B	100.00**	75.00*	45.00	3.80	3.70	1.39	5.96**	0.54	0.22	9.76**	4.24	1.61	976.00**	318.50	71.60
9	CMS-607B	92.50**	65.00	0.00	2.70	0.76	0.00	2.70**	0.41	0.00	5.40	1.17	0.00	498.75	77.00	0.00
10	CMS-850B	100.00**	95.00**	45.00	3.90	2.12	0.70	4.90**	0.82	0.28	8.80**	2.94	0.98	880.00**	280.00	43.88
11	CMS-852B	100.00**	95.00**	65.00	5.10	2.52	0.73	5.18**	0.75	0.32	10.28**	3.27	1.05	1030.00**	310.65	68.70
12	CMS-857B	100.00**	95.00**	90.00**	1.48	7.71**	6.16**	3.40**	0.68	0.54	4.88	8.39**	6.70	488.00	797.20**	603.00
13	R-12-2	100.00**	100.00**	80.00**	5.22	6.68**	2.91	4.50**	1.69	0.63	9.72**	8.37**	3.54	972.00**	837.00**	283.20
14	R 127-1	92.50**	87.50**	27.50	1.61	2.56	1.11	8.10**	1.36	0.30	9.71**	3.92	1.41	897.70**	342.80	39.03
15	R 64NB	100.00**	82.50**	60.00	3.71	3.56	1.49	3.76**	0.86	0.42	7.47	4.42	1.91	747.00**	364.90	114.55
16	R-78	100.00**	95.00**	87.50**	8.20**	7.85**	6.56**	7.10**	1.54	1.20	15.30**	9.39**	7.76**	1530.00**	891.90**	678.95
17	R-630	100.00**	87.50**	37.50	5.46**	4.95	2.94	4.74**	0.99	0.51	10.20**	5.94	3.45	1020.00**	519.20	129.25
18	GM-27R	77.50**	60.00	37.50	6.48**	2.35	1.65	5.96**	1.40	0.99	12.44**	3.75	2.64	963.85**	225.00	99.00
19	GM-37R	92.50**	75.00*	7.50	3.42	1.58	0.90	3.00**	0.83	0.40	6.42	2.41	1.30	593.10	179.95	9.50
20	GM-41R	100.00**	95.00**	50.00	3.22	2.62	1.89	3.80**	0.91	0.43	7.02	3.53	2.32	702.00*	336.20	116.00
21	GM-44R	82.50**	57.50	55.00	3.10	2.41	1.97	3.64**	0.94	0.66	6.74	3.35	2.63	555.55	193.00	145.00
22	GM-56R	90.00**	55.00	25.00	7.52**	4.88	0.30	3.72**	1.51	0.40	11.24**	6.39	0.70	1010.00**	352.00	18.50
23	GM-59R	90.00**	50.00	30.00	5.20	2.41	1.80	3.76**	1.40	0.54	8.96**	3.81	2.34	806.40**	191.05	70.20
24	GM-71R	90.00**	70.00	60.00	7.76**	3.97	2.90	5.36**	0.88	0.24	13.12**	4.85	3.14	1180.00**	339.50	188.40
25	GP_6-11	100.00**	90.00**	90.00**	6.68**	3.56	2.35	6.74**	1.03	0.75	13.42**	4.59	3.10	1340.00**	413.10	279.00
26	GP_6-18	100.00**	85.00**	15.00	6.04**	3.20	1.80	5.96**	0.71	0.38	12.00**	3.91	2.18	1200.00**	332.50	34.10
27	GP_6-18-1	95.00**	90.00**	20.00	8.03**	1.99	0.93	9.50**	0.60	0.46	17.53**	2.59	1.39	1670.00**	233.10	27.80
28	GP_6-54	100.00**	87.50**	75.00*	7.51**	6.87**	2.61	5.44**	0.80	0.57	12.95**	7.67*	3.18	1300.00**	670.60	238.50
29	GP_6-63	95.00**	60.00	22.50	6.04**	3.87	1.17	6.90**	0.54	0.33	12.94**	4.41	1.50	1230.00**	264.25	34.25
30	GP_6-83	95.00**	77.50**	27.50	5.22	5.42*	2.70	6.84**	0.87	0.82	12.06**	6.20	3.52	1150.00**	488.25	96.65

31	GP_6-109	100.00**	60.00	10.00	6.27**	2.26	0.45	8.04**	0.55	0.30	14.31**	2.81	0.75	1430.00**	168.60	7.50
32	GP_6-118	100.00**	90.00**	85.00**	10.16**	5.44**	3.88	7.18**	0.98	0.32	17.34**	6.42	4.20	1730.00**	577.80	357.00
33	GP_6-127	95.00**	72.50	30.00	5.34*	3.90	0.82	6.28**	1.74	0.45	11.62**	5.64	1.27	1100.00**	409.45	37.45
34	GP_6-135	95.00**	55.00	15.00	5.42*	3.04	0.85	4.60**	0.68	0.26	10.02**	3.72	1.11	951.90**	205.90	16.65
35	GP_6-139	90.00**	42.50	22.50	5.45**	4.13	1.19	6.70**	0.32	0.12	12.15**	4.45	1.31	1090.00**	188.80	29.75
36	GP_6-160	95.00**	40.00	0.00	3.05	1.30	0.00	5.40**	0.80	0.00	8.45**	2.10	0.00	802.75**	84.00	0.00
37	GP_6-173	100.00**	82.50**	65.00	7.71**	5.54**	2.05	6.24**	1.01	0.51	13.95**	6.55	2.56	1400.00**	539.75	166.40
38	GP_6-175	100.00**	67.50	22.50	4.62	1.88	2.34	4.86**	0.53	0.39	9.48**	2.41	2.73	948.00**	162.60	61.75
39	GP_6-176	82.50**	57.50	47.50	4.80	4.62	3.84	7.90**	1.10	0.59	12.70**	5.72	4.43	1050.00**	328.50	210.90
40	GP_6-181	100.00**	50.00	0.00	4.82	4.99	0.00	4.92**	0.25	0.00	9.74**	5.24	0.00	974.00**	262.40	0.00
41	GP_6-211	100.00**	100.00**	85.00**	7.72**	7.27**	3.43	7.10**	0.95	0.53	14.82**	8.22**	3.96	1480.00**	822.00**	336.60
42	GP_6-217	100.00**	90.00**	7.50	7.86**	7.11**	0.90	5.26**	0.39	0.35	13.12**	7.50	1.25	1310.00**	675.00	9.50
43	GP_6-219	100.00**	40.00	5.00	8.14**	3.53	1.50	8.84**	0.40	0.15	16.98**	3.93	1.65	1700.00**	156.05	8.25
44	GP_6-226	100.00**	85.00**	32.50	6.80**	2.68	1.61	8.18**	0.82	0.35	14.98**	3.50	1.96	1500.00**	298.20	63.60
45	GP_6-236	100.00**	67.50	55.00	9.27**	3.46	1.20	4.92**	0.67	0.45	14.19**	4.13	1.65	1420.00**	278.55	90.75
46	GP_6-250	95.00**	50.00	15.00	4.29	3.64	1.70	4.78**	0.80	0.40	9.07**	4.44	2.10	861.65**	222.00	31.50
47	GP_6-263	95.00**	60.00	7.50	6.79**	4.69	0.93	5.22**	0.82	0.35	12.01**	5.51	1.28	1140.00**	330.60	9.63
48	GP_6-271	100.00**	90.00**	65.00	5.32*	10.32**	3.84	5.52**	0.79	0.53	10.84**	11.11**	4.37	1080.00**	999.90**	284.05
49	GP_6-276	95.00**	85.00**	57.50	2.94	1.69	1.18	5.22**	0.77	0.55	8.16**	2.46	1.73	775.20**	209.10	99.85
50	GP_6-282	95.00**	37.50	0.00	5.00	2.73	0.00	8.02**	0.52	0.00	13.02**	3.25	0.00	1240.00**	122.65	0.00
51	GP_6-286	92.50**	67.50	40.00	6.20**	9.73**	3.64	6.28**	1.15	0.67	12.48**	10.88**	4.31	1150.00**	733.55**	172.20
52	GP_6-297	100.00**	82.50**	27.50	6.16**	5.82**	0.41	6.98**	0.95	0.25	13.14**	6.77	0.66	1310.00**	559.30	18.55
53	GP_6-303	85.00**	0.00	0.00	4.38	0.00	0.00	7.40**	0.00	0.00	11.78**	0.00	0.00	1000.00**	0.00	0.00
54	GP_6-305	100.00**	100.00**	95.00**	6.41**	9.06**	3.88	8.54**	0.83	0.33	14.95**	9.89**	4.21	1500.00**	989.00**	399.95
55	GP_6-310	100.00**	92.50**	87.50**	7.96**	2.89	1.83	11.04**	1.20	0.72	19.00**	4.09	2.55	1900.00**	378.95	222.80
56	GP_6-312	100.00**	52.50	27.50	6.64**	4.79	1.28	7.22**	0.60	0.38	13.86**	5.39	1.66	1390.00**	283.90	45.65
57	GP_6-313	90.00**	22.50	0.00	6.34**	0.91	0.00	7.50**	0.30	0.00	13.84**	1.21	0.00	1250.00**	27.60	0.00
58	GP_6-317	100.00**	27.50	0.00	4.53	1.66	0.00	4.88**	0.51	0.00	9.41**	2.17	0.00	941.00**	60.40	0.00
59	GP_6-324	95.00**	80.00**	47.50	7.80**	7.00**	1.47	7.60**	0.87	0.43	15.40**	7.87**	1.90	1460.00**	629.60	90.20
60	GP_6-325	100.00**	87.50**	62.50	3.82	7.46**	4.02	3.04**	0.30	0.16	6.86	7.76*	4.18	686.00	678.60	261.55
61	GP_6-326	100.00**	100.00**	80.00**	3.30	5.25	2.32	6.80**	2.31**	0.75	10.10**	7.56	3.07	1010.00**	756.00**	245.60
62	GP_6-327	100.00**	77.50**	52.50	5.00	4.89	1.79	7.32**	1.54	0.52	12.32**	6.43	2.31	1230.00**	497.80	120.95
63	GP_6-331	100.00**	82.50**	57.50	4.34	5.58**	1.96	3.50**	0.82	0.40	7.84**	6.40	2.36	784.00**	527.60	136.60
64	GP_6-332	95.00**	80.00**	47.50	13.00**	6.37**	2.69	6.20**	0.64	0.33	19.20**	7.01	3.02	1820.00**	562.05	142.95
65	GP_6-347	100.00**	90.00**	30.00	6.96**	2.23	0.65	7.26**	0.66	0.48	14.22**	2.89	1.13	1420.00**	260.58	33.75
66	GP_6-358	95.00**	32.50	12.50	5.76**	3.98	2.43	4.66**	0.67	0.62	10.42**	4.65	3.05	989.90**	151.40	37.55
67	GP_6-366	95.00**	82.50**	77.50**	5.41*	6.66**	4.34	5.62**	1.55	0.90	11.03**	8.21**	5.24	1050.00**	678.30	405.80

68	GP_6-370	90.00**	90.00**	67.50	11.42**	5.08	1.82	5.34**	0.89	0.49	16.76**	5.97	2.31	1510.00**	537.30	155.45
69	GP_6-371	100.00**	95.00**	92.50**	7.40**	8.88**	4.77	9.40**	1.39	0.41	16.80**	10.27**	5.18	1680.00**	975.65**	478.55
70	GP_6-374	80.00**	72.50	52.50	5.12	3.68	1.73	4.30**	1.12	0.67	9.42**	4.80	2.40	753.60**	348.05	125.75
71	GP_6-384	100.00**	72.50	52.50	13.80**	6.00**	2.40	8.84**	0.71	0.38	22.64**	6.71	2.78	2260.00**	486.00	146.30
72	GP_6-387	100.00**	90.00**	25.00	5.88**	4.03	2.32	6.20**	0.72	0.42	12.08**	4.75	2.74	1210.00**	427.50	69.70
73	GP_6-400	90.00**	77.50**	42.50	7.48**	4.57	2.37	9.44**	0.92	0.73	16.92**	5.49	3.10	1520.00**	424.75	131.20
74	GP_6-420	100.00**	70.00	55.00	8.84**	7.43**	3.73	10.62**	0.80	0.36	19.46**	8.23**	4.09	1950.00**	576.10	224.20
75	GP_6-424	100.00**	95.00**	92.50**	4.21	7.88**	2.80	9.34**	1.57	0.61	13.55**	9.45**	3.41	1360.00**	897.75**	314.90
76	GP_6-442	100.00**	100.00**	92.50**	4.30	8.00**	3.00	6.96**	1.42	0.69	11.26**	9.42**	3.69	1130.00**	942.00**	340.85
77	GP_6-451	95.00**	77.50**	37.50	5.83**	2.74	1.22	6.10**	0.83	0.51	11.93**	3.57	1.73	1130.00**	276.15	65.10
78	GP_6-459	100.00**	85.00**	37.50	7.70**	5.67**	1.28	8.60**	1.13	0.53	16.30**	6.80	1.81	1630.00**	578.00	68.35
79	GP_6-511	100.00**	60.00	10.00	6.36**	4.87	1.00	5.48**	0.61	0.38	11.84**	5.48	1.38	1180.00**	330.00	13.80
80	GP_6-517	100.00**	65.00	60.00	5.10	3.06	2.90	9.10**	2.50**	0.27	14.20**	5.56	3.17	1420.00**	360.70	190.20
81	GP_6-534	95.00**	22.50	0.00	3.40	1.38	0.00	7.18**	0.15	0.00	10.58**	1.53	0.00	1010.00**	34.63	0.00
82	GP_6-561	90.00**	80.00**	52.50	9.28**	5.83**	2.70	6.98**	1.56	0.60	16.26**	7.39	3.30	1460.00**	591.20	173.75
83	GP_6-570	100.00**	75.00*	55.00	6.28**	5.41*	2.50	8.22**	1.23	0.57	14.50**	6.64	3.07	1450.00**	499.40	167.80
84	GP_6-578	95.00**	87.50**	72.50	3.95	5.60**	2.35	10.40**	1.45	0.53	14.35**	7.05	2.88	1360.00**	617.50	208.40
85	GP_6-579	100.00**	90.00**	50.00	6.49**	2.37	0.90	5.32**	0.30	0.20	11.81**	2.67	1.10	1180.00**	240.30	55.00
86	GP_6-586	100.00**	65.00	65.00	3.14	4.70	3.45	3.44**	0.90	0.58	6.58	5.60	4.03	658.00	366.10	263.10
87	GP_6-589	90.00**	17.50	20.00	1.50	1.95	2.60	3.90**	1.22	0.47	5.40	3.17	3.07	486.00	54.90	61.40
88	GP_6-614	100.00**	97.50**	77.50**	6.71**	6.98**	4.58	9.28**	1.54	0.87	15.99**	8.52**	5.45	1600.00**	831.00**	422.10
89	GP_6-615	100.00**	67.50	5.00	6.82**	3.07	0.60	8.46**	0.76	0.15	15.28**	3.83	0.75	1530.00**	258.95	3.75
90	GP_6-656	100.00**	92.50**	22.50	4.50	3.55	1.40	4.48**	0.52	0.37	8.98**	4.07	1.77	898.00**	376.65	40.10
91	GP_6-657	100.00**	77.50**	0.00	4.18	6.22**	0.00	5.52**	0.47	0.00	9.70**	6.69	0.00	970.00**	519.55	0.00
92	GP_6-699	100.00**	77.50**	67.50	11.34**	7.83**	2.20	8.24**	0.92	0.46	19.58**	8.75**	2.66	1960.00**	679.05	179.95
93	GP_6-714	100.00**	85.00**	80.00**	3.39	6.95**	6.00**	5.00**	2.05	0.48	8.39**	9.00**	6.48	839.00**	763.00**	518.40
94	GP_6-734	96.67**	92.50**	55.00	6.91**	8.59**	4.77	7.94**	1.21	0.40	14.85**	9.80**	5.17	1430.00**	907.40**	284.35
95	GP_6-764	90.00**	65.00	20.00	7.76**	4.35	2.12	9.10**	0.59	0.29	16.86**	4.94	2.41	1520.00**	322.40	48.10
96	GP_6-792	100.00**	85.00**	37.50	7.34**	6.28**	1.57	6.50**	0.94	0.36	13.84**	7.22	1.93	1380.00**	615.90	73.25
97	GP_6-794	100.00**	75.00*	52.50	8.29**	5.39*	1.89	8.22**	0.27	0.24	16.51**	5.66	2.13	1650.00**	424.50	112.20
98	GP_6-819	100.00**	37.50	5.00	9.12**	1.12	0.90	9.90**	0.25	0.17	19.02**	1.36	1.07	1900.00**	51.35	5.35
99	GP_6-847	95.00**	50.00	12.50	5.14	2.42	0.49	4.30**	0.69	0.49	9.44**	3.11	0.98	896.33**	155.50	12.88
100	GP_6-854	100.00**	92.50**	67.50	7.56**	8.17**	4.62	5.64**	1.20	0.67	13.20**	9.37**	5.29	1320.00**	866.15**	357.75
101	GP_6-861	90.00**	30.00	0.00	9.52**	2.54	0.00	7.12**	0.19	0.00	16.64**	2.73	0.00	1500.00**	81.90	0.00
102	GP_6-863	100.00**	92.50**	92.50**	10.35**	12.65**	4.24	9.72**	1.44	0.95	20.07**	14.09**	5.19	2010.00**	1300.00**	480.60
103	GP_6-872	85.00**	50.00	0.00	4.15	2.58	0.00	4.52**	0.81	0.00	8.67**	3.39	0.00	736.95**	168.50	0.00
104	GP_6-875	100.00**	40.00	7.50	4.10	2.57	0.38	6.16**	0.30	0.20	10.26**	2.87	0.58	1030.00**	114.60	4.63

105	GP_6-883	100.00**	72.50	12.50	8.82**	3.85	1.13	7.50**	0.95	0.35	16.32**	4.80	1.48	1630.00**	347.60	18.88
106	GP_6-887	100.00**	70.00	0.00	4.13	5.86**	0.00	3.90**	0.68	0.00	8.03**	6.54	0.00	803.00**	459.20	0.00
107	GP_6-891	100.00**	52.50	52.50	6.75**	1.55	1.48	6.46**	1.05	0.70	13.21**	2.60	2.18	1320.00**	136.70	115.15
108	GP_6-899	95.00**	65.00	10.00	8.44**	3.65	0.30	8.20**	0.44	0.25	16.64**	4.09	0.55	1580.00**	265.85	5.25
109	GP_6-906	100.00**	40.00	40.00	9.90**	2.43	2.16	9.16**	0.62	0.66	19.06**	3.05	2.82	1910.00**	122.75	111.70
110	GP_6-912	100.00**	87.50**	72.50	5.70**	8.00**	3.88	5.94**	1.11	0.33	11.64**	9.11**	4.21	1160.00**	798.15**	304.65
111	GP_6-917	90.00**	80.00**	35.00	7.25**	5.43**	1.19	7.18**	1.73	0.44	14.43**	7.16	1.63	1300.00**	572.80	58.70
112	GP_6-951	80.00**	25.00	15.00	6.80**	3.97	1.75	6.68**	0.49	0.25	13.48**	4.46	2.00	1080.00**	109.80	29.25
113	GP_6-952	100.00**	65.00	10.00	7.72**	3.10	1.58	7.70**	0.56	0.33	15.42**	3.66	1.91	1540.00**	237.90	19.05
114	GP6-953	100.00**	70.00	20.00	13.90**	7.00**	1.59	8.50**	0.60	0.33	22.40**	7.60	1.92	2240.00**	532.60	37.53
115	GP_6-961	80.00**	42.50	37.50	10.57**	4.73	2.74	6.66**	1.26	0.49	17.23**	5.99	3.23	1380.00**	253.95	120.53
116	GP_6-965	100.00**	97.50**	65.00	9.30**	5.70**	3.99	10.60**	0.70	0.46	19.90**	6.40	4.45	1990.00**	623.15	288.90
117	GP_6-967	100.00**	90.00**	80.00**	3.82	5.43**	3.09	7.52**	1.19	0.52	11.34**	6.62	3.61	1130.00**	595.80	288.80
118	GP_6-969	100.00**	90.00**	90.00**	4.36	5.23	2.23	8.20**	1.72	0.58	12.56**	6.95	2.81	1260.00**	625.50	252.90
119	GP_6-990	100.00**	45.00	15.00	5.18	1.69	1.00	8.30**	0.70	0.45	13.48**	2.39	1.45	1350.00**	107.55	21.75
120	GP_6-1001	95.00**	77.50**	45.00	11.63**	4.49	1.96	8.88**	0.77	0.56	20.51**	5.26	2.52	1950.00**	408.60	114.70
121	GP_6-1020	100.00**	82.50**	17.50	8.39**	3.83	2.78	6.42**	0.44	0.33	14.81**	4.27	3.10	1480.00**	353.00	53.63
122	GP_6-1023	95.00**	85.00**	55.00	5.82**	7.60**	1.76	9.56**	1.35	0.40	15.38**	8.95**	2.16	1460.00**	760.75**	118.80
123	GP_6-1026	90.00**	67.50	0.00	3.61	1.17	0.00	5.50**	0.42	0.00	9.11**	1.59	0.00	819.90**	107.30	0.00
124	GP_6-1037	95.00**	77.50**	32.50	6.45**	9.95**	3.10	11.00**	1.04	0.45	17.45**	10.99**	3.55	1660.00**	852.60**	116.10
125	GP_6-1047	100.00**	90.00**	52.50	3.31	2.90	0.38	6.48**	0.73	0.31	9.79**	3.63	0.69	979.00**	326.70	36.40
126	GP_6-1060	100.00**	95.00**	80.00**	4.18	6.96**	3.11	7.20**	0.97	0.68	11.38**	7.93**	3.79	1140.00**	753.35**	303.20
127	GP_6-1063	100.00**	90.00**	20.00	5.74**	3.60	1.55	9.72**	0.49	0.33	15.46**	4.09	1.88	1550.00**	368.10	37.60
128	GP_6-1072	100.00**	80.00**	75.00*	9.40**	5.72**	5.80**	11.24**	0.85	0.51	20.64**	6.57	6.31	2060.00**	526.45	473.25
129	GP_6-1075	100.00**	90.00**	57.50	3.58	5.52**	2.77	6.64**	1.15	0.37	10.22**	6.67	3.14	1020.00**	600.30	181.20
130	GP_6-1089	100.00**	12.50	0.00	9.47**	0.68	0.00	6.40**	0.48	0.00	15.87**	1.16	0.00	1590.00**	14.68	0.00
131	GP_6-1101	100.00**	82.50**	40.00	5.41*	7.70**	3.06	5.08**	1.15	0.67	10.49**	8.85**	3.73	1050.00**	728.90**	149.20
132	GP_6-1102	100.00**	82.50**	75.00*	4.70	7.20**	4.35	5.20**	1.75	0.71	9.90**	8.95**	5.06	990.00**	739.50**	379.10
133	GP_6-1114	97.50**	75.00*	17.50	6.28**	4.18	0.60	2.80**	0.51	0.27	9.08**	4.69	0.87	885.30**	351.70	15.75
134	GP_6-1117	100.00**	87.50**	35.00	3.81	5.83**	1.22	4.46**	1.36	0.40	8.27**	7.19	1.62	827.00**	630.05	56.70
135	GP_6-1127	100.00**	57.50	7.50	5.66**	3.47	0.35	5.36**	0.47	0.50	11.02**	3.94	0.85	1100.00**	227.25	6.50
136	GP_6-1135	100.00**	57.50	0.00	11.40**	2.70	0.00	7.80**	0.49	0.00	19.20**	3.19	0.00	1920.00**	183.10	0.00
137	GP_6-1150	90.00**	50.00	17.50	7.96**	4.33	1.97	9.10**	0.74	0.63	17.06**	5.07	2.60	1540.00**	253.50	45.10
138	GP_6-1207	100.00**	90.00**	20.00	11.36**	5.94**	0.93	9.24**	0.83	0.30	20.60**	6.77	1.23	2060.00**	609.30	25.25
139	GP_6-1217	95.00**	87.50**	10.00	8.17**	3.20	0.13	9.56**	0.93	0.18	17.73**	4.13	0.31	1680.00**	360.85	3.05
140	GP_6-1227	90.00**	32.50	12.50	4.37	2.28	0.10	2.82**	0.45	0.20	7.19	2.73	0.30	647.10	88.50	3.75
141	GP_6-1228	95.00**	75.00*	67.50	14.24**	8.45**	3.22	8.92**	1.64	0.33	23.16**	10.09**	3.55	2200.00**	756.20**	239.20

142	GP_6-1254	90.00**	80.00**	62.50	10.60**	10.17**	6.37**	10.30**	1.42	0.75	20.90**	11.59**	7.12	1880.00**	927.20**	445.25
143	$GP_6$1350	77.50**	25.00	12.50	3.67	1.29	1.19	3.98**	0.71	0.40	7.65*	2.00	1.59	592.75	49.50	20.53
144	$GP_6$1436	77.50**	47.50	25.00	7.20**	4.99	1.00	5.38**	0.54	0.28	12.58**	5.53	1.28	974.70**	263.65	31.30
145	$GP_6$1450	100.00**	15.00	0.00	5.18	0.52	0.00	7.92**	0.28	0.00	13.10**	0.80	0.00	1310.00**	11.93	0.00
146	GP_6-1468	100.00**	87.50**	32.50	3.26	8.05**	2.53	4.58**	0.80	0.42	7.84**	8.85**	2.95	784.00**	774.80**	96.30
147	GP_6-1477	100.00**	80.00**	32.50	2.80	3.00	1.87	4.56**	0.89	0.45	7.36	3.89	2.32	736.00**	312.53	75.55
148	$GP_6$1482	90.00**	72.50	30.00	3.59	4.29	2.32	5.80**	1.71	1.19	9.39**	6.00	3.51	845.10**	435.35	106.75
149	GP_6-1509	90.00**	62.50	35.00	7.75**	6.70**	2.37	7.70**	1.20	0.48	15.45**	7.90**	2.85	1390.00**	493.20	101.00
150	GP_6-1518	100.00**	87.50**	35.00	11.34**	6.11**	0.97	7.84**	0.84	0.44	19.18**	6.95	1.41	1920.00**	608.85	49.35
151	GP_6-1533	90.00**	75.00*	57.50	5.64**	6.79**	3.29	6.44**	0.92	0.51	12.08**	7.71*	3.80	1090.00**	578.25	219.00
152	GP_6-1561	100.00**	82.50**	35.00	7.95**	6.05**	1.53	7.60**	1.11	0.42	15.55**	7.16	1.95	1560.00**	591.10	69.00
153	GP_6-1573	100.00**	95.00**	45.00	5.58**	5.34*	3.06	5.36**	0.62	0.38	10.94**	5.96	3.44	1090.00**	564.60	153.40
154	GP_6-1576	100.00**	95.00**	85.00**	4.36	5.21	2.19	4.08**	0.92	0.51	8.44**	6.13	2.70	844.00**	578.30	229.50
155	GP_6-1588	95.00**	52.50	7.50	6.34**	3.43	1.20	8.84**	0.77	0.70	15.18**	4.20	1.90	1440.00**	221.40	14.25
156	GP_6-1595	100.00**	75.00*	65.00	5.28*	2.72	2.15	7.46**	0.73	0.64	12.74**	3.45	2.79	1270.00**	257.40	181.35
157	GP_6-1616	100.00**	95.00**	70.00	4.29	3.64	2.85	5.30**	1.05	0.28	9.59**	4.69	3.13	959.00**	444.10	219.10
158	GP_6-1665	100.00**	95.00**	27.50	7.44**	2.59	0.50	6.40**	0.56	0.55	13.84**	3.15	1.05	1380.00**	299.90	29.25
159	GP_6-1725	100.00**	85.00**	60.00	6.46**	6.27**	3.91	4.62**	1.03	0.70	11.08**	7.30	4.61	1110.00**	620.50	277.15
160	GP_6-2255	100.00**	100.00**	95.00**	8.58**	7.81**	3.64	6.92**	1.44	0.83	15.50**	9.25**	4.47	1550.00**	925.00**	424.65
Range			12.50-100	5-95		0.52-12.65	0.10-6.56		0.25-2.50	0.12-1.20		0.80-14.09	0.30-7.76			
Mean		96.35	72.20	40.33	6.22	4.61	1.94	6.50	0.89	0.42	12.72	5.50	2.36	1228.00	428.06	128.63
CD 5%			7.13			0.48			0.06			0.65			81.77	
CD 1%			9.39			0.63			0.08			0.86			107.63	

Table 4. Comparative performance of 160 sunflower genotypes under control and moisture stress conditions using stress inducing chemical PEG-6000 at 20 and 25 per cent concentrations

Characters	Overall mean values GXT interaction			Changes in mean value		Percent change in mean		No. of significant genotypes recorded out of 160			Promising genotypes identified for moisture stress tolerance
	Control (Non-stress)		PEG-6000 (Moisture stress)								
	0 %	**20 %**	**25 %**	**20 %**	**25 %**	**20 %**	**25 %**	**0 %**	**20 %**	**25 %**	CMS 857B, CMS 104B,CMS 378B (3) R-78, R-12-2, R 630, GM 71R (4) GP_6-305, GP_6-371, GP_6-118, GP_6-714, GP_6-1072, GP_6-325, GP_6-2255, GP_6-424, GP_6-442, GP_6-863, GP_6-11, GP_6-969, GP_6-310, GP_6-211, GP_6-1576, GP_6-326, GP_6-967, GP_6-1060, GP_6-366, GP_6-614, GP_6-54, GP_6-1102, GP_6-1254, GP_6-1616, GP_6-420, GP_6-219, GP_6-517, GP_6-912, GP_6-734, GP_6-586 and GP_6-589 (36)
Germination per cent	96.35	72.20	40.33	-24.15	-56.02	-25.06	-58.14	159	94	24	
Root length (cm)	6.22	4.61	1.94	-1.61	-4.28	-25.88	-68.81	96	62	5	
Shoot length (cm)	6.50	0.89	0.42	-5.61	-6.08	-86.30	-93.53	160	2	0	
Seedling length (cm)	12.72	5.50	2.36	-7.22	-10.36	-56.76	-81.45	143	33	1	
Seed vigour	1228	428.06	128.63	-799.94	-1099.37	-65.14	-89.50	147	26	2	

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GENOME-WIDE ASSOCIATION OF OIL YIELD PLASTICITY TO DROUGHT, NITROGEN AND CHILLING STRESSES IN SUNFLOWER

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ABSTRACT

To face the climate change, the plasticity of genome is a great advantage that plant breeders could build on in order to adapt varieties to new environments. A way to accelerate the adaptation is by the discovery of new alleles involved in the plasticity responses and their introgression in elite varieties. So we conducted a genome wide association study (GWAS) in plasticity responses face to multiple abiotic stresses. We characterized 14 environments by their levels face to four abiotic stresses: nitrogen, drought, chilling and heat, thanks to the SUNFLO crop model. Three known varieties, used as controls and observed in the 14 environments, allowed computing the stress felt during key periods of the growth development. Among the 56 stress indices computed by SUNFLO, the best model to fit the oil yield, regarding the AIC criteria averaged on the three above control varieties, contained only three stresses: the nitrogen during the whole growth period, the drought from sowing to filling and the chilling before flowering. The observed oil yield of a panel of 371 sunflower lines was regressed by linear norm reaction model with the three stress indices of the best model. The slope of each stress norm reaction was used as plasticity phenotypes. Association mapping was based on a set of 65,534 SNPs with MAF >0.05 using the usual mixed model of association, including the maintainer or restorer status as fixed effect and the Alike in State relatedness matrix for the polygenic random term. A forward approach was performed to detect multiple associated SNPs. Homology study of detected SNPs with the annotated genes of Arabidopsis completed the analyses. The results concerning the plasticity face to chilling will be detailed in this talk

Key Words : Plasticity, multi-stress, abiotic, GWAS

BREEDING FOR SUNFLOWER HYBRIDS ADAPTED TO CLIMATE CHANGE: THE SUNRISE COLLABORATIVE AND MULTI-DISCIPLINARY PROJECT

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ABSTRACT

In the context of climate change, an increased variability is expected in timing and amount of water available for crop production. For sunflower crop, yield losses of 10 to 30 % have been predicted at 2030 horizon in Europe. During the past 10 years, genetic progress was lower than expected to improve yield, which imposes to the sunflower community to re-invest current breeding resources and methodologies. To reach high and stable yields across a wide range of environments a French project of 8 years, named 'SUNRISE' (SUNflower Resources to Improve yield Stability in a changing Environment) and supported by the French National Research Agency, is gathering 9 public and 7 private partners since 2012. It associates several approaches: (i) the sequencing and genotyping of the genetic diversity among cultivated and wild sunflowers, (ii) the development of appropriate and high-throughput phenotyping strategies to characterize the molecular, physiological and agronomical responses to variation of the abiotic environment, (iii) the discovery through genome-wide association, linkage mapping and genomic selection of the genetic factors involved in those responses, (iv) the integration of this genetic knowledge into a crop model (SUNFLO) to test in silico G by E interactions and design promising ideotypes in future environments, and finally (v) the evaluation of the outputs for the breeding sector and the transfer of knowledge to agriculture. This partnership will ensure that the developed knowledge, resources and methods will be translated into products and varieties supporting the adaptation of the agriculture to societal and ecological challenges.

Key Words : drought, genetic resources, breeding, crop model, ideotype, genome

CONTROL OF *VERTICILLIUM DAHLIAE* CAUSING SUNFLOWER WILT USING *BRASSICA* COVER CROPS

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ABSTRACT

Since 2010, sunflower in France has been severely affected by a vascular wilt disease caused by *Verticillium dahliae*. Disease is widespread and causes significant damage up to 30% yield loss. *V. dahliae* is a soil-borne fungus living in roots able to survive in the absence of a host in the form of microsclerotia (MS). *Brassica* crops used as cover crops can naturally suppress soilborne pathogen viability. This fumigation activity has been linked to volatile isothiocyanates (ITCs) released from glucosinolates (GSLs). In this study, *Brassica* cover crops of white and brow mustard, radish, rape and rapeseed were evaluated for their ability to reduce the viability and development of *V. dahliae*. Cultivars were selected by GSL side-chain and concentration, and *V. dahliae* strain for its aggressiveness on sunflower. Biofumigation was assessed in a laboratory assay. MS and developed *V. dahliae* on growing media were exposed for 20 days to volatile compounds released by fresh or freeze ground plant tissues. The toxicity of ITCs-GSLs on *V. dahliae* was assessed by the area under fungus progress curve relating its development on the media. The five *Brassica* reduced the development and germination of *V. dahliae* by 90% (brown-mustard) to 63% (radish), and the development by 90% (rape) to 69% (white-mustard) compared to the control in absence of tissues. Aliphatic GSLs in brown mustard and rape, and indole GSLs in rape and radish may explain the strong reduction of *V. dahliae* development and viability respectively. These results indicate that *Brassica* have potential for use as cover crops for the control of soilborne disease problems and sunflower wilt.

Keywords *Verticillium dahliae*, Microsclerotia, Biofumigation, Cover crops, *Brassica* crops, glucosinolates, Sunflower.

INTRODUCTION

Verticillium dahlia Kleb. is a destructive and vascular soil-borne fungus that infects many economically major agricultural crops and ornamental plants all around the world (Schnathorst, 1981; Pegg and Brady, 2002). In France, since 2010, sunflower (*Helianthus Annuus*) has been severely affected by this pathogen causing significant damage up to 30% yield loss (Mestries and Lecomte, 2012). Symptoms of sunflower Verticillium wilt appears around flowering, first on the lower leaves and move towards upper leaves. On leaf, brown necrosis surround by a yellow halo, vascular discoloration and wilting of sunflower are observed (Wilhelm, 1956). Verticillium wilt is difficult to control because the pathogen can survive in the soil as microsclerotia (MS), its resting structure, for nearly 13 years even in the absence of a suitable host (Bruehl, 1987 ; Griffiths, 1970). Thus, MS are regarded as the primary targets to control Verticillium wilt (Hawke and Lazarovits, 1994).

Since the prohibition of effective but harmful chemical fumigants as methyl bromide, techniques to manage *V. dahliae* in sunflower are limited and not very effective. Alternative methods including sustainable disease control options for managing soilborne fungus are needed (Davis et al., 2010 ; Ochiai et al., 2007 ; Rowe and Powelson, 2002). Thus,

biofumigation, performed by the incorporation of fresh biomass from *Brassica* plants into the soil, appears as an alternative promising method (Kirkegaard et al., 1993 ; Angus et al., 1994 ; Kirkegaard et al., 1998 ; Kirkegaard et al., 2000 ; Matthiessen and Kirkegaard, 2006 ; Larkin and Griffin, 2007 ; Njoroge et al., 2008; Omirou et al., 2011). *Brassica* crops used as cover crops have disease-suppressive effects against soilborne population of fungal pathogens, nematodes and weeds (Brown and Morra, 1995, 1997 ; Buskov et al., 2002 ; Mojtahedi, 1993 ; Olivier et al., 1999 ; Sarwar et al., 1998). It is based on the high concentration of glucosinolates (GSLs) which are secondary metabolites structurally categorized as aliphatic, aromatic, and indole GSLs (Brown and Morra, 1997 ; Fahey et al., 2001 ; Omirou et al., 2011). GSLs are biologically inactive molecules, but after tissues disruption, GSL are hydrolyzed by myrosinase to volatile compounds like indoles, nitriles, thiocyanates and isothiocyanates (ITCs). Among those, ITCs have a biocidal activity and are the most toxic for soil-borne pathogens (Chew, 1988 ; Fenwick and Heaney, 1983 ; Mithen, 2001 ; Omirou et al., 2011 ; Rosa, 1997 ; Sarwar et al., 1998). However, the profile, concentration and distribution of GSLs – ITCs varies greatly within Brassica species, plant tissues and even among cultivars (Kirkegaard et al., 1998 ; Mithen, 1992).

Few studies have investigated the potential of GSLs-containing brassicaceous cover crops for suppression of *V. dahliae*. Additionally, the role of ITC-related biofumigation often cannot be interpreted because no information is provide on the type or the concentration of GSLs present in the used biomass. Thus, predictions of the biofumigation potential of different *Brassica* species to *V. dahliae* based on GSLs concentration needs to be confirmed to evaluate the incidence of their potential biocidal activity *in vitro*. The adoption of biofumigation seems most likely to proceed if it is specified to suit the target pests and production systems. This preliminary study aimed at evaluating the potential biofumigation effects of *Brassica* cover crops on *V. dahliae* - sunflower pathosytem. The objective of this study was (1) to assess the potential biofumigation effects of five *Brassica* cover crops by following *in vitro* the relative toxicity of relevant GSLs on *V. dahliae* development and MS formation (2) to identify the most effective Brassica crops for *V. dahliae* control in future field trials.

MATERIALS AND METHODS

Production of biomass, sampling and sample preparation

Seeds of 5 different cultivars of brown mustard (*Brassica juncea* cv Etamine) (100 pl/m²), white mustard (*Sinapis alba* cv. Abraham) (100 pl/m²), radish (*Rhaphanus sativus* cv. Anaconda) (80 pl/m²), rape (*Brassica rapa* cv. Avalon) (80 pl/m²) and rapeseed (*Brassica napus* cv. Mosa) (80 pl/m²) were sown on February 2015 in 5 trays (0.6 m²; 50 cm depth) filled with potting compost into the greenhouse of INRA, Auzeville (Haute-Garonne, France). These five cultivars were selected among 22 after a field trial in 2014 for their GSL profiles and concentrations in the shoot and root tissue for each crop.

Photoperiod, temperature and air humidity were controlled in the greenhouse. A 13h photoperiod was applied from plant emergence to flowering stage with 400W High Pressure Sodium vapor lamp (SON-T AGRO, Philips). Supplemental lighting was turned off when global radiation was above 250 W/m². The temperature in the greenhouse was maintained at 13°C ± 3°C. Plants were fertilized with two applications of NPK (24 kg N, 28 kg P, 28 kg K /ha) and SO_3 (40 kg/ha) to provide nutriments for GSL synthesis in the plant. Plants were irrigated regularly to maintain adequate soil moisture until flowering. Powdery mildew (*Erysiphe cichoracearum*) was treated by triticonazole (POLYSOINS ULTRA SPRAY, Scotts France SAS) at 0.15 g /L.

The relative production of GSLs in the tissues usually reach a maximum around flowering (Sarwar and Kirkegaard, 1998). Accordingly, brown and white mustard, and radish were sampled at mid-flowering at 51 days after sawing (DAS), and rape and rapeseed at 61 DAS. Roots (RB) and shoots (SB) were sampled, washed and then separated. RB and SB of each cultivar were grinded separately using a ELIET primo mill. The mill was rinsed between each sample. Samples of a particle size <0.5 cm were either used fresh for the in-vitro assay or stored in sealed bags and immediately frozen and stored at -80 °C until processing.

Isolation and analysis of the desulfated GSL

Frozen plant materials were freeze dried and ground as fine as possible with a Tetsch MM 300 mixer mill at 30 Hz for 1 min. Aliquot of 50 mg were weighed in 2.0 ml Eppendorf tubes after witch 1ml 70% MeOH was added to the samples and boiled it for 10 min at 90°C. After boiling, samples were placed for 15 min in an ultrasonic bath and centrifuged at 6500 rpm for 10 min. The supernatant was added to 0,5ml DEAE Sephadex A-25 column. The pellet was kept, washed twice with 1ml 70% MeOH, vortex and placed in the ultrasonic bath for 15 min. After centrifugation at 6500 rpm for 10 min the supernatant was added to the same column. The column was washed twice with 1ml 70% MeOH, once with 1ml MilliQ and twice with 1ml 20mM NaOAC buffer (pH 5.5). Thereafter, 20 µl of aryl sulfatase (Sigma type H-1 of *Helix pomatia*) was added to the columns and flushed down with 50 µL NaOAc buffer (pH 5.5). The columns were covered with aluminium foil and incubated over night at room temperature. The next day, the resulting desulphoglucosinolates were elute from the column with 2 times 0,75ml MilliQ water. The elution was frozen with liquid nitrogen, freeze dried, dissolved in 1ml MilliQ water and measured on the HPLC (de Graaf et al., 2015).

Verticillium dahliae isolates and inoculum production

A *V. dahliae* strain obtained from one microsclerotia (MS) was used in this study and selected for its aggressiveness among 8 strains. The strain was isolated from an infected sunflower residue in a field located in Verfeil (Haute-Garonne, France), close to the trial site, and showing severe Verticillium wilt, root dislocation and high production of MS. Inoculum was plated on Petri dishes containing potato dextrose agar (PDA, Difco) (39 g/l, 150 mg of streptomycin, pH 6) and grown at 25 ± 1 °C in the dark. For *in vitro* assay, the fungus was either used developed (DV) after 10 days growing on PDA and containing mycelium, spores and MS, or as an agar plug of MS (MSV) transplanted on PDA plate.

Evaluation of biofumigation potential of Brassica plant biomass on V. dahliae.

In vitro assay was developed to evaluate the biofumigation potential of RB, SB and a mix of root and shoot biomass (RSB), fresh or frozen, of white and brown mustard, radish, rape and rapeseed to control *V. dahliae*. The capacity of biofumigant cover crop on *V. dahliae* mycelial growth and MS germination was tested on DV and MSV fungus. For each fungus treatment, jars containing either 5 g of grinded RB, SB or RSB (4 g SB + 1g RB), fresh or frozen, were closed with inverted PDA petri containing DV or MS fungus. Each treatment were replicated 5 times corresponding to 300 jars in total. Control jars with inverted DV and MSV were prepared but no biofumigant material was added. Control were replicated 15 times. Jars were sealed with Parafilm® and incubated at 24 °C in the dark for 21 days. The radial growth of DV and MSV fungus was determined weakly.

Data analyses

Area under the fungus development progress curve (AUDPC) was calculated based on the weakly measurement of the fungus growth diameter on Petri dish. AUDPC was calculated according to the equation of Campbell and Madden (1990):

$$AUDPC = \sum_{i}^{n-1} (y_i + y_{i+1})/2 * (t_{i+1} - t_i)$$

where *n* is the number of measurement, *y* the growth diameter of the fungus, and *t* the days between each evaluation. Variables were analyzed by analysis of variance (ANOVA) using Statgraphics Plus 5.1 statistical software (Rockville, MA, USA) with replicate as a random variable. For each ANOVA, homogeneity of variance by Levene's test (confidence level of 0.95) and the normality of the residuals by the Shapiro-Wilks test (confidence level of 0.95) were conducted. When the F ratio was significant ($P < 0.05$), differences between treatment means were determined using protected least significant difference (LSD).

RESULTS

GSL concentration in Brassica crops

The GSLs profile and concentration in the shoot and root tissues of each biofumigant crops are shown in the Table 1. The contrasting GSL profiles between the five Brassica species, and within RB and SB is significant. The GSL profiles of white mustard and rape had significant aromatic GSLs with main concentration of sinalbin in SB and gluconasturtiin in BR respectively. The brown mustard GSLs profiles were dominated by sinigrin (aliphatic GSL) in BS and radish by unknown indole 16.3 (indole GSL) in RB. The GSLs profile of the rape biomass was more diverse including appreciable concentrations of glucobrassicanapin (aliphatic GSL), gluconasturtiin (aromatic GSL) and neoglucobrassicin (indole GSL) mainly in RB.

Table 1. Type and mean concentration of main glucosinolates in the shoot (SB) and root (RB) in the tissues of *Brassicas* biofumigant crop varieties

	Glucosinolate concentration (µmol.g^{-1} dry weight tissue)									
	White Mustard		Brown Mustard		Rape		Rapeseed		Radish	
	SB	RB	SB	RB	SB	RB	SB	RB	SB	RB
Aliphatic										
Sinigrin	1,3	0,0	42,1	3,6	0,0	0,0	0,0	0,0	0,0	0,0
Glucoerucin	3,0	0,1	0,0	0,0	0,0	0,6	0,0	1,1	0,0	0,0
Glucoraphanin	0,2	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
Gluconapin	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,1	0,0	0,0
Progoitrin	0,0	0,0	0,0	0,0	0,9	2,6	0,1	0,3	0,0	0,0
Glucobrassicanapin	0,0	0,0	0,0	0,0	1,9	4,2	0,3	0,2	0,0	0,0
Aromatic										
Gluconapoleiferin	0,0	0,1	0,0	0,0	1,6	3,0	0,0	0,1	0,0	0,0
Gluconasturtiin	0,1	2,8	0,0	2,4	0,0	13,4	0,0	15,2	0,0	0,0
Glucotropaeolin	2,8	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Sinalbin	15,1	4,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0
indole										
4-hydroxyglucobrassicin	0,0	0,1	0,0	0,1	0,5	1,0	0,0	0,1	0,0	0,0
Glucobrassicin	0,0	0,1	0,2	0,0	0,7	0,8	0,7	0,4	5,2	0,1
4-methoxyglucobrassicin	0,1	0,2	0,0	0,0	0,5	1,1	0,1	0,2	0,3	0,2
Neoglucobrassicin	0,0	0,4	0,1	0,4	1,5	4,9	0,8	2,2	0,0	0,0
Unknown indole 16.3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,4	41,6

Biofumigation potential of plant biomass on V. dahliae development

Results from the *in vitro* assays to evaluate the incidence of different biofumigant crops showed that the *V. dahliae* development or germination, measured by the AUDPC, was significantly ($P < 0.05$) affected both for the fungus developed (DV) or the plug of microsclerotia (MSV) compared with the nonamended control (Fig. 1A and B). The AUDPC

was reduced by 63 to 90 % according to the species and a stronger impact on MSV fungus than DV was observed.

The biofumigant potential of the five *Brassica* toward *V. dahliae* differed according to the fungus stage (DV /MSV) (Fig. 1). From DV fungus, brown mustard and rape were more effective and radish was less effective to reduce mycelial growth of *V. dahliae* (Fig. 1A). From MSV, rape and radish were more effective than white mustard to reduce the MS germination of *V. dahliae* (Fig. 1B).

Regarding the type of biomass, there was no significant ($P > 0.05$) difference of RB, SB or RSB on fungus development for the different cover crop except for SB of brown mustard and RB of rape in DV, and for RB of the radish in MSV that reduced significantly ($P < 0.05$) the progression of the fungus. Because there was no significant effect of the cover crop biomass conditioning (fresh or frozen) on fungus development (DV and MSV), statistical analyses were performed on pooled data.

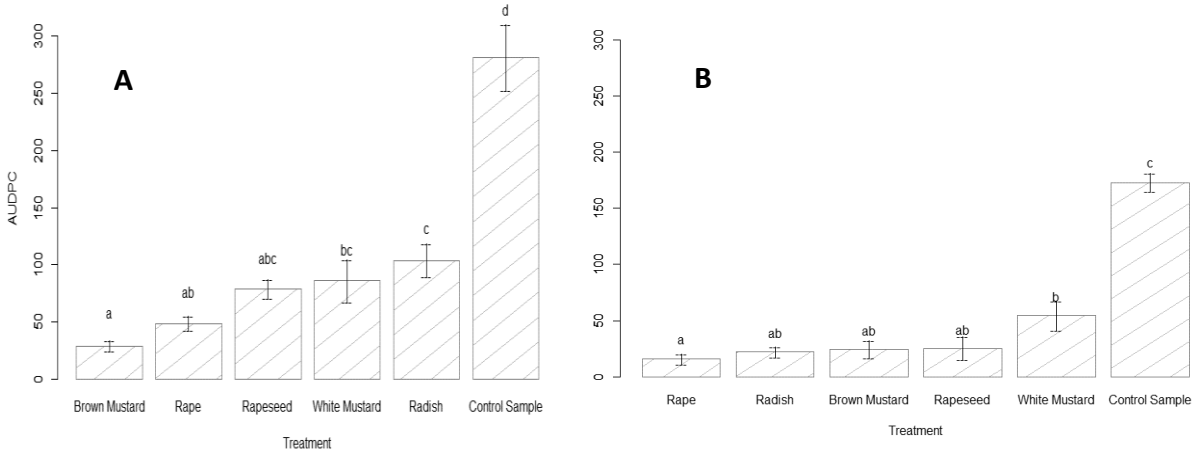

Figure 1. Effect of cover crop types and non-amended control on *V. dahliae* development and germination as measured by the area under the fungus development progress curve (AUDPC) for pooled data from conditioning and type of biomass from the fungus developed (DV) (**A**) and the plug of microsclerotia (MSV) (**B**). Within figures, means followed by letters are significantly different from one another based on LSD ($P < 0.05$).

DISCUSSION

The aim of using cover crop to manage soilborne diseases is mainly the elimination of the inoculum source to reduce yield losses caused by pathogens infections. In this study, assessing the potential of *Brassica* for their biofumigation potential toward *V. dahliae* mycelial growth and the ability of microsclerotia to germinate was evaluated *in vitro*. Optimizing this method for control of *V. dahliae* requires knowledges of the type and concentration of the different GSLs occurring in the respective plant tissues. Therefore, the relative toxicity of ITCs released by their precursor GSLs in root and shoot biomass of white and brown mustard, radish, rape and rapeseed toward *V. dahliae* was tested.

In this study, the five cover crop treatments resulted in statistically significant ($P < 0.05$) reduction of the fungus development from *V. dahliae* developed (DV) and from microsclerotia (MSV) compared with the non-amended control. The toxicity of biological

compounds induced by the grinded biomass and more specifically the ITC-liberating GSLs in the tissues of *Brassica* crops towards *V. dahliae* was confirmed, which is in accordance with other studies testing *in vitro* toxicity of ITCs to other soilborne fungi (Manici et al., 1997 ; Sarwar et al., 1998 ; Smith and Kirkegaard, 2002,). However, the toxicity of ITC showed contrasted effect depending on whether the fungus was mycelial developed or from microsclerotia, which has not been investigated in the literature before. From DV, the mycelial growth of *V. dahliae* was significantly reduced with brown mustard and rape. From MSV, rape, brown mustard and radish blocked the germination of MS.

Brown mustard produced amounts of 2-propenyl ITC from sinigrin GSL (Kirkegaard and Sarwar, 1998; Morra and Kirkegaard, 2002) and the toxicity of this aliphatic GSL could have a significant biofumigation potential as confirmed towards *V. dahliae* and other soilborne pathogens (Angus et al., 1994; Mayton et al., 1996 ; Olivier et al., 1999 ; Smolinska and Horbowicz, 1999 ; Larkin and Griffin, 2007 ; Neubauer et al., 2014). For the DV treatment, the radish could be rated as a poor biofumigation crops as concluded by Neubauer et al., (2014) who evaluated the biofumigation potential of the culture by the aliphatic and aromatic GSL concentrations. However, the radish blocked significantly the germination of MS, more than the brown mustard. The high concentration of unknown indole 16.3 (indole GSL) could be involved but the biofumigant potential of indole GSL has not been studied against soilborne pathogens but more toward plant-parasitic nematodes (Ruanpanun et al., 2010 ; Kruger et al., 2013). Contrary to the radish and brown mustard, the sensitivity of the fungus toward rape biomass was equivalent in DV and MSV who reduced the viable MS by 90 % and *V. dahliae* growth by 83 % compared with the control. Despite the total concentration of aliphatic GSL (10.2 $\mu mol.g^{-1}$ DW) and indole GSL (10.9 $\mu mol.g^{-1}$ DW) was significantly lower than brown mustard (45.6 $\mu mol.g^{-1}$ DW) and radish (54.8 $\mu mol.g^{-1}$ DW) respectively, the rape biomass released a wider diversity of GSLs with aliphatic, aromatic and indole GSLs whereas those were specifics in one type of GSL. This could thus be involved in the high biofumigant potential of the rape, and the high concentration of one type of GSLs would not be predominant to reduce or suppress the development of *V. dahliae*. Regarding the incidence of white mustard and rapeseed to reduce the pathogen growth compared to the other, their profile predominant in gluconasturtiin and sinalbin (aromatic) GSL could explained this lower toxicity due to their lower volatility (Sarward et al., 1998). Although their biofumigation potential has been demonstrated before, aromatic GSLs released from these *Brassica* crops did not seems as effective for biofumigation as those from brown mustard, rape and radish.

CONCLUSION

These results demonstrate that *Brassica* cover crop are able to reduce *V. dahliae* growth and microsclerotia germination. Moreover, the importance of identifying which GSLs type release the most toxic hydrolysis products toward the pathogen was underline. Inhibition of fungi development by grinded cover crops containing sinigrin (aliphatic), unknown indole 16.3 (indole) or a wider diversity of GSLs hydrolysis in *Brassica* tissue was superior to aromatic GSL, suggesting an important role for these compounds in the pest suppression potential of some *Brassica*. The variation in toxicity of different GSLs - ITCs to the fungi suggests there is significant scope to enhance the biofumigation potential of these crops by selecting those which produce a wider diversity of GSLs precursors to the most toxic ITCs such as rape to suppress Verticillium wilt in sunflower fields.

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STUDY OF THE GENOMIC DIVERSITY OF VERTICILLIUM SP. CAPABLE OF COLONIZING SUNFLOWER. HOW KNOWLEDGE OF PATHOGEN GENETIC STRUCTURE CAN BE COMBINED WITH CLASSICAL BREEDING APPROACHES TO GUIDE IT

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ABSTRACT

Plant disease management approaches are mainly represented by resistance genes and agrochemicals that are used repeatedly until their efficacy is overcome by the targeted pathogen. Despite no sexual cycle observed, comparative genomics show extensive chromosomal rearrangements and lineage-specific genomic regions that increase *V. dahliae* evolutionary potential. Yet, the complex relationship between spatial pattern of disease, plant genetics, crop practices and the evolutionary dynamics of *Verticillium* population remains poorly studied. The objective of this study was to investigate how uniform is the genetic make-up of the pathogen presents within a field. A spatial analysis was performed on a field with sunflower disease history to generate regions of high or low disease prevalence. Two sunflower genotypes were sampled 45 times in predefined areas according to the disease prevalence map previously established: i) a Symptomatic (S) and ii) an Asymptomatic (AS). Qualitative PCR were carried out to: i) point out the presence of *Verticillium* in stems (VdFE1/VdFE2 primers pair), and ii) to determine the defoliating and race profiles of the strains studied (Ave1F/Ave1R and D_NDf/D_NDr primers pairs used as markers for increased virulence and defined on tomato and cotton). Results showed i) a full colonization of S genotype by *Verticillium dahliae* and 75% of AS genotype colonized by undetermined *Verticillium* sp.; and ii) the exclusive presence of *Verticillium dahliae* strains that do not carry Ave1 gene. Deeper investigation of genomic diversity of *Verticillium dahliae* will be presented to better determine *V. dahliae* strains profile capable of colonizing sunflower.

Key Words : Verticillium, host-pathogen interaction, agrosystem, disease prevalence, cultivar specificity

EVALUATION OF SUNFLOWER (HELIANTHUS ANNUUS L.) HYBRIDS FOR PHOTOTHERMAL UNITS ACCUMULATION, OIL YIELD, OIL QUALITY AND YIELD TRAITS UNDER SPRING PLANTING CONDITIONS OF HARIPUR, PAKISTAN

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ABSTRACT

Field experiment was conducted at University of Haripur, Pakistan during spring 2014, to explore the role of photothermal units on oil contents, fatty acids profile, yield and yield traits of four sunflower hybrids viz SMH-0917, NKS-278, SMH-0907 and Hysun-33. Sunflower hybrids were sown in spring and arranged under randomized complete block design with 3 replications under field conditions. Significant variation ($p<0.05\%$) was found among the sunflower hybrids for photothermal units requirements for flower initiation, flower completion and physiological maturity. Highest photothermal unit accumulation was found in Hysun-33 followed by SMH-0917 and SMH-0907. Highest seed oil contents and oil quality (highest linoleic acid and oleic acid percentage while least percentage of palmitic acid) was recorded in Hysun-33, SMH-0917 and SMH-0907. Overall Hysun-33, SMH-0917 and SMH-0907 performed better for head diameter, number of achenes per head, total dry matter yield (kg ha-1) and economic yield (kg ha-1) under field conditions. It was also inferred that the temperature and moisture availability positively influence the oil quality of sunflower hybrids under spring planting conditions. Variability found among the tested sunflower hybrids for photothermal units accumulation, oil content, oil quality and yield traits could be exploited in the breeding program for development of early maturing and high yielding local sunflower hybrids.

Key Words : Sunflower, photothermal units, oil content, fatty acid profile, yield and yield traits

DETERMINING NEW AGGRESSIVE BROOMRAPE INFESTATION IN MEDITERRANEAN REGION OF TURKEY

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ABSTRACT

Orobanche L. is a large genus mainly distributed throughout subtropical and temperate regions of the northern hemisphere. The Mediterranean region is one of the most important centers of diversity. The genus Orobanche has been represented by 39 species due to this new record in Turkey. (Zare et al., 2009) Sunflower cultivation has gradually increased in the eastern Mediterranean region since 2004.). In 2011, sunflower broomrape began to appear in cultivated area in Adana and increased rapidly until today. Based on this research results and natural condition observations show that infested new broomrape races areas are increasing seriously year by year in the mediterranean region. As a result it will be required of new sources genetic resistance to the most virulent races or herbicide resistant hybrids for this region.

INTRODUCTION

Orobanche L. is a large genus mainly distributed throughout subtropical and temperate regions of the northern hemisphere. The Mediterranean region is one of the most important centers of diversity. The genus Orobanche has been represented by 39 species due to this new record in Turkey. (Zare et al., 2009)

Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm, totally devoid of chlorophyll, that infects the roots of sunflower (*Helianthus annuus* L.) plants, drawing water and nutrients from them. This parasitic plant is regarded as one of the most important constraints on sunflower production in areas of eastern and southern Europe, the Middle East, Russia, Ukraine and China (Parker,1994). According to Kaya *et al.* (2004), about 80% of sunflower areas in Turkey (Thrace region) are infested with the seeds of the parasite. According to these authors, epiphytotic occurrence of broomrape is registered in this region each 20 years. Furthermore, the parasite forms new, more virulent races which overcome the resistance of the varieties and hybrids commonly used in production (Kaya *et al*., 2004; Pacureanu-Joita *et al*., 1998; Alonso, 1996; Fernandez-Martinez *et al*., 2000). This impedes effective control of broomrape.

Sunflower cultivation has gradually increased in the eastern Mediterranean region since 2004. In 2005, sunflower acreage and production in the region were tripled compared with 2004. There has not been any record on broomrapes in sunflower fields in eastern Mediterranean region yet, but broomrapes are considered a possible threat for sunflower fields in this area. Orobanche cernua Loef. causes considerable damage in sunflower fields in other regions of Turkey where sunflower has been sown for years and it may spread from those regions to the eastern Mediterranean region (Bülbül et al. (2009). In 2011, sunflower broomrape began to appear in cultivated area in Adana and increased rapidly until today (Figure 1, 3) .

MATERIALS AND METOD

Sunflower hybrids in the official registration trials which commercial sunflower hybrids belong private companies were tested against to new broomrape races in natural conditions between 2013-2014.

Broomrape observations were evaluated as Frequency (F) Intensity (I) and Attacking Rate (AR) based on Pustovoit method. The plants were accepted as resistant having % 0-10 Frequency and 0-1 AR values and (Vranceanu *et al.*, 1980). The plants had % 10-20 frequency as accepted tolerant .

F = % The number of plant with orobanche (The plant number infested

orobanche / Total plants in the row x 100)

I = The number of orobanche in one infested plant (Total orobanche /

Total plants infested orobanche in the row).

AR = F x I / 100 = The number of orobanche in one plant in the row.

Figure 1. Broomraper in Adana Region in Turkey

RESULTS

High percentages of broomrape attack were registered in southeastern regions of Turkey during 2013 and 2014 growing seasons. According to 2013 observations, only sensitive varieties showed infection of broomrape (Table 1). In 2014 all plants in the set which contain official checks were susceptible or highly influenced and then We concluded that they could be new races (Table 2, Figure 2).

Table1. Broomrape observations in natural conditions				
		2013		
		Adana (Ceyhan)		
		F	I	SD
No	Varieties	%	(piece)	(piece)
1	Candidate 1	0.3	3.0	0.01
2	Candidate 2	7.9	3.42	0.27
3	Candidate 3	9.4	2.08	0.2
4	Candidate 4	9.4	2.43	0.23
5	Candidate 5	10.1	4.98	0.5
6	Candidate 6	11.1	3.66	0.41
7	Candidate 7	14.6	4.55	0.66
8	Candidate 8	15.8	2.94	0.46
9	Candidate 9	16.1	3.43	0.55
10	Candidate 10	17.1	2.3	0.39
11	Candidate 11	19.8	2.27	0.45
12	Candidate 12	21.2	3.17	0.67
13	Candidate 13	25.8	2.47	0.64
14	Candidate 14	29.2	7.47	2.18
15	Candidate 15	29.6	3.02	0.89
16	Candidate 16	30.8	4.53	1.4
17	Candidate 17	45.5	2.46	1.12
18	Candidate 18	57.9	3.1	1.79
19	Candidate 19	100	8.91	8.91
20	Candidate 20	100	10.23	10.23
21	Candidate 21	100	6.66	6.66
22	Candidate 22	100	8.45	8.45
23	Check1	1.5	3.33	0.05
24	Check2	1.8	1.86	0.03
25	Check3	2.8	3.73	0.1
26	Check4	11.0	2.0	0.22
27	Check5	24.8	2.4	0.6
28	Check6	35.4	3.64	1.29

Table2. Broomrape observations in natural conditions				
		2014		
		Adana (Sarıçam)		
		F	I	SD
No	Varieties	%	(piece)	(piece)
1	Candidate 1	18.0	1.35	0.24
2	Candidate 2	100	6.72	6.72
3	Candidate 3	78.4	1.22	0.96
4	Candidate 4	84.4	1.42	1.2
5	Candidate 5	100	2.11	2.11
6	Candidate 6	80.3	1.99	1.6
7	Candidate 7	100	12.43	12.43
8	Candidate 8	100	11.69	11.69
9	Candidate 9	100	8.11	8.11
10	Candidate 10	86.8	2.57	2.23
11	Candidate 11	100	3.91	3.91
12	Candidate 12	86.8	6.94	6.02
13	Candidate 13	100	6.25	6.25
14	Candidate 14	100	4.12	4.12
15	Candidate 15	100	11.98	11.98
16	Candidate 16	100	15.8	15.8
17	Candidate 17	100	8.08	8.08
18	Candidate 18	90.2	4.38	3.95
19	Candidate 19	100	10.86	10.86
20	Candidate 20	98.5	12.19	12.01
21	Candidate 21	100	11.55	11.55
22	Candidate 22	100	15.33	15.33
23	Check1	49.8	1.39	0.69
24	Check2	100	1.52	1.52
25	Check3	45.1	2.24	1.01
26	Check4	100	10.41	10.41
27	Check5	100	8.0	8.0
28	Check6	100	2.84	2.84

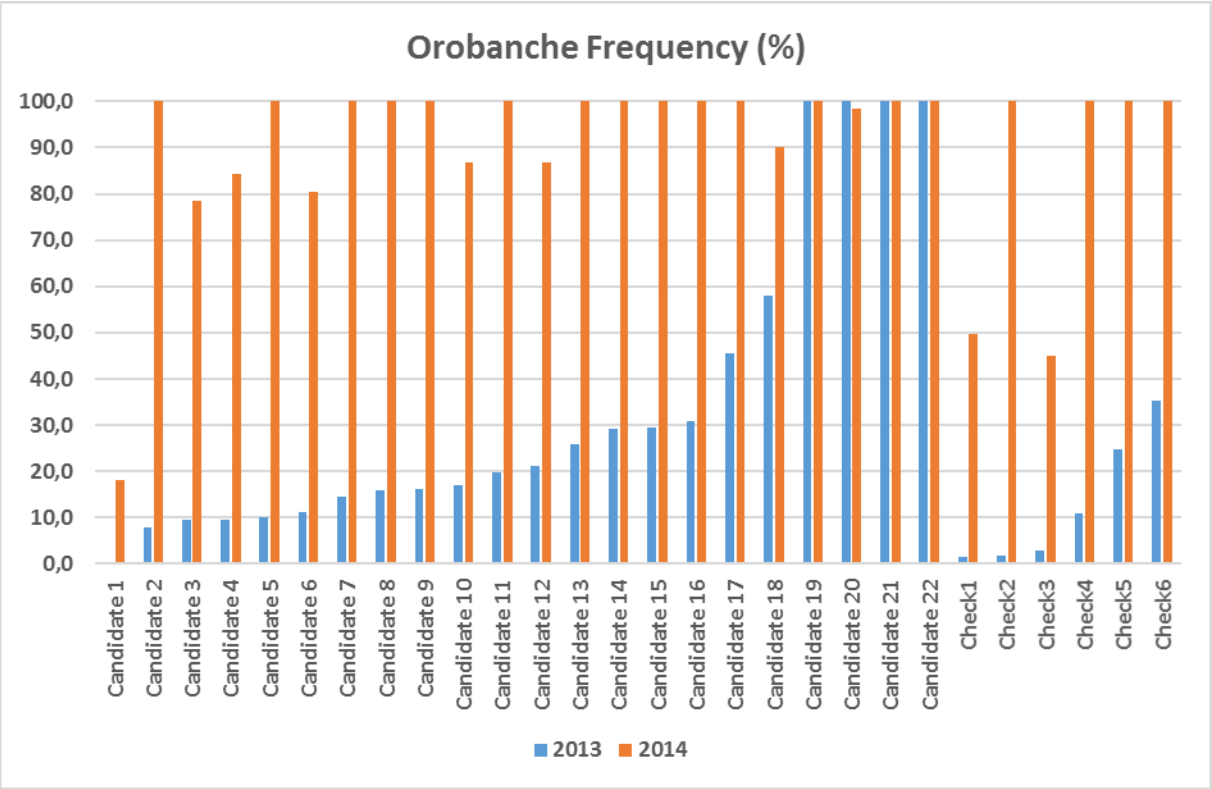

Figure 2: Broomrape density and frequency in trials

Figure 3: Broomrape in the fields.

CONCLUSIONS

In recent years, the parasite *Orobanche sp.* has developed new and virulent populations, in the sunflower crop in Europe, including Turkey.

Based on this research results and natural condition observations show that infested new broomrape races areas are increasing seriously year by year in the mediterranean region.

Control of this parasite remains extremely difficult, as thousands of tiny seeds produced by one single broomrape plant can be easily dispersed by wind, water, animals, humans, machinery or attached to sunflower seeds. Broomrape seeds may remain viable for 15-20 years and will only germinate in the presence of the host plant (Škorić, 1988).

As a result it will be required of new sources genetic resistance to the most virulent races or herbicide resistant hybrids for this region.

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STUDY OF *OROBANCHE CUMANA* GENETIC DIVERSITY

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ABSTRACT

The study of *Orobanche cumana* genetic diversity is critical to better understand the evolution of this sunflower parasitic plant and develop new resistant sunflower hybrids. A broad collect of *Orobanche cumana* populations has been organised since 2012 in major countries affected by broomrape (Spain, Turkey, Romania, Hungary, Ukraine, Russia and France) resulting in the harvest of more than 500 orobanche seed lots. A subset of 12 populations representing different level of aggressiveness and different countries has been submitted to transcriptome sequencing in order to performed SNP discovery. This approach lead to the discovery of 368,000 SNP bi-allelic among which 1536 were selected for genotyping of the entire set of collected seed lots. This large diversity study outlined contrasted level of fixity between populations, more specifically French and Spain populations are largely homozygous compared to populations coming from Eastern Europe greatly heterozygous. The principal component analysis based on the relationship matrix (PCoA), allows the discrimination of population according to their geographical origin with 2 opposed clusters on axis 1 representing French and Spanish populations and a third cluster representing eastern Europe populations that can be split by countries on axis 3. Finally, this study allowed the definition of a diversity kit of 200 SNP that can be routinely used for *Orobanche Cumana* diversity study, this SNP toolkit is freely available upon demand to Biogemma.

Key Words : broomrape, genetic diversity, SNP

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REACTION OF SUNFLOWER (HELIANTHUS ANNUUS L.) LINES TO DROUGHT STRESS BASED ON TOLERANCE INDICES

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ABSTRACT

In order to evaluation of genetic diversity of oily sunflower lines, screening drought resistance indices and identification of drought resistance lines, 100 lines of oily sunflower were evaluated in a simple lattice design with two replications under two conditions including normal irrigated and drought stress under filed condition in Salmas. Based on the potential (Yp) and stress (Ys) yield, quantitative drought tolerance criteria such as: mean productivity (MP), tolerance index (TOL), geometric mean productivity (GMP), harmonic mean (HM), stress susceptibility index (SSI) and stress tolerance index (STI) were calculated. Generally in both condition, line with code number of 8 with average yield of 81.25 g m-2 and line 66 with average yield of 5.425 g m-2 had the maximum and minimum values of yield. In normal and drought stress conditions, the highest value of MP, GMP and HM were possessed to genotype 8. Correlation analysis between drought resistance indices with potential and stress yields revealed that indices including MP, GMP, HM and STI are most suitable criteria for screening sunflower's genotypes. Line 8 was chosen as best drought resistant regarding to these four criteria and high values of Yp and Ys.

Key Words : Multivariate Analysis, Sunflower, Tolerance Index, Yield, Water Deficit

CADMIUM-POTASSIUM INTERRELATIONSHIPS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Cadmium (Cd) is a toxic heavy metal for all living organisms. In this study, tolerance and bioaccumulation of Cd and mitigation of its toxic effects with potassium (K) treatments in sunflower (*Helianthus annuus* L., cv. Sirena) were investigated. Five levels of Cd (0, 0.1, 0.3, 0.6 and 1.2 mM) and three levels of K (0, 200, 400 mg kg-1) applied to the soil. Increasing Cd levels depressed root length and shoots and roots dry weight (DW), total chlorophyll and carotenoid, resulting from its toxic effects. However, these decreases were slightly ameliorated by applied K. Increasing Cd levels significantly increased membrane permeability (MP). Also, the shoot and root Cd contents, uptakes and total accumulation rate (TAR) of sunflower plant were increased by Cd treatments. These parameters all showed a declining trend with applied K. Moreover, shoot and root K content and uptake of sunflower increased considerably with applied K. The shoot and root bioconcentration factor (BCF) and translocation factor (TF) of Cd were decreased by applied K.

Key Words : Cadmium toxicity, growth, bioaccumulation, translocation, Helianthus annuus L., potassium

RESPONSE TO SUNFLOWER (*HELIANTHUS ANNUUS* L.) PLANT AT EARLY GROWTH STAGE TO CADMIUM TOXICITY

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Cadmium (Cd) which is a non-essential element for plants, animals and humans has been a major pollutant in both terrestrial and aquatic environments for several decades. The effect of Cd toxicity was studied in sunflower (*Helianthus annuus* L., cv. Sirena) grown in greenhouse under natural light conditions. For this reason, the soil was treated with six levels of Cd (0, 0.05, 0.10, 0.25, 0.50 and 1.00 mM). Plant growth, photosynthetic pigments, relatively water content (RWC), bioaccumulation and translocation of Cd and uptake of zinc (Zn), potassium (K), and calcium (Ca) were investigated. Shoot and root growth and root elongation were depressed with increasing Cd levels and deleterious effect of Cd on plant growth was appeared shoot more than roots. Also, the contents of chlorophyll (Chl) and carotenoid (Car), RWC, growth tolerance index (GTI), uptake of Zn, K, and Ca in shoot and root were decreased with Cd application as well as bioaccumulation and translocation of Cd. Moreover, Cd treatments increased Cd content and uptake in shoot and root, total accumulation rate (TAR) of Cd, membrane permeability (MP), rate of Car/Chl caused by its toxic effects.

Key Words : Cadmium, toxicity, translocation, accumulation, growth, Helianthus annuus L.

THE VIRULENCE OF PLASMOPARA HALSTEDII IN THE SOUTHERN REGIONS OF RUSSIAN FEDERATION

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ABSTRACT

The population of oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni (sunflower downy mildew causal agent) has been monitored in Krasnodar Krai, Rostov region and Republic of Adygea more than 15 years. Prior to the beginning of the 2000s there were races 100, 300, 310 and 330 in the region. In the period from 2004 to 2007 races 100, 300, 310 and 700 met sporadically. The race 330 was the most common; in a number of agrocoenoses it was 100 % of samples. In some fields races 710 and 730 prevailed. In 2008-2011 only races 330, 710 and 730 were found; the race 330 still prevailed and was also found on *Ambrosia artemisiifolia* L. Since 2012 in the majority of fields races 710 and 730 prevailed, and the race 330 wasn't allocated in many of them; for the first time in Russia pathotype 334, that able to overcome Pl_6, was found in Krasnodar Krai. In the period of 2013-2015 increased distribution of the race 334 in Krasnodar Krai and Republic of Adygea was observed. At the same time, in 2014 in one field in the Rostov region only races 310 and 330 (prevailed) were identified. The virulence of the pathogen population is closely connected with the cultivated assortment of sunflower. Further spread and accumulation of P. halstedii race 334 and the emergence of new pathogen pathotypes in the said regions are predicted.

Key words: downy mildew, *Plasmopara halstedii*, races, sunflower, *Helianthus annuus, Ambrosia artemisiifolia*

INTRODUCTION

One of the most spread and harmful diseases of sunflower (*Helianthus annuus L.*) in Russia is downy mildew, caused by oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni. The population of the parasite has been studied in the south regions of Russian Federation (Krasnodar Krai, Rostov region and Republic of Adygea) more than 15 years.

Initially, in the territory of the former USSR there was only race 100. However, in the early 1980s all resistant sunflower varieties of domestic selection became affected in the Krasnodar region (Tikhonov, Zaychuk, 1984). Prior to the beginning of the 2000s there were identified races 100, 300, 310 and 330 in the region (Antonova, 2003). It should be noted that over the previous decade foreign sowing material of sunflower was freely delivered in the country and decline in terms of return of the culture in field has become the norm.

In the period from 2004 to 2007 seven races of *P. halstedii* were found in the said regions. The most common was the race 330; in several agrocoenoses it was 100 % of samples. Races 710 and 730 prevailed in some fields. Races 100, 300, 310 and 700 met

sporadically. Status of the pathogen population in the region in those years was described in detail previously (Antonova et al., 2008).

The aim of our study was to monitor the racial structure of *P. halstedii* population in the southern regions of Russian Federation (Krasnodar Krai, Rostov region and Republic of Adygea).

MATERIALS AND METHODS

The leaves from the infected by downy mildew sunflower plants were collected from the fields in Adigeya republic, Krasnodar and Rostov areas in 2009-2015 (table 1).

Table 1. The total numbers of identified *P. halstedii* isolates and fields, where isolates of *P. halstedii* were collected, in different years

Total number	Years		
	2009-2011	2012-2013	2014-2015
identified *P. halstedii* isolates	196	474	480
surveyed fields	11	16	19

For identification of pathogen races, according to the nomenclature system (Tourvieille et al., 2000), nine *P. halstedii* differential lines of *H. annuus* were used: set 1 – VNIIMK 8883 (D-1), RHA-265 (D-2), RHA-274 (D-3); set 2: DM-2 (D-4), PM-17 (D-5), 803-1(D-6); set 3: HA-R4 (D-7), HA-R5 (D-8), HA-335 (D-9). The line HA-304 (D1), that was not stable in reaction of resistance or susceptibility, has been changed on the universally susceptible variety VNIIMK 8883.

Pre-germinated seeds of differentials with radicle length 1,0-2,0 cm were placed in plastic growth trays with sterilized sand, covered by filter paper. The radicles of seedlings were covered by wet cotton wool. 150 ml of zoosporangial suspension of isolates (concentration about 10^6 zoosporangia/ml) were added in growth trays (one *P. halstedii* isolate per tray) and incubated 16-20 hours at the temperature 16 ^oC. Inoculated sunflower plants were grown at the temperature 25±2 ^oC (16 h photoperiod) and after 7-9 days were placed in darkness at 16 ^oC in 100 % humidity overnight for induction of *P. halstedii* sporulation. Plants with sporulation on leaves or with abundant sporulation on cotyledons only were classified as susceptible.

RESULTS

Until 2007 in the southern regions of Russia (Krasnodar Krai, Rostov region and Republic of Adygea) seven races of *P. halstedii* were found. Among them during 2004-2007 races 100, 300, 310 and 700 constituted together about 2,5 %, and the most common were races 330, 710 and 730 (about 65, 13,5 and 19 % respectively).

Since then, there have been significant changes in the structure of the pathogen population. They are shown in table 2, which presents the prevalence and frequency of occurrence of races in the region in different years.

Table 2. The distribution of *Plasmopara halstedii* races on sunflower in southern regions of Russia during 2004-2015*

Races	Years											
	2004-2007			2009-2011			2012-2013			2014-2015		
	F	I	Ifi	F	I	Ifi	F	I	Ifi	F	I	Ifi
100	0.05	0.2	3.3-7.1**	0	0	0	0	0	0	0	0	0
300	0.07	0.7	1.2-3.5	0	0	0	0	0	0	0	0	0
310	1.6	0.9	1.2-10.3	0	0	0	0	0	0	5.3	1.8	17.2
330	100	65.1	12.7-100	100	46.5	25.0-92.0	75.0	18.5	7.7-25.6	31.6	26.2	10-91.4
700	1.3	0.7	1.2-12.7	0	0	0	0	0	0	0	0	0
710	70	13.6	2.3-69.6	100	25	3.3-57.1	100	35.7	26.6-90.0	73.7	24.0	5.0-54.0
730	56	18.8	3.3-58.2	100	28.5	6.7-52.1	93.8	44.5	16.7-70.2	73.7	30.5	10.0-64.0
334	0	0	0	0	0	0	12.5	1.3	0.4; 2.2	47.4	17.5	20.0-100

* F - the frequency of the race occurrence in the fields, %;

I - race proportion in the total number of identified *P. halstedii* isolates, %;

Ifi - minimum and maximum percents (%) of the race in positive samples, %

**- the samples of *P. halstedii* isolates were small

Race 100, 300 and 700 were not found after 2007. However oospores of *P. halstedii* are capable of being viable in the soil up to 10 years (Viranyi and Spring, 2011). Therefore it is not excluded that these races still are present in the region as another one of the old races - 310, which was found in one field in 2014 and amounted to 17 % of the sample (table 2). The period of existence of these races in the pathogen population prolongs by cultivation of susceptible sunflower in separate fields.

Till 2011 race 330 was found in each of surveyed fields and dominated in the south of Russia. But from 2012 its part in the pathogen population has considerably decreased: it became less than 20 % in 2012-2013 and less than 30 % in 2014-2015. In 2012-2013, it was present in 75 % of samples, in 2014-2015 – only in 32 %. At the same time, race 330 has been found on plants of common ragweed (*Ambrosia artemisifolia* L.) in Krasnodar Krai in different years (2011, 2013 and 2015). All isolates collected by us from ambrosia belonged only to this race. Analyze of SNP DNK locuses proved identity of this isolates and isolates of the race 330 from sunflower (Iwebor et al., 2012). Thus the race 330 can persist in local population of *P. halstedii* on ambrosia. Even the widespread cultivation of sunflower, resistant to this race, will not lead to its complete disappearance, as in Russia *A. artemisiifolia* is ubiquitous in areas of sunflower cultivation.

Races 710 and 730 were found only in 70 and 56 % (respectively) of the surveyed fields in 2004-2007 and they were found almost in every field in 2009-2013. Since 2012 these two races (individually or together) prevailed over race 330 in pathogen population both in general and in the majority of separate agrocoenosises.

In 2012 one isolate of race 334 was discovered in Krasnodar Krai. In Russia it was the first time of detection of the pathotype that able to overcome the resistance gene Pl_6 of sunflower. In 2013 the race 334 has been found again in one field. In 2014-2015 increased distribution of this race was observed in Krasnodar Krai and in Republic of Adygea (tables 3 and 4). It was present in almost half of surveyed fields and reached 17,5 % of the total number of identified pathogen isolates. Race 334 ranged from 20 to 100 % in the samples from different field (table 2).

All changes which have happened in racial structure of *P. halstedii* population were closely connected with cultivated assortment of sunflower that was clearly demonstrated in the tables 3 and 4.

Table 3. Races of *P. halstedii*, found in the sunflower fields in the Republic of Adygea and Rostov region in 2011-2015

Location of the field	Year	Foreign hybrids of sunflower in the field*	The number of isolates					
			total	race				
				310	330	710	730	**334**
Rostov region	2011	-	19	0	6	6	7	0
	2014	-	55	0	40	7	8	0
		-	64	11	53	0	0	0
Republic of Adygea	2012	-	28	0	7	11	10	0
	2014	+	10	0	0	5	2	**3**
	2015	+	16	0	0	3	3	**10**

* - foreign hybrids of sunflower have been cultivated in the field in any of last 5 years (before the year of sampling): '+' – yes, '-' – no

In one of the sunflower fields in the Rostov region (2014), race 330 dominated and race 310 has been found. Races 710 and 730 have not been revealed there (table 3). From the history of the field it is known that only domestic sunflower varieties were cultivated there. In the other two fields of this region and in one of the fields in Adygea (2012) also domestic varieties and hybrids were grown only. There were identified races 330, 710 and 730. Race 334 was found in the Republic of Adygea in two fields, in which during several last rotations of sunflower foreign hybrids were cultivated.

The similar situation was observed in fields of Krasnodar Krai (table 4). In the fields, where only domestic varieties and hybrids have been cultivated (at least 5 last years before the year of sampling), races 330, 710 and 730 were isolated, but not the race 334.

Race 334 was revealed in the fields where in any of last 5 years (before the year of sampling) foreign hybrids of sunflower have been cultivated. It was such in the fields in Korenovsky and Tbilissky districts (Krasnodar Krai), where over the last 5 years foreign hybrids were sowed twice. In several samples race 334 made 100 %: these *P. halstedii* isolates were collected from the foreign sunflower hybrids with Pl_6 – the gene of resistance to all parasite landraces except 334.

Table 4. Races of *P. halstedii*, found in the sunflower fields in the Krasnodar Krai in 2011-2015

Districts	Year	Foreign hybrids of sunflower in the field*	The number of isolates				
			total	race			
				330	710	730	**334**
Belorechensky	2011	-	11	7	2	2	0
Gulkevichsky	2011	-	28	10	9	9	0
	2015	-	64	5	18	41	0
Novokubansky	2012	-	39	1	26	12	0
Labinsky		-	12	0	9	3	0
Kushchyovsky		+	3	0	2	0	1
Korenovsky	2013	+	50	24	2	40	34
Tbilissky	2014	+	70	11	5	5	49
Slavyansky	2015	-	12	7	4	1	**0**
Novopokrovsky		+**	15	0	0	0	15**
		-	7	0	1	6	**0**
Kanevskoy		+**	17	0	0	0	17**
Pavlovsky		+**	25	0	0	0	25**

* - foreign hybrids of sunflower have been cultivated in the field in any of last 5 years (before the year of sampling): '+' – yes, '-' – no; ** - *P. halstedii* isolates were collected from the foreign hybrids with Pl_6

Russia became the second European country in which race 334 has been revealed. This race was registered for the first time at the beginning of the 2000 in France and after 2007 - in the USA and Canada (Gulya,2007; Delmotte et al., 2008; Viranyi et al., 2015).

It is possible that race 334 was introduced into our country with the seeds of foreign sunflower hybrids. On the other hand, its appearance could become the result of evolutionary processes in local *P. halstedii* population, exerted by cultivating of resistant sunflower hybrids and elevated by crop rotation violations.

Experience of different countries showed that after the appearance of new races in the population of this parasite, the emergence of other races can be expected soon. As it was happened in France. After the race 100, there emerged pathotypes with virulence code 7xx which have overcome the resistance of differential lines RHA-274 (D-3): races 710 and 703 to which also lines PMI3/DM-2 (D-4) and HA-R4 (D-7) + HA-R5 (D-8) (respectively) are susceptible. Then, due to the massive deployment of new resistance genes (as Pl_6 and Pl_7), there were formed new races, that able to overcome resistance of differential lines RHA-265 (D-2) – races 3*xx*, PM-17 (D-5) – races *x*3*x*, HA-335 (D-9) – races *xx*4 и *xx*7 (Tourvieille de Labrouhe et al., 2005; Delmotte et al., 2008; Viranyi et al., 2015).

In the south of Russia after race 100, races with virulence code 3x0 (300, 310 and 330) appeared, then – 7x0 (700, 710 and 730) and the last to date – race 334. The pathotypes with

virulence to the genes of resistance in differential lines 803-1 (D-6), HA-R4 (D-7) and HA-R5 (D-8) still were not recorded here.

Thus, the racial composition of the *P. halstedii* population in the south of Russian Federation has changed due to the cultivated assortment of sunflower. Races 330, 710 and 730, which dominated last years, still were widespread but their parts in the parasite population decrease. In 2012, for the first time in Russia, the race 334 has been found. It is quickly distributes and has occupied a dominant position in some fields. Further spread and accumulation of *P. halstedii* race 334 and the emergence of new pathogen pathotypes in the southern regions of Russia are predicted.

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QUANTIFICATION OF DROUGHT TOLERANCE LEVELS OF SUNFLOWER INBRED LINES BY MEANS OF CHLOROPHYLL-*A* FLUORESCENCE

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ABSTRACT

Plants are often exposed to various environmental stresses such as drought. One of the major objectives in plant breeding programs for crops grown in arid/semiarid areas is selection of crop cultivars with remarkable resistance to drought stress. To select drought tolerant lines, chlorophyll *a* fluorescence (ChlF) measurements has used in addition to morphological and physiological analysis in recent years. Some sunflower (*Helianthus annuus* L.) inbred lines developed by Trakya Agriculture Research Institute (TARI) with National Sunflower Project were grown in Bahri Dagdas International Agricultural Research Institution in order to determine the drought tolerance levels using fast ChlF techniques. Fluorescence signals were recorded and analyzed using JIP-test. V_J, V_I, ABS/RC, ET_0/TR_0, DI_0/RC, RE_0/ET_0 and PI_{total} originated from JIP-test parameters were evaluated. Besides, drought factor index (DFI) was calculated using data of PI_{total} and lines were classified according to their drought tolerance levels. Results obtained from present study indicated that lines were markedly affected depending on the duration and severity of the drought. Additionally, sunflower inbred lines could be separated into four groups as highly drought tolerant (9814 A), drought tolerant (TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A) less drought tolerant (8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107 A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A) and drought sensitive (6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A) based on the DFI values.

Key words: Sunflower, Drought tolerance, Inbred lines, Chlorophyll *a* fluorescence kinetics

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important agricultural crops in the world and the main source of unsaturated vegetable oil (Baloğlu et al., 2012; Gholinezhad et al., 2015). While the world sunflower production has varied from 2010, production of Turkey has been increased (FAOSTAT, 2016). Turkey has among the top 10 in sunflower producer countries according to National Sunflower Association data. On the contrary of well-studied model plants, different water regimes adaptable plants could be valuable genetic sources to understand links between stresses and stress responses (Raineri et al., 2015). Due

to its drought tolerance and deep root system compared to other crops, sunflower has been an attractive alternative genetic source (Howell et al., 2015).

Among the various abiotic stresses, drought is the most significant environmental stress in agriculture worldwide and under the limited water conditions improving yield and yield capacity is major goal of plant breeder. Due to urbanisation, industrialisation, depletion of ground-water and global warming, the amount of available water is decresing day by day. dorught triggered by these conditions cause major constraints on the physiology, biochemistry, growth, development and productivity of plants (Bechtold et al., 2016; Szechyńska-Hebda et al., 2016) depending on the stres intensity and duration (Raineri et al., 2015). More than 80 years of breeding activities have caused some yield increase for crop plants grown in areas affected by drought (Cattivelli et al., 2008) and this situation would be the most economical approach to improving agricultural productivity and reducing agricultural use of substantial water resources (Sperdouli and Moustakas, 2012). Drought stress primarily influences photosynthesis, by multidimensional ways just as reduce in leaves expansion, decreased CO_2 diffusion to the chloroplast, impared photosynthetic apparatus with enzymes and expedite of leaf senecence (Farooq et al., 2009; Pinheiro and Chaves, 2011; Hasanuzzaman et al., 2014). Based upon limitation of CO_2 uptake and imbalance between absorbing and using of sunlight, the possibility of overexcitation of photosystem II (PSII) increases. This case induces a decrease of photosynthetic rate and an increase in the dissipation of absorbed energy through non-radiative processes (Faraloni et al., 2011). Under drought conditions, photosystem II (PSII) is more sensitive than photosystem I (PSI) (Deng et al., 2003), therefore PSII has a key role to analyze changes that occur in photosynthesis (Baker, 1991). ChlF is a non-invasive measurements of PSII activity and is a commonly used technique (Murchie and Lawson, 2013; Schansker et al. 2014). To determine the photosynthetic performance, ChlF kinetics can be considered as a biosensor tool. All oxygenic photosynthetic samples investigated so far using ChlF techniques show the characteristic polyphasic rise from the ground state value (F_0, 20μs) at the O step to its maximum value (F_M, approx. 300-500 ms) at the P step with J (F_J, 2 ms) and I (F_I, 30 ms) intermediate steps (Strasser et al. 2004). An analysis of the fast OJIP fluorescence kinetics, called JIP test, quantifies the in vivo energy fluxes passing through the reaction centres and photosystems (Strasser and Strasser, 1995; Strasser et al., 2000). An analysis of the fast OJIP fluorescence kinetics, called JIP test, links different steps and phases of the transient with the redox states of PSII, also correlates the phases with the efficiencies of electron transfer in the intersystem chain between PSII and PSI and to the end electron acceptors at the PSI acceptor side (Strasser et al., 2004).

The aim of this study was to evaluate the effects of drought on ChlF kinetics in sunflower female inbred lines developed in National Sunflower project (TÜBİTAK-113O926) conducted by TARI under controlled conditions in Konya, Turkey.

MATERIALS AND METHODS

The study was conducted in Bahri Dagdas International Agricultural Research Institution's research fields in 2014. A total of fifty female inbred lines, originated different genetic sources, were initially sown, however the measurements of 38 lines were used to analyse the drought tolerance, and the rest of lines were excluded. Trials were conducted in controlled environmental conditions with randomized complete block design with one row and three replications. In each row, there were 5 plants and the distance between rows 70 cm and in rows were 30 cm. Trials were planted by hand in 31 May and drip irrigation was

applied and as covering rain shelters. Chlorophyll fluorescence measurements were three times like below in the experiments.

Control: All plant water need were supplied by drip irrigation.

Stress group 1 (S_1): 65-day-old sunflower lines under natural condition without irrigation (R3 stage).

Stress group 2 (S_2): 75-day-old sunflower lines under natural condition without irrigation (R5-1 stage).

Stress group 3 (S_3): 85-day-old sunflower lines under natural condition without irrigation (R6 stage).

At the end of the treatments, polyphasic ChlF measurements were carried out from leaves. The polyphasic OJIP fluorescence transient was measured with a Handy PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) fluorimeter. Samples dark-adapted for at least 30 min were illuminated with continuous light (650 nm peak wavelength, 3000 μmol m^{-2} s^{-1} maximum light intensity, for 1 s) provided by 3 LEDs, and the Chl a fluorescence signals were recorded according to Strasser and Strasser (1995). The OJIP transient was analysed by JIP test, based on the energy flux theory for biomembranes in a photosynthetic sample, which leads to the equations and calculations for the efficiencies of the whole energy cascade from absorption to the reduction of end electron acceptors at the PSI acceptor side and the performance indexes (Tsimilli-Michael et al., 2000; Strasser et al., 2004; Strasser et al., 2010). The fluorescence parameters (Table 1) were calculated using the Biolyzer software package.

Drought factor index (DFI) was calculated from using data of performance index (PI_{total}) and sunflower lines were ranked. DFI was calculated according to Strauss et al. (2006) and Oukarroum et al. (2007) with minor modification and calculated with the formula: DFI = $logA + 2\ logB + 4\ logC$, where C is the average relative performance index (PI) during the first treatment of drought, B is the average relative PI_{total} during the second treatment, and A is the average relative PI_{total} of the third treatment. The relative PI_{total} was calculated as $PI_{drought}/PI_{control}$.

Experimental data were subjected to Analysis of Variance (ANOVA) using the statistical software SPSS Statistics. Means were compared with least significant differences (LSD) at 5% level ($P<0.05$).

RESULTS AND DISCUSSION

Photosynthesis, the key process of plant metabolism, is strongly influenced by environmental conditions (Kalaji et al., 2014). Measurements of photosynthetic efficiencies are an important component of agricultural, environmental, and ecological studies. ChlF measurements represent a simple, non-destructive, inexpensive and rapid tool allowing scientists to get information on the photosynthetic process without destroying the tested samples. The ChlF parameters are potentially useful for screening genotypes for drought tolerance (Oukarroum et al., 2007; Strasser et al., 2010; Boureima et al., 2012; Çiçek et al., 2015; Kalaji et al., 2016).

National Sunflower Project was conducted by TARI in Edirne. Many inbred female lines and F_1 hybrids producted within this project and registered for Turkey. To determine of the level of drought tolerance these lines were grown under field conditions in Konya,

Turkey. In this study, the effects of different drought stress on photosynthetic efficiency were examined by using the changes in some chlorophyll a fluorescence parameters (JIP-test parameters), such as V_J, V_I, ABS/RC, ET_0/TR_0, DI_0/RC, RE_0/ET_0 and PI_{total}. The means of these parameters were calculated across each treatment of all the sunflower hybrids and the values of stress groups were normalized by the values of the control plants (control value: 1) for each hybrid. In general, the changes in these parameters were observed compared to their controls.

The relative variable fluorescence at the J-step (V_J) increased almost all stress duration compared to control (Figure 1). V_J values of sunflower lines were prominently increased depending on stress duration. The highest increases were observed in 8959A, 9728A, 9907A (more than 50% or almost 60% increase) under severe drought stress (S3). It has been suggests that the fluorescence yield at the J-step is strongly determined by the redox state of the electron carriers in the electron transport chain (Haldimann and Strasser, 1999). Drought stress might blocked or inhibited the re-oxidation of the electron carriers in the sunflower lines.

The relative variable fluorescence at the I-step (V_I) was significantly increased compared to control for all stress treatments (Figure 2). Depending on the drought intensity, the highest increase was observed in 2478 A, 9728 A, 9412 A and 97181 A hybrids (44-46 %) for S3. It has been suggested that V_I values is as an approximate estimation of the fraction of QB-non-reducing PS II (Hsu and Lee, 1991). Drought stress significantly increased the fraction of Q_B-non-reducing PSII centers in almost all inbred sunflower lines. Electron transfer between Q_A^- and Q_B does not function in the Q_B-non-reducing reaction centers (Cao and Govindjee, 1990). This situation might affect the electron transport towards PS I. Moreover, an increased V_I was used as a probe for the inhibition of electron transport at the acceptor side of PSII under stress condition (Chen et al., 2004).

It has been suggested that Drought Factor Index (DFI) represents the relative drought induced changes of Performance Index (PI) during a freely time of drought stress (Kalaji et al., 2016). Sunflower inbred lines investigated in present study were ranked according to their DFI values given on Table 2. Drought-tolerant genotypes with the lowest reduction in the PI_{total} under drought stress had the largest (less negative) DFI values. Based on the DFI values, thirty eight sunflower inbred lines could be separated into four groups: 9814 A is the first group (highly drought tolerant; DFI: -1.19), TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A are the second group (drought tolerant; DFI: -1.58 - -1.90), 8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107 A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A are the third group (less drought tolerant; DFI: -2.01 - -2.48) and 6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A are the fourth group (drought sensitive; DFI: -2.53 - -3.01).

DFI is calculated from PI values, so it is closely related to PI as seen above. At all stages of development PI_{total} was decreased significantly compared to control and depending on the drought periods (S1, S2 and S3) the highest decreases were in 6626 A and 9942 A (approx.35%), 8435 A and 8543 A (approx. 55%), 8959 A, 6545 A and 9728 A (approx. 75%) respectively (Figure 3). The performance index (PI_{total}) are used to utilize the effects of the environmental constraints on the plant. It has been proved that these parameters are more sensitive to the environmental changes than other fluorescence parameters, such as F_V/F_M and they correlates well with plant vitality (Oukarroum et al., 2007; Tsimilli-Michael and Strasser,

2008, Kalaji et al., 2016; Siddiqui et al., 2016). PI_{total} is predicating the performance up to the PSI end electron acceptors.

ABS/RC is reciprocal of RC/ABS utilized to calculate PI. ABS/RC known as average antenna size, expresses the total absorption of PSII antenna chlorophylls divided by the number of active (in the sense of QA reducing) reaction centers (Strasser et al., 2000). ABS/RC parameter was decreased in S1 and S2 (except for 9942A, 6626 A and 8543A) stages, the parameter values was increased approximately half of the hybrids in the S3 stage and the other half was close to control value or higher than it (Figure 4). Under stress conditions ABS/RC was increased by the reason of inactivation of the PSII reaction centers (van Heerden et al., 2007; Mladenov et al., 2015).

Probability that a trapped exciton moves an electron into the electron transport chain beyond QA^- (Ψ_0, ET_0/TR_0) was significantly decreased in all stress groups compared to controls (Figure 5). In relation to the stress duration, the highest decrease in the Ψ_0 was observed in S3 treatment. Also, increase in V_I value supports results of Ψ_0 obtained from this study. Lower Ψ_0 values might exhibit that the activity of electron transport beyond Q_A was considerably inhibited. Jiang et al. (2006) and Oukarroum et al. (2015) reported similar relationship between these parameters.

RE_0/ET_0, efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors (δR_0), was significantly affected from drought stress durations dependent manner compared to control (Figure 6). As for ET_0/TR_0 and PI_{total}, RE_0/ET_0 values of sunflower inbred lines were decreased depending on duration and the severity of drought stresses. Schansker et al. (2005) stated that a lower RE_0/ET_0 level indicated a decrease of a traffic jam of electrons at the acceptor side of PSI caused by an inactivation of ferredoxin-$NADP^+$-reductase.

Chlorophyll fluorescence (ChlF) transients allow the evaluation of the physiological condition of photosystem II (PSII) and energy fluxes of thylakoid membranes. It also gives information on the cooperation of photochemical and nonphotochemical reactions. DI_0/RC which expresses the ratio of dissipation to the amount of active reaction center (Strasser et al., 2000), was decreased in S1 and S2 (except for 9942 A, 6626 A and 8543 A) stages. In addition, for 2453 A, 2517 A, 62001 A, 6545 A, 6626 A, 8435 A, 8454 A, 8543 A, 8959 A, 9209 A, 9444 A, 9907 A, 9942 A and CL078 A hybrids DI_0/RC was significantly higher in drought treated leaves than in controls (Figure 7). Dissipation of light energy per active reaction centers (DI_0/RC) can be thought of as the absorption of photons in excess of what can be trapped by the reaction centers as heat (Mathur et al., 2011). Reduction in energy dissipation would explain increase in the fluorescence emission by the excited antenna chlorophyll *a* molecules before the migration of excitation to the reaction centers (Falqueto et al., 2012). DI_0/RC might be increased to avoid photo-oxidative damage of photosynthetic apparatus.

CONCLUSION

Drought stress affected adversely the photosynthetic efficiency of examined sunflower hybrids. The tolerance levels of sunflower inbred lines were determined using ChlF techniques. Sunflower lines could be classified into four groups as highly drought tolerant (9814 A), drought tolerant (TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A) less drought tolerant (8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107

A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A) and drought sensitive (6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A). The drought factor index calculated from PI parameter can be used to classify the level of stress tolerances. The use of supplementary parameters like PI can be more useful than complex biophysical parameters to understand the photochemical processes, also to interpret the data correctly.

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Table 1. ABSTRACT of the JIP test formulae using data extracted from the polyphasic chlorophyll a fluorescence (OJIP) transient in this study (Han et al., 2009; Strasser et al., 2010).

Data extracted from the recorded fluorescence transient OJIP	
$F_0 = F_{20\mu s}$	Initial fluorescence intensity, when all PSII RCs are open
$F_K = F_{300\mu s}$	Fluorescence intensity at 300 ms
$F_J = F_{2ms}$	Fluorescence intensity at the J-step (at 2 ms)
$F_I = F_{30ms}$	Fluorescence intensity at the I-step (at 30 ms)
F_M	Maximal fluorescence intensity, when all PSII RCs are closed
V_J $(F_{2ms} – F_0)/(F_M – F_0)$	Relative variable fluorescence at the J-step (2 ms)
V_I $(F_{30ms} – F_0)/(F_M – F_0)$	Relative variable fluorescence at the I-step (30 ms)
$M_0 = 4(F_{300\mu s} – F_0)/(F_M – F_0)$	Approximated initial slope (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence F_V
Specific energy fluxes or activities expressed per reaction center (RC)	
$ABS/RC = M_0 (1/V_J)(1/\varphi_{Po})$	Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size)
$DI_0/RC = ABS/RC - TR_0/RC$	Dissipated energy flux per RC at t = 0
Quantum yields and efficiencies/probabilities	
$F_V/F_M = \varphi_{Po} = TR_0/ABS = [1− (F_0/F_M)$	Maximum quantum yield for primary photochemistry

$RC/ABS = \varphi_{Po} \times (V_J/M_0)$	The concentration of reaction centres per chlorophyll
$\Psi_0 = ET_0/TR_0 = (1-V_J)$	Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\delta R_0 = RE_0/ET_0 = (1 - V_I) / (1 - V_J)$,	Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors
Performance index (products of terms expressing partial potentials at steps of energy bifurcations)	
$PI_{total} = (RC/ABS) \times [\varphi_{Po} /(1 - \varphi_{Po})] \times [\Psi_0 /(1 - \Psi_0)] \times [\delta R_0/(1 - \delta R_0)]$	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors

Table 2. Drought factor index (DFI) values of 38 sunflower inbred lines grown under drought stress. The inbred lines were ranked according to their DFI values.

	Lines	**DFI Values**
1	9814 A	-1,19
2	TT 179 A	-1,58
3	0046 A	-1,80
4	CL 068 A	-1,84
5	9725 A	-1,85
6	2517 A	-1,90
7	8454 A	-2,01
8	9209 A	-2,01
9	9178 A	-2,09
10	9661 A	-2,15
11	8255 A	-2,18
12	CL 078 A	-2,23
13	9444 A	-2,26
14	TT 176 A	-2,26
15	96172107 A	-2,31
16	TT 188 A	-2,36
17	2478 A	-2,38
18	9718 A	-2,44
19	6388 A	-2,45
20	9942 A	-2,47
21	62001 A	-2,48
22	6626 A	-2,53
23	6163 A	-2,57
24	97181 A	-2,58
25	TT 187 A	-2,58
26	9907 A	-2,58
27	7751 A	-2,58
28	917574 A	-2,68

29	6545 A	-2,68
30	9726 A	-2,69
31	TT 198 A	-2,71
32	8428 A	-2,74
33	8435 A	-2,80
34	8543 A	-2,82
35	9412 A	-2,84
36	8959 A	-2,86
37	2453 A	-2,92
38	9728 A	-3,01

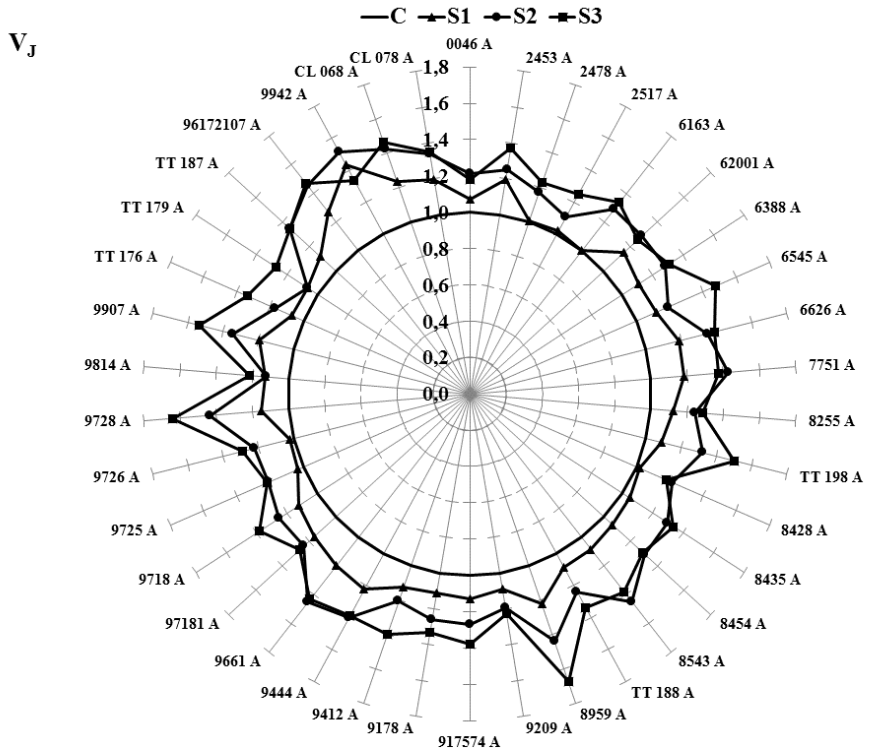

Figure 1. A radar-plot presentation of the changes in the relative variable fluorescence at the J-step (V_J) of sunflower inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

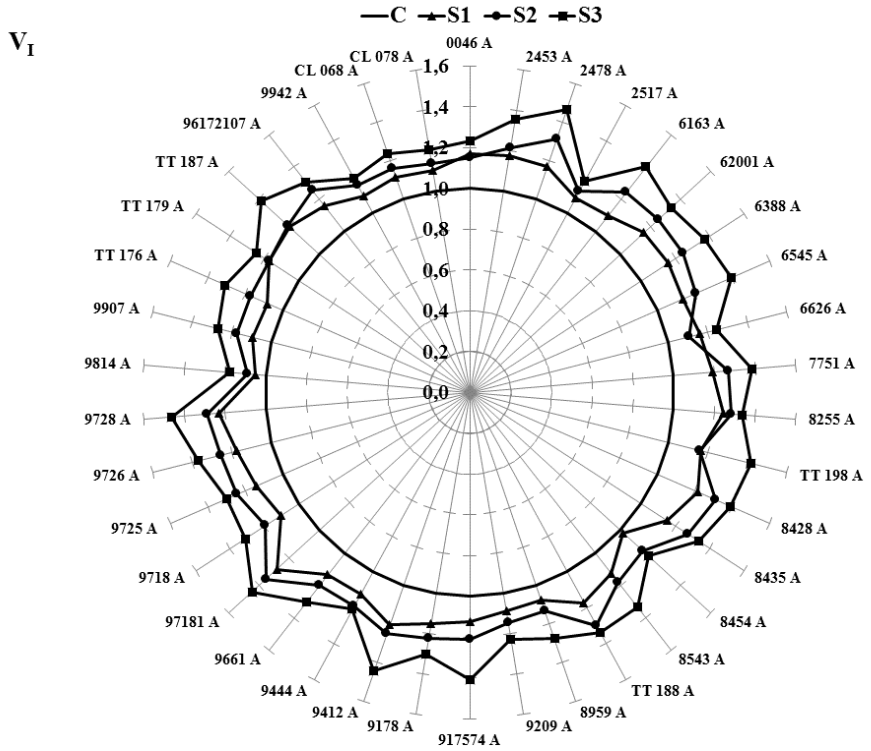

Figure 2. A radar-plot presentation of the changes in the relative variable fluorescence at the I-step (V_I) of sunflower inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

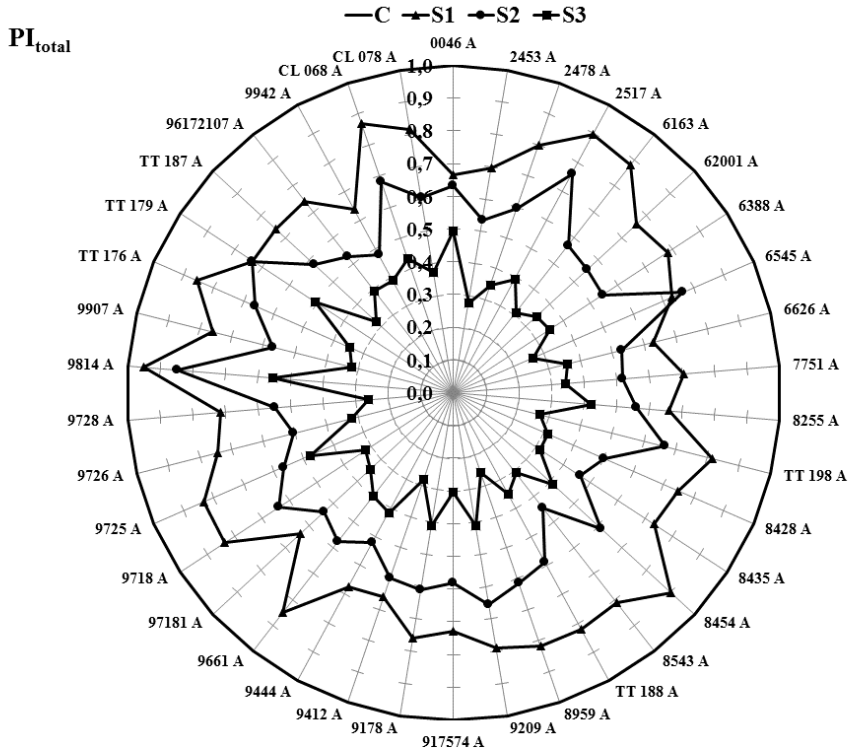

Figure 3. A radar-plot presentation of performance index (PI_{total}) parameter of dark-adapted sunflower leaves exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

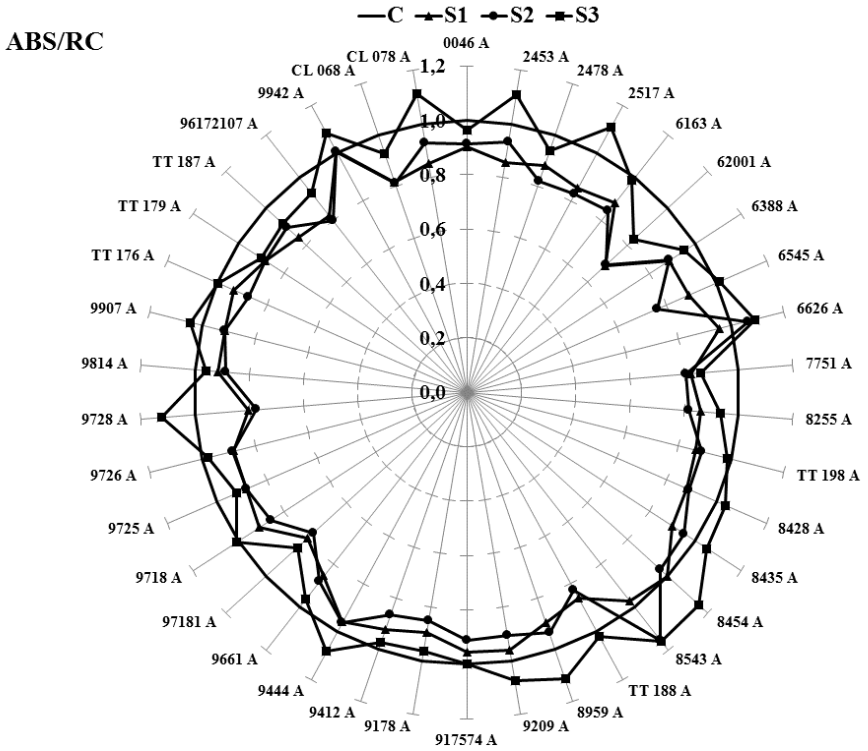

Figure 4. A radar-plot presentation of ABS/RC, effective antenna size of an active reaction centers, in the inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

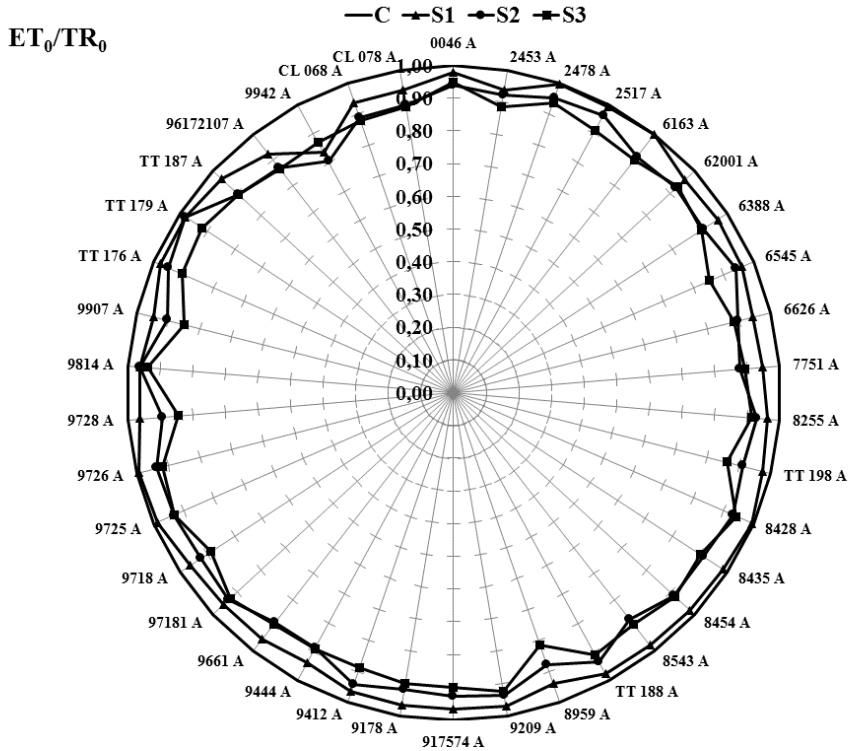

Figure 5. Drought stress effect on the efficiency with which the energy of a trapped exciton is converted into the electron transport beyond Q_A (ψ_o=ET_0/TR_0) of inbred lines. All values of stress treatments are normalized by the values of their controls (control value: 1).

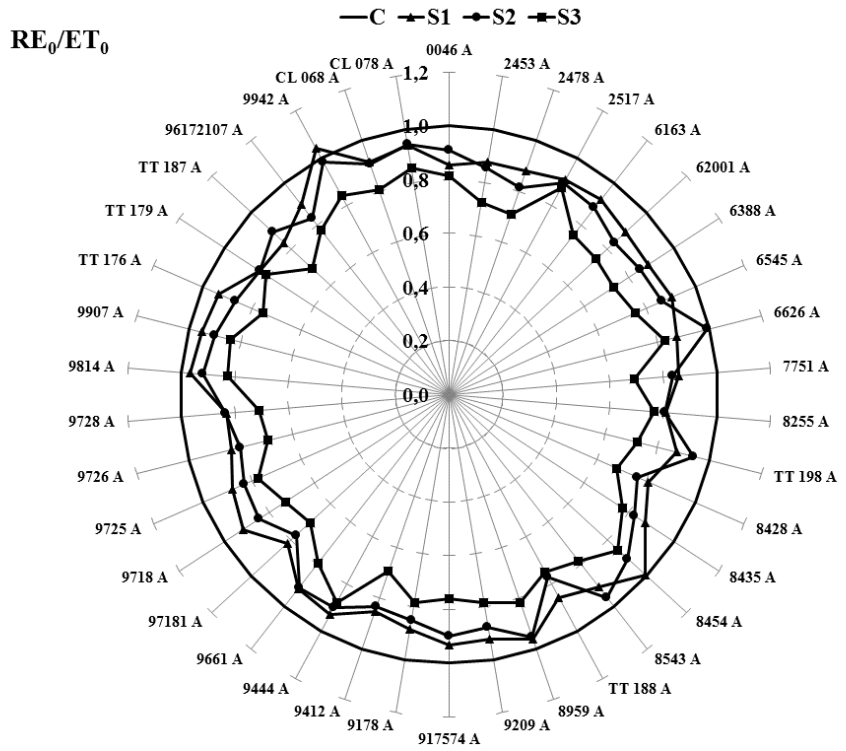

Figure 6. Drought stress effect on the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors ($\delta Ro = RE_0/ET_0$) of inbred lines. All values of stress treatments are normalized by the values of their controls (control value: 1).

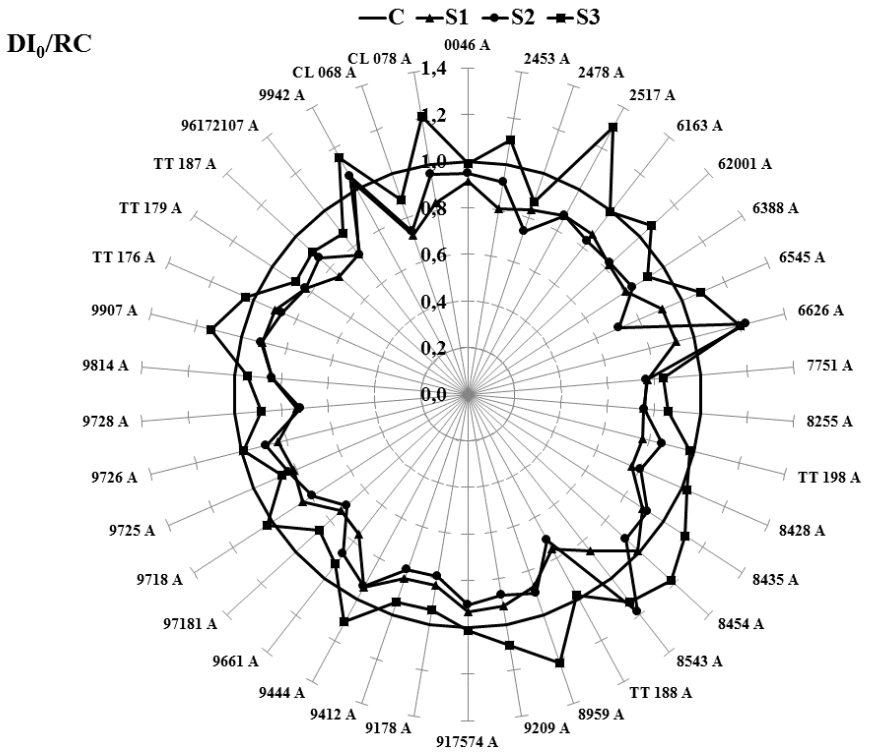

Figure 7. A radar-plot presentation of the flux of dissipated excitation energy per RC (DI_0/RC) of sunflower lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

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PHYSIOLOGICAL VARIABILITY OF SUNFLOWER DOWNY MILDEW CAUSAL AGENT, PLASMOPARA HALSTEDII, IN IRAN

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ABSTRACT

Sunflower downy mildew caused by *Plasmopara halstedii*, is considered as one of the most important diseases in majority of the crop production areas. The use of resistant varieties and hybrids is an effective method to avoid its damage. Because of obligatory characteristics of the pathogen, it is exposed to ecological and physiological pressures ending to appearance of new physiological races. To obtain reliable and consistent resistances to the disease, monitoring of such variations are required. In this study we collected the isolates of the fungal agent and mass-produced them employing whole seedling immersion method. By using the same inoculation technique, sunflower downy mildew differential lines were inoculated and then evaluated for systemic infection as susceptible reaction. The results demonstrated physiological similarity of the isolates and also existence of race number 100 in Iran.

Key Words : Sunflower, downy mildew, physiological races.

CHANGES IN THE PATHOGENIC COMPOSITION, ATTACKING THE OIL SUNFLOWER IN BULGARIA.

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ABSTRACT

The sunflower development has been greatly hindered by the sunflower diseases. In the past ten years, we have been monitoring a shift from diseases that have been labeled highly significant, to such with a more sporadic nature. Due to the purposeful breeding work, the scientific community has created hybrids, resistant to leaf pathogens such as: gray spots (Phomopsis helianthi Munt.-Cvet. et al.), mildew (Plasmopara helianthi Novot.) and parasite broomrape (Orobanche cumana Wallr.). By contrast, the extreme temperature heights, during the vegetation period, well reduced the development and distribution of the black spots (Phoma macdonaldii Boerema). The climate change led to a high peak in the brown spots (Alternaria sp.) and charcoal rot (Macrophomina phaseolina (Tassi) Goid advancement. Every past year we notice an increase on the macrophomina attacks. Research shows that the infection in some selection materials can go as high as 50%. This tendency of pathogenic adjustment requires a rapid restructuring of the selection program to prevent declines in the production of sunflower.

Key words: sunflower, climate, pathogens.

INTRODUCTION

The Earth's climate is in constant change, and so has the crops' development conditions. A crop moves from one phase to another in its development, as a result from reaching certain temperature sums. In the recent years we have been constantly speaking of a drastically changing climate, mainly referred to as the Global Warming. (Aleksandrov V. et all. 2010) has made an extensive research on the climate changes in the last few decades in Bulgaria. Some conclusions drown from this research are that the rise of air temperatures during the XX century has been the highest in comparison with previous centuries, as the 1906-2005 year period, the medium air temperature has been 0.74^{o} C higher. The year with the highest temperatures is 2009. From the beginning of XX century, the rain over North Europe has risen with 10 to 40%, while the rain over some regions in South Europe (Bulgaria amongst them) has declined up to 20%. The most notable drought was during the year 2000. In some regions the agrometherological conditions has caused a decline in the vegetation period up to and below 90 days. Those regions include Dobrudzha and the south regions of northwest Bulgaria. The data from the phenological observations suggests that plants vegetation gets ahead of its normal course with 7-15days in the different climate regions, which without a doubt, proves that the climate has warmed during the last 30 years. The rise of temperature and diminution of rain has greatly affected the pathogenic composition attacking the crops, sunflower included. Growth cycle of these pathogens is closely associated with both the temperature and the atmospheric and soil moisture (Mari M and C.Martini, 2015), (M.Pautasso et al. 2012). The purpose of this study is to track changes in the pathogenic composition, attacking the oil sunflower in Bulgaria over the past two decades.

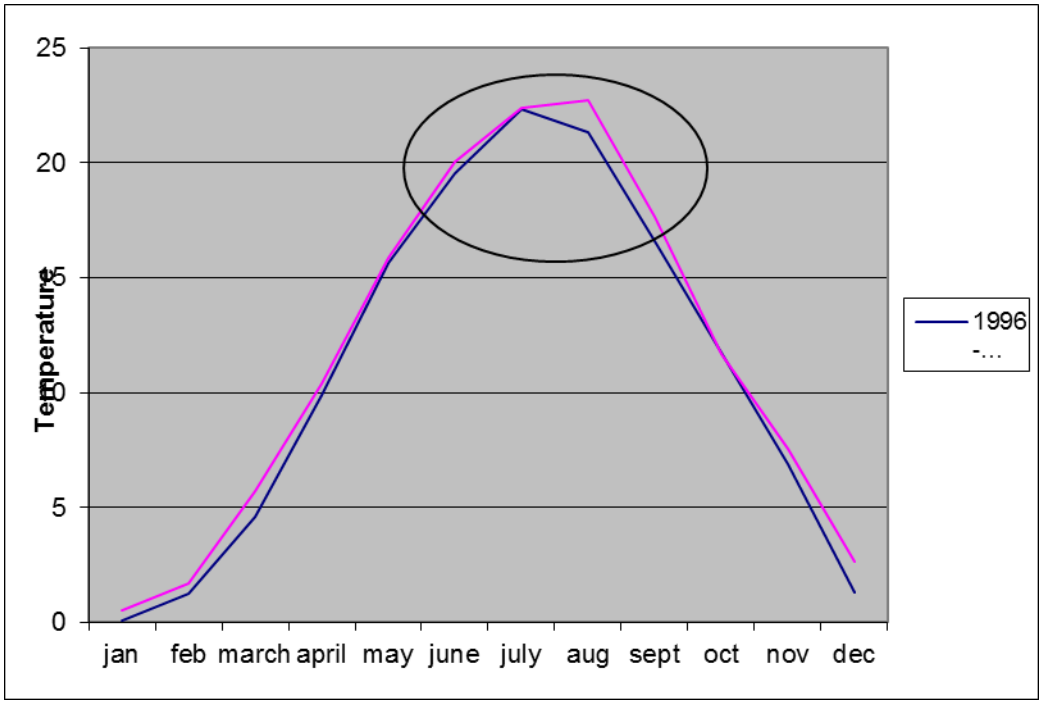
MATERIAL AND METHOD

The investigation was carried out in artificial infection field of Doubrudja Agricultural Institute. During the vegetation period we have established the extent and the type of damage caused by economically important diseases. When a new set of diseases appear, they are registered in the appearing country, and scientific community begins their reporting starting the next year. The data used for the period 1996-2015, is taken from the annual reports of the author (unpublished data). Data for temperature and precipitation fallen is divided into two decades 1996 – 2005 and 2006 – 2015. They are obtained from the weather station located on the territory of Doubrudja Agricultural Institute - General Toshevo.

RESULT AND DISCUSSION

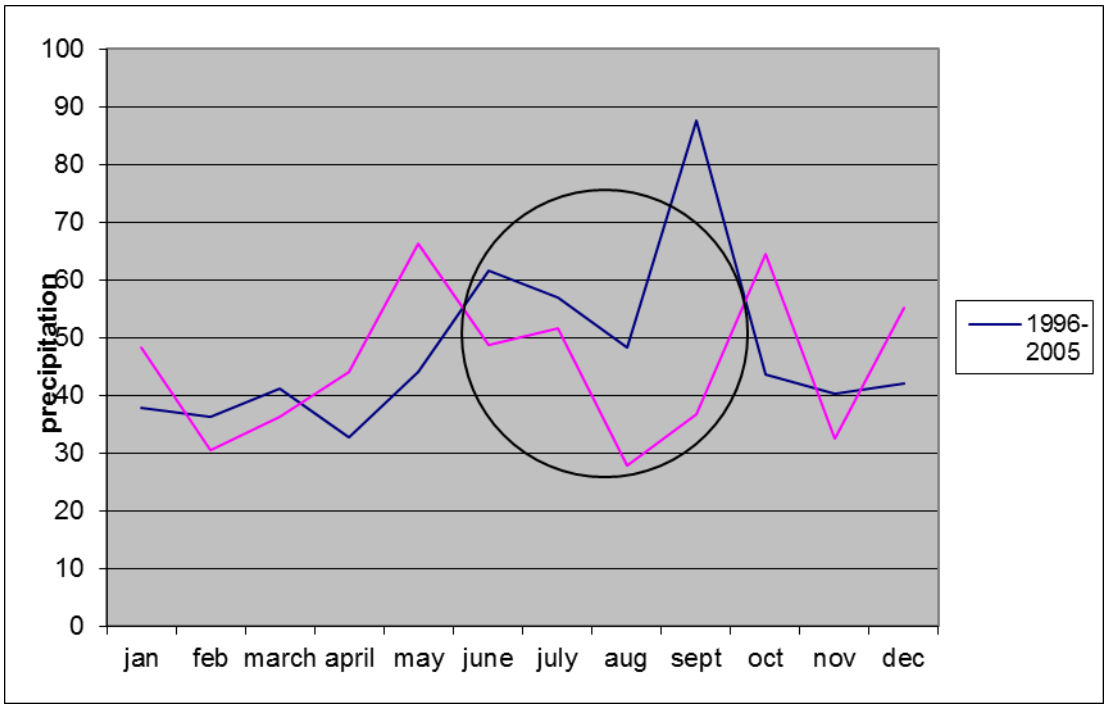
It is a fact that climate change has direct effects on the plant pathosystems. In the last two decades, the DZI science department has estimated a temperature increase (approximately 0.8°C-1°C) and soil moisture decrease, especially in the active vegetation period of sunflower when it is most prone to diseases attack. Plant pathologists have always considered environmental influences in their studies of plant diseases: the classic disease triangle emphasizes on the interactions between plant hosts, pathogens and the environment. (Garrett 2008; Klopfenstein et al. 2009; Grulke 2011 Coakley (1995) stated that disease development may increase, decrease or remain stable depending on the host-pathogen interaction. Any change in the ecosystem can affect plant diseases, as plant disease is the result of the interaction between a susceptible plant, and a virulent pathogen and the environment.

Fig.1 Average monthly temperature for the periods 1996-2005 and 2006-2015



When we examine two decades of data and compare the temperature and the precipitation during the sunflower's active vegetation (June, July, August), we clearly notice a tendency of temperature rise and reduction of precipitation in the 2006 – 2015 time period. This enviornmental change has let to shift in the sunflower's pathogenic composition.

Fig.2 Amount of precipitation in the periods 1996-2005 and 2006-2015



Moreover, some aspects associated with climate changes, such as the increase of temperature and changes in precipitation and moisture can have some effects on the fitness (number of generations, the sexual reproduction) of plant pathogens, extending the amount of time available for their reproduction and dissemination.

Table 1. Changes occurred in the distribution and aggressiveness of pathogens on sunflower

Fungy	1996 - 2005	2006 - 2015
Plasmopara helianthi	strong attack in the field	Decreased attack on pathogen
Phomopsis helianthi	Medium to strongly attack	Reduce the intensity of attack
Botritys cinerea	Average intensity of the attack usually at the end of the growing season	Long and hot autumn with a single infested plants
Alternaria sp.	Medium attack	Moderate to severe infestations in some years
Phoma macdonaldii	Medium attack	Moderate to severe infestations in some years
Albugo tragopogonis	Singal plants	Spread throughout the country
Puccinia helianthi	From low to middle attack	Medium to strongly attack
Rizopus sp	Low attack	Increased severity of pathogen
Macrophomima phaseolina	Low attack	Increased severity of pathogen
Verticilium dahliae	Low attack	Increased severity of pathogen

Plasmopara helianthi is an important disease on sunflower in Bulgaria. The last decade its primary appearance on sunflower fields has decreased because of the presence of effective fungicides, but the secondary infection, by the same pathogen, is commonly observed. Probably the climate change affects the host's biology and this indirectly influenced its response to pathogen attacks. Probably higher temperatures produce an elongation on the vegetative season and the consequent increase of secondary infections on leaves. The same result was observed by (Richerzhagen et al., 2011) in *Cercospora beticola* causing leaf spot on sugar beet in southern Germany. (Richerzhagen et al., 2011) suggest that due to an annual mean temperature increase by approximately 0.8°C-1°C in the last century the leaf spot attacks has risen.

According (Coakley et al., 1999) higher winter temperatures might increase pathogen survival on crop residues accumulating the amount of initial inoculum to infected subsequent crops. It is not excluded that this is due to severe attack by *Alternaria sp.* Our deductions and results are similar. We have observed amplification in the procent of attacks during the last two decades. (Encheva V, 2007).

The increase of temperature contributes to the spread of pathogens in some new geographical areas, where the pathogens can encounter new potential hosts. Initially we observed the disease *Albugo tragopogonis* Schr. (*Encheva and all*.2000) in *2000.* During the last decade we estimated its attack on the whole Bulgarian territory. The same goes to another disease spread in Bulgaria: *Rhizopus sp*. (Encheva V and N.Nenov 2004).

The increased temperatures in winter and spring can assist the maturation of ascospores and their release, forcing an early start of the disease management. The general increase in temperature produces an extension of the vegetative season, exposing crops to higher infections. (*Phoma macdonaldi* and *Puccinia sp*.). This leads to a divergence cycle of both the disease and the host plant development. The climate change largely influence the manifestations of one or more fungal diseases.

In recent years, almost all sunflower vegetation goes by extremely high temperatures. They directly affect the development of pathogens in crops. Such widespread diseases, mainly occurring in areas with hot climate, are *Macrophomima phaseolina, Verticilium dahliae , Rizopus sp* . etc. Meanwhile the new climate situation limits the emergence of diseases such as *Phomopsis helianthi, Botritis cinerea.* There are fungal diseases like *Alternaria* that do not respond notable to weather conditions. Models predict in the mid-term a lower impact of oilseed rape diseases such as *Leptosphaeria maculans* and *Pyrenopeziza brassicae* (Fitt et al. 2011). The climate increase in Northern Germany, for example, facilitates the oil seed rape pathogens such as *Alternaria brassicae*, and *Sclerotinia sclerotiorum.* Indeed these new conditions not only threaten plant health but may in some cases exterminate the plant itself. It is predicted that *Verticillium longisporum* will be favoured by average increase in temperatures, particularly when taking into account a long-term (2071–2100) view (Siebold and von Tiedemann 2012).

CONCLUSIONS

The impact of climate change on plant diseases requires more research. The change of climate could alter stages and rates of development of the pathogen, modify host resistance and thus lead to transitions in host-pathogen interaction. Climate changes mostly affect agricultural production. Research on the climate change impact on plant disease has led to a new aim: to create a drought-resistant sunflower hybrid with genes that control diseases, conducive to high temperatures and low soil moisture.

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VARIATION IN AGGRESSIVENESS OF *PHOMA MACDONALDII* ISOLATES FROM THREE BALKAN COUNTRIES AND UKRAINE

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ABSTRACT

Phoma macdonaldii is a ubiquitous pathogen, affecting sunflower by premature leaf senescence, stem cortical tissue necrosis and basal stem girdling. Sunflower genotypes expressing partial resistance have been reported. Currently, disease has reemerged as a threat to sunflower production in some sunflower cropping areas. To estimate variability in pathogen population, sunflower stems with disease symptoms from Serbia, Turkey, Romania and Ukraine were sampled. Total of 54 isolates was used for inoculation of four inbred lines differing in resistance to phoma black stem. Results from scoring of sunflower inbred lines seven days after inoculation showed significant differences in disease severity measured by percentage of cotyledon petiole necrosis. Significant difference was detected both among isolates and genotypes. Isolates were segregated in five clusters. Five isolates were found to be highly aggressive based on disease severity. The least aggressive were seven isolates, producing mild symptoms on all tested genotypes. Majority of tested isolates lead to complete necrosis of inoculated plant part of the most susceptible genotype and mild symptoms of other three genotypes. Isolate aggressiveness was not correlated with geographic origin. In conclusion, significant variability among pathogen isolates was confirmed with several isolates distinguished as highly aggressive. This research could assist in breeding process for resistance to phoma black stem.

Keywords: sunflower, *Phoma macdonaldii,* aggressiveness

INTRODUCTION

Diseases are a major constraint in sunflower production. Phoma black stem, caused by pathogenic fungus *Phoma macdonaldii*, is widely distributed disease, usually considered to have limited impact on sunflower yield and quality (Gulya et al., 1997). The most distinguishing symptoms of disease appear in form of black lesions on stem, elliptical in shape and commonly 5-10 cm in length (Marić and Schneider, 1979). Symptoms can develop at stem base and in time girdling of stem may result in premature ripening (Donald *et al*., 1987).

Severity of disease depends on sunflower genotype and up to date no complete resistance to phoma black stem has been found. Sunflower genotypes significantly differ in susceptibility (Roustee et al., 2000a; Bert et al., 2004). Tolerant genotypes were found in cultivated and wild sunflowers (Darvishzadeh et al., 2010; Larfeil et al., 2010). Disease development differs spatially and temporally as a result of influence of environmental factors and cultural practices (Sessau et al., 2010; El Sayed and Marić, 1981). In addition, variability among sunflower genotypes is complemented with differences in isolate aggressiveness

(Roustaee et al., 2000b). Most recently, virulence variability of pathogen was reported in Argentina (Lazzaro *et al.*, 2012).

The objective of this study was to determine aggressiveness of *P. macdonaldii* isolates collected in Serbia and compared these results with aggressiveness of isolates from three countries where sunflower is extensively cultivated.

MATERIAL AND METHOD

P. macdonaldii isolates were collected in 2012, across three regions in Serbia, and received from Ukraine, Romania and Turkey. Four sunflower inbred lines (CMS-1-122, ROD-DI-111, VL-A-8, DOP-32-08), differing in resistance to phoma black stem were selected based on previous research (Dedić *et al*., 2012). Sunflower seed was surface sterilized in 1% solution of NaOCl and sown in plastic containers 9x9x9 cm in size, and filled with peat. Four plants grown in each container served as replication. Experiment was set in three replications. Temperature during experiment was maintained at 22/18 °C during 16/8 h photoperiod. Inoculation of plants, with fully developed first pair of leaves, was done following method described by Roustaee *et al.* (2000b). Cotyledon petioles were inoculated with 20 µl of *P. macdonaldii* picnospore suspension, concentration 10^6 picnospores/ml. Seven days after inoculation disease severity was assessed using scale 1-9 (Roustaee *et al*., 2000b). For each line and isolate median value was calculated and isolates were clustered using software PAST (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Results after inoculation of four inbred lines shows significant difference in susceptibility to disease with median values ranged from 1 to 9 (Figure 1). Significant variation of disease severity was observed among isolates expressed in large interquartile range particularly for inbred lines ROD-DI-111 and VL-A-8.

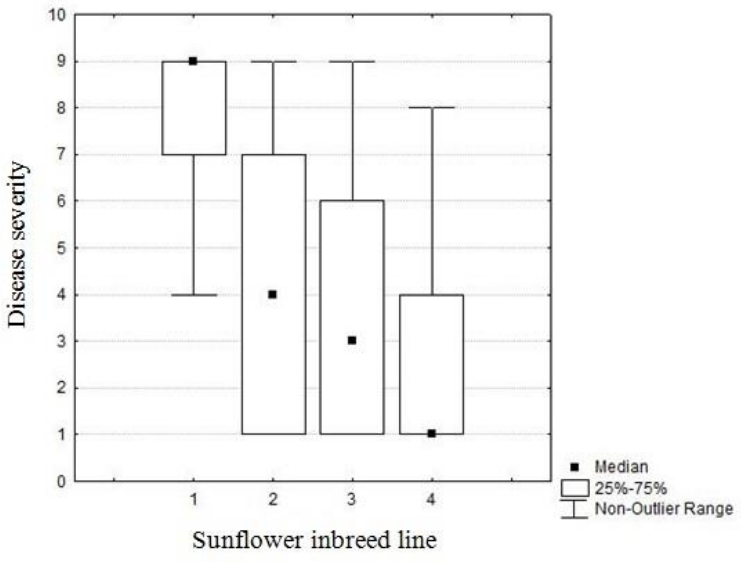

Figure 1. Median values and variation of Phoma black stem severity for all tested isolates on inbred lines CMS-1-122 (1), ROD-DI-111 (2), VL-A-8 (3) and DOP-32-08 (4)

Isolates were clustered in four distinct groups based on disease severity of inoculated inbred lines (Figure 2). The first group was consisted of five isolates which produced the most severe symptoms and consequently were considered to be highly aggressive. Out of this five isolates origins of two is same region in Serbia (SRB-R1S52, SRBR1S21), another two were sampled in Ukraine and one in Romania. The second group has two isolates which were characterized by high disease severity on inbred lines CMS-1-122 and DOP-32-08 and mild symptoms on other two inbred lines. The third group consisted of five low aggressive isolates. Isolates from the fourth and the largest group expressed high disease severity on genotype CMS-1-122, moderate disease severity on genotypes ROD-DI-111 and VL-A-8, and low disease severity on genotype DOP-32-08.

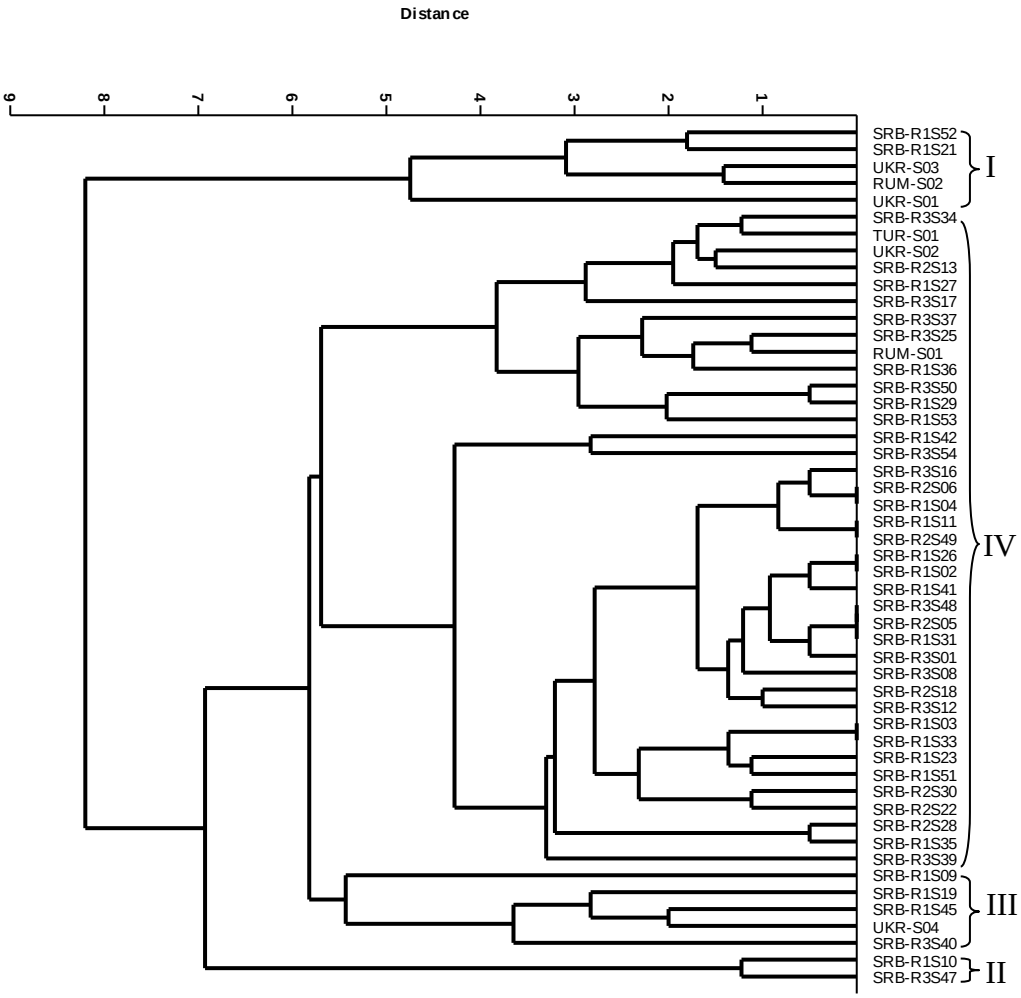

Figure 2. Dendrogram showing similarity and clustering of *P. macdonaldii* isolates based on their aggressiveness on four sunflower inbred lines

Considerable variability in aggressiveness was confirmed as a result of this research. Majority of tested isolates have similar pattern in disease severity on selected genotypes. However, a group of both high and low aggressive isolates was distinguished along with two isolates able of producing severe symptoms on the most resistant genotype. Isolates not originated from Serbia were not clustered based on aggressiveness. Most of these isolates proved to be highly aggressive. However, number of isolates from other countries was small

and this conclusion needs to be verified on larger sample of pathogen population from that areas. Difference in aggressiveness among *P. macdonaldii* proved in this research was also confirmed by other researchers. Most data comes from France where this disease is considered highly damaging (Mirleau-Thebaud *et al.,* 2011). Larfeil *et al*. (2002) determined five pathotypes based on stark differences in disease severity, following inoculation of ten sunflower inbred lines. Rostaee *et al*. (2000a) presented considerable variability among isolates in various traits including aggressiveness. Similar differences were found after testing isolates of pathogen in Argentina (Lazzaro *et al.,* 2012). In addition, highly significant genotype-isolate interaction in *P. macdonaldii* – sunflower pathosystem was reported (Darvishzadeh *et al.,* 2007; Maleki and Darvishzadeh, 2014).

In conclusion, patterns of reaction of four inbred lines to disease revealed differences in aggressiveness, with 5 out of 54 tested isolates regarded as highly aggressive. Isolates with similar aggressiveness did not cluster according to geographic origin. Determination of pathogen variability will provide selection of *P. macdonaldii* isolates suitable for breeding programs.

ACKNOWLEDGEMENTS

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SUNFLOWER DISEASES IN NORTHERN GREECE

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ABSTRACT

Sunflower has been cultivated extensively in Greece since 2010, mainly for the production of biofuels. The major region of cultivation is Northern Greece, covering approximately 60.000 hectares. Twelve diseases and three non-parasitic disorders were detected by the author during the 2011-2015 period. Based on incidence rates and severity, the diseases are divided into two categories: a) major diseases, which include Downy mildew, Septoria leaf spot, Alternaria leaf blight, Phoma black stem, Charcoal rot and Phomopsis stem canker and b) minor diseases, which include Sclerotinia wilt, Rust, Powdery mildew, Rhizopus head rot, Bacterial leaf spot and Bacterial stalk rot. Seed scorch, Leaf scorch and Bract necrosis were the non-parasitic disorders observed. The five-year survey showed that Phoma black stem and Charcoal rot were the most significant and prevalent (77% and 75% incidence rates respectively in the 2011-2015 period) diseases of sunflower in Northern Greece and they are considered– especially Charcoal rot – to be the cause of premature ripening syndrome. The leaf disease exhibiting the greatest incidence rate (31% during the 2011-2015 period) was Septoria leaf spot.

Key Words : sunflower

HELIAPHEN : A HIGH-THROUGHPUT PHENOTYPING PLATFORM TO CHARACTERIZE PLANT RESPONSES TO WATER STRESS FROM SEEDLING STAGE TO SEED SET

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ABSTRACT

Characterization of plant morphological and physiological responses is a limiting step to breed crops adapted to drought-limiting conditions. Automation of plant management on a phenotyping platform overcomes it by allowing large scale experimentation with yet accurate and individual plant monitoring. In response to both genetic and eco-physiological experimentation requirements, we developed the HELIAPHEN platform. This unique outdoor platform can host 1300 plants, such as sunflower, in 15L pots. It allows plant growth in climatic conditions similar to field, as well as a precise and automated monitoring of plant water consumption thanks to a prototype robot. Its primary functions are to move autonomously on the 600m² platform, and to treat each pot at its location (including weighing and watering up to a targeted weight). Beyond these functions, the robot takes at each handling, plant images from multiple angles with four cameras, to follow the evolution of morphological traits along with the description of the water status. In addition, a ultrasound radar measures automatically plant height and a laser measures stem diameter at the plant basis. These secondary functions are currently improved with new captors such as a light curtain and a 3D laser in order to reconstitute a 3D representation of the plant. To validate the meaning of the HELIAPHEN outputs, we confirmed the impact of drought stress managed with the robot on seed weight, number and thousand kernel weight (TKW). Furthermore, we observed a correlation between field and HELIAPHEN data for TKW and seed number observed on 45 sunflower hybrids.

Key Words : robot, drought, transpiration, growth, imaging

INDUCED RESISTANCE IN SUNFLOWER AGAINST WHITE ROT (*SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY) AND DOWNY MILDEW (*PLASMOPARA HALSTEDII* (FARL.) BERL. ET DE TONI)

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ABSTRACT

The main diseases of sunflower, such as white rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) and downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni) cause severe yield losses worldwide. Controlling these pathogens by available tools is rather difficult because of the poliphag character (*S. sclerotiorum*) and the high genetic variability *(P. halstedii*) of the parasites. Recently, a major interest has focused on the relevance of alternative protection methods, such as induced resistance against pathogens. In our work we tested the effects of some chemical inducers (benzothiadiazole /BTH/, isonicotinic acid /INA/, beta-aminobutyric acid /BABA/) as well as biological activators (arbuscular mycorrhizal fungi /AMF/ and Trifender /*Trichoderma asperellum* T1/) against white rot and downy mildew in four sunflower genotypes (cv. Iregi szürke csíkos, P63LE13, PR64H41 and Croplan DMR) in glasshouse. BTH was also investigated in a field experiment. Applied alone and in combination, Trifender, AMF and most efficiently BTH decreased downy mildew symptoms (sporulation) in cv. Iregi. Inducers significantly reduced white rot development in cv. Iregi (AMF fungi) and in Croplan (BABA, INA, BTH). In the field experiment with BTH the development of sclerotinia rot was restricted at the beginning in cv. Iregi and hybrid Croplan but not in hybrid PR64. Ratio of dead plants, however, was significantly lower in all sunflower genotypes treated with BTH and infected with the fungus compared to control plants. According to our results, application of these activators may be considered in future plant protection against sunflower diseases.

Key Words : SAR, *Sclerotinia* rot, sunflower downy mildew.

A REEVALUATION OF MYCELIOGENIC GERMINATION OF SCLEROTIA FOR *SCLEROTINIA SCLEROTIORUM* STRAIN SUN-87

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ABSTRACT

Basal stalk rot of sunflower is an economically important and rather unique disease among crops that are susceptible to *Sclerotinia sclerotiorum*. This disease is the result of myceliogenic germination of sclerotia whereby the vegetative hyphae infect the sunflower below the soil level. In contrast, sunflower head rot and similar diseases of susceptible crops result from carpogenic germination to produce airborne ascospores that infect above ground senescent or wounded tissues. We initiated research on several factors reported to affect sclerotia germination as a prelude to comparing transcriptomes associated with myceliogenic and carpogenic germination. Specifically, we reevaluated the effects of inoculum development temperature, sclerotia development temperature, conditioning temperature, conditioning of hydrated and desiccated sclerotia, and the duration of sclerotia desiccation on germination of Sun-87 sclerotia, largely as outlined by Huang (1991), Huang and Kozub (1993), and Huang et al. (1998). We were not able to use conditioning temperature to clearly differentiate myceliogenic and carpogenic germination (-20 vs. $\geq 0.5^0$C), as reported by Huang (1991), using either hydrated or desiccated Sun-87 sclerotia. Additionally, we were not able to verify that a low inoculum production temperature was the main factor affecting carpogenic germination of Sun-87. Rather, a low temperature during inoculum and/or sclerotia production enhanced germination. Finally, we were not able to verify that myceliogenic germination of Sun-87 occurred most readily when sclerotia formed at 20-25^{0}C were desiccated prior to germination. Desiccation almost always resulted in carpogenic germination, albeit at a low level relative to germination of hydrated sclerotia. Additional experiments are in progress to discover a reliable and non-confounded method that clearly differentiates myceliogenic and carpogenic germination.

Key Words : Sclerotinia sclerotiorum; white mold; stalk rot; head rot; myceliogenic germination; carpogenic germination; disease; pathology

SEED PRIMING APPLICATION EFFECT ON ALLEVIATION OF DROUGHT STRESS IMPACTS DURING GERMINATION IN SUNFLOWER HYBRIDS (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

In order to study the effect of different seed priming techniques on germination and early growth of sunflower under drought stress conditions, a factorial experiment based on completely randomized design with 3 replications was conducted in the laboratory of Seed and Plant Certification and Registration Institute. Seeds of two sunflower cultivars; Azargol and Hysun-36 were pre-treated with 5 treatments including: 2 osmopriming concentrations of Potassium Nitrate (KNO3); 500 and 1000ppm, two hydro-priming with distilled water in two durations of 12 and 18 h and a control treatment without priming. After priming, seeds were placed on different osmotic drought conditions for germination test and early growth evaluation. Osmotic conditions were provided by PEG-6000 in 3 osmotic potential levels; -0.3, -0.6 and -0.9 MPa and one control condition of 0 MPa. Results showed that, The lowest seed germination percentage and early growth occurred at -0.9 MPa for both cultivars and priming with 1000 ppm KNO3 increased seed adaptation to osmotic conditions because the highest germination and growth under osmotic condition observed in this treatment. Hysun-36 showed to be more drought tolerant so that highest germination and growth in osmotic dry condition demonstrated for this cultivar. There were no significant difference in seed germination and early growth performance under osmotic drought between hydro-priming 18h and non-primed control. This results revealed that to gain a better germination and seedling stablishment in dry cultivation, osmo-priming with 1000 ppm KNO3 may be beneficial.

Keywords: sunflower, seed priming, germination percentage, drought stress and seedling vigor index

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil seed crops in Iran. It is a high yielding oilseed crop, but under scarce conditions, the yield is very lower than its real potential. Among the factors responsible for the low yield, imbalance use of fertilizer, improper plant protection, poor growth and sub optimum plant population are rather important. Suboptimum plant population generally results from poor and erratic germination (Barsa et al., 2003). Recently, salt and drought stress are perhaps the two most important abiotic stresses that limit plant growth and development(Elhafid et al., 1998). A good strategy is the selection of cultivars and species for salinity and drought conditions(Ashraf et al., 1992). But an alternative strategy for the possibilities to overcome salt and drought stresses is by seed treatments with hydro priming or other treatments(Yagmur and Kaydan, 2008). Seed priming is now a widely used commercial process that accelerates the germination rate and improves seedling uniformity in many crops(Halmer, 2003; Taylor and Harman, 1990). Priming allows some of the metabolic processes necessary for germination to occur without germination take place. The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, rice, soybean and sunflower (Parera and Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini, 2003; Yari and Sheidaie, 2011; Sadeghian

and Yavari, 2004). Seed priming with Potassium Nitrate (KNO_3) had shown good potential to enhance germination, emergence and seedling dry weight of Sunflower(Kaya et al., 2006; Singh and Rao, 1993**)**, corn(Basra et al., 1989**)** and soybean(Saadateyan et al., 2009). Hydropriming method has also been used successfully in sunflower(Kaya et al., 2006; Saadateyan et al., 2009), wheat(Harris et al., 2001), Rice(Yari and Sheidaie, 2011) and cotton(Casenave and Toselli, 2007). Moreover hydro priming increased germination and seedling growth under salt and drought stresses(Kaya et al., 2006; Saadateyan et al., 2009).

The present study was, therefore, carried out with the objective to evaluate the effects of seed priming treatments under drought stress conditions on germination and the seedling growth of hybrids sunflower.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the department of seed and plant certification &registration Institute, Iran in 2011. Experimental units were arranged factorials in a completely randomized design (CRD) with three replications.

Seed material: Seed materials of Sunflower hybrids(Hysun-36 and Azargol), which selected on the basis of their area that planted in Iran.

Priming techniques: Priming media were used such as distilled water, 1000 ppm Potassium Nitrate (KNO3); 500ppm Potassium Nitrate (KNO_3); and control. All priming media were prepared in distilled water. Seed was fully immersed in KNO_3 priming media at a temperature of 25°C for durations of 2 hours and immersed in distilled water of 25°C for durations of 12 and 24 hours under dark conditions. Seed were removed from priming media at the same time, then rinsed thoroughly with distilled water and lightly dried using blotting paper and then allowed to dry on paper towels at room temperature. Control treatment consists of untreated seed.

Osmotic stress of PEG_{6000}: Three drought stresses with different osmotic potentials of -0.9,-0.6 and -0.3MPa were arranged as described by Michel and Kaufmann(1973). The osmotic potential of control solution was 0 MPa. The osmotic potentials of PEG_{6000} were read with a Wescor Vapour Pressure Osmometer-5520.

Seed germination test and seedling growth: The treated and untreated seeds were then transferred to Petri dishes (50 seeds per Petri dish with three replications) containing two (whatman No.2) filter paper moistened with 10ml of control solution or the same solution added with PEG6000**.** Petri dishes were placed in germinator at 25±1°C under dark conditions. The Petri dishes were controlled in one day intervals for solutions content. Germination was recorded daily up to day 7 after the start of the experiment. A Seed was considered germinated when radical emerged by about 2mm in length (ISTA, 2003).

Mean germination time, germination percentage, germination rate and vigor seedling index were calculated as described in Ellis and Roberts(1981).

$$MGT\ (day) = \sum NiDi/N \qquad (1)$$

Where *Di* is the number of days after sowing, *Ni* is the number of seeds germination on i^{th} day, N is the total number of germinated seeds.

Germination Percentage (GP) was calculated as $GP = \frac{\text{Total seeds germination}}{\text{Total number of seeds}} \times 100$ (2)

$$\text{Germination Rate (GR)} = \sum Ni/\sum TiNi \qquad (3)$$

Where Ni is the number of newly germinated seeds at time Ti.

The Seedling Vigor Index(SVI) was calculated as the product of seedling dry weight by germination percentage.

Seedling Vigor index(VI)=SDW×GP (4) Where SDW is seedling dry weight at the end of test and GP% is the final germination percentage. Final germination percentage, seedling length, radical length, stems length, seedling dry weights were recorded 7 days after cessation of the experiment. MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983**)**. The significance between the means were compared using Least Significant Difference(LSD) values($P<0.05$).

RESULTS

Germination: Germination percentage was influenced by seed priming treatments and drought osmotic stress conditions. The results indicated that Hysun-36 hybrid showed a significantly less decline in germination percentage under drought stress conditions in comparison to Azargol. Germination percentage under drought stress (-0.3, -0.6 and -0.9MPa of PEG) was increased by Seed primed with KNO_3 and water(12h) compared to untreated seeds(Table1). However, hydro priming seeds for 18h indicated no significant differences with untreated ones in percent germination(Table1).

Mean Germination Time: A significant three-way Interaction (hybrid, seed treatment and stress) was found for MGT ($P<0.01$)(Table2). There was no significant difference in MGT among primed seeds under control drought stress condition (0 MPa PEG) for both Azargol and Hysun-36hybrids. Seed primed with 1000ppm KNO_3 reduced the time to start germination and MGT under -0.6 and -0.9 MPa osmotic potential of PEG compared with other treatments in Azargol hybrid. Also seed treated with 500 ppm KNO_3 and 1000ppm KNO_3 shortened the time to seed germination compared with other treatments in Hysun-36 hybrid. Whereas, hydro primed seeds for 18h had negative significant effect on MGT at (-0.3, -0.6 and -0.9 MPa) and -0.9 MPa of PEG in Azargol and Hysun-36hybrids, respectively (Table2).

Germination Rate (GR): A significant three-way Interaction(hybrid, seed treatment and stress) was found for GR ($P<0.05$). Seeds primed with 1000ppm KNO_3 and hydro priming enhanced rate of germination under control stress condition (0 MPa of PEG) in Azargol hybrid. Also seed treated with KNO_3(especially 1000ppm KNO_3) improved rate of germination at higher concentration of PEG for both Azargol and Hysun-36hybrids.

Root length: Results of comparison means of hybrid and osmotic stresses showed that Azargol hybrid had longer root length than Hysun-36 hybrid under control drought stress condition(0 MPa of PEG). In contrast; increasing concentration of PEG improved length of root in Hysun-36 hybrid. The result of this experiment showed that priming with 1000ppm KNO_3 and hydro priming(12h) increased root length under drought osmotic stress conditions when concentration of PEG increased.

Shoot length: Shoot length was significantly influenced by hybrid and osmotic stresses ($P<0.01$). Results of comparison means of hybrid and drought osmotic stress conditions showed that the highest shoot length was attained from Azargol hybrid under control drought stress condition(0MPa of PEG), but Hysun-36hybrid had longer Shoot length than Azargol hybrid under drought osmotic stress at -0.3 MPa of PEG. The results of comparison means of seed priming treatments and osmotic stresses indicated that in the conditions of 500 ppm KNO_3 and 1000ppm KNO_3 treatments under control drought stress condition(0 MPa of

PEG) the longest status was shown, but by increasing concentration of PEG, there were no significant differences in Shoot length among seed priming treatments(Data not shown).

Seedling dry weight: Results of comparison means of hybrid and drought osmotic stress conditions showed that Hysun-36hybrid had more Seedling dry weight in comparison to Azargol hybrid under drought osmotic stress conditions ($P<0.01$). Seedling dry weight was significantly affected by seed priming treatments. Seed subjected with 500ppm KNO_3, 1000ppm KNO_3 and hydropriming(12h) increased seedling dry weight compared with untreated seeds while hydro priming for 18h had negative effect on Seedling dry weight compared to untreated seeds(Table3).

Seedling Vigor Index: The results of comparison means of hybrid and osmotic stresses indicated that seedling vigor index of Hysun-36hybrid at -0.3 and -0.6MPa concentration of PEG was higher compared to Azargol hybrid. The results showed that by increasing drought osmotic stress, Hysun-36 hybrid have more potential resistance in germination stage compared with Azargol hybrid, under drought osmotic stress conditions. Results of priming media comparison means showed that higher seedling vigor index was recorded from applying 1000ppm KNO_3 priming treatment. The lowest Seedling Vigor Index was observed in untreated seeds. These findings indicated that primed sunflower seeds with KNO_3 could positively affect on seedling Vigor Index(Table 3).

DISCUSSION

In many crops pre-soaking or priming causes improvement in germination and seedling establishment (Harris et al. 2001). Increases in the seedling correlated with higher water uptake by primed seed resulted in higher seedling growth. The beneficial effects of KNO_3 on germination were found in this study .Final germination was higher from1000ppm KNO_3, Suggesting no toxicity of KNO_3 due to ion accumulation in the embryo, which is in support with the earlier findings (Demir and Venter, 1999; Kaya et al., 2006; Singh and Rao, 1993). Also seeds primed with 1000ppm KNO_3 and hydropriming(12h) improved rate of germination under drought osmotic stress conditions. these finding are line with Mvale et al(2003) reported that osmopriming seed improved germination rate in sunflower seeds. Also osmopriming has been shown to activate processes related to germination, through affecting the oxidative metabolism such as increasing superoxide dismutase(SOD) and peroxidase (POD)(Jie, 2002). Moreover, the present study revealed that seed treated with 500ppm KNO_3 and 1000ppm KNO_3 shortened the time to seed germination compared with other treatments. These finding are in line with Demir and avaenter(1999) who states that seed primed with KNO_3 reduced MGT and had positive effect on germination percentage in sunflower seeds. In contrast, hydro priming seeds for 18 hour had negative effect on MGT under -0.9 MPa osmotic potential of PEG. This could be explained by more rapid water uptake than the amount of water for germination in these hybrids. Also the results showed that by increasing drought osmotic stress, Hysun-36hybrid have more potential resistance in germination stage compared with Azargol hybrid. Increasing concentration of PEG improved length of root in Hysun-36hybrid.These findings support the earlier work of Beckman et al.(1993), who reported that increasing in length of root in switch grass by seed priming treatments.

Table1. Effect of seed priming treatments on germination percentage of hybrids sunflower under drought stress conditions

Seed priming treatments \ Osmotic potentials(MPa)	0	-0.3	-0.6	- 0.9
Control	96.17 a	72.00 d	41.67 g	18.67 j
500ppm (KNO_3)	92.33 ab	73.67 cd	54.33 e	34.33 h
1000ppm (KNO_3)	96.17 a	78.83 c	54.33 e	46.17 fg
distilled water(12h)	94.67 a	78.50 c	49.67 ef	27.67 i
distilled water(18h)	91.83 ab	73.33 cd	45.5 fg	19.50 j

*Means with same letter are not significantly different at *LSD (P<0.05*)

Table2. Effect of seed priming treatments on Mean Germination Time of hybrids sunflower under drought stress conditions

		Osmotic potentials(MPa)			
Hybrid	treatments	0	-0.3	-0.6	-0.9
Azargol	Control	1.69 P	2.25 l	3.24 h	4.63 d
Azargol	500ppm (KNO_3)	1.64 P	2.19 lmn	3.22 h	4.70 cd
Azargol	1000ppm (KNO_3)	1.67 P	2.13 lmno	2.95 i	3.93 ef
Azargol	distilled water(12h)	1.63 P	2.21 lm	3.21 h	4.68 cd
Azargo	distilled water(18h)	1.68 P	2.61jk	3.75 fg	5.11 b
Hysun-36	Control	1.98 o	2.75 ij	4.0 1e	5.31 b
Hysun-36	500ppm (KNO_3)	1.98 o	2.53 k	3.76 fg	4.84 c
Hysun-36	1000ppm (KNO_3)	1.99 mno	2.53 k	3.64 g	4.71 cd
Hysun-36	distilled water(12h)	1.98 no	2.92 i	4.04 e	5.3 b
Hysun-36	distilled water(18h)	1.01mno	2.95 i	4.01 e	5.65 a

*Means with same letter are not significantly different at *LSD (P<0.05*)

Table3. Effect of seed priming treatments on seedling dry Weight (g) and Vigor index of sunflower

Seed priming treatments	Seedling dry Weight(g)	Vigor index
Control	25.79 b	19.45 c
500ppm (KNO_3)	28.15 a	22.5 ab
1000ppm (KNO_3)	28.44 a	23.84 a
distilled water(12h)	27.64 a	22.38 ab
distilled water(18h)	26.93 ab	21.22 b

* Means with same letter are not significantly different at LSD ($P<0.05$)

CONCLUSIONS

Overall it could be concluded that suitable priming the sunflower seeds was 1000ppm KNO_3 resulted in higher germination percentage and seed vigor under drought osmotic stress conditions. Therefore, priming with KNO_3 may be an efficient method to overcome seed germination problems and to improve seedling growth in field, especially under drought conditions. Hydro priming for 18 h had negative effect on Seedling dry weight compared to untreated seeds. It was concluded that increasing of hydro priming time may have negative impact on germination and seedling growth in hybrids of sunflower used in this experiment. Also Hysun-36hybrid have more potential resistance under drought stress conditions in germination stage compared with Azargol hybrid and it could be suitable for planting at this conditions.

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THE BEHAVIOUR OF SOME SUNFLOWER CULTIVARS TO THE MAJOR PEST AGENTS IN THE SOUTH-EASTERN AREA OF ROMANIA

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ABSTRACT

The behavior of some sunflower cultivars against major pests were studied under natural contamination conditions at SC Sport Agra SRL, Amzacea, Dobrogea County. The pathogens which cause diseases during vegetation period were: *Sclerotinia sclerotiorum, Phomopsis helianthi (Diaporthe), Phoma macdonaldi, Alternaria helianthi* and *Sclerotium bataticola.* Climatic conditions during vegetation period in 2014, favored the manifestation of the pests in sunflower crops. One of the most dangerous parasites on the plants in Dobrogea county area was Broomrape (*Orobanche cumana)*. It was shown a significant dissemination, especially in the south and south-eastern area of Romania. In experimental plots were studied 24 sunflower hybrids. The best results for the major pest agents have showed NK ALEGO,NK MELDINI , ES EUROMIS, ES TERRAMIS, LG 55.42 CL, LG 56.61CL and FAVORIT. For the control of weeds on sunflower trial it used a specific herbicide - PULSAR- 40 (imazamox40g/l) at rate of 1,2 l/ha.
Key words: *sunflower,behavior major pest, control*

INTRODUCTION

Sunflower crop is in Romania the 3rd agricultural crop after maize and wheat. In 2014, 990.000 ha were cultivated with sunflower and average production of 2129 kg / ha(NIS - Crop production for main crops in 2014). The behavior of some sunflower cultivars against major pests were studied (500mp/plot) *under* natural contamination conditions at SC Sport Agra SRL, Amzacea- Dobrogea area. The pathogens which cause diseases in vegetation periods were: *Sclerotinia sclerotiorum, Phomopsis helianthi (Diaporthe), Phoma macdonaldi and Alternaria helianthi* (Jinga et al. 2005) . One of the most dangerous parasites on plants in Dobrogea area was Broomrape (*Orobanche Cumana Wallr.).* (Pacureanu et al., 1998) It was shown a significant dissemination, especially in the south and south-eastern area of Romania. This specie has shown a significant dissemination, especially in the south and south-eastern area of Romania (Parker, 1994 , Vranceanu et al.1995.). On sunflower crops, the losses can reach 30- 70% of the harvest. (Iliescu et al.1995, Jinga et al., 2010).

MATERIALS AND METHODS

Experience has been placed on S.C. SPORT AGRA S.R.L. Amzacea, Constanta. The studied crops were sunflower pest. The experience was situated on a land belonging to the South Dobrogea plateau, represented by cambic chernoziom with a profile deeper than other chernozioms, a blackish-brown soil of 40-50 cm thickness, medium texture (Demeter, 2009). The content of nutrients was: mobile P index - 72; N index - 4; Humus - 3.11; K index - 200; Neutral pH - 7.2. The climate is deeply temperate continental, with an average annual temperature of 10.7-11.7°C, with a high temperature in the period 20th June to 15th August. Meteorological data are presented in

Table 1. Sowing (70 m^2/plot) was carried out on 6 April 2014 and observations in August. Due to heavy rains in the entire vegetation period, the attack of pathogens that cause diseases was very aggressive.

Table 1: Meteorological data 2013-2014

Month	Temp monthly average ºC	Temp Min ºC	Temp Max ºC	Rainfall mm	Humidity %
Aug	21.7	16.1	23.1	1.9	59.4
Sept	18.6	14.5	22.7	2.1	60.1
Oct	13.2	9.9	16.4	3.0	77.9
Nov	11.2	8.7	13.6	1.5	84.4
Dec	2.9	-0.1	5.9	0.4	85.4
Jan	3.9	1.2	6.6	113.0	88.4
Feb	4.6	2.0	7.2	2.0	83.8
Mar	8.8	5.6	12.0	40.5	64.6
Apr	12.0	8.9	15.1	42.0	74.2
May	16.8	12.8	20.8	61.5	72.1
June	21.0	17.0	25.0	22.8	72.9
July	23.5	26.0	21.0	30.0	75.0
Aug	22.3	17.1	22.7	2.6	60.4

For the control of weeds on sunflower trial it used a specific herbicide - PULSAR- 40 (imazamox40g/l) at rate of 1,2 l/ha.

RESULTS AND DISCUSSIONS

Table 2: The behavior of sunflower cultivars to the major pest agents in the Amzacea plots (F%)

Hybrids/Pest agents	*Sclerotinia sclerotiorum*	*Phomopsis helianthi*	*Phoma macdonaldi*	*Sclerotium bataticola*	*Alternaria helianthi*	*Orobanche comana*
NK ALEGO	0	10	35	20	25	2
NK NOEMA	10	20	40	30	90	10
NK ADAGIO	11	5	30	25	30	21
NK MELDIMI	16	23	27	20	75	0
TALENTO	12	9	29	20	32	12
8 H 288 CLDM	20	26	50	60	48	25
8 N 358 CLDM	10	40	85	50	80	10
8 N 421 CLDM	4	20	90	20	85	12
8 H 449 CLDM	11	50	92	30	70	9
8 H 463 CL	11	40	68	41	75	22

ES EUROMIS CL	12	21	70	32	88	0
ES NOVAMIS CL	2	10	42	30	80	5
ES TERRAMIS CL	7	2	72	21	75	0
ES H 91.61 CL	3	15	50	20	58	2
ES BALISTIC CL	18	20	68	40	70	11
LG 56.33 CL	0	6	71	21	90	21
LG 56.63 CL	4	20	90	21	72	18
LG 55.42 CL	11	10	85	52	85	0
LG 56.61 CL	0	0	38	42	41	2
LUCIA CL PLUS	2	30	82	32	80	18
MORENA CL	4	21	85	30	79	13
PARAISO 1000 CL	12	38	93	48	65	15
SUNFLORA	15	21	72	30	91	24
FAVORIT	5	6	63	16	30	0

Data presented in Table 2 it is observed that this year agriculture received numerous sediments that favored the emergence and development of the pest in sunflower crops.

The demonstration plots SC SPORT AGRA SRL Amzacea were observations made on the phytosanitary status of 24 sunflower hybrids from different companies, as shown in the attached photos.(1,2,3,4).

The pathogen *Sclerotinia sclerotiorum* has decreased in most showed hybrids attack, attack frequency being 5- 20%.

Phomopsis helianthi pathogen presented the higher appeals to most hybrids, between 20 and 50%. Lower frequency was found at hybrids NK ADAGIO, ES TRRAMIS and LG 5661CL .

Phoma macdonaldi frequency had intensity of 30-90 % .

Sclerotium bataticola presented the frequency between 20 and 50% in most hybrids.

Alternaria helianthi was also present on most hybrids, with a rate of up to 90%.

This high percentage of pathogens due to favorable weather conditions this year has particularly with very heavy rainfall and strong winds, which affected sunflower healthy crop .

Regarding parasite *Orobanche cumana* stands a few hybrids with a low attack:HK ALEGO , LG 5661 CL. Noted four hybrids without appeal, namely:NK MELDIMI, ES EURAMIS CL, ES TERRAMIS, LG 5542 CL and FAVORIT.

CONCLUSIONS

- In Romania, on sunflower crops in areas heavily infested with, pathogens and the broomrape especially in the south and south-eastern area of the country, as are those in Dobrogea, losses reach 30- 80% of the harvest.

- This high percentage of pathogens due to favorable weather conditions this year has particularly with very heavy rainfall and strong winds, which affected sunflower healthy crop.

- In natural inoculation on Amzacea location, good results show only 7 hybrids:

NK ALEGO, NK MELDIMI, ES EURAMIS CL, ES TERRAMIS, LG 5542 CL, LG 5661CL and FAVORIT.

- Sunflower cultivars taken under investigation show a different behavior against the attack of the parasite *Orobanche cumana*. Four hybrids without appeal, namely: NK MELDIMI, ES EURAMIS, ES TERAMIS ,LG 5542CL and. FAVORIT.

Figure 1. Experimental sunflower field

Figure 2. Hybrid 8 H 288 CLD– highest *Orobanche*. attack

Figure 3. Hybrid Alego -Zero *Orobanche* attack

Figure 4. Hybrid Favorit -Zero *Orobanche* attack

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APPLICATION OF GEOSTATISTICS ON PHENOMIC AND PHENOTYPING DATA: AN A POSTERIORI DIAGNOSTIC OF DISEASE SPATIAL PATTERN UNDER NATURAL INFESTATION

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ABSTRACT

Effective disease evaluation relies on a better understanding of specific interactions between host and pathogen. One problem related to soilborne diseases is the non-homogeneous nature of soilborne pathogens in terms of distribution and/or genetics that could lead to misinterpretations with respect to the presumed host resistance. The objective of this study was to analyze the *V.dahliae* distribution by looking at the spatial pattern of the sunflower Verticillium Wilt in a native system. A set of experiments were conducted from 2013 to 2015 in fields with sunflower Verticillium Wilt history. One symptomatic and one asymptomatic sunflower genotypes were introduced at specific locations (defined by spatial coordinates) to establish a grid arrangement for the disease spatial pattern evaluation. Two different sets of traits were recorded: i) reference methodology for Verticillium Wilt phenotyping (disease incidence and severity) to characterize regions of disease prevalence from 2013 to 2015, and ii) phenomic index (NDVI, passive method) to integrate senescence components in the disease evaluation in 2015. Geostatistical analyses were performed on both sets of traits and both controls to evaluate part of the micro-environment variability within the field that can interact with disease expression. The control scores were then interpolated to unsampled points through the Ordinary Kriging method. Results showed significant variation in the disease expression at field level. This confirmed that the pathogen components play a major role in the plant probability to develop the disease. No significant losses of biomass were observed, leading to the conclusion that the senescence factor did not interact with disease expression.

Key Words : Sunflower, *Verticillium dahliae*, geostatistics

IMPROVING GENE-TO-PHENOTYPE PREDICTIONS WITH CROP SIMULATION MODELS: WORK IN PROGRESS FOR SUNFLOWER YIELD STABILITY UNDER WATER DEFICIT

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ABSTRACT

The efficiency of the breeding process benefits from accurate gene-to-phenotype predictions to assign a breeding value to genotypes.Recently, hybrid modeling approaches leverage both statistical modeling (e.g. linear mixed models, LMM) and crop simulation modeling (CSM) to account for gene x environment interactions in complex traits. For example, depending on how the models are organized, (1) genomic selection frameworks can be augmented with predictors computed from crop simulation models or (2) the genotype-dependent inputs of crop simulation models can be predicted as a function of allelic composition (gene-based simulation modeling). Here, we illustrate two prediction approach es with preliminary results from the sunflower SUNRISE project, which focus on improving sunflower yield stability under water deficit. The first one is a genome-wide association study of phenotypes that were computed using field and simulated datasets on a MET of 17 locations x year x management combinations. The second is the development of a gene-based model using the SUNFLO model as crop simulation model, using data from 400 hybrids phenotyped in 5 locations.
We showed that in both approaches, statistical and numerical models complement each other to improve the prediction of non-additive gene effect and gene x environment interactions. In the association study, the characterization of water deficit at the plant level allowed to improve detection accuracy, as compared to using field measurements only. The gene-based modeling feasibility study allowed the prediction of 7 SUNFLO parameters using 48 detected SNPs.

Key Words : gene x environment interactions, modeling, linear mixed model, crop model, sunflower

INVESTIGATIONS AND THE DESCRIPTION OF VIRUS DISEASES IN SUNFLOWER GROWING AREAS IN THE TRAKYA REGION OF TURKEY

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ABSTRACT

Being an important source of vegetable oil for human consumption and the source of raw material for food industry, sunflower (*Helianthus annuus* L.) production has been increased steadily in the World and as well as in Turkey. However there are some important virus and virus-like diseases on sunflower reducing oil seed yield and quality. Genetic disorders, herbicide injuries and downy mildew disease exhibiting systemic oak leaf pattern type mosaic, dwarfing and the sterile seed formation symptoms caused by *Plasmopara halstedii* Farlow were observed during the survey studies in 2015 in the Trakya Region of Turkey. In order to determine viruses on symptomatic sunflowers and weed hosts, 244 leaf samples were collected. For the identification of *Potato virus* Y (PVY), Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) test, for *Tobacco streak virus* (TSV) Triple Antibody Sandwich-Enzyme Linked Immunosorbent Assay (TAS-ELISA) and for the identification of *Potyvirus*'es Plate Trapped Antibody-Enzyme Linked Immunosorbent Assay (PTA-ELISA) test methods were employed. For the determination of the effect of virus infections on sunflower seed yield criteria, seeds were harvested from both infected and healthy plants separately which compared for their 1000 seed weight, hectoliter seed weight and their oil content. According to DAS-ELISA test results PVY never present in the sunflower fields of Trakya Region. Depending on symptomatic observations and the results of TAS-ELISA tests 11 out of 244 plant samples had TSV with the rate of 4.51 %. As the results of PTA-ELISA tests and the symptomatic field observations 25 of 244 plants were found infected with *Potyvirus*'es with the rate of 10.25 %. Totally 36 out of 244 plant samples revealed the presence of viruses with the rate of 14.75 % in the sunflower growing areas. This is the first report of the presence of virus infections on sunflowers in Turkey. Virus infections cause reductions of 1000 seed and hectoliter seed weights of oil seeds as the oil content was found slightly high.

Key words: Sunflower, *Helianthus annuus* L., PVY, TSV, *Potyvirus*

IDENTIFICATION OF GENETIC AND MOLECULAR FACTORS INVOLVED IN SUNFLOWER PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL VARIATIONS: AN ARCHETYPE OF INTEGRATIVE SYSTEMS BIOLOGY APPROACH

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ABSTRACT

Adaption of plants to their environment is complex and involves the genetic control of molecular, developmental and physiological processes that are largely unknown in sunflower. This complexity is even greater in hybrids where both parental genomes interact to produce heterotic phenotypes. Therefore, understanding the molecular systems involved in stress tolerance in a hybrid context is of primary importance for sunflower breeding. The SUNRISE (SUNflower Resources to Improve yield Stability in a changing Environment) project - *a French project of 8 years supported by the French National Research Agency and gathering 16 public and private partners since 2012* - aims to develop a systems biology approach to describe and model links between genes, phenotype and physiological functions at the epigenetic, transcriptomic, proteomic, enzymatic and metabolomic levels at different plant developmental stages. This integrated approach will lead to the improvement of natural genetic resources exploitation and selection of complex molecular and physiological processes involved in sunflower responses to environmental variations, especially to water constraint and in heterosis.

Key Words : SUNRISE, integrated approach, water contraint, hetorosis

EXPLOITATION OF THE KNOWLEDGE ON OOMYCETE EFFECTORS TO DRIVE THE DISCOVERY OF DURABLE DISEASE RESISTANCE TO DOWNY MILDEW IN SUNFLOWER

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ABSTRACT

Plasmopara halstedii is an obligate biotroph oomycete causing downy mildew disease on sunflower, *Helianthus annuus*, an economically important cultivated crop. Disease symptoms observed in fields, plant dwarfism, leaf bleaching, sporulation and production of infertile flowers, impair strongly seed yield. *P. halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different *Pl*resistance genes, not yet cloned. Number of pathotypes increased from 1 to 16 during the last 25 years in France, concomitantly with the breakdown of *Pl* resistance loci used in fields. Finding broad-spectrum *a priori* durable resistance against pathogens would open the doors to efficient, environmentally friendly and cost-effective disease control. In oomycetes, two classes of effectors are translocated into the host plant, RXLRs and CRNs, but oomycete avirulence genes described so far are RXLRs. Through high throughput genomic sequencing of 17 *P.halstedii*pathotype isolates, we selected by stringent *in silico* methods, 74 putative RXLR effectors. 33 show polymorphism with at least one pathotype whereas 41 are conserved in sequence among the 17 pathotypes. Analysing the pathotype effector polymorphism in regard to the content in *Pl* resistant genes of sunflower lines should help us to identify candidates for pathogen avirulence genes. Triggering of defense reactions (Hypersensitive Response) through their transient expression in sunflower lines carrying known resistance genes will be used to validate them. Subcellular localization experiments of selected candidate effectors fused to GFP should give hints to their function in the plant cell. In addition, polymorphic effectors will be used to design molecular markers for rapid pathotype identification. Thirty conserved effectors corresponding to highly expressed genes upon sunflower infection are suspected to be essential genes for the pathogen. They have been cloned and are tested by agroinfiltration on various resistance sources of *H. Annuus* and some of them induce plant cell death. Co-segregation of resistance with cell death activity caused by the effector will have to be tested on segregating populations. If true, these effectors should accelerate the identification, the functional characterization and the mapping of broad-spectrum sunflower resistances potentially sustainable.

Key Words : downy mildew, disease resistance, oomycete effectors

SUNFLOWER BREEDING STRATEGY FOR RESISTANCE TO DOWNY MILDEW DISEASE IN INDIA

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ABSTRACT

Sunflower downy mildew disease is caused by *Plsamopara halstedii* (farlow) and was first reported in 1986 in Marathwada region of Maharashtra state (India) on well adapted cultivar Morden with 1-36 % intensity. The occurrence of disease was reported in other states of India *viz*., Karnataka, Andhra Pradesh and Punjab. For effective screening of the sunflower germplasm, varieties and hybrid for the downy mildew disease, under the controlled condition the sick plot technique was developed at Oilseeds Research Station Latur in 1988. Hence, the centre has been identified as facilitator for the screening of the advance material evaluated under All India Coordinated Research Project (AICRP) on Sunflower for downy mildew at National level. The results of sick plot revealed that the disease reduces sunflower seed yield up to 89 % and negatively affects the other traits. The race identification studies of Indian isolate of *Plsamopara halstedii* revealed that it belongs to race-1 (European race). Breeding for downy mildew resistance is one of the major goals in sunflower breeding programme in India. The research work carried out since1988 to 2015 at the centrer under AICRP on sunflower screened the advance breeding material in both field and sick plot condition. Till date 5408 sunflower accessions against downy mildew were evaluated and reported 1075 disease free having high level of resistance to the pathogen. This resulted into the release of 15 sunflower hybrids and populations at national level. The identified resistance sources have been effectively utilized in the introgression of the resistant genes from identified sources for the improvement of parental lines. The centre has identified 14 parental lines (8 CMS, 6 restorer lines) possessing downy mildew resistant (Pl genes). The center has released three state level hybrids LDMRSH-1, LDMRSH-3 and LSFH-35 and LSFH-171 at national level for commercial cultivation.

INTRODUCTION

In India different diseases are the main limiting factor in the production of sunflower (*Helianthus annuus* L.) and they cause poor realization of genetic yield potential of sunflower hybrid. Downy mildew (DM) is an economically significant disease. It is caused by the fungus *Plsamopara halstedii (Farl.).* Downy mildew is widespread in all sunflower growing countries with the exception of Australia.With regards to India, during early 1980's Ram Nath *et al* (1981) detected oospores of *Plsamopara halstedii* on sunflower seeds imported from Bulgaria. Mayee and Patil (1986) reported its occurrence in Marathwada region of Maharashtra state on *cv* Modern with 10.0 percent intensity for first time. The DM occurrence was immediately brought to the notice of Oilseeds Researchers in the Annual *kharif* workshop held at Dr. Punjabrao Deshmukh Agrilculture University, Akola of Maharashtra State (Anonymous, 1985). Similarly suggestions to restrict the disease with wide publicity were given through regional and national news papers for keeping watch on disease spread. (The Hindu, January 1st 1986, Indian Express, April, 23rd 1985). A Committee was constituted to go in the details of DM occurrence and the committee felt that the disease has been introduced through infected seeds and probable failures of quarantine detection has resulted in the introduction of disease. Recently a survey was conducted during 1995-96 to find out the present status of the disease in Marathwada region of Maharashtra State in India. Sixty fields in six districts were visited and out of those, 22 fields (36.67 %) had DM incidence with

varying intensity ranging from 1 to 30.00 per cent (Shrishikar, 1995). The cv Modern was highly infected by the disease as compared to hybrids.

The extent of damage depends on infection type i.e. whether it is primary (systemic) or secondary infection, while primary or systemic infection causes significant yield reductions. Primary infection is effected during seed germination in the soil and the emergence of sunflower seedling. It may caused by Fungus mycelium or oospores present on infected seeds or by oospores present in the infected soil in to which healthy seeds were sown. No matter if primary infection starts from seeds or soil, the causes of disease development in infected plants is identical.

The fungus develops in unison with the development of young plants. It penetrates the root, stem cotyledons and reaches the meristematic tissues at the top of young plants. The fungus developments inside the infected plants intercellular in all plant parts, pervading the young tissues and depriving the infected plants of assimilates and water. This is why infected plants lag behind healthy ones in growth and development. This way of fungus expansion inside the plant tissue is called a systemic infection. It begins with the infection of the germ and ends with the infection of the head and seeds. The fungus penetrates all parts of the seed (Husk, endosperm and germ) which then produces a new infected seedling. Infected plants in addition to having stunted growth i. e. short internodes are chlorotic and with a platform head which gives a smaller yield than the normal head. On the infected plant parts, the roots, cotyledons, the stem and especially the leaves, there occurs abundant white mycelium, which is typical for this disease. The mycelium occurs also on the reverse side of the leaves and it contains the vegetative organs of the fungus conidiophores and conidia (Zoosporangia). On the upper side of the leaf there occurs clorotic spots. Infected plants collapse and remain in the field after harvest. Measures of protection against Downy mildew include cultivation practices, chemical measures and the use of resistant hybrids.

The most effective chemical measures of Downy Mildew control is seed treatment with metalaxyl based preparations. These measures protect the sunflower crop at the time of the primary infections i.e. early stage of development of sunflower. Breeding for downy mildew resistance is one of the major goals in sunflower breeding programme in India. The research work carried out since1988 to 2015 at the Latur centrer under AICRP on sunflower screened the advance breeding material in both field and sick plot condition. Use of genetically resistant hybrids is definitely the most effective way of controlling Downy mildew in sunflower. Therefore research work set up with the objective of developing sunflower genotype genetically resistant to dominant race of Downy mildew in India gained prime importance in sunflower breeding programme.

MATERIAL AND METHODS

To determine the variability and level of resistance available in cultivated sunflower (1988-2015) 5408 accessions which include CMS, inbreds, restorer, open pollinated varieties, germplasm lines and hybrids collected from NBPGR, IIOR, Hyderabad and different AICRP (All India Coordinated Research Project) sunflower centers in India were evaluated in field along with Downy mildew sick plot unique developed at Oilseeds Research Station, Latur, Maharashtra.

Inoculation Technique

Many inoculation techniques viz. soil inoculation, seed inoculation, radical inoculation, foliar spray method, whole seedling immersion and disc method have been described for artificial screening of sunflower genotypes against DM disease. Patil *et al* (1993) have compared all these methods to find out effective technique for artificial screening of sunflower genotypes against DM under controlled conditions. The methodology for seed inoculation and Radical inoculation technique is given below

1. **Seed inoculation:** Seed of cv Modern were soaked in water for five hrs and kept in rolled towel paper. Next day these seeds were immersed in zoosporangial suspension (2.5 x 10^4 propagules/ml of distilled water) for five hrs and sown in pots).
2. **Radicle inoculation:** In this method the germinated seeds of cv Morden were inoculated with zoosporangial suspension, when radical was 4 to 5 mm long by spraying of sporangial suspension @ (2.5 x 10^4 propagules/ml of distilled water).

RESULT AND DISCUSSIONS

From the table 1 it can be conclude that the radical inoculation method is the best method for screening sunflower genotypes against DM. Similarly Wehtje and Zimmer (1978) have reported that in sunflower seedling infected with P. halstedii, the zoospore encystment and infections occurred primarily, within or adjacent to zone of elongation of radical and up to 1000 infection sites / mm of root length were observed.

Table : 1 Incidence of Downy mildew as influenced by different inoculation techniques (1986-89)

S.No	Method	Percent disease incidence			Mean
		1986-87	1987-88`	1988-89	
1	Soil inoculation	52	55	42	49.66
2	Seed Inoculation	52	56	60	56.00
3	Radicle inoculation	100	95	90	95.00
4	Foliage Spray	60	58	57	58.33
5	Whole seedling immersion				
	a) Cotyledon method	65	64	69	66.00
	b) First two leaf stage	64	64	69	63.66
6	Disc method	*	*	*	

- Only local lesions were observed

Race situation and sources of resistance

The fungus completes its sexual life cycle annually, affording maximum opportunity for recombination of genes responsible virulence and / or other pathogenic characters. Initially two races of the fungus were differentiated, race- 1, originally referred to as the European race and race -2, known as Red River race, referring to the Red River vally area of North Americ. However, presently a total eight races are known worldwide (Viranyi, 1990). With regards to India, Patil and Mayee (1990) conducted the studies to find out the race situation of India isolate of *Plasmospara halstedii* and study revealed that (Table 2) it belongs to race - 1. The reaction to different isolates observed on various 'R' lines which resulted in identification of five resistant restorer lines in India.

Table : 2 Reaction of *Plasmospara halstedii* Indian isolate to Downy mildew sunflower host differentials

SN	Differential Line	Percent disease incidence			Known reaction to race			Reaction to Indian isolate
		1986-87	1987-88	1988-89	1	2	3	

1	RHA-272	2.00	0.56	0.90	R	R	S	R
2	RHA-273	0.80	0.83	0.43	R	R	S	R
3	RHA-274	0.00	0.00	0.00	R	R	S	R
4	RHA-801	0.00	0.00	0.00	R	R	S	R
5	RHA-265	0.66	0.56	0.83	R	S	S	R
6	Progress	58.66	63.33	60.00	S	S	R	S

R = Resistant; S = Susceptible

The progress cultivar is known to resistant to race -3, but it is susceptible to Indian isolate, hence the Indian isolate does not belong to race -3. The cultivar RHA-265 is resistant to race -1 and susceptible to race -2 and 3. The same cultivar is also resistant to Indian isolate; hence the Indian isolate could be race -1 category. A regular field screening programme for sunflower lines is being conducted at Oilseeds Research Station, Latur (MS) to find out the reaction of sunflower lines against DM under sick soil condition, since from 1988. Two DM resistant hybrids *viz*., LDMRSH-1 (CMS-338-A x MRHA-2) and LDMRSH-3 (CMS-207-A x MRHA-1), LSFH -35 (234 A x RHA-1-1), LSFH-171 (CMA-17A x RHA-1-1) developed at Oilseeds Research Station, Latur have been released.

Disease management

The DM disease is seed, soil and air borne in nature, it is necessary to adopt various control strategies like regulatory measures, cultural management, seed treatment and use of resistant varieties etc. to combat the disease under field condition.

Host resistance

This includes use of resistant varieties (LDMRSH-1 and LDMRSH-3) to combat DM problem (Patil et al 1992). Use of hybrid varieties should be encouraged sine they are found tolerant compared to population. Similarly at Oilseeds Research Station, Latur many DM resistant hybrids have been identified through screening in DM plot and based on yield potential and DM resistance, ICAR has released such hybrids for the commercial cultivation (Shrishikar, 2005) (Table 4).

Table 4: List of sunflower Downy mildew resistant / tolerant hybrid s identified by ICAR varietal release committee (2002- 15)

Name of sunflower hybrids / variety	Year	Remarks
Sungene – 8	1996	This variety has been released during AICRP workshop held at JNKKV, Jabalpur in April 1996
LS-11	1998	This variety released by varietal identification committee meeting held at TNAU, Coimbtore in April 1998
MSFH-47	2000	This hybrid was highly resistant to DM and it was released by ICAR varietal identification committee meeting at PAU, Ludhiana in April 2000

Pro-009 (Prosun-09)	2003	This hybrid has been releases during AICRP sunflower workshop held at TNAU, Coimbtore
SH-416	2003	This hybrid has been releases during AICRP sunflower workshop held at TNAU, Coimbtore
DRSF-108	2003	This hybrid has been releases during AICRP sunflower workshop held at TNAU, Coimbtore
PCSH – 243	2004	This hybrid has released during AICRP sunflower workshop held at ANGRU, Hyderabad
RPO-011	2004	This hybrid has released during AICRP sunflower workshop held at ANGRU, Hyderabad
SCH-35	2004	Released by M. S. State, ORS, Latur Hybrid
XF-4132	2005	This hybrid has been released during AICRP sunflower workshop held at H. P. Krishi Vishwa Vidhyala, Palampur
PAC-334	2008	This hybrid has been released during Annual Group meeting held at GAU, Junagarh on 21-23 May 2009`
LSFH-171	2012	This hybrid has been released during Annual Group meeting held at UAS, Bangaluru on 27-29 April 2012

Chemical control

Seed treatment with fungicide like Apron 35 S. D. (Metalaxy fungicide) found very effective for the control of DM disease. The efficiency of Apron 35 S.D. fungicide and they reported that the fungicide is quite effective in reducing DM incidence under field conditions when used @ 6 g / kg of seed. However, a new formulation Apron XL 35 ES- @ 3 ml / kg as seed dresser has also been recommended (Shrishikar, 2005) (Table 3).

Table 3: Management of sunflower DM disease through Apron XL 35 ES fungicide under sick plot condition 2002-04 (Pooled)

SN	Details	DM incidence (%)	Yield kg/ha	BC ratio
1	Apron1 ml / kg	20.40	726	1.81
2	Apron 2 ml / kg	14.86	861	2.14
3	Apron 3 ml / kg	5.46	1106	2.74
4	Apron 6 ml / kg	8.56	1043	2.57
5	Control	85.5	263	0.65
	SE ±	1.1	106	
	CD at 5 %	3.4	326	

CONCLUSION

The race identification studies of India isolate of *Plasmopara halstedii* confirms that it belongs to race -1 (European race). The multiyear work carried out at ORS, Latur centre under All India Coordinated Research Project on sunflower screened 5408 lines and identified 1075 disease free entries with high level of resistance to DM pathogen. This resulted into the release of 15 sunflower hybrids and populations at national level. The identified resistance sources have been effectively utilized in the introgression of the resistant genes from identified sources for the improvement of parental lines. The centre has identified 14 parental lines (8 CMS, 6 restorer lines) possessing downy mildew resistant (Pl genes). The center has released three state level hybrids LDMRSH-1, LDMRSH-3 and LSFH-35 and LSFH-171 at national level for commercial cultivation.

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THE BEHAVIOR OF SUNFLOWER HYBRIDS IN DIFFERENT ENVIRONMENTAL CONDITIONS IN ROMANIA

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ABSTRACT

Sunflower breeders, working for a model (idiotype) of sunflower, must to know the main characteristics of the environment for which they are developing the hybrids, starting from soil type, potential growing season length, mean, minimum and maximum temperatures (per month) and the amount and distribution of rainfall, during the year. In the practical selection, which is part of the production of hybrids with high potential of productivity, as well as high adaptive potential, a strong influence belongs to the adaptive reactions to the ecological environment they are located in. The dry periods are more frequently, with negative effect on yield, including sunflower. We studied a set of 10 sunflower hybrids, in two years (2014 and 2105), in two locations, situated in different areas in Romania: Braila (eastern Romania and Fundulea (south Romania). The hybrids have been cultivated in three randomized replications. Comparing the two years, 2014 and 2015, regarding the air temperature and the amount of rainfall, in sunflower vegetation period generally, year 2015 was more dry, specially in Braila. The amount of rainfall was quite high, in Fundulea location, in 2014 year. Taking into consideration these data, the results regarding the seed yield for the ten hybrids, are showing that in Braila location it was registered a low seed yield comparing with Fundulea, in 2014 year. In 2015 year the highest seed yield was released by the hybrids, in Braila location. The hybrids Fundulea 708 and PR64LE20 had a good behavior, regarding the seed yield. The oil content, for all hybrids, was very good, in both years, in Braila location, the soil and climatic conditions in this location, being favorable for this characteristic. Regarding plant height, in both location and both years, the taller hybrids released the highest seed yield.

Key Words : sunflower, environmental conditions, hybrids, seed yield, oil content

HISTORY AND PRESENT STATE OF DOWNY MILDEW IN ARGENTINA

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ABSTRACT

Until 1998, races of *Plasmopara halstedii* (Farl.) Berl. *et* de Toni present in Argentina were 300 and 330, and almost all the sunflower hybrids sown in the country were resistant by the introgression of *Pl*2 resistant gene. Since that year resistance conferred by Pl2 was broken and races 710, 730 and 770 were determined. Resistance genes to these new races were introgressed from public lines from USA and Argentinean populations (*Pl5, Pl6, Pl7, Pl8, Pl15, Pl17, Pl18* and *PlArg*). Seed treatment with metalaxyl has been also widely used in different sunflower areas, but strains tolerant to this fungicide were found in all these races. Since 2013, downy mildew has been found in hybrids containing *Pl*15 gene, indicating the presence of a new race which is not possible to be classified by the international set of differential lines. Sustainable management of this disease should be based on reducing the selection pressure over the pathogen by combining practices as the introgression of several resistance genes simultaneously, using different active ingredients for seed treatment, crop rotations and avoiding contaminated seed exchange.

Keywords: metalaxyl tolerance; *Plasmopara halstedii;* new races; differential inbred line; *Pl* 15 resistant gene.

INTRODUCTION

Sunflower (Helianthus annuus L.) downey mildew (DM) caused by the oomycete *Plasmopara halstedii* (Farl.) Berl. & de Toni is one of the major diseases of this crop worldwide. It is potentially destructive when early infections occur through the roots causing damping-off and dwarfism. Usually, *P. halstedii* can generate variants of pathogenicity (pathotypes) with high frequency. Seed exchange between countries and / or production regions may favor the spread of these new pathotypes. Currently, there are at least 36 pathotypes of *P. halstedii* worldwide, but the number of races is increasing rapidly (Virányi and Spring, 2011). Furthermore, during the 80´s and 90´s many cases of *P. halstedii* tolerant to metalaxil were report from Spain, Hungary (Oros and Virányi, 1984), Turkey (Delen *et al*., 1985), France (Lafon *et al*., 1996) and USA (Gulya *et al*., 2000) among others.

Until 1998, the races of *P. halstedii* present in Argentina were 300 and 330 (Bazzalo, *et al*., 1996), and almost all the sunflower hybrids were resistant by the introgressed *Pl*2 resistant gene. Since that year, resistance conferred by *Pl*2 was broken by new races (710, 730 and 770 races) (ASAGIR, 2003). Resistance genes to these races were introgressed from public lines from USA and Argentinian populations (*Pl*5, *Pl*6, *Pl*7, *Pl*8, *Pl*15, *Pl*17, *Pl*18 and *Pl*Arg) (Bertero de Romano, *et al*. 2010). Cultivars with genetic resistance and/or seed-coated with metalaxyl or metalaxyl–M (mefenoxam) are strategies widely used for disease control in Argentina. Crop rotation with low presence of sunflower, a delay in sowing dates in high soil density (high clay content and no tillage) can also contribute to disease control. However, between 2012 and 2015 the prevalence and

intensity of DM has increased in different production regions of Argentina, indicating changes in pathogenicity of *P. halstedii*. The aim of this study was to identify new pathotypes of *P. halstedii* associated with the occurrence of epiphytotics of DM in Argentina. With this objective we have identified DM epiphytotic in different production regions of Argentina and then collected *P. halstedii* inoculum (sporangia) to determinate race and / or tolerance to metalaxyl.

MATERIALS AND METHODS

Between 2012 and 2015 seasons the presence of DM epiphytotics (> 5% incidence) were identified in different production regions of Argentina. The geographical location and the implemented strategies for DM control (genetic resistance and/or seed-coated with metalaxyl) were recorded for each case. Isolates of *P. halstedii* were collected. For inoculum multiplication, susceptible sunflower genotypes (without *Pl* genes or only *Pl*2; Paraiso 20®, Nidera Argentina; Cauquen®, El Cencerro, Argentina; HA 89 public line) were inoculated with each isolate using a protocol adapted from Viranyi and Gulya (1995). The resulting inoculum was briefly stored at -20ºC.

Twenty-eight isolates were selected and used to carry out the tests of tolerance to metalaxyl and/or race determination. Seeds of susceptible genotypes were treated with metalaxyl (46 mg per seed) to determine the tolerance of each isolate. A control without metalaxyl was included to determinate de level of tolerance. A randomized complete block design was used with two or three repetitions. Each experimental unit consisted of ten seeds planted in speedlings in a soil:perlite (1:1) substrate. The inoculation was performed according to the protocol of Viranyi and Gulya (1995). For the disease evaluation (incidence: plants with signs of DM per total plants), susceptibility was considered when sporulation on cotyledons and the first true leaves became evident to the naked eye. Occasionally, damping-off could be seen among the inoculated seedlings. Data was analyzed with ANOVA and LSD Fischer for disease incidence media comparison. For the race determination the sets of sunflower differential lines proposed by Tourvieille de Labrouhe, *et al*. (2000) were used. Seeds inoculation, seedling cultivation and disease evaluation were carried out as described above.

RESULT AND DISCUSSION

DM epiphytotics were identified in the three sunflower production regions of Argentina (Figure 1). North Santa Fé province and south Buenos Aires province were the regions with major number of cases (6 and 20 cases, respectively) between 2012 and 2015 crop seasons. In north Santa Fé, five *P. halstedii* isolates were tolerant to metalaxyl (Barro Pazos, Ceibal, Malabrigo, Reconquista and Villa Ocampo). These isolates were identified as 710, 730 and 770 races (Figure 1) (Bazallo 2014, Bazzallo and Piubello, 2015). Also, the presence of 713 race was detected near Santa Fé city (Figure 1; Table 1). It is the first registry in Argentina of *Pl*13 and *Pl*14 break (Table 1).

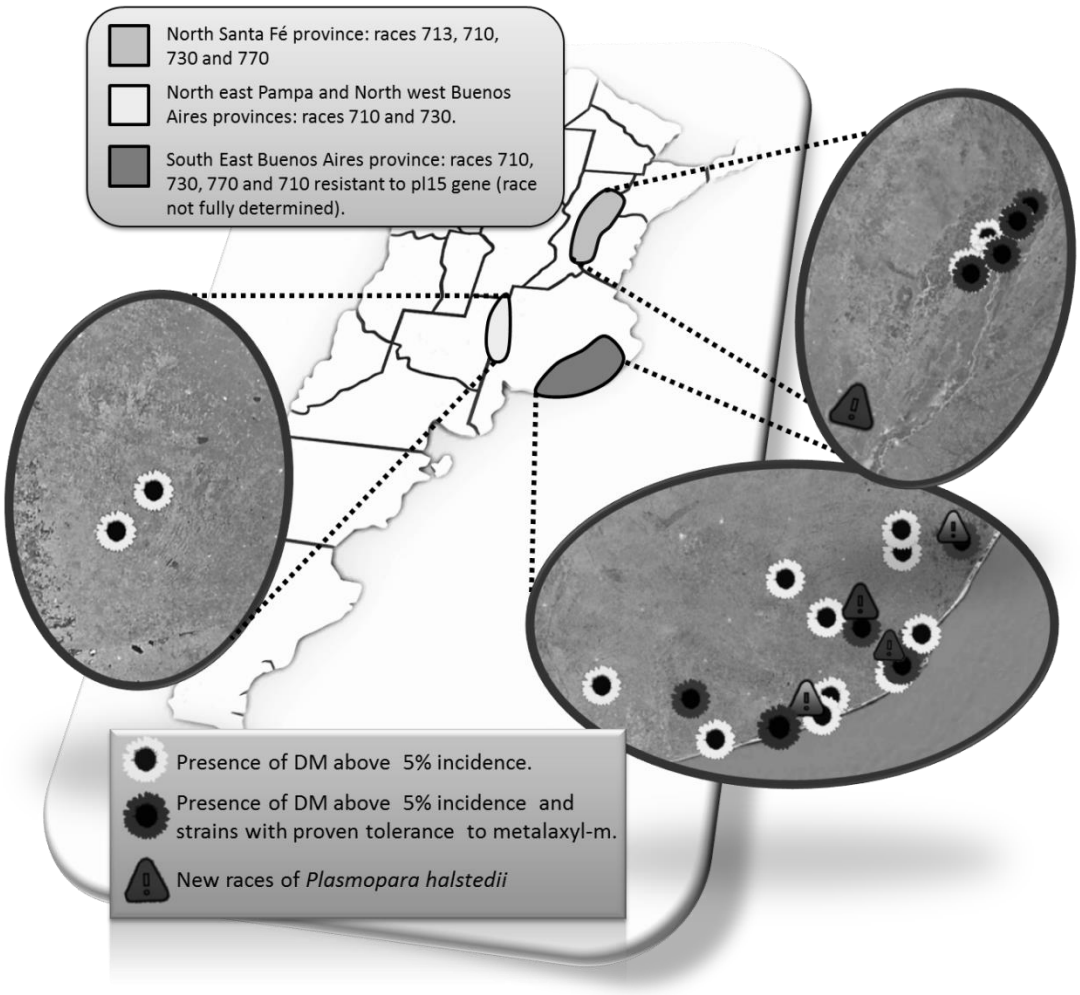

Figure 1. Location of downy mildew epiphytotics (more than 5% of incidence) caused by *P. halstedii* in Argentina between 2012 and 2015. Torelant isolates to metalaxyl and/or new races are discriminated.

In south of Buenos Aires province, twelve *P. halstedii* isolates were corroborated as tolerant to metalaxyl (Balcarce, Mar del Plata, Otamendi, Tres Arroyos among others) and were identified as 710, 730 and 770 races (Figure 1) (Erreguerena *et al*., 2013; Bazallo 2014, Bazzallo and Piubello, 2015). In this region, in 2014-2015 seasons it was found that some hybrids with resistant (*Pl*15 gene) to widespread Argentinian races (710, 730 y 770) were affected by DM. In these cases, the *P. halstedii* isolates were characterized as 710 race, although these variants compared with ordinary 710 races broke *Pl15* resistance gene (Table 1).

Table 1: New *P. halstedii* races in Argentina characterized by their reaction of sunflower differential line suggested by Tourvieille de Labrouhe, *et al.* (2000) and by an inbred line with *Pl*15 resistance gen.

					New races in Argentina	
Diferencial set		Line	*Pl* Gen	Race 710 ordinary variant (Argentina)	Race 710 variant not fully discriminated	Race 713
SET ONE	D-1	HA89		S	S	S
	D-2	RHA265	1	S	S	S
	D-3	RHA274	2	S	S	S
SET TWO	D-4	PMI-3	4	S	S	S
	D-5	PM-17	5	R	R	R
	D-6	803-1	803	R	R	R
SET THREE	D-7	HAR-4	14	R	R	**S**
	D-8	HAR-5	13	R	R	**S**
	D-9	HA-335	6	R	R	R
Inbred line with Pl 15		NI-Pl15	15	R	**S**	R

The present context of sunflower production in Argentina shows a growing dynamics towards to the generation of pathogenic variants of *P. halstedii* than the historically observed. In recent years there has been determined the tolerance to metalaxyl and the appearance of at least two new races (713 and 710 race that break the *Pl*15 resistance). *Plasmopara halstedii* pathotypes with tolerance to metalaxyl are distributed mostly in the regions of sunflower production, except for center region (east of La Pampa and west of Buenos Aires provinces). The loss of effectiveness of metalaxyl as a seed control requires its replacement with other molecules. The *Pl15* break by a new race exposes some of the Argentinian commercial hybrids to DM increasing the risk of an epyphitotic occurrence in the southern region of Buenos Aires province. For an accurate discrimination of this new race in relation to 710 ordinary race is required to expand the differential set of inbred lines suggested by Tourvieille of Labrouhe, et al. (2000). In this context, the *Pl6* and *Pl8* genes widely introgressed in Argentinian hybrids remains efficient for controlling DM. Is important to implement molecular techniques for *P. halstedii* identification in seed and avoid the incorporation of the pathogen or its pathotypes in the crop regions free of DM. Sustainable management of disease should be based on reducing the selection pressure over the pathogen by combining practices as the simultaneously introgression of several resistance genes, using alternative active ingredients for seed treatment, crop rotations and avoiding early sowing date and contaminated seed exchange.

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A REVIEW ON THE SEED-BORNE MICROFUNGI OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower (*Helianthus annuus* L.), is one of the major oilseed crop grown for edible oil in all over the world and is prone to seed-borne fungi during harvesting and storage. These saprophytic and parasitic fungi may affect the seed quantity and quality. They can cause reduction of the amount of fatty acid in the seed and/or the formation of mycotoxins during storage. Fungal species can vary depending on storage conditions. In this review, the researches carried out on seed-borne microfungi of sunflower were evaluated. In studies on seed-borne fungi of sunflower, *Aspergillus, Penicillium, Fusarium, Alternaria, Rhizopus, Mucor, Curvularia, Cladosporium, Trichoderma, Macrophominia, Emericella*, *Stemphylium*, *Chatemium* and *Phoma* were reported as the genera most commonly found. And, the most frequently isolated species from seeds were *Aspergillus flavus, Aspergillus niger, Alternaria alternata, Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus ochraceus, Curvularia lunata, Fusarium solani, Fusarium moniliforme, Aspergillus nidulans, Mucor mucedo, Cladosporium cladosporioides*and *Penicillium chrysogenum*. In addition, the presence of mycotoxins such as aflatoxins (B1, B2, G1 and G2), sterigmatocystin, ochratoxin A, zearalenone, T2-toxin, diacetoxyscirpenol, alternariol and alternariol monomethyl ether were recorded from various researches.

Key Words : sunflower, seed, seed-borne microfungi

EPIPHYTOTIC DISEASE OF SUNFLOWER STEM CANKER IN ARGENTINA

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ABSTRACT

The canker on sunflower stem was diagnosed in America, Europe and Australia (Marisevic & Gulya, 1992; Thompson *et al*., 2011). Although it was detected in Argentina the disease was sporadic (1985) while in Uruguay, from 2003, has been found as a serious disease (Huguet 2006, Stewart 2005). In the 2015 and 2016 growing seasons high-incidence outbreaks of the disease have been important in the Northwestern of La Pampa Province, Southern of Córdoba Province and Northeastern of Buenos Aires Province. The symptoms on sunflower stems presented pale brown cankers developed around petioles insertions. The leaf blades in connection with cankers shown V-shaped necrosis. The leaves laterally disposed above the canker presented intervein necrosis. On the bases of capitula brown rotten areas were observed affecting receptacles and even achenes (discoloured seeds). The receptacles presented necrosed bracts, expanded V-shaped necrosis pointing to and even involving the peduncles. The degree of susceptibility was recorded on 36 commercial sunflower hybrids under naturally conditions. The incidence of stem canker ranged from 1.25 % to 33.75 %. In some cases sunflower fields presented rot incidence of 100 % on capitula (R8 phenological stage). Yield losses are still under evaluation. From the symptoms described on sunflower stems and capitula several fungal isolates were obtained according to the methodology described by Muntañola et al. (1981, 1985). Isolates were morphologically determined as *Phomopsis* cf. *helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov. *Nova Hedwigia* 34: 433 (1981). Molecular studies in connection with isolates from stems (and capitula) are being carried out by Dr. Sue Thompson (DEEDI, Australia). Koch's postulates were completed on healthy sunflower plants. The isolates have presented pycnidia semi-immersed, dark brown, separate or confluent, subglobose to ampulliform, 480-630 x 440-530 μm, with exuding pale yellow drop-like slime. Conidiogenous cells were cylindrical, gradually tapering into necks, hyalines, 9.6- 15.4 x 1.4-1.9 μm. Alpha conidia were not observed. Beta conidia were filiform, sigmoid, hamate, 17-32 x 0.96 μm. *Phomopsis* cf. *helianthi* was also isolated from stem's cankers on the common weed *Helianthus petiolaris* Nutt. Other isolates are under evaluation on other weeds in order to detect potential pathogen hosts. The results obtained could reflect an expanding outbreak of the sunflower stem canker in Argentina.

Keywords: sunflower, phomopsis, diseases, stem canker, hybrids

INTRODUCTION

The canker on sunflower stem was diagnosed in America, Europe and Australia (Marisevic & Gulya, 1992; Thompson *et al*., 2011). Although it was detected in Argentina the disease was sporadic (1985) while in Uruguay, from 2003, has been found as a serious disease (Huguet 2006, Stewart 2005). In the 2015 and 2016 growing seasons high-incidence outbreaks of the disease have been important in the Northwestern of La Pampa Province, Southern of Córdoba Province and

Northeastern of Buenos Aires Province. Symptoms were observed on leaves, stems and capitulas. There are no antecedents of epiphytotic disease of sunflower stem canker in this region.
The objectives of study were to describe the symptoms in plants and determine a causal agent of disease, determine alternative hosts of sunflower stem canker, and characterize the health behavior in sunflower hybrids in this region.

MATERIALS AND METHODS

In northern of La Pampa Province (S 35° 34' 43.4" W 63° 41' 19.75"), 36 sunflower hybrids in 3 trials grouped according to their characteristics were seeded: a) resistant to imidazolinone (IMI resistant, 18 hybrids) b) Normal and high oleic (10 normal hybrids and 2 high oleic hybrids), c) Confectioner (6 hybrid). The plots consisted of three rows with row spacing of 0.52 m and 9 m long. A density of 57,000 plants ha^{-1} was used in test a and b. In trial c, density was 40,000 plants ha^{-1} with equal row spacing. All cultivars were planted at 23/10/2015 in cero tillage on soybean predecessor, in a sandy loam soil. In each trial design it was done in randomized blocks with 4 repetitions.
Health behavior was evaluated on each experimental unit in 20 plants under natural infection. The incidence of plants with canker on the stem in the phenological state of R7 (Schneiter an Miller 1981) was determined.
From the symptoms on sunflower stems and capitula several fungal isolates were obtained. Fungal material was isolated and established in pure culture on Malt Extract Agar prepared according to Booth (1971) (Muntañola-Cvetković et al., 1981, 1985) using Malt Extract Oxoid LP 0039; pH = 6 before sterilization without any hydroxide addition. Vancomycin (250 ppm) was incorporated into the isolating media in order to suppress bacterial development. Incubation was carried out at 26-28 ºC under 16 h UV light (345-400 nm)/8 h obscurity cycles. Sporulation starts after ten incubation days. Measures were taken from two weeks cultures picking up pycnidia holding pale yellow slime (5 pycnidia, 10 conidiogenous cells, 10 conidia). Specimens were mounted on cotton blue 0.1 % w/v in lactic acid 85 % w/w and Shear's mounting fluid.

RESULT AND DISCUSSION

The symptoms on sunflower stems presented pale brown cankers developed around petioles insertions. The leaf blades in connection with cankers shown V-shaped necrosis. The leaves laterally disposed above the canker presented intervein necrosis. On the bases of capitula brown rotten areas were observed affecting receptacles and even achenes (discoloured seeds). The receptacles presented necrosed bracts, expanded V-shaped necrosis pointing to and even involving the peduncles.
The incidence of stem canker ranged from 1.25 to 33,75 % for sunflower IMI resistant hybrids. The normal and high oleic hybrids ranged from 6.25 to 33.75 %, while confectioner hybrids have presented incidence from 13,75 to 30 %.
In some cases sunflower fields of farmers presented rot incidence of 100 % on capitula (R8 phenological stage). Yield losses are still under evaluation.
From the symptoms described on sunflower stems and capitula several fungal isolates were obtained according to the methodology described by Muntañola et al. (1981, 1985). Isolates were morphologically determined as *Phomopsis* cf. *helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov. *Nova Hedwigia* 34: 433 (1981). Molecular studies in connection with isolates from stems (and capitula) are being carried out by Dr. Sue Thompson (DEEDI, Australia). Koch's postulates were completed on healthy sunflower plants. The isolates have presented pycnidia semi-immersed, dark brown, separate or confluent, subglobose to ampulliform, 480-630 x 440-530 μm, with exuding pale yellow drop-like slime. Conidiogenous cells were cylindrical, gradually tapering into necks, hyalines, 9.6- 15.4 x 1.4-1.9 μm. Alpha conidia were not observed. Beta conidia were filiform,

sigmoid, hamate, 17-32 x 0.96 μm. *Phomopsis* cf. *helianthi* was also isolated from stem's cankers on the common weed *Helianthus petiolaris* Nutt.
Other isolates are under evaluation on other weeds in order to detect potential pathogen hosts.
The results obtained could reflect an expanding outbreak of the sunflower stem canker in Argentina.

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INVESTIGATIONS AND THE DESCRIPTION OF VIRUS DISEASES IN SUNFLOWER GROWING AREAS IN THE TRAKYA REGION OF TURKEY

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ABSTRACT

Being an important source of vegetable oil for human consumption and the source of raw material for food industry, sunflower (*Helianthus annuus* L.) production has been increased steadily in the World and as well as in Turkey. Edirne, Kırklareli and Tekirdağ provinces in the Trakya region have been important sunflower growing areas in Turkey. In order to determine virus diseases reducing oil seed yield and quality survey studies were conducted in two different periods during 2015 growing season. In order to determine viruses on symptomatic sunflowers and weed hosts, 244 leaf samples were collected. For the identification of *Potato virus* Y (PVY), Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) test, for *Tobacco streak virus* (TSV) Triple Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (TAS-ELISA) and for the identification of Potyvirus'es Plate Trapped Antibody-Enzyme Linked Immuno Sorbent Assay (PTA-ELISA) test methods were employed. For the determination of the effect of virus infections on sunflower seed yield criteria, seeds were harvested from both infected and healthy plants separately which compared for their 1000 seed weight, hectoliter seed weight and their oil content. According to DAS-ELISA test results PVY never present in the sunflower fields of Trakya Region. Depending on symptomatic observations and the results of TAS-ELISA tests 11 out of 244 plant samples had TSV with the rate of 4.51 %. As the results of PTA-ELISA tests and the symptomatic field observations 25 of 244 plants were found infected with Potyvirus'es with the rate of 10.25 %. Totally 36 out of 244 plant samples revealed the presence of viruses with the rate of 14.75 % in the sunflower growing areas. This is the first report of the presence of virus infections on sunflowers in Turkey. Virus infections cause reductions of 1000 seed and hectoliter seed weights of oil seeds as the oil content was found slightly high.

Key Words : Sunflower, *Helianthus annuus* L., ELISA, PVY, TSV, Potyvirus

BIPOLARIS AUSTRALIENSIS ON SUNFLOWER IN RUSSIA

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ABSTRACT

For the first time in the Russian Federation *Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama (teleomorph *Cochiobolus australiensis* (Tsuda & Ueyama) Alcorn) was found in sunflower seeds. This fungus causes human and animal diseases (allergic chronic sinusitis, dermatitis, etc.). Also *B. australiensis* is able to infect plants of different families (Fabaceae, Poaceae, etc.). On sunflower, it was previously found in India (in seeds and leaves) (Kumar and Dwivedi, 1981; Chavhan et al., 2008) and Pakistan (in seeds) (Sharfun-Nahar et al., 2005). Pathogenicity tests of our single-spore *B. australiensis* isolate under greenhouse conditions demonstrated ability of this fungus to infect healthy sunflower plants (at list in stages from seedlings to the beginning of flowering): it caused brown leaf spots. Phytotoxic property of *B. australiensis*, which was not earlier recorded for any plants, has been established. Its cultural filtrate was highly toxigenic for the 10-days-old seedlings of sunflower, having caused abnormal development of roots (up to full suppression of their growth). It has been shown that *in vitro* this dark-pigmented hyphomycete actively grew and developed on various food substrates at temperatures ranging from 4 to 40 °C, regardless of illumination (as on the light and in the darkness).

Key Words : Sunflower , Bipolaris australiensis, Pathogenicity, Phytotoxic property

METABOLOMIC PROFILING OF SUNFLOWER SEEDS IN RESPONSE TO WATER STRESS DURING GERMINATION

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ABSTRACT

Climate change is now recognised as one of the most serious challenges facing the world. In particular, climate change is a challenge for farming, and water limitation is a major abiotic stress which affects seed germination, stand establishment and *in fine* crop yields. Therefore, agricultural adaptation is necessary for the future and there is a need to better understand the molecular and cellular bases of tolerance to water stress during seed germination. In this context, tolerance to water stress during sunflower (*Helianthus annuus* L.) seed germination was studied. Seeds from one tolerant and one sensitive sunflower hybrid were selected with regards to their ability to germinate under water limitation, using a polyethylene glycol (PEG) solution (- 0.6 MPa, 20°C). A non-targeted metabolomic study was then carried out using seeds imbibed for 15 h at 20°C on water and on the PEG solution in order to identify seed metabolites associated with tolerance to water stress during the germination phase. We used liquid chromatography coupled to mass spectrometry (LC-MS) and proton nuclear magnetic resonance spectroscopy (1H-NMR). 1H-NMR spectra and the main compounds of MS spectra were annotated. Thus, 47 major compounds were selected and univariate and multivariate statistical analyses were carried out on these compounds. Statistical analyses were also performed on the entire MS profiles. Our analyses demonstrate that the metabolic profiles differ more between the two hybrids than between the two treatments. The effect of PEG imbibition was also investigated for each hybrid. We observe more response markers for the tolerant hybrid than for the sensitive one, suggesting that the metabolism of seeds from the tolerant hybrid is more affected by water stress.

Key Words : Sunflower, water stress, metabolomics, LC-MS, NMR

OIL AND MEAL QUALITY

AGRONOMIC PERFORMANCE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) IN AN ORGANIC CROP ROTATION SYSTEM IN THE HUMID TROPICS

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ABSTRACT

The demand for organic sunflower seeds is very high in the international market. Sunflower is a rustic plant that is cultivated under different production systems across several agro-ecological zones in the world. A locally adapted and late maturing sunflower variety ('Funtua') was sown after soybean, sesame and maize between 2008 and 2012 to assess its agronomic performance under continuous, rotational and conventional cropping systems in the forest – savanna transition zone. The field trials were carried out during the late cropping season (June – Nov.) in a randomized complete block design and replicated four times. Data were collected on plant height at maturity, seed yield and yield attributes of sunflower each year. Varying results were obtained on the effects of cropping systems on the agronomic parameters measured across the years. However, cropping system significantly (*P<0.05; F-test*) affected seed yield of sunflower in 2009, 2011 and 2012. The conventional cropping system only significantly (*P<0.05*) produced seed yield (1642.6 kg.ha^{-1}) higher than the continuous (778.0 kg.ha^{-1}) and rotational cropping (1262.0 kg.ha^{-1})systems in 2009. Thereafter, as the system stabilized, the rotational cropping system recorded higher seed yield than the continuous and conventional cropping systems in 2010, 2011 and 2012. The difference was significant (*P<0.05*) in 2012 with the rotational cropping system producing seed yield higher by 7.3 and 31.3% than the conventional and continuous cropping systems, respectively. Adoption of rotational cropping system is hereby recommended for sustainable organic crop production system in the humid tropics.

Key words: crop rotation, sesame, sunflower, yield, yield characters

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oilseed crop that has a very wide range of adaptation ability, low labour requirement for its cultivation and also very suitable for mechanization (Ozer *et al.,* 2004; Kaemeini *et al.,* 2009). Consequently, sunflower can be described as a suitable crop for crop rotation scheme in the tropics where water and not temperature is the major growth limiting factor. It also exhibits erect growth habit, comparable resistance to lodging, short duration, limited ground cover and has easily harvestable heads (Robinson, 1984; Kamal and Bano, 2009). Sunflower is grown principally for its seed that contains oil (36–52%) and protein (28–32%) as reported by Rosa *et al.* (2009). According to NSA (2016), the world's average yield and total land area statistics of sunflower increased appreciably by 11.3% and 9.3% between 2007/2008 and 2012/2013, respectively.

Crop rotation is a planned order (sequence) of specific crops from different genus, species, subspecies or varieties on the same field over a given period of time (Helm, 1993). The advantages of crop rotation include: prevention of soil depletion; improvement of soil fertility, internal resource utilization; reduction of soil erosion, reliance on synthetic chemicals, allelopathic or

phytotoxic effects and environmental impact; control of diseases and pest infestation; enhancement of workload distribution and distribution of economic risks (Helm, 1993). According to Kamal and Bano (2009), over 200 natural allelopathic compounds have been discovered and isolated from different cultivars of sunflower. However, the responses of crops that follow sunflower in a sequence of as companion crops vary (Farooq *et al*., 2011; Nikneshan *et al.*, 2011). It was recently reported that the α-pinene in essential oil of sunflower head is very critical to the inhibitory effect of head extract (Kaya *et al.*, 2013). Consequently, it was suggested that removing the head of sunflower could be beneficial for alleviating the allelopathic effect. Unfortunately, this crop is rarely cultivated in rotation with other crops in the tropics. Therefore, in a bid to develop a production package for some staple and commercial food crops with high export potentials a crop rotation scheme was initiated consisting of four component crops with export potentials (soybean, sunflower, sesame and maize) in 2008. The objective of the study was to evaluate the performance of the component crops in rotation relative to continuous and conventional cropping systems.

MATERIALS AND METHODS

The mean monthly rainfall data during the late copping season of 2008 – 2009 are presented in Table 1. Year 2010 was the wettest year (791.2 mm) during the late cropping season and 2008 was the driest (328 mm). Although, the highest rainfall (288.1 mm) was recorded in 2011 during the most critical month for sunflower (October) which coincided with grain filling. The crop rotation scheme involved four component crops (soybean, sesame, sunflower and maize as shown in Table 2) and the study was carried out at the Organic plot of the Teaching and Research Farm of the University of Agriculture, Abeokuta (7^o 15' N, 3^o 25' E, altitude 140 m.a.s.l). The soil of the experimental field is oxic Paleudulf (Adetunji, 1991). The test variety of sunflower was Funtua (a local adapted and late maturing variety). The experimental design was randomized complete block design (RCBD) with four replicates. Treatments evaluated were continuous, rotational and conventional cropping systems. The plots of the conventional cropping system were located about 15 m away from the organic plots to avoid commingling. The row spacing adopted for sunflower under the three cropping systems was 60 x 30 cm and each plot measured 6.5m by 6.0m ($39m^2$). Sowing of sunflower seeds was done on August 15, 2008, July 2, 2009, August 15, 2010, July 18, 2011 and July 20, 2012 based on the onset of rains in the late cropping season. No herbicides or inorganic fertilizers were applied on the continuous and rotation plots. However, pre-emergence herbicides (Galex and Gramoxone) and fertilizer combination (60 kgN/ha, 56 kg P_2O_5/ha and 100 kgK_2O/ha) were applied on the conventional plots at sowing and 4 weeks after sowing (WAS), respectively. Manual weeding was done on all plots at 3 and 6 WAS. The organic fertilizer (Aleshinloye Fertilizer (Grade B) contained 1.2%N, 76 ppm P, 13.75 cmol K, 10.28 cmol Na) was applied at the rate of 25 tonnes/ha to the continuous and rotational cropping systems plots at 4 WAS. This rate was equivalent to 60 kg N ha/ha of the inorganic fertilizer recommended for sunflower in the transition zone (Olowe *et al.*, 2005). Application of organic fertilizer commenced in 2009 a year after the rotation scheme took off. Harvesting was done at physiological maturity (R8) as described by Schneiter and Milner (1981). Five randomly selected plants per plot were tagged from the net plot for plant height measurement and yield attribute analysis. Data were collected on plant height at physiological maturity, head weight and diameter, number and weight of seeds per head and seed yield on plot basis. All data collected were subjected to analysis of variance and means of significant treatment were separated using the least significant difference method as described by Steel and Torrie (1984).

RESULTS

Effect of cropping systems on plant height, seed yield and yield attributes of sunflower

Cropping system only significantly ($P \leq 0.05$; *F*-test) affected plant height in 2012 with sunflower plants on rotational and conventional plots significantly taller than plants on under continuous cropping system (Table 3). However, the pooled mean indicated that the plant height of sunflower

under the conventional and rotational cropping systems were at par. Average head diameter and weight of sunflower were significantly ($P \leq 0.05$; *F*-test) affected by cropping system in 2009 and when pooled, and the plants under continuous cropping system recorded significantly lower head diameter and weight than those under rotational and conventional cropping systems (Table 4 and 5). Weight of seeds per head was significantly ($P \leq 0.05$; *F*-test) affected by cropping system in 2009, 2012 and when pooled (Table 6). Similarly, the effect of cropping system was only significant ($P \leq 0.05$; *F*-test) for number of seeds per head in 2009 and when pooled (Table 7).However, sunflower seed yield was significantly ($P \leq 0.05$; *F*-test) affected by cropping system in 2009, 2011, 2012 and when pooled. Sunflower under continuous cropping system produced lower (significant at $P \leq 0.05$) seed yield than the plants under rotational and conventional cropping systems during the three years, except when yield values were pooled and the continuous was at par with rotational system (Table 8).

DISCUSSION

Rainfall distribution which is the main growth limiting factor in tropical agriculture varied markedly during the five year period of experimentation. The total rainfall during the late cropping season of 2008 – 2012 ranged between 328.0 and 791.2 mm and these values compared favorably with the rainfall amount (500 – 750 mm) reported to be adequate for optimum performance of sunflower (Weiss, 2000). Year 2008 with the smallest amount of rainfall (328.0 mm) also recorded the lowest seed yield (540.8 kg/ha). This could be attributed to the low rainfall in October (84.5 mm) which coincided with the grain filling period. The rotational and conventional cropping systems recorded grain yields above 1,000 kg/ha between 2009 and 2012, except conventional cropping system in 2010 and 2011. These yield values must have been enhanced by the rainfall in the months of September and October, and the availability of nutrients supplied through fertilizer application and they compared favorably with Nigerian (1000 kg/ha), African (812 kg/ha) averages (Olowe *et al.,* 2013) and world average (1520 kg/ha) according to USDA (2012), and the more recent forecast (1410 kg/ha) for 2012/2013 by NSA (2016). The consistently higher seed yield recorded under rotational cropping system in 2010, 2011 and 2012 could be due to the gradual stabilization of the system following application of organic fertilizers and rotation of soybean and sesame as preceding crops to sunflower.

The main agronomic traits that critically contribute to seed yield of sunflower include number of heads per hectare, weight of seeds per head and number of seeds per head (Robinson, 1978). However, in our study, the pooled mean revealed that cropping system significantly affected grain yield with the conventional and rotational cropping systems recording higher values for weight and number of seeds per head relative to sunflower under continuous copping system. Furthermore, the relatively lower values for plant height, number and weight of seeds per head, head weight and diameter on sunflower under continuous cropping system could also be attributed to depleted nutrients in the soil and accumulation of pest and disease organisms following continuous cropping of sunflower for the fourth year on the same plot. However, no serious disease or pest problem was recorded during our study.

CONCLUSION

Based on the pooled results of this study, the agronomic performance of sunflower that received organic fertilizer under rotational cropping system confirmed the huge potential for sunflower being a crop with high adaptability and low labour requirement as a viable component in organic crop rotation system in the tropics.

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Table 1: Mean monthly rainfall (mm) during the late cropping season (July – November) of 2008 - 2012

Year	**July**	**August**	**September**	**October**	**November**	**Total**

2008	299.2	106.7	136.8	84.5	0.0	328.0
2009	160.0	162.1	151.6	180.1	64.6	718.3
2010	322.9	266.6	257.6	172.3	94.7	791.2
2011	349.5	88.7	204.1	288.1	3.6	584.5
2012	155.4	36.3	181.4	184.7	49.6	607.4

Table 2: Crop rotation scheme involving soybean, sesame, sunflower and maize (2008 -2012)

2008	**2009**	**2010**	**2011**	**2012**
Sunflower	Sesame	Maize	Soybean	Sunflower
Sesame	Soybean	Sunflower	Maize	Sesame
Maize	Sunflower	Soybean	Sesame	Maize
Soybean	Maize	Sesame	Sunflower	Soybean

Table 3: Effect of cropping systems on plant height (cm) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	**2008**	**2009**	**2010**	**2011**	**2012**	**Mean**
Continuous	-	184.7	125.8	206.1	218.0	183.7
Rotational	208.0	209.0	224.3	255.8	235.0	226.4
Conventional	192.0	206.4	216.7	243.7	237.8	219.3
LSD 5%	ns	ns	ns	ns	10.19	32.32

Notes: **, * Significant at P $\leq$ 0.001 and 0.05, respectively, ns – non-significant

Table 4: Effect of cropping systems on head diameter (cm) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	**2008**	**2009**	**2010**	**2011**	**2012**	**Mean**
Continuous	-	9.6	9.1	14.1	17.1	12.5
Rotational	9.8	12.1	10.5	16.2	18.0	13.3
Conventional	8.6	12.8	11.3	16.4	18.1	13.4
LSD 5%	ns	2.17	ns	ns	ns	0.69

Notes: **, * Significant at P $\leq$ 0.001 and 0.05, respectively, ns – non-significant

Table 5: Effect of cropping systems on head weight (g) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping	**2008**	**2009**	**2010**	**2011**	**2012**	**Mean**

systems						
Continuous	-	32.4	39.9	28.5	112.5	53.3
Rotational	60.3	58.0	57.2	41.3	123.5	68.1
Conventional	43.4	68.0	79.1	41.6	122.5	70.9
LSD 5%	ns	26.50	ns	ns	ns	13.36

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 6: Effect of cropping systems on seed weight (g) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	20.2	21.9	19.7	41.3	25.8
Rotational	21.5	33.1	31.9	37.9	57.7	36.4
Conventional	28.3	42.2	35.1	36.2	53.4	39.1
LSD 5%	ns	9.53	ns	ns	3.02	9.93

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 7: Effect of cropping systems on number of seeds per head of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	319.0	580.0	385.0	580.5	466.0
Rotational	540.8	680.0	853.0	547.0	607.0	645.4
Conventional	664.9	715.0	659.7	520.0	591.5	630.2
LSD 5%	ns	257.2	ns	ns	ns	57.44

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 8: Effect of cropping systems on seed yield (kg/ha) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	778.0	1000.0	584.7	981.1	835.9
Rotational	540.8	1262.0	1150.0	1348.5	1428.2	906.0
Conventional	664.9	1642.6	750.0	808.9	1324.0	1038.0
LSD 5%	ns	366.75	ns	579.8	75.23	145.0

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

ESSONS FROM TEN YEARS OF AN INTERPROFESSIONAL SURVEY PLAN ON OILSEEDS FOOD SAFETY

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ABSTRACT

French oilseeds food chain operators are coordinated through a food safety survey plan, in order to get a realistic picture of the contamination in oilseed products (seeds, oilseed meal, and vegetable oil). Concerned crops are those cultivated or processed in France: rapeseed, sunflower and soybean. Grain storage companies, feeding industries and oil industries participate voluntarily, and send their self-data that are pooled in a database. Thirty-three companies are actively involved, providing each year about 60000 to 180000 analytical results coming from about 2000 to 3000 samples of seeds, meals and oils (note: on one sample, several contaminants can be analyzed giving several analytical results). Pesticide residues represent more than 90% of the analytical results of this database as the laboratories can determine a large number of active substances with multi-methods. Other sought contaminants are: trace elements (cadmium, lead, arsenic, mercury, fluorine), mycotoxins (mainly aflatoxin B1 and total aflatoxins), toxic organic compounds (polycyclic aromatic hydrocarbons, dioxins and PCBs), microbiological contaminants (salmonella in meals), botanical impurities (eg seeds of *Datura spp.* in sunflower seeds), mineral oils or compounds likely to be formed during refining such as esters of 3-MCPD and glycidyl esters in oils. The food safety of oilseeds survey plan allows to identify which are main concerns, for instance post-harvest insecticide residues from cross contamination during storage. Results of this monitoring plan were transmitted to the French government and the European Commission in cases of regulatory threshold revisions (eg for cadmium in oilseeds, for the revision of pirimiphos-methyl thresholds).

Key words: Oilseeds, vegetable oil, survey plan, contaminants, pesticide residues

INTRODUCTION

The French oilseed food supply chain got together with food safety issues since the early 2000s that correspond to the establishment of a set of European regulations called “Hygiene Package” (Dauguet et al, 2006). In this context, the food safety survey plan (called PSO) was implemented from the 2005 campaign, helping to control the quality of products (seeds, meal and oil) in a interprofessional framework. Since PSO was launched, more and more operators of the oilseed supply chain have become active partners. This article gives a review of the seven years of the PSO.

Today, each operator of the food chain is facing a legal obligation:

- to implement a HACCP approach, based on sound analysis of health risks inherent in its business,
- to ensure the sanitary compliance of products that it puts on the market,
- to carry out self-monitoring.

The PSO, set up by Terres Inovia, ITERG and Terres Univia since 2005, is an observatory of the sanitary quality of oilseed products in France (Lacoste et al, 2005). This survey plan is based on

a shared private database on oilseed contaminants. This base is fed by self-monitoring data from industries (crushing industry and feed industry) and storage agencies that join this PSO, as well as by series of analyzes on seeds, meal and oil by Terres Inovia, ITERG and Terres Univia (figure 1).

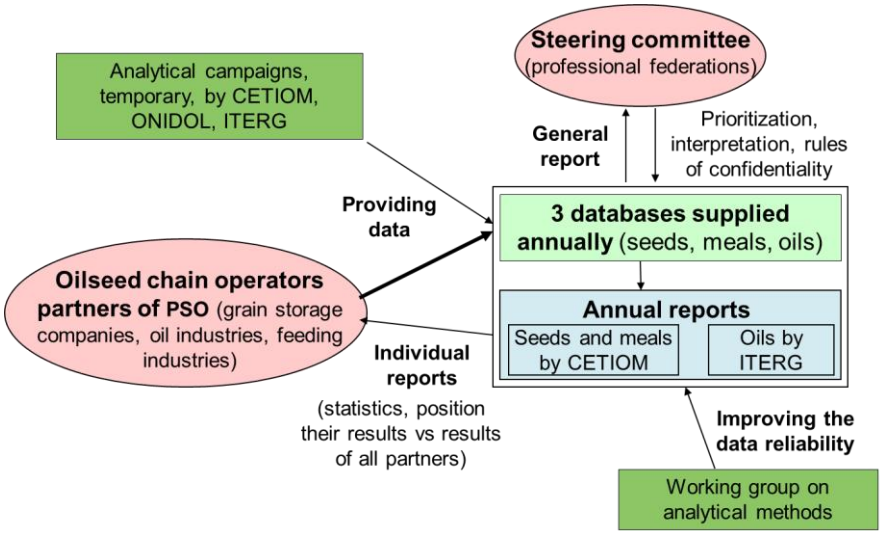

Figure 1. Organization of the Oilseed Survey Plan (PSO)

Intended for storage agencies to industrial oil processors and feed manufacturers, the PSO deals with:

- oilseeds: rapeseed, sunflower, soybean
- products: seeds, meals, crude and refined oils, byproducts of refining
- contaminants: residues of plant protection products, trace metals, mycotoxins, toxic organics, salmonella, botanical impurities ...

The confidentiality of data is guaranteed for partners, and no commercial exploitation of this database is made. The database on seeds and meals is managed by Terres Inovia, and the database on crude and refined oils is managed by ITERG.

So, the PSO is a tool of the oilseed supply chain, allowing a collective coordination on the safety aspects, highlighting progress and contributing to setting realistic regulatory thresholds. It represents also a forum for exchange of information between the operators in the sector, where are identified relevant research avenues.

A GOOD REPRESENTATIVENESS

To date, the PSO has 33 active partners: 28 grain storage agencies distributed throughout France, which represents 30-40% of the of the French oilseed harvest, 4 oil industrials (the main groups in France) and 1 partner in the feed industry, the OQUALIM association, which brings together 57 feeding companies (over 71% of the feed production). The representativeness of the PSO partners is correct. This plan is open to all interested companies and new members join it every year. Thus, each partner provides analysis data from its own self-monitoring data, and annually receives an individual report with its results compared to regulatory limits and to the overall PSO results: a moderate analytical investment gives access to a rich database, allowing refining its risk analysis.

For the last ten years, the PSO collected data annually from about 2,000 to 3,500 samples of seeds, cake, oils, and providing between 40000 and 120000 analytical results per year (several contaminants checked in each sample). Plant protection products residues (pesticides) represent over 90% of the results. The other investigated contaminants are: metal and mineral trace elements (cadmium, lead, arsenic, mercury, fluorine), mycotoxins (aflatoxin B1 and total aflatoxins essentially), toxic organic (PAHs, dioxins and PCBs), microbiological contamination (salmonella

cakes), botanical impurities (seeds of *Datura spp*. in sunflower seeds), mineral oils or compounds likely to form during refining such as the esters of 3-MCPD and glycidol esters in the oil.

THE PSO RESULTS

PSO allows us to check that almost all oilseed products comply with the regulations. Regulatory limits on oilseeds are defined in different texts: maximum limits for pesticide residues (MRLs, EC Regulation No. 396/2005 and Regulations amending it), maximum levels in feed (Directive 2002/32 / EC and texts the modifying) maximum levels in foodstuffs for human consumption (Regulation No. 1881/2006 and other regulations amending it).

However, PSO provides the observation that oil refining is necessary to remove some pesticide residues from crude oils. These are mainly insecticide residues, coming from post-harvest treatments (pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin), applied on the empty storage cells or on cereal grains stored in the same sites, and being incidentally found on oilseeds by cross-contamination (Dauguet, 2007; Dauguet, 2009). These molecules are then removed at various steps of oil refining, and therefore marketed refined oils are pesticide free.

Through the PSO, the real effort provided by crushing plant in order to control the microbiological quality of the meal could be checked: today salmonella nearly disappeared in rapeseed and sunflower meals produced in France.

The PSO suggests that mycotoxins are a danger almost inexistent for oilseeds, considering the regulated toxins. Only aflatoxin can be detected occasionally in sunflower, but at very low levels, far below the regulatory threshold. But monitoring of aflatoxins should as a regulatory threshold for aflatoxins in human food has been fixed for oilseeds intended for direct human consumption (2 mg/kg for aflatoxin B1 and 4 mg/kg for total aflatoxins) without industrial processing (confectionery sunflower), with a much higher maximum levels in feed (20 mg/kg for aflatoxin B1). For other not yet regulated toxins such as toxins of Alternaria, EFSA recommends Member States to acquire data in food. Within PSO, analyzes of these toxins have been carried out and their presence can be seen occasionally on sunflower. However, toxicological studies are not sufficiently substantiated to date to conclude on the risk posed by the toxins of Alternaria.The trace metals are not a family at risk as oilseeds never exceed these regulatory limits. In the case of cadmium, the concentrations found in the sunflower seeds and meal can be sometimes close to the threshold in animal feed (1 mg/kg).

A contaminant was identified recently in the PSO: *Datura spp* seeds, which are botanical impurities that can be found in sunflower seed crops. This weed is toxic, since it contains tropane alkaloids, and the presence of *Datura spp*. seeds is regulated in the raw materials for animal feed (1000 mg/kg of whole seeds of *Datura*). Indeed, the alkaloids contained in these impurities will be transferred in the meal after the oil extraction process.

Organic toxic substances, such as polycyclic aromatic hydrocarbons (PAHs) and dioxins and PCBs, are specifically monitored in crude and refined oils. The levels measured for these substances show that these substances do not pose a problem in the French oilseed sector. Recently, the presence of esters of 3-MCPD and glycidol esters has been reported in refined vegetable oils, and in formulated food products containing vegetable fats (Zelinková, 2006). Palm oil is the oil with the highest infection rates likely related to the high temperatures used during deodorization of physical refining, while the seed oils are generally less prone to the formation of this contaminant (Kuhlmann , 2011). The few results collected via the PSO confirm the low contamination of refined rapeseed and sunflower oils.

Following a sunflower crude oil contamination from Ukraine by mineral oils (Lacoste, 2010), manufacturers have established since 2008 a systematic verification of import sunflower oil. The data collected within the PSO showed that the contamination in 2008 was an isolated case.

PSO, A TOOL FOR THE OILSEED FOOD CHAIN

The results of PSO therefore enable operators in the sector to carry out an analysis of health hazards in oilseed products. Thus, the subject of post-harvest insecticide residues appeared

important. This encouraged the operators to carry out specific actions to identify the sources of cross-contaminations of oilseeds by these pesticide residues in storage facilities. Surveys conducted in collaboration with companies have enabled the identification of these situations leading to cross-contamination (Dauguet, 2007; Dauguet, 2009), and recommendations were relayed by the federations. According to the latest PSO results, the contents of these pesticide residues tend to decline.

The PSO has also been involved to argue for re-examine the maximum residue level of pirimiphos-in oilseeds, taking into account the phenomenon of cross-contamination during storage. This data were studied by EFSA which issued an opinion (EFSA, 2011) in which European food safety authority says that an MRL of 0.5 mg/kg would be suitable for oilseeds (while the current MRL was 0.05 mg/kg).

As part of the review of the regulatory thresholds of cadmium in food, PSO partners have also mobilized to provide the public authorities with data so that future limits are not an obstacle to trade in oilseeds. This issue mainly concerns the sunflower, which accumulates cadmium in its seeds. Today, none sunflower sample exceeded the regulatory threshold for feed, but a lower threshold could be a problem. Finally, this regulation does not apply to oilseeds. PSO data were transmitted to the French authorities in the context of the European discussions on the revision of cadmium thresholds, with the agreement of all PSO's members. This also illustrates the value of reliable data to assist in setting realistic regulatory thresholds.

CONCLUSIONS

The PSO is now considered a sustainable action for the benefit of operators in the French oilseed sector, which has no equivalent in other countries. In 2016, new means of communication and information are available for PSO members, with a dedicated and protected website. This provides more responsiveness and flexibility: more ease for online data entry and data reading.

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THE EFFECTS OF VACUUM AND ATMOSPHERIC DEEP-FAT FRYING PROCESS ON TOTAL FRYING-USE TIME OF SUNFLOWER OIL AND ON FRENCH FRIES QUALITY

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ABSTRACT

Deep-fat frying, which is one of the oldest and popular food preparation methods, is a process of immersing food in hot oil at a high temperature. In this study a vacuum cooking equipment prototype which could work both atmospheric pressure and under vacuum was developed for deep-fat frying process. The effect of vacuum and atmospheric frying temperature and number of frying in the same sunflower oil on the quality of French fries and sunflower oil was evaluated. Potato pieces was fried in ratio 1:6 (potato:oil) at atmospheric pressure and under vacuum at 135 and 180°C, respectively, for 10 min in every frying interval for a total of 7 (atmospheric pressure) and 15 (under vacuum) times of frying in the same oil.

The free fatty acid content of the frying oil at atmospheric condition was determined to be excessively high compared to that of vacuum frying oil. TPM of oil at the atmospheric frying after the 3th frying rapidly reached to TPM content of the 15th vacuum frying oil. It was observed that peroxide value of the oil at atmospheric frying was higher than that of vacuum frying oil. Viscosity of the oil at atmospheric condition increased rapidly with an increase in exposure time compared to that of vacuum frying oil. The color values of vacuum and atmospheric fried French fries were not significantly different from each other. No significant changes in texture of French fries were determined with oil utilization time in the both of frying process.

Key Words : Deep-fat frying, vacuum frying, oil utilization time, sunflower oil, oxidation

EFFECT OF CURCUMIN NANOPARTICLES ON OXIDATIVE STABILITY OF SUNFLOWER OIL-IN-WATER EMULSIONS

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ABSTRACT

Curcumin is a natural polyphenolic compound that is obtained from the root of *Curcuma longa* Linn (turmeric). Oil oxidation is an undesirable series of chemical reactions involving oxygen that degrades the quality of oil. The aim of the present study was to develop a method to nano-particularize curcumin in order to increase its antioxidant efficiency against oxidation of sunflower oil. For this purpose, curcumin was dissolved in dichloromethane, injected in heating water (60 ºC) including tween 80 and then stirred. After characterization of the particle size and distribution of the fabricated curcumin nanoparticles, they were lyophilized. In formation of the oil phase of emulsion with nanocurcumin (ENC), nanocurcumin was added into oil-in-water system in which sunflower oil was used as the oil phase. Oxidation stability of oil-in-water emulsions including curcumin nanoparticles was measured by oxidation test reactor. As a result, 98 % of the particles were in mean diameter of 9-10 nm. The formed nanoparticles were characterized by scanning electron microscope, Fourier Transform Infrared Spectroscopy and thermogravimetric analysis. Unlike curcumin, nanocurcumin was found to be freely dispersible in the presence of the surfactant. The chemical structure of nanocurcumin was the same as that of curcumin, and no remarkable change was observed during nanoparticle preparation. Thermal degradation of the nanocurcumin was similar to that of curcumin. It was found that emulsion with nanocurcumin (ENC) was more effective than those with and without curcumin against oxidation of the sunflower oil, as revealed by the longer induction periods (IP) for ENC (1 hr 20 min) than those for emulsions with and without curcumin (60 min. and 53 min.) The results demonstrated that the water solubility and antioxidant activity of curcumin was markedly improved by particle size within the nano-range.

Key Words : Sunflower oil, nanocurcumin, nanotechnology, oxidative stability, molecular and thermal characterization.

DETERMINATION OF TEXTURAL, RHEOLOGICAL PROPERTIES AND SFC, SMP VALUES OF OLEOGELS PREPARED USING SUNFLOWER OIL

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ABSTRACT

In Recent years, food products which is designed to provide development for human health and researches is to improve such products have been intensively carried out all over the World. Oils Reduced Trans and saturated fatty acids levels have come firstly. To this end, oleogels, which have a spreadable elastic structure, by adding organic or polymer gelling agents (oleogelators) to oils, have been used. In our country, sunflower seed provides about 45% our total oil seed production and sunflower oil comes first in mostly consumed edible oils. Oil obtained from sunflower seed is rich in linoleic acid. Also recently, production of high oleic sunflower oil, by reducing linoleic acid content of sunflower oil, has been started. In this study, creating of oleogels formulations include sunflower and high oleic sunflower oil, have low amount of trans and saturated fatty acids, alternate to margarines and determination of textural, rheological, SFC and SMP values of this samples was purposed. For 6 samples (1 reference and 5 new formulations) Textural properties according to Ogutcu and Yilmaz, 2015; rheological properties according to Lupi et. Al., 2013 (with some modifications); SFC values according to AOCS Official Method Cd 16b-93:2009 and SMP values according to ISO 6321:2002 have been proceeding.

Key Words : oleogels, sunflower oil, rhelogical, SFC

ASSESSMENT OF SUNFLOWER OIL ADULTERATION

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ABSTRACT

The aim of this study is to estimate the sunflower oil adulteration with cheaper oils as raw cottonseed and canola oils. Sunflower oils, raw canola oil and raw cottonseed oil samples were supplied from market to investigate the possibility of adulteration. Main fatty acid composition of samples was detected by using GC-MS. L^*, a^* and b^* color values of the samples were also determined to detect the correlation with fatty acid composition. Increase of linolenic acid and palmitic acid percentages of sunflower oils samples was a good indicator for estimation of canola oil and palm oil addition, respectively. Some of the sunflower oil samples were suspected to be adulterated. L^*, a^* and b^* color values were also discussed on prediction of the possibility of adulteration. b^* values were detected to be higher in suspected oils. Detailed statistical modeling studies may be recommended for estimation of cheaper raw oil addition in sunflower oil.

Key words: Adulteration, Fatty acid, Sunflower oil, Color

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INTRODUCTION

Sunflower is botanically classified as *Helianthus annuus* and is an annual plant. It is thought to have been domesticated around 1000 B.C. by Native Americans. People in many regions began to process vegetable oils, from many oil sources for cooking purposes, before thousands of years ago. In 1860, Russia farmers cultivated sunflower. At that time, they became the world's largest producer of sunflower seeds. (Anonymous, 2010).

Sunflower oil is rich in linoleic acid and it is one of the most economically important vegetable oil source, especially in Turkey. Also, the widely usage of cake/meal of sunflower, obtained after oil extraction, as livestock increases the economic value of sunflower (İncekara, 1972, Dayal et al., 2011).

The tendency of adulteration on olive oil is higher in comparison to other oils, so there are many researches on detection of the adulteration in olive oil (Gegiou and Georgouli, 1983; Mannina et al., 1999; Blanch et al., 1998, 1999, 2000; Salivaras et al., 1992; Dionisi et al., 1995; Flor et al., 1993). Sunflower oil is cheaper than many other oils and sometimes used for adulteration of olive oil (Savaş, 1969) but, in recent years sunflower oil is also subjected to be adulterated with some other cheaper oils.

The fatty acid composition of sunflower oil may vary by the effect of many reasons. Republic of Turkey Ministry of Food, Agriculture and Livestock published a regulation on the 12ht April 2012, called as 'Bitki adı ile anılan yağlar tebliği'. The Ministry announced the ranges of fatty acid

composition of many vegetable oils (Anonymous, 2012). It is attractive that the lower and the higher limits of the ranges are at their maximum and in accordance with the literature.

Raw canola oil and raw cottonseed oil are cheaper than sunflower oil was subjected to this study for our suspect of their use in adulteration of sunflower oil. The ranges of fatty acid composition of sunflower, canola and cottonseed oil's, mentioned in regulation, are shown in Table 1.

Table 1. The ranges of fatty acid composition of sunflower, canola and cottonseed oil in Turkish Food Codex on vegetable oils (%)*

Fatty acids		Sunflower oil	Canola oil	Cottonseed oil
Caproic	(C6:0)	nd[a]	nd	nd
Caprylic	(C8:0)	nd	nd	nd
Capric	(C10:0)	nd	nd	nd
Lauric	(C12:0)	nd - 0.1	nd	nd – 0.2
Myristic	(C14:0)	nd - 1.0	nd - 0.2	0.6 - 1.0
Palmitic	(C16:0)	4.0 - 7.6	2.5 - 7.0	21.4 – 26.4
Palmitoleic	(C16:1)	nd - 0.3	nd - 0.6	nd – 1.2
Margaric	(C17:0)	nd - 0.2	nd - 0.3	nd - 0.1
Heptadecenoic	(C17:1)	nd - 0.1	nd - 0.3	nd - 0.1
Stearic	(C18:0)	2.1 - 6.5	0.8 - 3.0	2.1 – 3.3
Oleic	C18:1	14.0 - 71.8	51.0 - 70.0	14.7 - 21.7
Linoleic	C18:2	18.7 - 74.0	15.0 - 30.0	46.7 - 58.2
Linolenic	C18:3	nd - 0.5	5.0 - 14.0	nd - 0.4
Arachidic	C20:0	0.1 - 0.5	0.2 - 1.2	0.2 - 0.5
Eicosenoic	C20:1	nd - 0.3	0.1- 4.3	nd - 0.1
Behenic	C22:0	0.3 - 1.5	nd - 0.6	nd - 0.6
Docosahexaenoic	C22:1	nd - 0.3	nd - 2.0	nd - 0.3
Lignoceric	C24:0	nd - 0.5	nd - 0.3	nd - 0.1
Nervonic	C24:1	nd	nd – 0.4	nd

[a]: not detected (≤ % 0,05); *Anonymous, 2012.

The aim of this study is to estimate the sunflower oil adulteration with cheaper oils as raw cottonseed and canola oils by the aid of fatty acid composition.

MATERIALS AND METHODS

Thirtysix sunflower oil, one conola oil and one cottonseed oil samples were obtained from market from many regions of Turkey. Names of companies were hidden.

Color measurement:

Color measurements of the oil samples were carried out using a Minalto CR400 colorimeter. The instrument was standardized each time by a white (L=93.01, a=1.11, b=1.30) tile. The color values were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness) (Hunter, 1948). 20 ml oil samples were poured in a petric plate on a white tile for measuring the color values (Morello et al., 2004; Sikorska et al., 2007).

Determination of fatty acid composition:

Fatty acid composition was carried out by Agilent 6890 series GC system (Agilent Technologies, USA) fitted with a capillary column packed with 100% cyanopropyl methyl polysiloxane (Supelco SP-2380 model, 60 m × 250 μm × 0.2 μm i.d.; Bellefonte, PA, USA) and equipped with a flame ionization detector. Before injection, oil samples were converted to fatty acid methyl esters (FAMEs). 0.1 g of oil sample was weighed in a sample tube and dissolved in 10 mL hexane. Then 1 mL of 2 N potassium hydroxide in methanol was added and shaken for one minute before the centrifugation procedure. After centrifugation, the clear supernatant was transferred to a GC auto-sampler vials for injection. One μL FAMEs were injected into the GC-FID system using an auto-sampler with a split ratio of 100:1. The oven's initial temperature was set to 50°C for 2 mins and then increased at a rate of 4°C/min up to 240°C, where it was held for 10 min. Both the injector and the detector temperatures were set to 250°C. The flow rate of carrier gas (hydrogen) and make-up gas (nitrogen) were set to 1 mL/min^{-1} (AOCS, 1984). The data were recorded by using the Agilent ChemStation data processor. FAMEs peaks were identified by comparison with retention times of known standards (Sigma Chemical Co.) and quantification was determined as the percent area of each peak relative to the sum of all peak areas. All analyses were conducted in duplicate and results are provided as average values.

Statistical analysis:

Data were subjected to analysis of variance with mean separation by Duncan's multiple range tests. Differences were considered statistically significant at the $P < 0,05$ level. Statistical analysis was performed using SPSS 10.0 for Windows. The statistical results were evaluated according to Düzgüneş et al., 1987.

RESULTS

The detected *L**, *a** and *b** value ranges for 36 sunflower oil samples were 69,177-70,670, (-1.903) - (-4.233) and 7.597-16.060, respectively. *L** value of the samples were changed in a narrow range but the range for *a** and *b** were wide that reflects the sensitivity on them. *a** value of 30th sample were higher in comparison with other sunflower oil samples. And, the value of *a** was similar to values obtained for cottonseed and canola oils. *b** value was the lowest for 12th sunflower sample and was the highest for the 30th sunflower oil sample (Table 2).

10th and 26th sunflower oil samples were found to be higher in myristic acid content than the other sunflower oil samples as 1,529 % and 4,055 %, respectively. Myristic acid content of the samples doesn't give any confirmative idea on suspicion of adulteration of sunflower oils by the use of canola and cottonseed oil. Other fatty acid profile of these samples was belonging to fatty acid profile of sunflower oil. Especially the detection of high myristic acid content may cause a formation of doubt of adulteration with palm, coconut and babassu oil, but lauric acid was not detected in these samples which may be a parameter for removal of doubt (Table 2).

Table 2. Main fatty acid composition (%) and *L**, *a**, *b** values of oil samples

Samples	Myrstic acid	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	Behenic acid	L^*	a^*	b^*
1	0.045 e*	4.924 ö	31.523 ghıijk	62.173 ab	0 c	0.321 k	0.741 efghı	70.090 efgh	-2.720 l	10.613 l
2	0.059 e	6.782 klm	29.675 jklmno	61.371 abc	0 c	0.814 efghıij	1.037 cd	70.460 abcde	-2.757 l	10.790 jk
3	0 g	5.701 mnoö	32.482 fghıi	59.487 bcde	0 c	0.640 hıijk	0.954 cde	70.563 abc	-2.350 f	8.417 u
4	0 g	9.200 ı	28.039 oöp	59.398 bcdef	0 c	1.459 abc	1.535 ab	69.187 m	-2.417 g	8.947 sş
5	0 g	8.375 ıij	34.538 ef	56.430 fghı	0 c	0.334 k	0.380 jk	70.260 defg	-2.870 n	10.873 j

6	0 g	8.304 ıijk	30.959 ıijklm	57.496 efgh	0 c	1.255 bcde	1.628 a	69.693 jkl	-2.507 h	9.840 n
7	0.223 d	8.683 ıi	34.470 ef	55.405 hıi	0 c	0.417 jk	0.646 ghı	70.003 ghıi	-2.677 k	9.530 ö
8	0.044 e	6.368 lmno	29.976 ijklmno	62.506 ab	0 c	0.514 ijk	0.744 efghı	69.910 hıij	-2.940 o	11.193 i
9	0.022 efg	5.656 mnoö	34.957 ef	58.452 cdefg	0 c	0.405 jk	0.748 efghı	70.650 ab	-2.587 ij	9.137 pr
10	1.529 b	7.657 ijkl	40.863 d	48.427 mnoö	0 c	0.348 k	0.660 ghı	70.050 fghı	-2.720 l	9.763 no
11	0.056 e	5.351 oö	44.656 c	49.105 mno	0 c	0.568 ıijk	0.640 ghı	70.670 a	-2.150 c	7.653 v
12	0 g	8.487 ıij	33.855 fg	56.419 ghı	0 c	0.561 ıijk	0.644 ghı	70.527 abcd	-1.857 a	6.783 y
13	0 g	8.635 ıij	34.467 ef	55.406 hıi	0 c	0.524 ıijk	0.649 ghı	70.533 abcd	-2.530 hı	9.447 ö
14	0.038 ef	7.125 jkl	30.536 ijklmn	61.267 abcd	0 c	0.528 ıijk	0.704 fghı	70.403 cdef	-2.297 de	8.830 t
15	0 g	5.330 oö	29.133 klmno	64.480 a	0 c	0.648 ghıijk	0.657 ghı	69.827 ıijkl	-3.230 s	11.997 h
16	0 g	16.219 de	24.608 rs	57.652 efgh	0 c	0.941 defghı	0.730 fghı	70.430 abcdef	-1.903 a	7.597 v
17	0.040 e	13.417 fg	26.297 öpr	59.149 bcdefg	0 c	0.638 hıijk	0.610 hı	70.660 ab	-2.610 j	9.703 o
18	0.053 e	7.416 jklm	37.286 e	54.279 ıij	0 c	0.464 jk	0.632 ghı	69.887 hıijk	-2.407 fg	9.213 p
19	0 g	8.232 ıijk	31.736 ghıij	58.568 cdefg	0 c	0.548 ıijk	0.698 fghı	69.597 l	-3.010 ö	11.090 i
20	0 g	12.344 gh	30.751 ıijklmn	55.303 hıi	0 c	1.084 bcdefg	0.727 fghı	70.420 bcdef	-2.563 ıi	9.780 no
21	0 g	14.707 ef	28.657 mnoö	54.583 ıij	0 c	1.112 bcdef	0.732 fghı	70.163 efg	-2.720 l	10.037 m
22	0 g	16.465 d	33.662 fgh	47.653 noöp	0 c	1.265 bcd	0.740 efghı	69.773 ijkl	-3.643 ü	14.553 d
23	0 g	17.237 d	30.753 ıijklmn	50.470 klm	0 c	1.027 cdefgh	0.752 efghı	70.440 abcdef	-2.730 l	10.093 m
24	0 g	14.232 ef	31.222 hıijkl	52.481 jk	0 c	1.426 abc	0.682 fghı	70.513 abcd	-3.133 r	12.210 g
25	0 g	21.377 bc	23.522 s	52.640 ijk	0 c	1.345 bcd	0.810 defgh	69.660 kl	-2.013 b	8.257 ü
26	4.055 a	11.530 h	33.265 fghı	49.721 lmn	0 c	0.733 fghıijk	0.669 fghı	70.297 cdef	-2.843 m	10.573 l
27	0 g	22.614 b	28.394 noö	46.557 oöp	0 c	1.210 bcde	0.874 def	70.100 efgh	-2.940 o	10.737 k
28	0.054 e	5.678 mnoö	46.585 c	46.406 öp	0 c	0.549 ıijk	0.664 ghı	70.567 abc	-2.753 l	9.740 no
29	0 g	14.781 ef	33.259 fghı	50.478 klm	0 c	0.899 defghıi	0.709 fghı	70.550 abc	-2.347 ef	9.053 rs
30	0 g	6.622 klmn	58.456 b	26.634 r	5.251 b	2.058 a	1.169 bc	69.917 hıij	-4.233 y	16.060 c
31	0 g	14.427 ef	25.669 prs	58.113 defgh	0 c	1.110 bcdef	0.659 ghı	70.523 abcd	-2.413 g	9.767 no
32	0 g	22.327 b	28.820 lmno	46.459 oöp	0 c	1.123 bcdef	0.744 efghı	69.177 m	-3.403 t	12.967 e
33	0 g	21.618 bc	30.509 ijklmno	45.538 p	0 c	1.104 bcdef	0.816 defg	70.630 abc	-2.263 d	8.850 şt
34	0 g	20.440 c	23.464 s	49.369 mn	5.295 b	1.115 bcdef	0.339 l	70.573 abc	-3.333 ş	11.773 ı
35	0 g	11.518 h	28.450 mnoö	53.400 ij	5.400 b	0.734 efghıijk	0.357 k	70.233 defg	-3.470 u	12.530 f
36	0 g	9.199 ı	37.226 e	52.344jkl	0 c	0.578 ıijk	0.564 ıi	70.217 defg	-3.077 p	11.827 ı
Cottonseed	0.502 c	28.268 a	16.533s	53.392ij	0 c	0.818	0 m	66.903 n	-4.120	32.057

oil						efghıij			v	b
Canola oil	0 g	5.475 noö	68.251a	17.414s	7.557 a	1.547 ab	0 m	64.933 o	-5.893 z	53.973 a

*Means with different superscript letters differ significantly.

The samples, 1, 2, 3, 8, 9, 10, 11, 14, 15, 18, 28 and 30 were found to be in the range in palmitic acid as mentioned in the regulation (4,0 - 7,6 %) announced by the Ministry. The palmitic acid content was ranged between 8,232 % - 9,200 % for the samples 4, 5, 6, 7, 12, 13, 19 and 36. The samples, 17, 20, 21, 24, 26, 29, 31 and 35's palmitic acid content were detected to be from 11.530 % to 14.781 %. It was surprising to detect the palmitic acid content of the samples 16, 22, 23, 25, 27, 32, 33 and 34 in between 16,219 % and 22,327 %. This classification aroused the suspicion of adulteration of sunflower oil with cottonseed oil for the last group, due to higher amount of palmitic acid.

Oleic acid content of sample 30 was 58,456 % which was found to be higher than other sunflower oil samples. Oleic acid content of samples 10, 11 and 28 were from 40,863 % to 46,585 %. The lower range of oleic acid content was from 23.464 % to 26.297 % for the samples 16, 17, 25, 32 and 34. The range for oleic acid content in sunflower oil, canola oil and cottonseed oil was announced as 14,0 - 71,8 %, 51.0-70.0 % and 14,7 - 21,7 %, respectively. Estimation of adulteration by the aid of data on oleic acid content of sunflower oils looks too hard to evaluate the suspicion of addition of canola and cottonseed oil.

The lowest linoleic acid content of sample 30 was 26,634 %. The linoleic acid content of sunflower oil, canola oil and cottonseed oil in the regulation announced by the Ministry was ranged as 18,7 - 74,0 %, 15,0 - 30,0 % and 46,7 - 58,2 %, respectively. Linoleic acid content of sample 22, 27, 28, 32, 33 and 34 was from 45,538 to 49,369 %. The other sunflower oil samples were detected to have a linoleic acid range in between 48,427 and 64,480 %. In general, the linoleic acid content of sunflower oil and cottonseed oil is similar and it is not possible use the linoleic acid data as estimation parameter on adulteration of sunflower oil by cottonseed oil. But the addition of canola oil in sunflower oil may cause a little decrease in linoleic acid content of sunflower oil.

Linolenic acid may be a good estimation parameter for addition of canola oil in sunflower oil due to apparent increase in percentage. In the announce of the Ministry's regulation, the range for linolenic acid was from 0 to 0,5. The detection of linolenic acid in sample 30, 34 and 35 was from 5,251 to 5,400 % that increases the suspect of canola oil addition in sunflower oil. If linolenic acid content is taken in to account, the possibility of estimation of cotton seed oil addition in sunflower oil is very poor due to low ranges of linolenic acid content in cottonseed oil (0 - 0,4 %). Detection of linolenic acid in sunflower oil arouses the suspicion of adulteration of sunflower oil with canola oil due to a visible increasement. Linolenic acid was not detected in the sunflower oil samples except for the samples 30, 34 and 35.

Arachidic acid content of sample 30 was found as 2, 058 % and was higher than the other sunflower oil samples. It was the sample that was highly suspected to be adulterated with canola oil by the data on linolenic acid. The arachidic acid data was the second hint to strength this suspicion for the sample 30. The arachidic acid content of samples 4, 6, 20, 21, 22, 23, 24, 25, 27, 31, 32, 33 and 34 was from 1,084 to 1,459 %. These data on arachidic acid are higher than the announcement of the Ministry (0,1 - 0,5 %) for sunflower oil. According to these results, it may be offered to the Ministry to increase the limits of arachidic acid content up to 1,5 % in sunflower oil.

Behenic acid content of all tested samples was in the range that Ministry announced. Behenic acid is not a good parameter for estimation of adulteration of sunflower oil with the addition of canola and cottonseed oil.

DISCUSSION

Raw canola and cottonseed oils are cheaper than sunflower oil. By this study the suspense of adding these cheaper oils in sunflower oil was inspected by the evaluation of the possibility of the usage of fatty acid composition as a verification parameter.

Detection of linolenic acid in sunflower oil may strength the suspense of adding canola oil in suflower oil. Palmitic acid content increases by the addition of cottonseed into sunflower oil.

Sample 30 is a special example that may be announced to be the most suspected sunflower oil to be adulterated by the addition of canola oil, individually. Linolenic acid was detected in sample 30 and also the amount of oleic acid was relatively higher enough to strength the possibility of suspicion.

The palmitic acid and linolenic acid content of sample 34 and 35 were higher in comparison to other sunflower oil samples those shift the tendency of suspense on addition of both canola and cottonseed oils.

Especially *b** value was found to be the highest for the sample 30. b* was also high in samples 34 and 35. Those oils were thought to be most suspected ones among the other samples which could be adulterated.

Detailed statistical modeling studies may be recommended for estimation of cheaper raw oil addition in sunflower oil.

Detection of linolenic acid in sunflower oil may be a good indicator for addition of any other oil, especially the addition of canola oil. Palmitic acid may be a parameter for estimation of cottonseed addition but it is not a strong indicator individually.

b* value was found to be high in the sample which was the most suspicious to be adulterated with the addition of raw canola oil. *b** value of other suspected samples were also high in comparison to the other sunflower oil samples.

Additionally, revision of the arachidic acid range of sunflower oil in the related regulation may be referred to Ministry to increase the upper limit up to 1,5 %.

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EFFECT OF DIFFERENT STORAGE CONDITIONS ON QUALITY PROPERTIES OF RAW AND ROASTED SUNFLOWER KERNELS

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ABSTRACT

Quality of raw sunflower kernels changes due to the biochemical changes throughout the storage period. Thus, quality of sunflower kernels (SK) roasted after different storage periods may have different shelf lives. Relative humidity and temperature are the main factors affecting the quality of raw SK, whereas packaging material (O2 and water vapour barrier) properties and the gas composition in the package are the main factors affecting the quality of roasted sunflower kernels. The purpose of the present study was to explore the influences of storage conditions (room conditions-LOCAL and 10°C, Relative Humidity<%65 - MAM) on the quality of raw SK and to extend our knowledge concerning the changes in oxidative stability of roasted sunflower kernel processed at various storage periods (just after harvest, 8 and 12 months after harvest). Roasted products were packed in packaging material with high oxygen barrier (<0.008 ml/m²/day at 23°C) properties and kept at 10, 20 ve 30°C storage conditions under normal atmospheric conditions and nitrogen gas (>95%). Peroxide value, free fatty acids, contents of hexanal and vitamin E were determined at 2 months intervals during the storage for 12 months. Oxidative quality of the raw SK was similar when stored at cool (10°C, RH<65%) and local conditions (avg. 51 %RH, 19°C). SK roasted at 8th and 12th month storage periods lost quality more rapidly than the kernels roasted just after the harvest. Packaging under nitrogen gas rather than cold storage had the strongest influence in the prevention of oxidative changes of the roasted products.

Key Words : Sunflower kernel, oxidation, rancidity, peroxide value, free fatty acid, hexanal, vitamin E

QUALITY CHARACTERISTICS OF ROASTED SUNFLOWER SEEDS DURING STORAGE

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ABSTRACT

Sunflower seed being a part in both oil and dried nut industry is a highly nutritious oil seed. The oil and unsaturated fatty acids content plays an important role in determining the shelf life of seeds depending on lipid oxidation while increasing the nutritional value of the seeds. The oil seeds, like sunflower seed, which have high unsaturated fatty acid content, are exposed to oxidation during the long time storage that cause off-flavour, taste and rancidity. This may result in reduced overall sensory score when consumed. The packaging material properties (oxygen and water vapour permeability) have important effects on the shelf life of roasted dried nut products. The main objective of this study is to investigate the quality changes of sunflower seed in different packaging conditions and to optimize storage conditions for longer shelf life. In this study, the sunflower seeds obtained from different planting areas (Ankara, Kayseri, Bursa-İnegöl) were first roasted and then packaged under atmospheric and nitrogen gas conditions, and stored at 20°C for estimation of the shelf life. Peroxide value, free fatty acids, hexanal content, Vitamin E content and sensory quality properties were monitored during the shelf life study. As a result of this study; bio-chemical and sensory qualities of the stored products decreased within 2 months of storage period. It was observed that the product which is obtained from Bursa-İnegöl planting area packaged under nitrogen has the best chemical and sensory quality properties.

Key Words : Sunflower seed, oxidation, peroxide value, hexanal, Vitamin E, sensory

ACCEPTABILITY OF CHAPATI MADE WITH SUPPLEMENTATION OF SUNFLOWER (HELIANTHUS ANNUS L.) SEED MEAL

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ABSTRACT

The nutritional value of lab processed sunflower seed meal prepared from different sunflower seed cultivares i.e HSFM-848 and Morden as well as commercially processed cake (CPC) of sunflower seeds.*Chapati* was standardized in the lab by addition of sunflower seed meal and protein isolates (obtained from CPC) at 10,20 and 30% level. Nutritional evaluation revealed that lab processed seed meal of HSFM-848, Morden and CPC contained crude protein 42.51,51.44 and32.66%, fat 1.48, 0.86 and 0.55% crude fibre 4.16,2.48 and 14.56%, calcium 170.00,224.00 and 192.33mg/100g and iron 4.28, 25.12 and 22.13 mg/100g, respectively. Lab processed meals had significantly lower amount of polyphenols and higher amount of saponins as compared to the value of CPC. *in vitro* protein digestibility of lab processed seed meal as well as CPC was found to be improved after processing. *Chapatis* were found to be organoleptically acceptable. All the developed *chapatis* were rated in the range of like moderately to like very much category on Nine-Point Hedonic scale. Incorporation of sunflower seed meal and protein isolates at 10% level with wheat flour was the desirable level without altering the organoleptic traits and can be used for preparation of other traditional products like halwa suhali,cake & biscuits. These sunflower seed meal supplemented products if added in children diet can help in over coming protein energy malnutrition among infants & children in india.

Key Words : supplements, nutritional value, sunflower seed meal, acceptability, chapatti

SOME ANTINUTRIENTS AND IN VITRO PROTEIN DIGESTIBILITY OF HOME PROCESSED SUNFLOWER SEED MEAL

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ABSTRACT

Lab processed seed meals obtained from HSFM-848 was prepared by decorticating the seeds manually followed by grinding and extracting the oil with Hexane. Commercially Processed Meal contained average polyphenol content of 1675.00, 2001.66 and 1945.00 mg per 100g, saponin content of 1935.35, 1420.75 and 1112.25 mg per 100g respectively. Polyphenol content of CPC was significantly higher than those of Lab processed seed meals, whereas saponin content of CPC was significantly lower than those of lab processed seed meals Lab processed seed meals prepared from HS-1 and Morden cultivars and commercially processed cake contained on the average crude protein content (41.75, 50.68 and 31.75%), fat (1.45, 0.95 and 0.45%), crude fibre (3.75, 2.12 and 14.85%), respectively. But the polyphenol content of commercially processed cake (1936.00 mg/100g) was found to be significantly higher than those of both the lab processed seed meals. Saponin content of lab processed seed meal prepared from HSFM-848 variety (1922.68 mg/100 g) was significantly higher than that of Morden variety whereas the saponin content of commercially processed cake (1112.65 mg/100g) was found to be significantly lower than that of both the lab processed seed meals.It may be concluded from the study that the seed meal obtained from sunflower seeds after laboratory processing is nutritionally superior, in the preparation of various traditional food products. These food products if added in the diet will improve the nutritional quality of home diet .Processing has a significant effect on lowering antinutrients present in sunflower seeds which results in increase of *in vitro* digestibility of proteins and availability of minerals from sunflower seed meal.

Key Words : In vitro protein digestibility, home processed, sunflower seed, saponins, polyphenol

CONTENT AND OIL PRODUCTIVITY IN SUNFLOWER GENOTYPES PRODUCED IN CAMPO NOVO DO PARECIS – MT, BRAZIL

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ABSTRACT

This study aimed to evaluate genotypes of sunflower seeded second harvest in the year 2014 in Campus Campo Novo do Parecis, in the experimental field of the Instituto Federal de Educação Ciência e Tecnologia de Mato Grosso. The experimental design was a randomized block design with treatments 16 (16 genotypes) and four replications. The experimental plots consisted of four rows 6.5 m long with row spacing of 0.45 m, containing area of 11.7 m², totaling an area of 748 m². The population of 45000 plants per hectare is used. Data were subjected to analysis of variance and the Scott - Knott test at 5 % probability. The genotypes that stood out in relation to achenes productivity were the MG 360, AGUARÁ 06, MG 305, AGUARÁ 04, CF 101, SYN 045, GNZ NEON, HELIO 251 and SYN 3950HO. For the achenes oil content and productivity, the MG 360 genotype was the highest value and stands in relation to other genotypes.

Keywords: spectroscopy, *Helianthus annuus* L., lipids, oilseeds, achenes productivity.

INTRODUCTION

Among oilseeds grown in the world, the sunflower stands out among the main, both in production and in planted area. Sunflower (*Helianthus annuus* L.) is an annual cycle plant and its rapid growth characteristics, resistance to drought, cold and heat, more than most species of economic cultivation in Brazil and can be used for various purposes (Leite et al., 2005) as high quality oil extraction for human consumption or as raw material for biodiesel production, among others.

In general, sunflower seed it has about 45 to 65% oil in its composition (Grunvald et al., 2014A). Sunflower oil essentially consists of triglycerides (98 to 99%). It has a high content of unsaturated fatty acids (about 83%) and Vitamin E (alpha-tocopherol), but a reduced content of linolenic acid (≤ 0.2%). Sunflower oil is essentially rich in essential fatty acid (EFA) linoleic acid, about 60% that helps in reducing serum cholesterol and LDL. Thus contributing to the prevention of arteriosclerosis and cardiovascular problems (Turatti et al., 2002).

Changes in oleic are the result not only of the genotype, but also of climatic differences during their cultivation. Thus, among the various technologies developed for sunflower production, the appropriate choice of the genotype that has high yield and / or oil is important to ensure the success of the culture as a component of the production system (Porto et al., 2007).

In the region of Campo Novo do Parecis, sunflower is grown second summer harvest from February/March, due to the occurrence of rainfall conditions and temperatures suitable for its cultivation (Castro and Farias, 2005). However, despite being the main growing region in the country, little information is available on the agronomic characteristics of genotypes as content and productivity of oil, to facilitate the cultivation practices, reducing risk and increasing profitability.

MATERIAL AND METHODS

The work was carried out at the experimental fields and facilities of the Instituto Federal de Educação Ciência e Tecnologia de Mato Grosso - Campo Novo do Parecis in second-crop system in succession to soybeans in the agricultural year 2013/2014. The soil, according to the American System of Soil Classification (USDA, 1960) is the Typic Tropudox. The initial characterization of fertility, for the first layer of 0-0.20 m, presented the following values: pH ($CaCl_2$) = 5,7; MO = 26 g dm^{-3}; P (resina) = 5,9 mg dm^{-3}; K, Ca, Mg e H+Al = 1,5; 32; 11 e 40 $mmol_c$ dm^{-3}, respectively; with V = 54,8%.

Average temperatures occurred during the experimental period were: 30.3; 23.2 and 18.9 °C for maximum temperature, medium and minimum, respectively, and 570 mm rainfall, meeting the water demands required by sunflower between 500 and 700 mm distributed along its growing cycle (Castro and Farias, 2005).

The experimental design was a randomized complete block design with 16 treatments (genotypes) and four replications, as follows: ADV 5504, AGUARÁ 04, AGUARÁ 06, BRS 323, BRS G42, CF 101, GNZ NEON, HELIO 250, HELIO 251, HLA 2012, M734, MG 305, MG 360, PARAISO 20, SYN 045 and SYN 3950HO. The experimental plots consisted of 4 rows with 6.5 m long, with row spacing of 0.45 m, containing area of 11.7 m² (1.8 x 6.5 m). Only the two 5 meters central rows of each genotype were considered for data collection. The plotted area comprises 4.5 m².

The plot of the rows, was done on March 7, 2014, and the previous application of fertilizers was carried out with the aid of a sowing machine and was distributed at a depth of 0.10 m, 45 kg ha^{-1} Potassium Chloride + 267 kg ha^{-1} NPK 10-30-20, totalizing: 26.7 kg ha^{-1} N; 80 kg ha^{-1} P2O5; 80 kg ha^{-1} K_2O, according to the results of soil analysis and recommendation (EMBRAPA, 2004). Further, beside the row fertilization at 0.04 m deep, three seeds were placed in each hole, each 0.495 m, by manual planter.

The desiccation and the application of boron was performed on March 07, using trawl trailed sprayer with an application volume of 150 L ha^{-1} using glyphosate (648 g a.i. L^{-1}) at a dosage of 2 L ha^{-1} + Prometryn dosage 2 L ha^{-1} + mineral oil (0.5 L ha^{-1}) + boric acid dosage of 3 kg ha^{-1} (600 g ha^{-1} Boron).

Thinning was done 10 days after emergence (DAE) with a scissor, leaving only one plant per hole, reaching a population of 45,000 plants ha^{-1}.

The following coverage fertilizations were made: 1) 32 DAE with a dosage of 50 kg ha^{-1} N (urea); 2) foliar application of boron, with knapsack sprayer at 35 DAE using a dosage of 3 kg ha^{-1} (600 g ha^{-1} Boron), and 43 DAE with a dosage of 11 kg ha^{-1} (1.1 kg ha^{-1} of Boron). The source of Boron used was boric acid 150 L ha^{-1} according to the requirement of sunflower of 2 kg ha-1 B, Control of weed, pests and diseases have been carried out according to the recommendations of EMBRAPA (2004).

To avoid birds attacks, the plotted sections of the central rows were protected (stage R6) by using polypropylene based bags (30 x 30 cm) and fixed with clips.

The following agronomic characteristics were evaluated: productivity achenes (**PR**; kg ha-1), determined based on two central lines 5 meters, which is corrected for moisture condition of 11% (wet basis) obtained by reading the humidity value of the achenes; oil content (**OC**; %), predicted by near infrared spectroscopy (NIR) according to the methodology described by Grunvald et al. (2014b); and oil yield (**OY**, kg ha-1), calculated by multiplying the achenes oil content (%) and productivity achenes (kg ha-1) / 100.

The harvest of the capitulums was performed manually in the two of 5 meter central rows in R_9 with pruning shears aid. Later the capitulum inflorescence were the natural dryied, cleaned and weighed.

The results were submitted to analysis of variance followed by the average test Scott-Knott, both 5% probability, with the statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

All variables showed significant differences ($p < 0.05$) in the analysis of variance (Table 1). The data from the achenes productivity variables, oil content and oil yield are shown in Table 2. For the achenes productivity, genotypes were stood SYN 3950HO (2205.5 kg ha^{-1}) and HELIO 251 (2204.1 kg ha^{-1}), but not statistically different genotypes GNZ NEON, SYN 045, CF 101, AGUARÁ 04, MG 305, AGUARÁ 06 and MG 360, which had average productivity ranging from 1836.8 e 2132.5 kg ha^{-1}. However, it appears that even the lowest yields were found in genotype HLA 2012 e BRS G42, with average 40% lower than those observed in the most productive genotypes.

Table 1. ABSTRACT of the analysis of variance for the sunflower productivitiy parameters (Campo Novo do Parecis, MT, 2014).

Parameters[1]	F[2]	CV (%)[3]	GA[4]
PR (kg ha^{-1})	6.4*	12.4	1846.9
OC (%)	27744.6*	0.1	43.2
OY (kg ha^{-1})	6.8*	12.4	796.5

[1] PR = achenes productivity, OC = oil content, OY = oil yield; 2 * significant at 5%; 3 CV = Coefficient of variation; 4 GA = General average.

Values higher than this study were found by Backes et al. (2008) for HELIO 250 genotypes (1849.0 kg ha^{-1}), M734 (2052.0 kg ha^{-1}), AGUARÁ 04 (2252.0 kg ha^{-1}) and below to HELIO 251 (1882.0 kg ha^{-1}) in second-crop cultivation in northern Santa Catarina. Additionally, Vogt et al. (2010), in sunflower crop sown in November in northern Santa Catarina, reported higher yields for genotypes AGUARÁ 04 (1916.0 kg ha^{-1}) e M734 (1962.0 kg ha^{-1}) and means inferior to HELIO 250 (1450.0 kg ha^{-1}). Already Capone et al. (2012) evaluated the performance of cultivars in southern Tocantins state reported productivities 2834.1 e 2997.6 kg ha^{-1} para os genótipos HELIO 250 e HELIO 251, respectively. Poletine et al. (2013) reported an assay developed in the northwestern region of the state of Paraná, for genotypes BRS G42, SYN 3950HO, M734 and MG 305, with productivities 715.5 kg ha^{-1}, 1215.0 kg ha^{-1}, 1225.0 kg ha^{-1} e 1592.0 kg ha^{-1}, respectively. These variations in productivity reveal the importance of evaluation of genotypes in different producing regions to verify the feasibility of its use.

Analyzing the oil content of genotypes, the MG 360 genotype had the highest oil content, 47.8% (Table 2), differing from the other investigated genotypes.

Table 2. Mean values for productivity achenes (PR), oil content (OC) and oil yield (OY) from different sunflower genotypes.

Genotypes	PR (kg ha^{-1})	OC (%)	OY (kg ha^{-1})
ADV 5504	1446.9 c	47.1 b	681.5 b
AGUARÁ 04	2084.1 a	45.9 d	956.6 a
AGUARÁ 06	1859.5 a	41.6 n	773.7 b
BRS 323	1782.0 b	42.1 l	750.2 b
BRS G42	1425.9 c	42.0 m	598.9 b
CF 101	2104.4 a	45.1 f	949.1 a
GNZ NEON	2132.5 a	37.8 p	806.1 a

HELIO 250	1694.7 b	43.5 h	737.2 b
HELIO 251	2204.1 a	39.1 o	861.8 a
HLA 2012	1313.0 c	46.7 c	613.2 b
M734	1673.7 b	37.6 q	629.3 b
MG 305	1993.8 a	43.3 i	863.3 a
MG 360	1836.8 a	47.8 a	878.0 a
PARAISO 20	1685.3 b	43.2 j	728.5 b
SYN 045	2108.5 a	43.6 g	919.3 a
SYN 3950HO	2205.5 a	45.2 e	996.9 a

Different letters differ by Scott-Knott test at 5% probability.

However, the ADV 5504 genotypes (47.1%) and HLA 2012 (46.7%) also showed considerable oil content. In contrast, the M734 genotype was presented the lower oil content, with the representative average 37.6%. Some industries have been remunerating the sunflower producers from the oil content contained in achenes and no longer by simple mass achenes, since not always the genotype with the highest productivity of achenes per area results in greater productivity of oil in the same area , and the oil product of greater interest at the end of the manufacturing process and currently the main commercial sunflower crop product.

Watching the oil yield data, the averages of the genotypes SYN 3950HO, AGUARA 04 CF 101, SYN 045, MG 360, MG 305, HELIO 251 and GNZ NEON were the ones that showed the highest values (Table 2), getting between 806.1 (GNZ NEON) and 996.9 kg ha^{-1} (SYN 3950HO), but all belonging to the same statistical group. Thomas et al. (2012), testing different planting dates mentioned lower oil yield for AGUARA 04 genotypes, with 928.0 kg ha^{-1}, and HELIO 250, with 717.0 kg ha^{-1}. For the M734 genotype, the value was 864.0 kg ha^{-1}.

CONCLUSIONS

For achene productivity variable stood out the AGUARA 04 and 06 genotypes, CF 101, GNZ NEON, HELIO 251, MG 305 and 360 and 045 and SYN 3950HO, whose values were ranging between 1836.8 and 2205.5 kg ha^{-1}. However, for the oil content of the MG 360 was the one with the highest percentage, especially also in the group of genotypes with the highest oil productivity values, confirming its high potential for use in production systems Brazilian savannah.

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DETERMINATION OF FATTY ACID COMPOSITION FOR FRYING SUNFLOWER OIL USING GAS CHROMATOGRAPHY

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ABSTRACT

Frying of sunflower oil has been carried out for 7 running days at 175°C±2 in this study. The aim of this study is to determine fatty acid composition of sunflower oil under real domestic frying conditions. In the frying processes, potato has chosen for food and the processes have continued during seven days. The composition, trans fatty acid (TFA) amount and average molecular weight of sunflower oil have been determined by gas chromatography (GC) technique. This work focuses on finding changes in free fatty acid after repeated batch potato frying. Unsaturated fatty acid (UFA) contents of sunflower oil have been decreased and saturated fatty acids (SFA) have also been increased during frying process. When the frying times run on, the analysis of oil samples have showed that trans fatty acids such as elaidic and linoelaidic acids occur quite slowly. At the end of the repeated frying series, the elaidic acid (C18:1 trans) has been determined in oils for sunflower 1.5%. And also linoelaidic acid (C18:2 trans) has been detected 0.3%. At the end of the seventh day of frying, the relative contribution of oleic acid has been decreased.

Key words: Sunflower, frying, fatty acid composition

INTRODUCTION

Today, frying is one of the most popular methods for the preparation of food stuff, because the method is fast and relatively cheap and results in yellow brown products with a typical taste and smell, preferred by the consumer. The oil plays a critical role as a heat transfer and impregnation medium, and it is the crucial component of the frying process. For the quality of products being fried the quality of the frying medium is very important, because during frying the food takes up the oil becoming a significant part of the product. (Taha et al., 2014). Many factors affect the deterioration of a frying oil, such as the presence of unsaturated fatty acids, the oil temperature, oxygen absorption, the presence of metals, and the type of food (Arroyo et al. 1992). During frying, oil or fat is subjected to high temperatures in the presence of air and water from the food, thus producing a wide range of compounds resulting from thermal, oxidative, and hydrolytic reactions (Chatzilazarou et al. 2006, Dobarganes et al. 2013). As a result of the deterioration, the oil sustains some physical changes: the colour darkens, the viscosity increases, and smoke appears (Paul and Mittal, 1997).

The fatty acid composition of the frying oil is an important factor affecting fried food flavor and its stability; therefore, it should be low level of polyunsaturated fatty acid such as linoleic or linolenic acids and high level of oleic acid with moderate amounts of saturated fatty acid (Kiatsrichart et al., 2003, Mehta and Swinburn, 2001). Partial hydrogenation decreases polyunsaturated fatty acid but increases saturated fatty acid and trans-fatty acid to produce more stable frying oil. However, trans-fatty acids (TFA) adversely effects on cardiaovascular health (Rehab and Anany 2012). One approach to increasing the stability of unsaturated oil is partial hydrogenation (Li et al., 2008; Bysted et al., 2009), but hydrogenation also results in the formation

of SFA and trans fatty acids. Trans isomers of fatty acids have been reported to increase the ratio of low-density-lipoprotein (LDL) to high-density-lipoprotein cholesterol (HDL) in the plasma and increase the risk of coronary heart disease (CHD), and play a part in atherosclerosis development (Willett et al., 1993; Dalainas and Loannou, 2008). Low levels of trans fatty acids and saturated fatty acids that are basis of nutritional and diet physiological aspects also play important roles in selecting a frying oil. Since the fatty acid composition alone is not enough to explain the stability of oils, a variety of minor components, such as tocopherols, polyphenols, phospholipids, caretonoids and certain sterols are also beneficial to oil stability during frying (İnanç and Maskan 2012).

Oil and fats are one of the important components of human diet and ingredients of food industry. Oils and fats are preferred as carriers of fat soluble vitamins (A, D, E and K) and source of essential fatty acids and energy (Öğütcü et al., 2015). Vegetable oils are recognized as important compounds of our life. Sunflower is between the five biggest oilseeds in world production (Anwar et al., 2008). Sunflower oil contains a wide range of unsaturated fatty acids and is rich in essential fatty acids. Sunflower oil is considered nutritious due to high content of polyunsaturated fatty acids (PUFA), mainly linoleic acid (18:2). However, due to high PUFA, it is more susceptible to oxidative degradation leading to rancidity, off-flavors, and discoloration (Gordon 1991). And also sunflower oil is characterized by high content of tocopherols (up to 935 ppm) higher than those of other oils such as soybean and peanut. It is considered an oil of high stability due to its high content in natural antioxidants (Bramley et al., 2000; Shahidi, 2005). The nutritional aspects of edible oils associated with the presence of minor and major components play an important role in preventing diseases and improving health. It is important to formulate vegetable oil blends with special composition in order to enhance their stability and nutritional value (Frankel et al., 1994; Shiela et al., 2004).

The objective of the present study was to obtain the fatty acids combination of refined sunflower oil under normal frying conditions. Frying processes were done with potato repeating seven days.

MATERIALS AND METHODS

Frying Process

At the beginning of frying, the fryers have been stuffed with 2 L of fresh oil samples, and then oils have been heated to 175 ± 2 °C. The frying temperature has been controlled using a probe joined to the thermometer. An electrical domestic deep-fat fryer has been used for frying experiments. Prior to frying, potato slices have been dried on both sides on filter paper to remove any excess water. The frying process started 30 minutes after the temperature reached at 175 ± 2 °C. The frying time has been 6 minutes for potato slices. One frying has been done per day for seven consecutive days. All physical and chemical analyses of oils have been performed immediately after the frying. During frying process, fresh oil has not been added to frying pans.

Determination of Fatty Acids Composition

Gas chromatography has been used for the qualitative and quantitative determinations of the fatty acids reported in relative area percentages. Fatty acids have been methylated prior to analysis by gas chromatography. Analysis have been performed on Agilent 9C 6890N gas chromatograph (CA, USA) equipped with a DB-23 capillary column (60 m, 0.32 mm, 0.25μm film thickness) and a flame ionization detector. The oven temperature has been arranged from 160°C to 185°C at a rate of 7 minutes, later programmed from 195°C to 220°C for 3 minutes, finally kept 20 minutes at the last temperature. The injector and detector temperatures have been 230°C and 255°C, respectively. Nitrogen has been used as carrier gas at a flow rate of 1.0 ml/min. FAME has been identified by comparing their retention time with known commercial standard mixtures.

RESULTS AND DISCUSSION

The fatty acid compositions of sunflower oils are shown in Table 1. Composition of fatty acid in sunflower oil contained palmitic acid (7.1 %), stearic acid (4.3 %), oleic acid (19.0 %), linoleic acid (67.5 %) and linolenic acid (0.8 %). These results belong to before starting fryings. Linoleic acid (C18:2) is determinated the most abundant unsaturated fatty acid in the sunflower oil. Linolenic acid (18:3) is highly sensitive to oxidation because it contains three double bonds, while oleic acid (18:1) is less reactive as it contains only one double bond. At the end of the frying processes, composition of fatty acid in sunflower oil contained palmitic acid (11.4 %), stearic acid (4.9 %), oleic acid (9.1 %), linoleic acid (47.9 %) and linolenic acid (0.0 %). It is observed that there is a decrease in polyunsaturated fatty acids and resulting increase in the saturated acids content. When the frying times run on, the analysis of oil samples have showed that trans fatty acids such as elaidic and linoelaidic acids occur quite slowly. The elaidic acid ($C18:1_{trans}$) has been determined in oils for sunflower %1.5. And also linoelaidic acid ($C18:2_{trans}$) has been detected 0.3%. At the end of the seventh day of frying, the relative contribution of oleic acid has been decreased for sunflower oils.

Table 1 Changes in fatty acid composition (%) during frying processes.

Fatty Acids	Fresh oil	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
$C_{14:0}$	0.1982	0.2472	0.4000	0.5772	0.7579	0.9120	1.0700	1.2035
$C_{15:0}$	-	-	-	0.1859	0.3580	0.4727	0.5674	0.6498
$C_{15:1\ cis}$	0.2683	0.2037	0.1229	0.0680	0.0391	0.0253	0.0157	0.0102
$C_{16:0}$	7.0560	7.4007	8.0065	8.4157	9.2316	10.0710	10.8954	11.3895
$C_{16:1\ cis}$	0.1731	0.1710	0.1698	0.1687	0.1654	0.1619	0.1559	0.1463
$C_{16:1\ trans}$	-	-	-	-	-	-	-	-
$C_{17:0}$	-	-	-	-	-	-	-	-
$C_{17:1\ cis}$	-	-	-	-	-	-	-	-
$C_{18:0}$	4.3061	4.4458	4.5834	4.7435	4.8403	4.9146	4.9414	4.9502
$C_{18:1\ cis}$	18.9617	18.1325	17.2031	16.3104	15.1967	13.5689	11.2314	9.1256
$C_{18:1.\ trans}$	-	-	0.139	0.4793	0.7193	0.9486	1.2546	1.4876
$C_{18:2\ cis}$	67.5091	63.1032	59.9364	56.0213	53.4558	51.0132	49.0135	47.9356
$C_{18:2\ trans}$	-	0.0601	0.1147	0.1625	0.2053	0.2421	0.2748	0.3059
$C_{18:3\ cis}$	0.7778	0.5364	0.4915	0.3221	0.2287	0.2032	0.0913	-
$C_{18:3\ trans}$	-	-	-	-	-	-	-	-
$C_{20:0}$	0.2939	0.3192	0.3605	0.3989	0.4408	0.4854	0.5073	0.5231
$C_{20:1\ cis}$	0.1552	0.1187	0.0983	0.0812	0.0706	0.0567	0.0364	0.0286
$C_{20:1\ trans}$	-	-	-	-	-	-	-	-
$C_{20:2}$	-	0.0102	0.0243	0.0411	0.0618	0.0825	0.1026	0.1168
$C_{20:3}$	-	-	0.0306	0.0052	-	-	-	-
$C_{20:5}$	0.062	0.0245	0.0056	-	-	-	-	-
$C_{22:0}$	0.6325	0.6726	0.7094	0.7532	0.7831	0.8029	0.8203	0.8316
$C_{22:1}$	0.0153	0.0102	0.0044	-	-	-	-	-
$C_{23:0}$	0.0447	0.0635	0.0976	0.1368	0.1732	0.2123	0.2419	0.2604
$C_{24:0}$	-	0.1201	0.2032	0.2713	0.3404	0.3941	0.4402	0.4657
$C_{24:1}$	-	0.0223	0.0445	0.0614	0.0727	0.0802	0.0889	0.0901

Poor frying stability in sunflower oil comes primarily from the high level of linoleic acid. Therefore, sunflower oil must also be hydrogenated to reduce its linoleic acid content to 35% or lower for industrial frying. On the other hand, fatty acid compositions do not fully explain frying stability of oils. For understanding of the frying stability of oil, there are so many parameters. Stability of oil indicates that the oil must be low in free fatty acids, peroxide value, conjugated

dienes, anisidine value, monoacylglycerols, diacylglycerols, and trace impurities, such as iron, phosphorus, calcium, and magnesium. All of these quality parameters have specific significance in influencing the performance of the frying oil.

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BIOPELLET PRODUCTION FROM WASTE MATERIALS OF THE SUNFLOWER IS A MAJOR INDUSTRIAL PLANT

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ABSTRACT

Sunflower is the most important industrial plant with oil content and consumption percent in Turkey. The highest production of sunflower with 44% is made in Thrace and a large content of waste materials (core-shell, sunflower bat etc.) are obtained after harvest and processing. These materials have alternative assessment opportunities. Untreated agricultural waste is generally used for heating directly. However, this method is not economical, efficient and suitable for environmental point. Harmful gases such as CO2 release during the combustion process occur. These waste materials leave to the field and return to the land again because of the difficulties and the lack of economic benefits with usage of heating material. However, it is possible that the waste materials can be converted into heating material, biopellet, is not harmful and has higher energy value. Biopellet is important heating material for farmer and sunflower oil industry. Farmers have a large amount of waste after sunflower harvest. Besides, high content of core-shell and solid material also get to stay in oil factories and cooperatives. Sunflower oil industry only annually produces 800 000 tons of solid waste in Turkey as a byproduct. Failure in evaluation of sunflower waste materials is too big to ignore is a serious economical loss. There are various studies about converting the sunflower waste materials after harvest and/or oil extraction. All of them say that biopellet production is valuable method for both environmental and economical. At the same time, the waste materials used as a heating material directly but inefficient combustion and excess content of volatiles were determined. All for these reason, biopellet is environmental friendly waste is a great need to improve fuel production. Although the ban, a significant amount of agricultural waste are burned in the field or using as fuel in homes in our country for each year. However, biopellet is a modern technic for heating offers integrated solutions for sustainable development in developed and industrial countries. Besides, it also serves the purpose of preventing climate change, erosion and efficiency, ecosystem health and loss of biodiversity. So, biopellet production is an ecological solution.

Key Words : Biopellet, core-shell, sunflower bat, sunflower waste.

FACTORS AFFECTING THE NUTRIENT COMPOSITION OF SUNFLOWER MEAL

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ABSTRACT

Sunflower (*Helianthus annus L.*) is a high oil-yielding seed crop cultivated worldwide that adapts very well to a wide range of climates. Sunflower seed meal is a by-product of the oil extraction of sunflowers and it is produced in large quantities. Sunflower meal (SFM) is mainly used as feed source that offer cheap, eco-friendly substrates for the animal nutrition. The meal is initially used as a protein complement in ruminant diets, and also monogastric animal rations in appropriate amounts. The chemical compositions of SFM have been extensively evaluated and it has been found that the chemical composition of SFM is varied greatly. The mean moisture and dry matter contents of SFM were reported as 9.0 % and 91.0 %, respectively. SFM is composed basically on lignocellulosic fiber and proteins. The content of crude protein in SFM ranges from 23.0 to 42.0 % and the crude fiber level varies between 13.0 % and 35.0 % depending on the extent of dehulling. The concentration of ether exracts in SFM varies from 0.50 to 13.0 % depending on the extraction process. The large variation of ether exract level was mainly related to the different extraction process. The differences in production methods, such as heating temperature, pressure and time during the process might lead to the changes in ether exract values. The different production techniques also caused the variation of the other chemical components of SFM. The content of phenolic compounds such as chlorogenic acid and caffeic acid in SFM ranges from 3 to 4 %. The average ash composition of sunflower meal was reported to be 6. 0 %. In conclusion, the processing techniques is one of the major factor affects the nutritional composition of SBM. Processing techniques are initially effective in the levels of ether exracts, the crude fiber levels and other nutrients therefrom. The variations of nutrient composition in SFM might result from dehulling process too. SFM composition can vary somewhat according to extrinsic factors such as genetic, seed varieties, climate and soil conditions. In addition, the chemical concentration of SFM is also affected in each plant and collecting typical samples in person and the analysis method used.

Key Words : Crude protein, crude fiber, nutrient composition, processing techniques, sunflower meal

EFFECT OF HIGH OLEIC SUNFLOWER OIL INCLUDING OLEOGEL ON THE TEXTURAL AND SENSORY PROPERTIES OF CAKE

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ABSTRACT

The existence of the relation between health and diet has motived people to consume food products with lower adverse health effects. As known consumption of excessive saturated fatty acid increases the risk of cardiovascular disease. Therefore, decreasing saturated fatty acid content of the food materials without damaging the quality of the food products is important issue in the food industry. When considering the importance of fats in the quality of the products, liquid oils are structured to transform them to solid fats. Oleogelation is one of the way which has been recently used for this aim. In the present study, probable usage of oleogels prepared from high oleic sunflower oil (HOSO) in the formulation of cake was investigated. For this aim three different oleogel formulations were studied: (i). 50 % cottonseed oil (CSO) + 25 % shortening + 25 % HOSO, (ii). 50 % HOSO + 50 % CSO and (iii) it is the same with second formulation however, this oil blend was oleogelled with dehydrated wax. Textural and sensorial properties of oleogel including cakes and control sample were investigated. Hardness, chewiness and gumminess values of the cakes prepared by oleogels were found to be higher than those of control sample. According to sensory analyses, the sample prepared from third formulation had the highest overall acceptability value. Wax type used in the formulation as well as oil types significantly affected textural and sensory properties of cakes. The findings of the present study highlighted that oleogels rich in unsaturated fatty acid content could be used in the cake formulation instead of shortening rich in saturated fatty acids.

Key Words : Oleogel, cake, high oleic sunflower oil, texture, sensory

CROP PRODUCTION AND MANAGEMENT

USE OF POLYMER HYDROGEL IN SOIL MOISTURE CONSERVATION FOR SUNFLOWER CULTIVATION IN RAINFED SITUATIONS OF NORTHERN KARNATAKA, INDIA: A CASE STUDY

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ABSTRACT

Impacts of climate change on food security are global and local. The agriculture production is being affected by change in mean rainfall and temperature. The inter-annual and inter-seasonal variation in rainfall pattern interms of distribution and quantum are projected change drastically in larger part of drylands in India. Sunflower being a drought tolerant crop, its productivity greatly affected by rainfall. With this background an experiment was mounted in deep black soils at University of Agricultural Sciences, Raichur for two consecutive seasons of kharif 2012 and 2013 in order to identify the appropriate moisture conservation techniques that would help to cope both under high and low rainfall situations. In comparison to first year of experimentation (2012), head diameter (cm), 100 seed weight (g) and seed yield (Kg/ha) recorded incremental change during second year due to more number of rainy days coupled with high rainfall. However, there was no drastic reduction in seed yield of sunflower due to use of agronomically sound moisture conservation techniques. Oil content (%) of sunflower seed was more under stress conditions (2012) than under non-stress conditions (2013) as crop received 469 mm in 2012, to 730 mm rainfall in 2013 between July to October which was most effective period for sunflower. Despite this large variation between rainfall, pooled data indicates that application of hydrogel @ 2.5 kg/ha along with Vermicompost @1 t/ha gave significantly highest sunflower seed yield (1815 kg/ha) as compared to sole application of hydrogel (@ 2.5 kg/ha (1642 kg/ha) or Vermicompost (@1 t/ha (1532 kg/ha) and this is on par with the application of hydrogel @ 2.5 kg/ha along with Gypsum @100 kg/ha (1740 kg/ha) and this was highly correlated with the soil moisture availability at different growth stages. However, the economics shows highest BC ratio was recorded with 2% $CaCl_2$ along with Gouch treatment (4.12) as compared to other treatments. There was strong correlation between head diameter against seed yield (r=0.9), gross returns (r=0.9), net return (r=0.85) and 100 seed weight (0.85). The 100 seed weight had significant correlation with gross and net return. Whereas, seed yield significantly correlated against net return which signifies increase in seed yield of sunflower increases the net profit margin for sunflower farmer.

Key Words: Hydrogel, Moisture Conservation, Sunflower, Yield, Oil percent

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed that originally belonged to subtropical and temperate zones (Demir et. al., 2010) crop and it is widely adaptable and more drought tolerant than most other grain crops (Usman et.al., 1994). It is well adjusted to soil that has high water-holding capacity but is easily adapted to a range of soil conditions (Ahmad et. al.,

1992). Water and nutrients play an important role in sunflower growth and development. It was introduced to India as an oil seed crop during seventies. It gained importance and popularity as a commercial oilseed crop of India under rainfed conditions. This is due to its suitability to many agro ecological regions, short duration, good quality oil and market price. This crop is mainly cultivated in rainy season and post rainy seasons of vertisols but can be grown in any season of the year since this crop is considered as day neutral plant because of its low photoperiod sensitivity. The rainfed sunflower crop witnesses wide fluctuation in productivity due to erratic rainfall distribution and less availability of nutrients. Karnataka is the leading sunflower producing state in the country and contributes nearly 52 per cent of the total area and 40 per cent of the total production in the country.

In India during 1993-94 sunflower occupied an area of 26.7 lakh ha. 13.5 lakh tons production and drastically decreased to 5.5 lakh ha with a production of 4.15 lakh tons during 2014-15 (Anonymous, 2015). Though the crop has gained important place among farmers, the productivity of sunflower is very low. The low productivity is mainly due to the crop growing under rainfed conditions on poor fertility soils with non-availability of cultivars under moisture and nutrient stress situations. To increase production, it is important for decreasing of effects of factors reduced seed yield utilizing from higher production techniques in addition to develop higher yielding cultivars (Kaya et al., 2012 and Skoric, 2012). This crop is often considered as a soil nutrient depleting crop, which puts heavy demands on soil and applied nutrients (Thavaprakash et.al., 2002). Due to its high uptake of nutrients sunflower responds very well to applied nutrients. Application of nutrients increased the seed yield of sunflower by 50 per cent (Chorey and Thosar, 1997). The rainfed sunflower experiences erratic and undependable rainfall, excess and deficient of moisture within the same season. The critical analysis of production factors to increase the productivity of sunflower under different agro ecological situations of India revealed that moisture and nutrient are the key inputs to realize higher and sustainable production of sunflower cultivars under rainfed conditions. The farmers of this region are resource poor and use very little fertilizer. Identification of best agronomic practices suited to moisture and nutrient stress conditions are vital to the farmers of this region. Hence, the hydrogel along with FYM, vermicompost and gypsum were evaluated under the influence of moisture and nutrient management practices in sunflower.

MATERIAL AND METHOD

Water is the most important factor limiting crop productivity at different growth stages of crop growth and development. Water stress is likely the most important factor that adversely affects plant growth and development. In this direction, a field experiment was conducted during the kharif seasons of 2012 and 2013 at Main Agriculture Research Station (MARS), University of Agricultural Sciences, Raichur, Karnataka, India. Geographically the experiment place is located in North Eastern Dry Zone (Zone-2) of Karnataka State, which falls between 16^{o} 15' N latitude and 77^{o} 20' E longitude with an altitude of 389 meters above mean sea level. The soil of the experimental site belongs to medium black with clay loam texture. The soil of the experimental field in both years was sandy clay to clay with pH ranging from 8.0 to 8.7, organic carbon from 0.37 to 0.61%, available phosphorus 3 to 21 kg/ha and available potassium 293-588 kg/ha. The soil has field capacity of 16.6% to 28.2% and permanent wilting point of 7.47% to 13.1% with available water holding capacity of 10.24 to 16.96 cms at 75cm depth. The potential sunflower hybrid, RSFH-130 was used with eight treatments viz., 2% $CaCl_2$ + Gouch treatment, Vermicompost seed line application (1 t/ha), FYM seed line application (2.5 t/ha), Gypsum (100 kg/ha), Hydrogel (2.5 kg/ha) seed line application, Vermicompost (1 t/ha) + Hydrogel (2.5 kg/ha), Gypsum + Hydrogel (2.5 kg/ha) and control was 100 % NPK. The experiment was laid out in completely randomized block design with three replications. The observations were recorded on growth, yield attributes and yield. Economics were computed based on the prevailing market price. The oil

content of sunflower seed was estimated by using Nuclear Magnetic Resonance (NMR) method (Model: Oxford MQA 6005). Finally the results were analyzed with suitable statistical analysis.

RESULTS AND DISCUSSION

Rainfall and Number of Rainy Days during Growth Period

Rainfall between July and October, the most effective rainfall period for sunflower growth varied from 361 mm in 2012, to 565.5 mm in 2013 against the past 30 years average of 730 mm, which were received in 25 and 34 days, respectively. However, entire year recorded hardly 468.9 mm in 31 days during 2012 and 729.9 mm in 48 days during 2013 (Table 1).

Table 1: Rainfall (Mm) And No. Of Rainy Days Observed During Sunflower Growth Perıod

Sl. No.	Month	2012		2013	
		Rainfall (mm)	No. of Rainy Days	Rainfall (mm)	No. of Rainy Days
1	July	103.6	06	116.4	10
2	August	50.0	03	102.2	06
3	September	126.0	09	250.0	13
4	October	81.4	07	96.9	05
Total		361.0	25	565.5	34
Total (Entire Year)		468.9	31	729.9	48

The data revealed that during rainy seasons of 2012 and 2013, application of hydrogel @ 2.5 kg/ha along with Vermicompost @1 t/ha gave significantly highest head diameter (18.3 cm and 19.9 cm, respectively) as compared to control i.e. 100% RDF (15.6 cm and 17.5 cm, respectively), sole application of hydrogel (@ 2.5 kg/ha (17.8 cm and 19.3 cm, respectively) or Vermicompost (@1 t/ha (16.9 cm and 19.3 cm, respectively) and this is on par with the application of hydrogel @ 2.5 kg/ha along with Gypsum @100 kg/ha (17.7 cm and 19.4 cm, respectively). Similar trend was also observed with 100 seed weight and oil content (Table 2). The results emphasizes that the combined effect of both hydrogel for moisture conservation and vermicompost or gypsum for nutrient supply have gave significantly higher values of, head diameter, oil content and 100 seed weight than the individual effect and as compared to 100% RDF. The moisture conservation effect and rainfall observed during the crop growth indicates that due to hydrogel use, the effect of deficit in rainfall during 2012 is reduced on the growth performance of the crop. The benefits of soil moisture conservation and nutrient supply in sunflower during post rainy season is also reported by Reddy et.al., 2003, Bakery et.al., 2009 and Aravinda Kumar et.al., 2010.

Seed and Oil Content

The two years rainy season pooled data (Table 2) revealed that the seed yield and oil content of sunflower was influenced significantly by integrated moisture conservation and nutrient source. Application of hydrogel @ 2.5 kg/ha along with Vermicompost @1 t/ha resulted in higher seed yield (1815 kg/ha) which was increased to the extent of 29% (1281 kg/ha) and 20% (1452 kg/ha) than 100% RDF (control) and FYM seed line application (2.5 t/ha), respectively. The various

moisture conservation (2% $CaCl_2$ and hydrogel) and nutrient sources (RDF, FYM, vermicompost and gypsum) did not vary much in oil content. However, hydrogel @ 2.5 kg/ha along with Vermicompost @1 t/ha recorded higher oil content (36.8%) than 2% $CaCl_2$ + gouch treatment (36.1%). The oil content values during 2012 are higher than the 2013, this is because of rainfall occurred during flowering in 2013 causes pollen wash thereby reduction in oil content was noticed. Irrespective of the treatments, the seed yields recorded during 2012 are lower as compared to 2013. This is due to the variation in both rainfall amount (361mm and 565mm, respectively) and rainy days (25 and 34 days, respectively). However, between the years the quantity of sunflower yield reduction in 100% RDF (control plot) was higher (15%) than the 3% recorded with 2% $CaCl_2$ + gouch treatment, 5.5% with sole application of hydrogel (2.5 kg/ha) and 8% with hydrogel @ 2.5 kg/ha along with Vermicompost @1 t/ha. This is mainly due to the moisture conservation by both $CaCl_2$ and hydrogel and its utilization by the crop during requirement. Similar results have been observed by Megur et.al., 1993, Devidayal and Agarwal 1998.

The rainfed sunflower is sometimes more hungry than the thirsty which adds to its low productivity. There is strong interaction between nutrient source and moisture availability for crop yield. Application of nutrients facilitates root growth, which can extract soil moisture from deeper layers and moisture conservation practices ensured the better availability of moisture to the plants. Furthermore, supply of nutrients facilitates early development of canopy that covers the soil and intercepts more solar radiation and thereby reduces the evaporation component of the evapotranspiration. The moisture conservation effect and nutrient source for sunflower were found not significant for oil content.

Economics

The individual years and mean of two years data pertaining to the gross returns, cost of cultivation, net returns and B C ratio are given in Table 3. Pooled data reveals that the maximum gross returns of Rs.62586/ha was recorded under hydrogel @ 2.5 kg/ha along with Vermicompost @1 t/ha where as lowest gross returns (Rs.44147/ha) was recorded under control (100% RDF). Among moisture conservation options, maximum gross returns (Rs.56631/ha) was recorded under hydrogel (2.5 kg/ha) as compared to 2% $CaCl_2$ + gouch treatment (Rs.52624/ha). The moisture conservation with nutrient sources significantly recorded higher net returns (Rs.42354/ha) than rest of the treatments, while 100% RDF (control) recorded lowest net returns (Rs.31657/ha). The highest BC ratio was recorded with 2% $CaCl_2$ along with Gouch treatment (4.12) as compared to other treatments. Similar results were observed by Kazen et.al, 2013 and Singh et.al, 2005. The higher gross returns, net returns and B C ratio of moisture conservation and nutrient sources might be due to higher seed yield coupled with higher market price during both the years.

CONCLUSION

The results of two years experiment clearly indicated that adoption of moisture conservation techniques like use of polymer hydrogel and treating seeds with 2% $CaCl_2$ along with Gouch and supply of nutrients through organics like FYM, vermicompost, gypsum to nutrient exhaustive crops like sunflower are proved to be best one in Vertisols of Semi Arid Tropics of Karnataka for obtaining higher yield and monetary returns besides having higher production sustainability of sunflower.

Table 2: Effect af Moisture Conservatıon and Nutrient Source on the Performance of Sunflower Yield and Yield Parameters

Treatment Details	2012				2013				Pooled data			
	Head Diameter (cm)	100 Seed Wt (g)	Oil Content (%)	Seed Yield (kg / ha)	Head Diameter (cm)	100 Seed Wt (g)	Oil Content (%)	Seed Yield (kg / ha)	Head Diameter (cm)	100 Seed Wt (g)	Oil Content (%)	Seed Yield (kg / ha)
Control (100 % NPK)	15.6	2.63	41.1	1175	17.5	3.37	31.7	1387	16.5	3.00	36.4	1281
2% $CaCl_2$ + Gouch treatment	17.4	3.13	39.9	1502	18.2	3.53	32.2	1550	17.8	3.33	36.1	1526
Vermicompost seed line application (1 t/ha)	16.9	3.09	41.1	1389	19.3	3.50	31.5	1674	18.1	3.30	36.3	1532
FYM seed line application (2.5 t/ha)	16.7	3.04	40.9	1315	19.1	3.47	32.6	1589	17.9	3.26	36.7	1452
Gypsum (100 kg/ha)	17.5	3.00	40.4	1366	19.1	3.43	32.8	1555	18.3	3.22	36.6	1461
Hydrogel (2.5 kg/ha) seed line application	17.8	3.03	40.6	1595	19.3	3.53	32.2	1689	18.6	3.28	36.4	1642
Vermicompost (1 t/ha) + Hydrogel (2.5 kg/ha)	18.3	3.14	40.3	1740	19.9	3.57	33.0	1890	19.1	3.36	36.8	1815
Gypsum + Hydrogel (2.5 kg/ha)	17.7	3.05	39.6	1646	19.4	3.57	32.9	1835	18.6	3.31	36.2	1740
SEm $\pm$	0.71	0.21	0.76	99.28	0.65	0.12	0.81	113.31	0.38	0.07	0.61	72.34
CD at 5%	2.16	0.62	2.33	301.12	1.97	0.38	2.44	343.69	1.14	0.23	1.86	219.43
CV %	7.17	11.08	3.28	11.73	5.92	6.17	4.31	11.92	3.60	3.96	2.92	8.05

Table 3: Effect of Moisture Conservation and Nutrient Source on the Performance of Sunflower Economics

Treatment Details	2012				2013				Pooled data			
	Gross Returns (Rs/ha)	Cost of Cultivation (Rs/ha)	Net Returns (Rs/ha)	B C Ratio	Gross Returns (Rs/ha)	Cost of Cultivation (Rs/ha)	Net Returns (Rs/ha)	B C Ratio	Gross Returns (Rs/ha)	Cost of Cultivation (Rs/ha)	Net Returns (Rs/ha)	B C Ratio
Control (100 % NPK)	41137	12265	28872	3.35	47158	12715	34443	3.71	44147	12490	31657	3.53
2% $CaCl_2$ + Gouch treatment	52570	12565	40005	4.18	52683	13015	39668	4.05	52624	12790	39834	4.12
Vermicompost seed line application (1 t/ha)	48603	16265	32338	2.99	56924	16715	40209	3.41	52765	16490	36275	3.20
FYM seed line application (2.5 t/ha)	46037	14765	31272	3.12	54032	15215	38817	3.55	50037	14990	35047	3.33
Gypsum (100 kg/ha)	47810	12915	34895	3.70	52876	13365	39511	3.96	50340	13140	37200	3.83
Hydrogel (2.5 kg/ha) seed line application	55825	16765	39060	3.33	57438	17215	40223	3.34	56631	16990	39641	3.33
Vermicompost (1 t/ha) + Hydrogel (2.5 kg/ha)	60923	20765	40158	2.93	64248	21215	43033	3.03	62586	20990	41596	2.98
Gypsum + Hydrogel (2.5 kg/ha)	57610	17415	40195	3.31	62385	17865	44520	3.49	59994	17640	42354	3.40
SEm $\pm$	3473	--	3473	0.24	3852	--	3852	0.26	2491	--	2491	0.18
CD at 5%	10535	--	10534	0.73	11685	--	11685	0.79	7555	--	7555	0.55
CV %	11.72	--	16.78	12.32	11.92	--	16.66	12.71	8.04	--	11.37	9.13

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EFFECTS OF MICRONUTRIENTS ON OIL QUALITY OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Abstract

Oils, proteins and carbohydrates are essential nutrients for human consumption. Oils have an important role as energy source. A large amount of edible oils is obtained from plants. Sunflower oil, which has high nutritional value with higher unsaturated oil (69%) and lower saturated oil (11%) ratio, is considered an important oil among the plant based oils. It is, therefore, accepted as a good and healthy edible oil source. Storage conditions of the oil and also applied cultural techniques highly influence the quality properties of the oil. Especially, micronutrient applications may alter oil quality. This study aims to investigate the effect of micronutrients use on oil quality in sunflower.

Introduction

Sunflower (*Helianthus annuus* L.) is considered as one of the four important annual crops in the World for edible oil. Sunflower, with 41.3 million tones production in 2013/14, is the forth wildly produced oil crop in the World, right after soybean, rapeseed, and cotton seed (FAO, 2015). The seed yield and oil concentration are very exclusive characteristics for sunflower breeders due to being a vegetable oil source. Oil content of sunflower, which might range from 260 to 720 g/kg among the genotypes, is the most important property for marketing. (Hu et. al., 2010). The 24-49% of sunflower seeds contain oil, when the cake contains 25-35% of protein, which is used for livestock feed (Farokhi et. al., 2014). Sunflower oil is characterized by its high content of unsaturated fatty acids such as oleic and lenoleic which represent 90 % of total fatty acids (Al-Qubaie, 2011, Arshad et al., 2013). Also, sunflower oil is quite palatable and contains soluble vitamins A, D, E and K. It is used in manufacturing of margarine (Iqbal et al., 2009). The oil content of sunflower is shown in Figure 1.

Oilseed crops are very sensitive to Fe, B, Mn ve Zn micro elements loss (Rahimi et al., 2012). The most consumed micronutrients during sunflower development are iron, manganese, boron and copper (Kaya, 2008). The absence of these elements will cause some deficiencies on sunflower plants and oil quality. Using sufficient amount of micronutrient will increase the quality and quantity of the products, at the same time will decrease the amount of contaminants elements (Rahimi, 2014). Sunflower oil consist of different types of saturated and unsaturated fatty acids (palmitic, stearic, oleic and linoleic acids etc.). Palmitic and stearic acids are the major saturated fatty acids, whereas oleic and linoleic acids are unsaturated. Fatty acid composition of sunflower in particular and that of other oil seed crops in general are influenced by fertilizing managements.

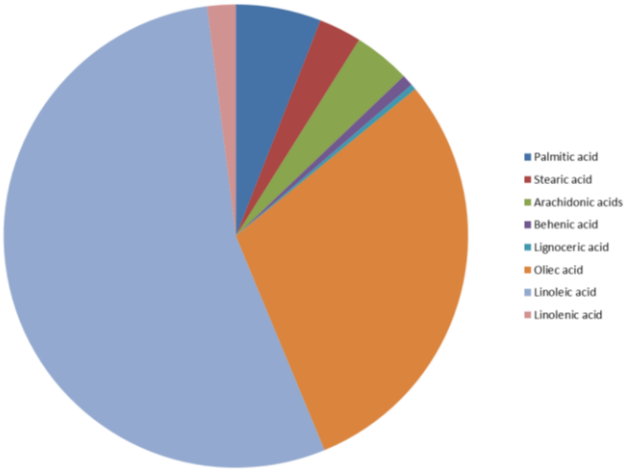

Figure 18: Oil content of sunflower (Nas, 2010).

Micronutrients in sunflower

Micronutrients, also called as trace elements, are nutrients, needed by organisms in small quantities. Micronutrients, used by sunflower, can be listed as; boron, iron, zinc, and manganese.

Boron (B): Sunflower is one of the most sensitive field crops to low B supply, and B deficiency in this crop has been reported from around the World (Blamey et al., 1997). Boron is absorbed by the roots of sunflower and accumulates the other organs and tissues of the plant. Most of B is accumulated in the larger leaves (Husa,1965). Boron has role on plant reproduction and pollen spikelet formation (Bolanos et. al., 2004). Besides, B is important for water relations, sugar translocations, cation and anion absorption and metabolism of N, P, carbohydrates and fat (Stiles, 1961; Shkolnik et. al., 1970). Boron deficiency occurs firstly in young leaves with bronze color and hardiness (Oyinlola, 2007). During fertilization of the sunflower field, the B concentration should be optimizated. In the study conducted by Oyinlola (2007), the optimized B concentration is 5.60-8.40 $kg.ha^{-1}$; on the other hand, there is a sharp decrese in oil content when the concentration up to 12 $kg.ha^{-1}$. Brighenti and Castro (2008) demonstrated that oil yield were increased by B consumption, and stated that B consumption increased the pollen fertility. Thus, with increase of the number of filled grain, yield is increased. Bahaa El-Din (2008) reported that application of 300 ppm B resulted in an increase of palmitic, stearic and oleic acids as compared to the treatment with 600 ppm B and control plants but the linoleic acid increased gradually with increasing B up to 600 ppm and cleared that B plays a vital role for increasing the productivity and quality of sunflower plants, especially when grown under B deficient soil. Significant decline in stearic acid and oleic acid contents while considerable increase in palmitic acid and linoleic acid contents was recorded by individual use of nitrogen and boron supplements. Farokhi et al. (2014) who found that oil yield and oil percantage were increased with B applicatian in sunflower. Tahir and his collauges (2014) conducted a study to show effect of B on sunflower yield (Table 1). They (2014) reported that the maximum oil contents were observed when B was applied at a rate of 8 kg ha-1 at the time of bud initiation.

Table 5: Effect of different boron concentrations on sunflower oil yield

Boron content	0 kg/ha	2 kg/ha	4 kg/ha	6 kg/ha	8 kg/ha	10 kg/ha	12 kg/ha
Oil yield (%)	40.82	41.53	42.46	42.77	42.85	41.92	41.36

Iron (Fe): Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants (Havlin et all., 1999). Iron is cofactor of many antioxidant enzymes. Fe^{+3} reduced to Fe^{+2} by O^{-2} reacting with H_2O_2 to form OH^- through Fenton Reaction. Besides, iron involves in different metabolic reactions like; catalase, phenolic depend peroxidases, ascorbate peroxidase and Fe superoxide dismutase (Raniery et. al., 2001). Iron fertilizer is considered as an important element in plant nutrition. According to Farokhi (2014), iron sulfate fertilization decreases oil percentage and increase oil yield of sunflowers (Table 2).

Table 6: Effect of iron sulfate on oil concentration of sunflower

$FeSO_4$ (kg/h)	**Stem diameter (mm)**	**Oil percentage (%)**	**Oil yield (kg/ha^{-1})**
0	9.56	55.07	1406.76
4	10.79	50.35	1607.20
6	11.50	50.89	3703.98

Ebrahimian et all. (2010) said that oil content increases by use of Fe microelement and Fe foliar application significantly increased total unsaturated fatty acids in sunflower. Besides, they concluded that Fe application significantly decreased POD (peroxidase) and CAD (catalase) but increased significantly SOD (superoxide dismutase) activity in sunflower. Ghavami et al. (2015) also reported that Fe application significantly increased protein and oil content compared to the control treatment

Zinc (Zn): Zinc nutrient is very important to plants due to the role on membrane integrity of root cells. Besides, zinc is assigned on protein synthesis such as auxins, which is a very important growth regulator. On the other hand zinc could decrease the toxic effect of boron, sodium and chloride (Mirzapour and Khoshgoftar, 2006). Zinc sulfate ($ZnSO_4$) is commonly used for Zn fertilization due to high solubility in water (Mordvedt and Gilkes, 1993). 4.5-34 $kg.ha^{-1}$ zinc, which depends on the soil need, is enough for inhibiting zinc deficiency in the field (Martens and Westermann, 1991). Zn fertilization with 10 to 20 kg per hectare increases oil content of the sunflower seed. In contrast, increasing in Zn concentration reduced oil content of the sunflower seeds (Mirzapour and Khoshgoftar, 2006). According to Khurana and Chatterjee (2001), more than 0.65 $mg.L^{-1}$ zinc supply inhibits oil content of sunflower seeds (Table 3).

Table 7: Oil content of sunflower according to Zinc supply

Zinc supply ($mg.L^{-1}$)	**0.00065**	**0.0065**	**0.065**	**0.65**	**6.5**	**65**
Oil content (%)	18.0	19.2	22.9	23.4	18.5	16.8

Ebrahimian et al. (2010) reported that oil content increases by use of Zn microelement and soil application of Zn micronutrients is more beneficial to oil biosynthesis. In addition, they concluded that foliar application of Zn microelement significantly increased POD (peroxidase) and SOD (superoxide dismutase) but decreased significantly CAT (catalase) activity and Zn foliar application significantly increased palmitoleic, linolenic, oleic and myristic acid content in sunflower. In another study, conducted by Eslami and colleagues (2015), spraying zinc sulfate to sunflowers effected oil content of the plants. The variations of oil content are showed in Table 4.

Table 8: Sunflower oil contents under different zinc sulphate concentration

	Oil percent (%)	**Oil yield (%)**	**Linoleic acid (%)**	**Oleic acid (%)**	**Stearic acid (%)**	**Palmitic acid (%)**
Z1*	34/55	1512/84	62/81	16/49	6/99	5/60
Z2*	35/20	1437/00	64/41	16/80	7/62	6/18
Z3*	34/9	1406/34	66/05	16/97	7/73	6/41

*__Z1__: 0 kg/ha, __Z2__: 30 kg/ha, __Z3__: 60 kg/ha.

Copper. Copper is assigned with carbohydrate and protein metabolism, chlorophyll synthesis and activation of some enzymes. Cu deficiency could be listed as; reducing in growth, distortion of younger leaves and necrosis of the apical meristem (Gibson et. al., 2013). Solubility of Cu^{+2} in the soil depends on pH of the soil. Unfortunately, when the soil pH reaches 5, the Cu^{+2} toxicity occurs by binding of copper elements to cation exchange sites of the root (Lin et. al., 2003). Besides, using micronutrients individually, they can also be used as combination. Thus, the effects of micronutrient on oil content of sunflower can be increased. According to Rahimi (2014), combination of micronutrients increases oil content of sunflower (Table 5).

Table 5: The effects of fertilization treatment on oil content of sunfllower.

Fertilizertreatment	Linoleic fatty acid (%)	Oleic fatty acid (%)	Stearic fatty acid (%)	Oil yield (ha/kg)
T_1	54.70	33.61	4.95	854.81
T_2	45.35	34.29	5.24	969.37
T_3	51.76	38.22	4.62	114.79
T_4	59.13	30.18	5.37	123.48
T_5	60.04	31.47	3.29	125.6

T_1: N, P, K, Mg; **T_2**: N, P, K, Mg, Fe; **T_3**: N, P, K, Mg, Fe, B; **T_4:** N, P, K, Mg, Fe, B, Mn; **T_5**: N, P, K, Mg, Fe, B, Mn, Z

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PERFORMANCE OF SUNFLOWER HYBRIDS IN BLACK COTTON SOILS OF NORTHERN KARNATAKA, INDIA

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ABSTRACT

Sunflower (*Helianthus annuus* L.), was introduced during seventies as an oil seed crop to India, It gained importance and popularity as a commercial oilseed crop of India under rainfed conditions. This is due to its suitability to many agro ecological regions, short duration, good quality oil and market price. The productivity of Sunflower in India at present is far lower (500 to 800 kg/ha) than the world average (1380 kg/ha). This is to a large extent due to several biotic and abiotic factors that the crop is invariably subjected in different regions. The availability of heterotic hybrids for cultivations has phenomenally increased during the last decade (2000-2010), which is expected to improve yield and disease control of the crop significantly. Drought and incidences of diseases and insect pests are the major constraints in sustaining the higher productivity of sunflower, in India. In this direction, a study was conducted during the *Kharif* season of 2014 to evaluate different sunflower hybrids (both public and private) on rainfed Vertisols of Raichur district in peninsular India. The study results revealed that significantly highest plant height was recorded by DRSH-1 (177.5 cm) over other hybrids and shortest hybrid was KSFH-11-384 (134.25 cm). Head diameter was significantly high for NSFH-1009 and RSFH-130 (9.1 cm) over KSFH-011-384 and KBSH-72. Significantly highest test weight was recorded by KBSH-44 (5.25 g) over remaining hybrids. Significant variation was observed with volume weight and the highest value was recorded by KSFH-11-384 (45.8 g/100 ml) followed by NSSH-1201 (42.55 g/100 ml). The performance of hybrids such as KBSH-44 and RSFH-130 was superior and significant for seed yield (2509 and 2485 Kg/ha, respectively). Correlation between plant height and days to 50% flowering and maturity was significant. High significant correlation was recorded between days to 50% flowering and days to maturity and former had significant correlation with head diameter. The hybrid RSFH -130 has surpassed the test of trial over other hybrids in terms of seed yield and oil content, which may be promoted for cultivation under black cotton soils of northern Karnataka.

Key words: Sunflower, Hybrids, Yield, Oil content

INTRODUCTION

Among the oilseed crops, sunflower (*Helianthus annuus L.*) occupies fourth position in area and production in the world after soybean, rape-seed-mustard and groundnut. World harvest of sunflower was 31.72 mt from 22.92 mha (FAO statistical Year book, 2013). World sunflower production has remained almost stable over the last decade. Russian Federation, Ukraine, Argentina, India and the China are the top five countries in the world with respect to production. Europe alone accounts for about 16 per cent of world area and 21 percent of total production. India is among the top four countries in area after Russian Federation, Ukraine and Argentina. Notwithstanding spectacular spurt in area, the productivity is only 42 per cent of world average (1380 kg/ha). In India during 1993-94 sunflower occupied an area of 26.7 lakh ha. 13.5 lakh tons production and drastically decreased to 5.5 lakh ha with a production of 4.15 lakh tons during 2014-15 (Anonymous, 2015). Despite phenomenal growth rate in area expansion, the productivity of sunflower crop in India has remained low. Karnataka, in South India, is called the "Sunflower State" as it alone accounts for more than 45 per cent of the total sunflower area in the country. However, the productivity is only 5 q/ha. In general productivity is low as sunflower moves from North India to South India. Rainfed cultivation, biotic and abiotic stresses and poor quality seed are some of the important factors responsible for low productivity (Ghani *et. al.,* 2000). The decline in acreage and production during 1996-98, has been also due to the incidence of a new viral disease (necrosis) in Southern and Central peninsula comprising Karnataka, Andhra Pradesh, Maharashtra and Tamil Nadu. Of late, soybean has emerged as a remunerative crop because of higher productivity and as a consequence, a part of sunflower area was diverted to soybean planting in rainy season.

Wide variation exists in flowering and maturity periods of sunflower across environments. On an average, crop matures in about 95 days in the south to about 120 days in the north. High productivity in north can be attributed to longer duration of the crop and high-applied inputs. Sunflower cultivation and hybrid seed production has gained new dimension after the launch of New Seed Policy in 1988. It resulted in the import of exotic hybrids and germplasm lines, which subsequently helped to diversify genetic base of sunflower cultivars grown in India (Devidayal and Agarwal, 1998). In coming years, major emphasis is to be placed on increasing productivity and yield stability across environments (Kaya et.al., 2012). This necessitates to study the performance evaluation of high seed yield cultivars with inbuilt resistance to biotic and abiotic stresses.

METHODOLOGY AND DATA

The field experiment was conducted during the *kharif* season of 2014 at Main Agriculture Research Station (MARS), University of Agricultural Sciences, Raichur, Karnataka, India. Geographically the experiment place is located in North Eastern Dry Zone (Zone-2) of Karnataka State, which falls between 16º 15' N latitude and 77º 20' E longitude with an altitude of 389 meters above mean sea level. The soil of the experimental site belongs to medium black with clay loam texture. The soil analysis results reveals that available nitrogen was low, therefore addition of 12.5 kg ha^{-1} to the recommended level of nitrogen (90 kg ha^{-1}) was done (103 kg ha^{-1}) and medium in available phosphorous (90 kg ha^{-1}) and potassium (60 kg ha^{-1}). The experiment has nine different sunflower hybrid treatments, The trial was laid out in a

Randomized Complete Block Design (RCBD) with four replications. The fertilizers were applied as per the recommended package (90:90:60 kg NPK/ha). Analysis of the results was done using SAS program, orthogonal contrasts studies for treatments and interactions (Robert and James, 1980 and Peterson, 1985) were also done.

RESULTS AND DISCUSSION

Weather during crop growth period

Rainfall between July and October, the most effective rainfall period for sunflower growth was 634.70 mm, which was received in 26 days (Table 1). No single irrigation was given, the trial was toatally rainfed. The average maximum temperature recorded during the cropping season wadsNo single irrigation was given, the trial was toatally rainfed. The average maximum temperature recorded during the cropping season was 32.54 C^o and minimum was 22.64 C^o, which is very conjenial for sunflower growth (Kazemini *et.al*., 2009; Reddy *et. al*., 2003 and Singh *et. al.,* 2005). The maximum relative humidity recorded during the crop season was 82.88 per cent and minimum was 58.25 per cent, which favours the build-up of pests and diseases like necrosis and leaf eating caterpillars for their control sparyaing of Chloropyriphos & Imidacholprid @2 ml and 0.3 ml/ltr of water, Trisophos @ 1.5 ml/ltr and Emamectin & Imidacholprid @ 1 ml and 0.3 ml/ltr of water was done as per the recommendation (Thavaprakash et. al., 2002; Tolga and Lokman, 2003).

Growth and Yield Attributes

The analyis of variance for growth parameters viz., plant height, days to 50 % flowering, days to maturity, head diameter, 100 seed weight, volume weight, plot yield, seed yield and oil content indicated significant difference among hybrids (p=0.05). The coefficient of variation (CV %) was high (11.30 %) for plot yield (Kg/Net Plot) and low (2.30 %) for Volume weight (g/100 ml). High CV indicates hybrids are highly variable for plot yield and least variation was observed for Volume weight. Different sunflower hybrids have a significant effect on growth attributes, *viz*. Plant height (cm), days to 50 per cent flowering and days to maturity. Among the sunflower hybrids, significantly highest plant height was recorded by DRSH-1 (177.5 cm) over other hybrids and shortest hybrid was KSFH-11-384 (134.25 cm) similar results were reported by Megur *et al*. (1993). KBSH-71 and KSFH-011-384 (54 days) and KBSH-72 and NSSH-1201 (55 days) were earliest to complete days to 50% flowering and early maturing hyrids. The results also revealed taht different sunflower hybrids have a significant effect on yield attributes, *viz*. head diameter (cm), 100-seed weight (g) and volume weight (g/100 ml). Among sunflower hybrids, head diameter was significantly high for NSFH-1009 (19.15 cm) followed by RSFH-130 (19.1 cm) and KBSH-44 (19 cm) over KSFH-011-384 (17.65 cm) and KBSH-72 (16.85 cm). Among hyrids, highest volume weight (g/100 ml) was recorded by KSFH-011-384 (45.80 g) and NSSH-1201 (42.55 g), both differed significantly from regional check and lowest volume weight (g/100 ml) was recorded by Laxmi-225 (39.05 g). Highest 100 seed weight was recorded by KBSH-44 (5.25 g) and lowest by Laxmi-225 (4.10 g). Hyrids viz., KBSH-71, DRSH-1,KBSH-72,NSFH-1009, and KBSH-44 differed significantly from regional check (RSFH-130) for 100 seed weight (Aravinda Kumar. *et al*., 2010; Teama and Mahmood, 1994). Similar results were observed by Barmaki *et al*. 2009, revealed that the yield and yield components of sunflower were

differed due to performance of different sunflower hybrids to weather and management practices. Similar type of synergetic effect was also reported by Patil *et al*. (2006).

Seed Yield and Oil Content

The hyrids have not differed for plot yield in comparison to regional check. However, KBSH-44 recorded highest plot yield (2.94 Kg/net plot) and lowest by Laxmi-225 (2.10 Kg/net plot).Most of the hyrids have differed among themselves for seed yield but in comparison to regional check, none of the hybrids have differed. The highest seed yield (2509 Kg/ha) was recorded by KBSH-44 and lowest by KSFH-011-384 (1923 Kg/ha). Hybrids have differed among themselves for oil content but in comparison to regional check none of them exhibited significant difference. However, highest oil content was recorded by KFSH-011-384 (41.76 %) and lowest by NSFH – 1009 (32.37 %). Similar results were observed by Kazem *et. al.,* 2013 and Mihaelaand Valeriu, 2010 in sunflower crop.

Correlation study results

The correlation among different agro-morphological and oil content has revealed highly significant positive correlation was recorded between days to maturity and days to 50 % flowering (r=0.98) indicating selection of hyrids of early maturity types may lead to correlated selection for early flowering types (Ahmad *et. al.,* 1992; Singh *et. al.,* 2005). Days to maturity also significantly correlated with plant height and head diameter (r=0.86; 0.67, respectively). Days to 50 % flowering was significantly correlated with plant height (r=0.85).

CONCLUSION

The new self fertile hybrids in sunflower both from public and private institutes in india, creates problem for the farmers in selection of perticular hybrid for their own land. Hence, the present study results gives some guidelines for the farmers based on their objective in selection of a perticular hybrid. Based on the study it is suggested that if farmer is interested for higher yield, then he should goi for long dration hybrids, as the long duration hybrids are highely correlated with higher yield and oil content. And if farmer is facing acute shortage of water and due to shift in rainfall pattern in this case farmer has to go for early maturing hybrids. Sunflower being a nutrient exhaustive crop, the new hybrids respond to both nutrients and water. Hence, the farming community has to be careful in selection of hybrids for their cultivation.

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Table 1. Weather parametrs recorded during the sunflower crop season at MARS, Raichur (July to October 2014)

Sl. No	Meteorlogical week	Weeks	Max. T (°c)	Min. T (°c)	RF (mm)	# Day	RH I (%)	RH II (%)	Evaporation	S. S Hrs	Win Speed
1	July 2-8	27	37.1	23.9	73	3	73	42	11.2	3.5	14.2
2	July l9-15	28	32.8	22.4	31.7	2	86	70	3.9	0.5	14.7
3	July 16-22	29	32.1	23.2	3.8	0	85	48	5.2	1.4	19.5
4	July 23-29	30	33.3	22.6	10.6	2	84	59	4.6	1.6	17.4
5	July 30-5	31	32.0	22.6	8.8	2	83	55	5.4	2.8	16.6
6	August 6-12	32	34.2	23.6	0	0	79	47	6.7	7.1	14.9
7	August 13-19	33	34.1	23.0	7.9	1	78	50	6.4	5.5	10.0
8	August 20-26	34	33.7	22.3	187	3	89	62	3.5	3.2	6.0
9	August 27-2	35	28.0	21.6	189.9	6	93	83	2.6	1.4	10.1
10	September 3-9	36	30.0	21.7	19.8	2	87	68	4.0	3.7	12.1
11	September 10-16	37	32.2	22.5	20.4	2	83	57	4.9	6.1	9.6
12	September 17-23	38	29.8	22.3	45.8	2	90	79	3.6	0.7	7.2
13	September 24-30	39	32.4	23.7	0	0	83	55	4.9	0.0	5.5
14	October 1-7	40	34.2	22.4	35.4	1	75	46	5.0	7.0	4.1
15	October 8-14	41	31.8	21.8	0.6	0	83	60	3.2	4.5	4.1
16	October 15-21	42	32.9	22.7	0	0	75	51	5.1	7.4	6.7
Avg/ Total			32.54	22.64	634.7	26	82.88	58.25	5.01	3.53	10.79

Date of Sowing: 08.07.2016 Date of Harvest: 18.10.2016

Table 2. AHT ANOVA Table

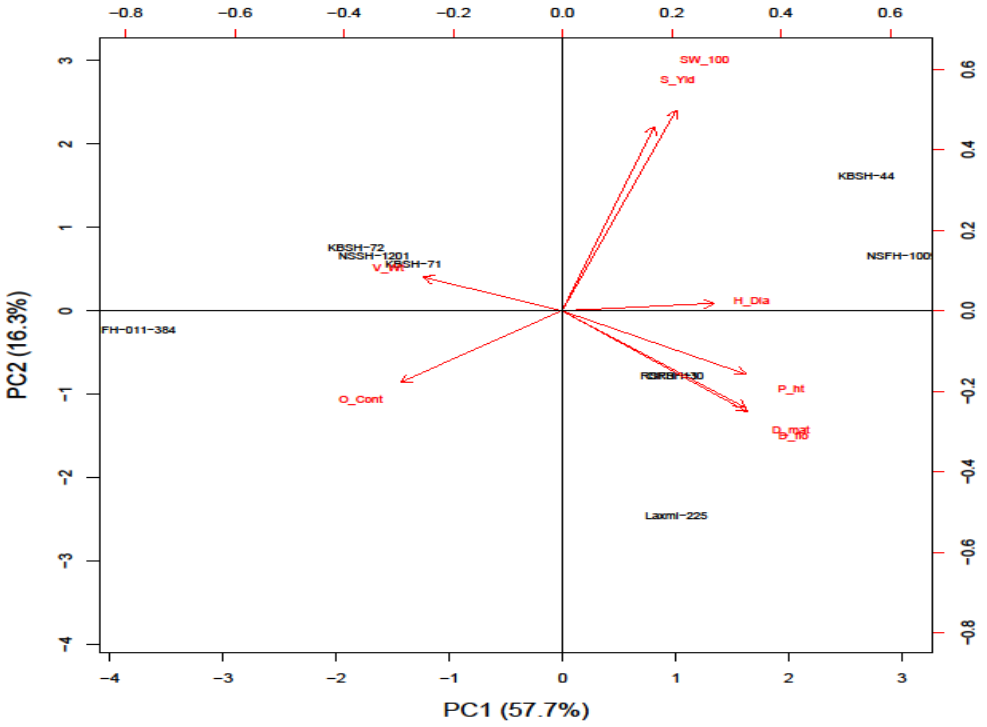
#	Treatment	Plant Height (cm)	Days to 50% flowering	Days to Maturity	Head diameter (cm)	100 Seed Weight(g)	Volume weight (g/100ml)	Plot Yield (Kg/net plot)	Seed Yield (Kg/ha)	Oil content (%)
1	KBSH-71	158.75	54	94	18.30	4.45	41.18	2.60	2225	40.49
2	DRSH-1	177.50	59	97	18.15	4.58	41.38	2.56	2184	39.87
3	KSFH-011-384	134.25	54	94	17.65	4.13	45.80	2.25	1923	41.74
4	KBSH-72	145.75	55	94	16.85	4.65	39.70	2.55	2180	39.66
5	NSFH-1009	173.50	60	97	19.15	5.00	40.40	2.70	2306	32.37
6	KBSH-44	167.00	59	97	19.00	5.25	40.15	2.94	2509	34.59
7	Laxmi-225	168.50	60	97	18.35	4.10	39.05	2.10	1797	37.13
8	NSSH-1201	146.75	55	94	18.40	4.30	42.55	2.68	2293	39.96
9	RSFH-130	163.25	59	97	19.10	4.03	40.30	2.91	2485	41.01
SEm±		3.98	--	--	0.40	0.12	0.49	0.15	122.05	1.14
C.D. at 5 %		11.60	--	--	1.18	0.35	1.43	0.59	356.25	2.35
C.V. %		4.96	--	--	4.40	5.34	2.38	11.30	11.04	4.16

Table 3. Decoded of sunflower hybrids with the respective institutes

Entry No.	Institutions
KBSH-71	UAS, Bangalore
DRSH-1	IIOR, Hyderabad
KSFH-011-384	Kaveri Seeds, Hyderabad
KBSH-72	UAS, Bangalore
NSFH-1009	Nuziveedu Seeds, Hyderabad
KBSH-44	UAS, Bangalore
Laxmi-225	Yaganti Seeds, Hyderabad
NSSH-1201	Nuziveedu Seeds, Hyderabad
RSFH-130 (Regional Check)	UAS, Raichur (Regional Check)

Table 4. Sunlfower correlation study

	S_Yld	*O_Cont*	*P_ht*	*D_flo*	*D_mat*	*H_Dia*	*SW_100*	*V_Wt*
S_Yld	1.00							
O_Cont	-0.23	1.00						
P_ht	0.27	-0.58	1.00					
D_flo	0.18	-0.65	0.85	1.00				
D_mat	0.23	-0.58	0.86	0.98	1.00			
H_Dia	0.53	-0.52	0.62	0.64	0.67	1.00		
SW_100	0.50	-0.75	0.38	0.27	0.26	0.22	1.00	
V_Wt	-0.24	0.49	-0.65	-0.61	-0.54	-0.25	-0.31	1.00



Graph 1. Principal component analysis of Sunflower hybrids performance

DEVELOPMENT OF SUNFLOWER PRODUCTION IN TURKEY

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ABSTRACT

The sunflower is the most important raw material of the oil sector among oilseeds produced in Turkey. Although adaptation areas of the sunflower that can be cultivated in dry or irrigated conditions almost in every region of Turkey are very large, cultivation areas have remained at the level of 500 000-600 000 hectars for many years. For this reason, it is necessary that support and incentives carried out should be increased in order to spread production in the potential areas after these areas are determined, yield should be increased, new species whose oil content is high should be developed, produced and spread. As a result, in order to enlarge cultivation areas of the sunflower, more areas, both dry and irrigated, should be opened to the sunflower agriculture through especially technical support and incentive of the government or the private sector.

Key words: Oilseeds, sunflower, cultivation

INTRODUCTION

The oilseeds which are among agricultural products can be counted as sunflower, cotton seed, soybean, rapeseed, safflower, groundnut, sesame and poppy (seed). However, the sunflower whose seed nearly %38-50 oil is obtained from among oil plants has considerable significance in the production and consumption of vegetable oil in Turkey.

The sunflower that is our traditional plant is the first thing that comes to mind in terms of oilseeds in Turkey. The sunflower which can be cultivated nearly in every region of Turkey and whose seeds include high rates of qualified oil is placed on the top in terms of cultivation areas of oil plants and amount of production. The sunflower meets the need of %68 of the production of oil plant in our country and %32 of the total use of oil (BYSD 2016).

In parallel to the rapidly increasing population in the world, the consumption of food stuffs and thus the consumption of vegetable oil is increasing day by day. In 2015, 527 million tonnes of oilseeds were produced in the world, and %7,6 of this consisted of the sunflower (USDA 2016). In Turkey, however, 3,2 million tonnes of oilseeds were produced in 2015, and %46 of this consisted of the sunflower. Our production of sunflower (for oil) which was 1 million tonnes in 2006 reached 1,5 million tones in 2015 with an increase by %50 in ten years (TUIK 2016). It can be said that this increase resulted from the increase in yield rather than cultivation areas. %50 of the production under discussion is made in Thrace-Marmara Region.

THE PRODUCTION OF SUNFLOWER IN TURKEY

Although adaptation areas of the sunflower that can be cultivated in dry or irrigated conditions almost in every region of our country are very large, cultivation areas have remained at the level of 500 000-600 000 hectars for many years.

There has not been much change in cultivation areas of sunflower (for oil) in Turkey for the last ten years. On the other hand, the amount of its production has increased from 1 million to 1,5 million owing to the increase in yield. 1,5 million tones of oil sunflower seeds were produced in the area of 569 000 ha in the season of 2015. The seed yield of the sunflower increased from 198 kg/da to 264 kg/da between 206 and 2015 (Table 1).

Table 1. Area, Production and Yield of Sunflower for Oil in Turkey

Years	**Area (ha)**	**Production (tons)**	**Yield (kg/da)**
2006	510.000	1.010.000	198
2007	485.700	770.000	159
2008	510.000	900.387	177
2009	515.000	960.300	186
2010	551.400	1.170.000	212
2011	556.000	1.170.000	210
2012	504.616	1.200.000	238
2013	520.260	1.380.000	265
2014	552.465	1.480.000	268
2015	568.995	1.500.000	264

TUIK 2016

Nearly %50 of the production of sunflower for oil (754 000 tonnes) in Turkey is made in Thrace-Marmara Region. Tekirdag, Edirne, Konya, Kirklareli ve Adana cities are respectively those in which the production of sunflower is mostly made (TUIK 2016). Thrace Region is followed by Central Anatolia Region. In terms of not yield per unit area but the length of cultivation aras, Tekirdag is on the first rank in Turkey (Table 2). In this region, the sunflower is cultivated in dry conditions. On the other hand, the city that has the highest yield is Konya (457 kg/da). This difference of yield per decares results from irrigated farming in Konya region.

Table 2. Area, Production and Yield of Sunflower in Turkey Cities in 2015

Cities	**Area (da)**	**Production (tons)**	**Yield (kg/da)**	**Percentage (%)**
Tekirdag	1.284.677	267.012	208	17,8
Edirne	984.061	226.573	230	15,1
Konya	460.376	210.307	457	14,0
Kirklareli	733.520	188.998	258	12,6
Adana	440.400	134.361	305	8,9
Corum	198.952	51.984	261	3,5
Aksaray	106.351	43.985	414	2,9
Tokat	134.962	41.593	308	2,8
Other Cities	1.346.651	335.187	-	22,4
Turkey	**5.689.950**	**1.500.000**	**264**	**100**

TUIK 2016

IMPROVEMENT OF PRODUCTION OF SUNFLOWER IN TURKEY

There are two basic ways that need to be followed in order to increase the production of sunflower. The first one is to increase its cultivation areas, and the second one is to increase yield. Certainly, carrying out both of these at the same time will be a more effective and quick way of increasing the production. Yet in real terms, the biggest potential in increasing the production of sunflower can be achieved first of all with the increase of cultivation areas and extending of sunflower agriculture in irrigable areas. Thus, increasing cultivation areas and other matters should be handled separately.

Adding New Areas to Sunflower Agriculture

The sunflower agriculture is carried out commonly in Thracian Region in Turkey. But the sunflower yield of the region is under the general average in Turkey. Significant attempts must be made in order to increase yield in this region. On the other side, it is observed that cultivation areas of the sunflower have remained constant for long years and that the production has increased due to high yield. The reason that the production costs of the sunflower are kept lower than those in the world in order to increase its cultivation areas and that it can compete with the products in areas where it is cultivated is very important and effective. Thus, projects that aim to expand its cultivation areas need to be put into effect. After the sunflower finds new cultivation areas for irrigation in the areas in the regions of especially GAP (Southeastern Anatolia Project) and KOP (Konya Plain Project), the total cultivation areas will increase, and its yield will also increase. Moreover, the cultivation of the sunflower has been common in Mediterranean Region in recent years. In this region, the sunflower is cultivated in February-March, and it is harvested

in July. The fact that it is harvested early is very important for oil factories that are inactive especially in that period in terms of meeting their needs. Also, since it is the first sunflower of the production season (early grown), its prices are usually high (Kolsarici et al. 2015).

Irrigation Opportunities in Dry Cultivated Area

Considerable decreases in the proportion of yield and oil of the production of sunflower could be seen in dry years in Turkey. For instance, drought in 2014 in Thracian Region caused yield and oil content of sunflower to decrease, and thus it decreased the total production of vegetable oil in Turkey. For this reason, investments in irrigation should be increased, the sunflower agriculture should be encouraged in irrigable areas, and areas (arid and semi-arid areas) where annual rainfall is under 500-600 mm should be irrigated et least once or twice flowering priod (Baydar 2011). Especially in Thracian Region where the sunflower agriculture is carried out, irrigation should be made with appropriate methods, and drip irrigation should be focused on. If the aim in our country where an irrigation area of 5,9 million ha is elevated to that of 8,5 million ha and if the sunflower agriculture is carried out in new areas being opened to irrigation, increases in yield will be observed to climb to %100 and this increase will contribute considerably to our production of vegetable oil.

Use of Species Resistant to Diseases and Pests

Orobanche (*Orobanche cumana* Wallr.) is a parasitic plant which leads to a decrease of %100 proportion in yield of the sunflower in our, European and Balkan countries (Kaya 2013). On the other hand, another problem is concerned with broad leaves weeds that can not be controlled with herbicides before sowing. After sunflower hybrids that are resistant to IMI (Imidazolinone) due to CLEARFIELD applications have emerged, it is now quite easy to control, through herbicides for weeds, both orobanş and weeds that pose serious problems in the production of sunflower (Anonim 2013). Therefore, the use of species that are developed for that purpose should be extended in all of the areas where sunflower agriculture is carried out.

Encouraging Oleic Type Species of Sunflower

Breeding of the native seed and its production should be accelerated and supported by the government. Also, new species of sunflower (High oleic) which includes high proportions of oleic acids (omega-3) should be developed. This is because prospering the level of welfare and self-awareness of nutrition canalize the society into preferring healthy oils that are of good quality. Research has shown that oleic acid as unsaturated fatty acid diminishes the risk of hypertension and provides protection against heart and coronary diseases by balancing cholesterol (Karacor and Cam 2015). Also, oils being obtained from oleic type sunflower can be used more than once (twice-eight times) in hotels, restaurants and catering firms, and this can lead a great deal of saving (Kolsarici et al. 2015). Only through such a measure, a notable stability or decrease in our need of vegetable oil that is met with importing could be supplied.

Improving Support

Support of sunflower for oil per kilogram increased from 0,20 to 0,30 liras in the last ten years of 2006-2015. For this reason, producers in Thracian- Marmara Region cultivate wheat for two years and sunflower for one year in crop rotation instead of wheat for one year and sunflower for one year as they are not satisfied with the support for sunflower, and this causes

significant deviations in values of sowing and production. In order to prevent this, price parity of sunflower/wheat should be kept in 2.5-3.0 in favour of sunflower so that sunflower can compete with wheat (Kolsarici et al. 2015). In addition, input costs of producers should be decreased by increasing amounst of support for fuel and fertilizers.

CONCLUSION

It is observed that cultivation areas of sunflower have remained constant for years in Turkey and that production has increased with yield. Thus, projects that try to enlarge cultivation areas should be put into action. The potential cultivation area of sunflower in Turkey is 1 450 000 ha. However, nearly 600 000 ha out of this potential is made use of. If sunflower is cultivated in the area of 850 000 ha that is not used and its yield reaches average 170 kg/da, the annual production of sunflower will reach the value of 1 450 000 tones, and thus the gap of vegetable oil that is supplied with importing will be closed in Turkey (Anonim 2015). Investments in sunflower agriculture should be risen, agriculture of this plant should be focused on, and areas (arid and semi-arid areas) where annual rainfall is under 500-600 mm should be irrigated at least once or twice in flowering period, Moreover, species which are resistant to diseases and pests, whose seed yield and oil content are high, and which have oil content of good quality should be devloped, produced, and their consumption should be made more widespread.

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CONFECTIONARY SUNFLOWER IN IRAN

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ABSTRACT

Cultivation area of confectionary sunflower has been increased to about 50,000 ha in Iran recently. The main production area is located in Khoy, West Azerbaijan with more than 20,000 ha average yielding 1500 Kg/ha. Kermanshah, Qazvin, Zanjan and Hamadan providences are followed by Khoy as the other main areas for cultivation of this crop. Regardless of having more than a century of cultivation history, there is no report for improvement of this crop and, farmers use only self provided seed annually. This condition has resulted in lower seed yield and also increase of the crop main destructive diseases such as Sclerotinia, Rust and Downey mildew. Moreover, due to open pollination nature of the plant, undesirable characteristics including heterogeneity, late maturity, higher plant height and lower seed set are still observed in the local land races. Despite these deficiencies, there is a wide genetic diversity which could be used as main sources for improvement of confectionary sunflowers. There are four main types of land races with different characteristics and different economic demand in local market. Development of genetic materials is a necessity for improvement of confectionary sunflower in Iran and international collaboration with reciprocal profit could accelerate this procedure.

Key Words : Confectionary sunflower, Disease, Land races, protein content

RELATIONSHIPS BETWEEN GERMINATION AND VIGOR TESTS WITH FIELD EMERGENCE OF SUNFLOWER IN IRAN

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ABSTRACT

In order to estimate the seedling emergence of sunflower cultivars, this research was conducted in both laboratory and field. The treatments were four sunflower cultivars (Record, Euroflor, Hysun and Azarghol) and three levels of seed germination(below standard or 80%, standard or 80% and above standard or 90%).The results indicated that increase of seeds germination in standard germination test caused the increase of normal seedlings percent and also the seedling weight and length vigor indices. Record cultivar and With high germination percent had a significant difference with other cultivars in most measured traits in laboratory and the reason is probably their genetic structure and less seed deterioration of these cultivars. The final seedling emergence at field was correlated with seedling weight and length vigor indices at laboratory. The normal seedlings number (germination percent) in standard germination test was correlated with final field emergence of seedlings. Therefore, by calculation the normal seedlings percent, we can predict the seed's potential for seedling production and establishment at field.

Key words: Germination, seed vigor, sunflower.

INTRODUCTION

The planting of high quality seed is an important factor of successful agriculture due to rapid and uniform seedling emergence and also higher establishment and achieving proper plant density which results in higher yield (TeKrony and Egli, 1993). The influence of seed vigor on seedling field emergence and establishment was assessed and specified that seed vigor affects the seedling field establishment, seedling emergence rate and it's uniformity which all of these factors, potentially can influence the accumulation of dry matter in plant population and therefore the yield (Heydecker, 1977).

The germination test determines the germination ability of seeds in a seed lot which it's results can be used for comparison of different seed lots quality and also estimation of required seed rate for planting (Anonymus, 2011). The study of germination test of 94 soybean's seed lots in laboratory and the results of seedling field emergence of same seed lots indicated that low germination caused the low seedling emergence and percent at a field (Delouche and Baskin, 1973). This research was conducted in order to estimate the seedling emergence of sunflowers cultivars with germination test.

MATERIALS AND METHODS:

In order to evaluation the correlation of seed germination with seed seedling field emergence of sunflower cultivars, a research was conducted in both laboratory and field in 2012. The treatments were four sunflower cultivars including Record, Euroflore, Hysun and Azarghol with three levels of seed germination as standard seed germination (85%) , above standard (the highest germination percent of each cultivar) and below standard level (80%) which were produced in 2011. The study was conducted as a factorial experiment based on completely randomized design for laboratory tests and as a factorial experiment with 2 factors based on randomized complete block design with 3 replications for field tests. The standard germination test was conducted according to rules of the international seed testing association (ISTA) (Delouche and Baskin, 1973). Two indices including seedling length vigor index and seedling weight vigor index were also were determined by Abdol-baki and Anderson approach (Abdul-Baki and Anderson, 1973). In order to estimate seedling field emergence percent and related traits; the seeds were sown and evaluated in experimental field of seed and plant certification and registration institute. The seedling field mergence index was determined by approach of Ram *et al*., 1998. The resulted data were analyzed by MSTAT-C software and the mean comparison was done by Duncan multiple range test. The correlation of measured traits was calculated by SPSS software and the charts were drawn by Excel software.

RESULTS:

The analysis of variance results of the standard germination test (table 1) indicated that the interaction of cultivar× germination ability was highly significant in all the traits. The normal seedlings from seeds of Record cultivar with 90 percent had the highest normal seedlings of 79%. However the lowest normal seedlings of 31 percent obtained from seeds of Record and Uroflor with 80 percent germination ability.

Table 1- The analysis of variance (mean squares) of studied traits at standard germination test.

S.O.V	df	Normal seedlings percent	Abnormal seedlings percent	Final germination percent	Seedling length vigor index	Seedling weight vigor index
Cultivar	3	1102.69**	835.657**	84.889**	634535.16**	100.416**
Germination	2	2692**	708.861**	10.33ns	361738.17**	761.967**
Cultivar × Germination	6	346.66**	158.713**	57.889**	81488.10*	56.54**
Error	24	9.361	10861	12.222	11526.19	9.124
Coefficient of variation) %(		5.49	13.62	3.76	12.6	12.96

ns,* and ** , respectively are non significant and significant at 1% and 5% level of probability

The maximum number of normal seedlings was obtained from seeds with 90 percent germination and the lowest was recorded in seeds with 80 percent germination (table 3). The highest abnormal seedlings of 49 percent observed from seeds of Hysun with 80 percent germination. The seeds of Record with 90 percent germination ability had the lowest abnormal seedlings percent of 8% (table 3). The maximum final germination percent (98%) belonged to seeds of Hysun with 80 percent germination ability. the lowest percent of final germination of 83 percent was recorded for Urofor with 90 percent of germination (table 3). The highest seedling length vigor index of 1220.200 was obtained from Azargol with 85 germination percent and the lowest was 374.800 from seeds of Hysun with 80 percent germination (table 3). The seeds of Record cultivar with 90 percent germination indicated the highest seedling weight vigor index of

34.6 and the lowest amount of 10.9 was observed in seeds of Uroflor with 80 percent of germination (table 3).

Table 2- The analysis of variance (mean squares) of studied traits at field.

S.O.V	df	seedling vigor index	Seedling emergence index	Final seedling emergence
Block	2	360.821 ^{ns}	22.886 ^{ns}	181.963^{ns}
Cultivar	3	623.215 **	2.861 ^{ns}	16.671^{ns}
Germination	2	968.160 **	26.258 ^{ns}	41.790 ^{ns}
Cultivar × germination	6	246.281**	7.265 ^{ns}	64.744 ^{ns}
Error	22	2436.838	9.278	74.162
Coefficient of variation(%)		15.37	11.59	11.40

ns,* and ** , respectively are non significant and significant at 1% and 5% level of probability

Table 3- The mean comparison of studied traits at standard germination test.

Cultivar	Germination (%)	Seedling weight vigor index	Seedling length vigor index	Final germination percent	Abnormal seedlings number	Normal seedlings number
Azargol	80	21.4de	1172.53ab	90^{cd}	14^{d}	63de
	85	26.7cd	1220.20a	94^{abc}	22^{c}	67^{cd}
	90	28.8bc	1150.53ab	98^{ab}	16^{d}	70^{bc}
Record	80	13.5fg	558ef	93^{abc}	35^{b}	31^{h}
	85	30.9abc	1000.73bc	87de	18^{cd}	72^{b}
	90	34.6a	865.06cd	95^{abc}	8e	79^{a}
Hysun	80	14.0fg	374.80f	98^{a}	49^{a}	31^{h}
	85	16.7ef	499.14f	95^{abc}	36^{b}	39^{g}
	90	26.6cd	726.53de	97^{a}	30^{b}	51^{f}
Euroflor	80	10.9g	531.86f	94^{abc}	33^{b}	31^{h}
	85	22.5d	886.66cd	91^{bcd}	15^{d}	61^{e}
	90	33.2ab	1211.20a	83^{e}	15^{d}	73^{b}

The results of field's variance analysis (table 2) specified that the interaction of cultivar×germination ability was highly significant for all of the measured traits except of final seedling emergence and seedling emergence index. The highest seedling vigor index at field was 501.4 that observed at seeds of Record with germination ability of 80 percent and the lowest seedling vigor index at field recorded 163.6 at seeds of Azarghol with 80 percent germination (Table 4).

Table 4- The mean comparison of interaction of measured traits at standard germination test

cultivar	germination	Azargol	Record	Hysun	Euroflro
Vigor index	80	163.6^{e}	501.4^{a}	434.8^{a}	343.9^{cd}
	85	279.5^{d}	293.2^{d}	401.3^{bc}	282.3^{d}
	90	332.6^{cd}	264.8^{d}	262.6^{d}	295^{d}

The final field seedling emergence had high positive correlation with seedling weight vigor and seedling length vigor indices. The seedling vigor index at field showed positive and high significant correlation with seedling length vigor index and normal seedlings percent (table 5).

Table 5-The correlation of seedling emergence and seed vigor assessment at standard germination test.

	1	2	3	4	5	6	7	8
1.final seedling emergence	1							
2.seedling emergence index	0.82**	1						
3 .seedling field vigor index	0.126	0.136	1					
4.normal seedling	0.105	0.253	0.64**	1				
5.abnormal seedling	-0.005	-0.116	0.64**	-0.88**	1			
6.final germination	0.229	-0.208	0.232	0.370*	0.416*	1		
7.seedling length vigor index	0.387**	0.382**	0.95**	-0.55**	0.610**	0.299	1	
8..seedling weight vigor index	0.52**	0.62**	0.178	0.032	0.194	0.21	0.382**	1

* and ** , respectively are significant at 1% and 5% level of probability

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GREEN AND BROWN BRIDGES AID SURVIVAL OF MULTIPLE DIAPORTHE/PHOMOPSIS SPECIES WITH A RANGE OF VIRULENCES ON SUNFLOWER, SOYBEANS, MUNGBEANS AND OTHER CROPS IN AUSTRALIA

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ABSTRACT

Multiple species of *Diaporthe/Phomopsis*, some well recognised, others new and undescribed have been identified from live sunflower, sunflower residues, other live crops plus crop and weed residues in the eastern Australian summer cropping regions. Pathogenicity tests have revealed that many of these species are virulent on other crops such as soybean, chickpeas and mungbeans. Some crops are susceptible to multiple species and other species are pathogenic on a range of hosts. In Australia, after thirty years of zero and minimum tillage practices where crop and weed residues are left on the surface in an effort to retain moisture, a significant inoculum reservior of many *Diaporthe/Phomopsis* species is present in the cropping system regardless of the presence of a favoured crop host. Although green bridges are well recognised in aiding surivival of many pathogens, the role of dead weeds left standing after herbicide applications, then acting along with crop stubble as a brown bridge, has largely gone unstudied. It is considered likely that other cropping systems worldwide would also be harbouring reservoirs of diverse pathogenic *Diaporthe/Phomopsis* species in the green and brown 'non-hosts' bridges associated with their individual cropping systems. Additionally, there is evidence that reservoirs of other pathogens and opportunitistic colonisers such as *Fusarium* and *Colletotricum* species are also surviving in the 'non-host' residues.

Key Words : Sunflower, Diaporthe, Phomopsis, pathogenicity, soybeans, mungbeans, green bridge, brown bridge, survival, weeds

PULSAR® PLUS AND EUROLIGHTNING® PLUS - HERBICIDES FOR ENHANCED WEED CONTROL IN CLEARFIELD® PLUS SUNFLOWER

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ABSTRACT

The Clearfield Sunflower Production System was introduced first in 2003 in Turkey and in the following years in the other key sunflower countries in Europe. The system combines high yielding sunflower hybrids with effective post-emergence herbicides such as Pulsar 40 or Eurolightning. Since the introduction, growers benefit from the possibility to control main broadleaf and grass weeds relying on the respective herbicide solutions. Starting from 2015 onwards, BASF and several seed partners started to introduce the so-called Clearfield Plus Sunflower Production System. Within that system, a new class of hybrids carrying the CLHA-Plus gene are combined with highly effective and reliable herbicide solutions such as Pulsar Plus and Eurolightning Plus. These herbicides, which have been developed especially for the use within the Clearfield Plus Production System, have been tested in field and laboratory trials starting from 2009 onwards showing robust performance. Laboratory tests proved, that the retention as well as the plant uptake of theses herbicides is enhanced compared to current market standards. In field trials, the herbicides provided a higher level of efficacy and reliability, an improved application timing flexibility and in combination with Clearfield Plus hybrids, an improved selectivity pattern compared to the available market solutions. With that, the growers can further improve the weed control in the crop, being the basis to use the full yield potentials of the modern sunflower hybrids.

Key Words : herbicide tolerance, sunflower, efficacy, formulations, weed control

CHEMICAL BROOMRAPE (*OROBANCHE CUMANA*) CONTROL IN CLEARFIELD® SUNFLOWER WITH DIFFERENT IMAZAMOX CONTAINING HERBICIDE FORMULATIONS

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Broomrape (*Orobanche cumana* Wallr.) in sunflower is one of the most important constraints in sunflower production in Europe. In the last twelve years, the chemical control option with the Clearfield Sunflower Production System in sunflower and the relevant Clearfield herbicides has been an important control strategy and complemented the genetic resistance against *broomrape*. The commercial formulation Pulsar® (Imazamox 40 g/l) exhibits besides broad-spectrum weed and grass control a unique efficacy on broomrape in sunflower. Season long broomrape control is, not at least, depending on a lethal concentration of Imazamox in the host plant over the time broomrape is attacking sunflower. The uptake of Imazamox and therewith the herbicidal concentration in sunflower is strongly influenced by the herbicide formulation. Between 2012 and 2015, field trials were conducted at locations in Bulgaria, Hungary, Romania and Spain to evaluate and compare the broomrape efficacy of Pulsar Plus, a new improved formulation, for the recently introduced Clearfield Plus tolerance. The Pulsar Plus formulation was evaluated at 30, 40 and 50 g ai/ha and compared to Pulsar, at the same rates. Pulsar Plus outperformed Pulsar in *O. cumana* control and sunflower yield.

Key Words : Broomrape, Clearfield, Clearfield Plus, Dose-response, Helianthus annus, Imazamox, Orobanche cumana

THE EFFECT OF CLIMATE FACTORS ON THE YIELD OF SUNFLOWER AND SUNFLOWER YIELD PREDICTIONS BASED ON CLIMATE CHANGE PROJECTIONS: EXAMPLE OF MARMARA REGION

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ABSTRACT

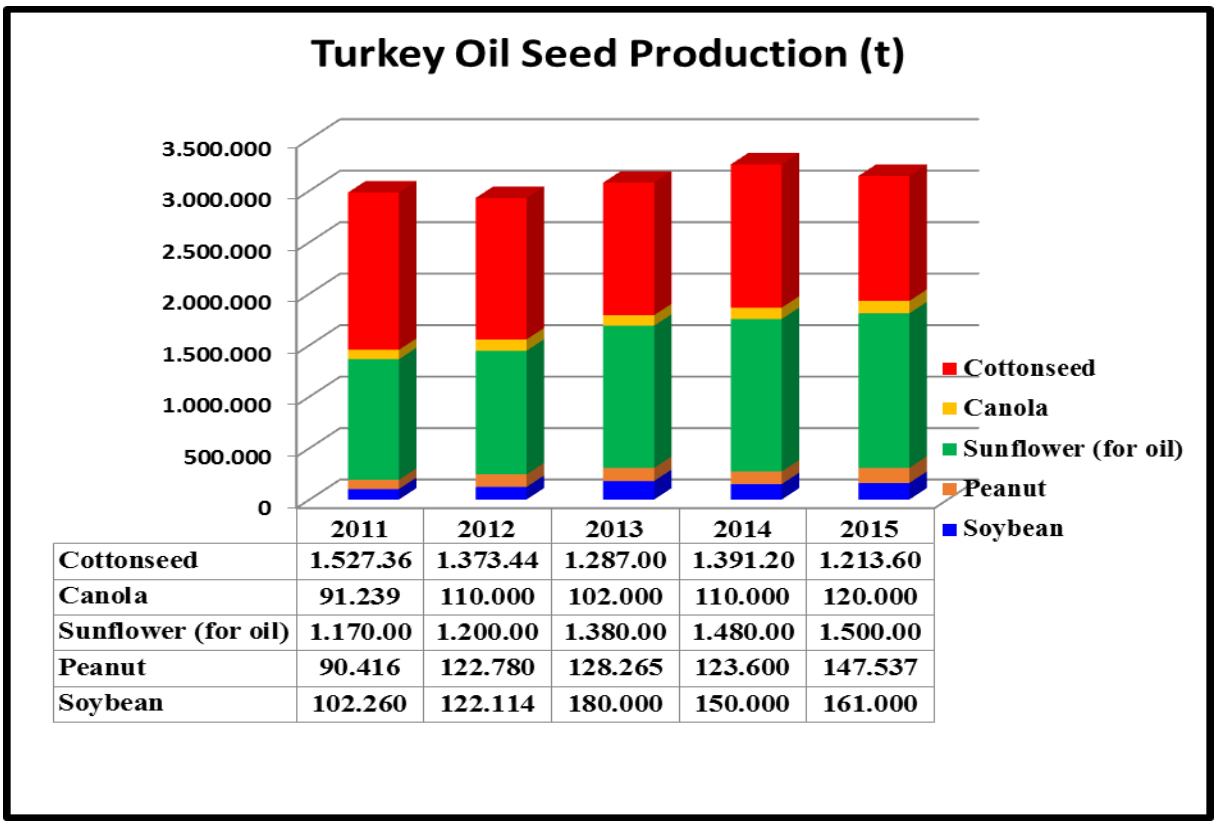
Sunflower is the raw material of vegetable oils sector in Turkey. Production is not sufficient even for domestic consumption. Therefore, it is necessary to carry out the projects to increase the yield and production areas. This study was carried out in order to identify the relationship between yield of sunflower and climate factors, also to determine the possible effects of the future climate changes on the sunflower yield. In the study, 10 provinces in Marmara region were evaluated. The following materials were used for this study; sunflower production values and meteorological data for the years of 1985-2014, climate projections that are based on HadGEM2-ES Global Climate Model with 20 km resolution, and RCP8.5 scenario that covers the period of 2016-2099. Climate parameters used in this study are number of days that minimum temperature below -5°C, monthly average temperature, number of days that maximum temperature above 35°C, monthly average relative humidity, number of days that average relative humidity above % 70, monthly total sunshine duration, monthly total precipitation. Firstly; single and multiple correlation analyses, the least-squares method with linear regression analyses were conducted between observation values and production data. Then, the potential impact of climate changes, that are projected for the future periods (2016-2040, 2041-2070 and 2071-2099), on yield of sunflower have been put forward with by using the generated high-rate regression equations and climate projection data. According to the results, it was determined that there is an important characteristic effect of climate factors on productivity With reference to the yield prediction analyses, Marmara region will be negatively affected.

Key Words: The yield of sunflower, climate factors, HadGEM2-ES, RCP8.5, the effects of climate change

INTRODUCTION

There are three main groups in human nutrition. These are oils, proteins and carbohydrates. Oils are an important source of calories in the human diet (Hatırlı et al., 2002). Oil seeds are raw material for the vegetable oil industry at the same time also are the raw material of many different sectors. Oilseed meals having relatively high protein content are preferred in animal nutrition (İlkdoğan, 2008). Vegetable oils that are used in food, energy and chemical industries sectors are strategic products (Taşkaya Top and Uçum, 2012).

According to the Association of Vegetable Oil Industries (AVOI) report 2014; while sunflower takes the fourth place after soybean, canola and cottonseed in the world oilseed production, it takes the first place in Turkey (AVOI, 2015). The most intensive sunflower farming region is Marmara region in Turkey.



	2011	2012	2013	2014	2015
Cottonseed	1.527.36	1.373.44	1.287.00	1.391.20	1.213.60
Canola	91.239	110.000	102.000	110.000	120.000
Sunflower (for oil)	1.170.00	1.200.00	1.380.00	1.480.00	1.500.00
Peanut	90.416	122.780	128.265	123.600	147.537
Soybean	102.260	122.114	180.000	150.000	161.000

Figure 19: Turkey oil seed production (tons)

According to the Turkey Statistical Institute (TSI) reports in 2014, the top five most produced oil seed plants are in the order of sunflower, cottonseed, soybean, peanuts and canola (TSI, 2015). The production of 2.7 million tons from these top five plants in 2010 increased by 19% with a production of 3.25 million tons in 2014. In 2010-2014 periods, the sunflower (for oil) production growth rate is approximately realized as % 26.4. In the same period, the rate of increase in sunflower production of the world is approximately 20.3 %. According to the data from USDA published by AVOI, world crude vegetable oil production is around 176 million

tones. Crude sunflower oil production in the years 2010-2014 has increased to 15 million tons from 12 million tons (AVOI, 2015).

Table 1: Turkey sunflower oil production data (TSI, 2016)

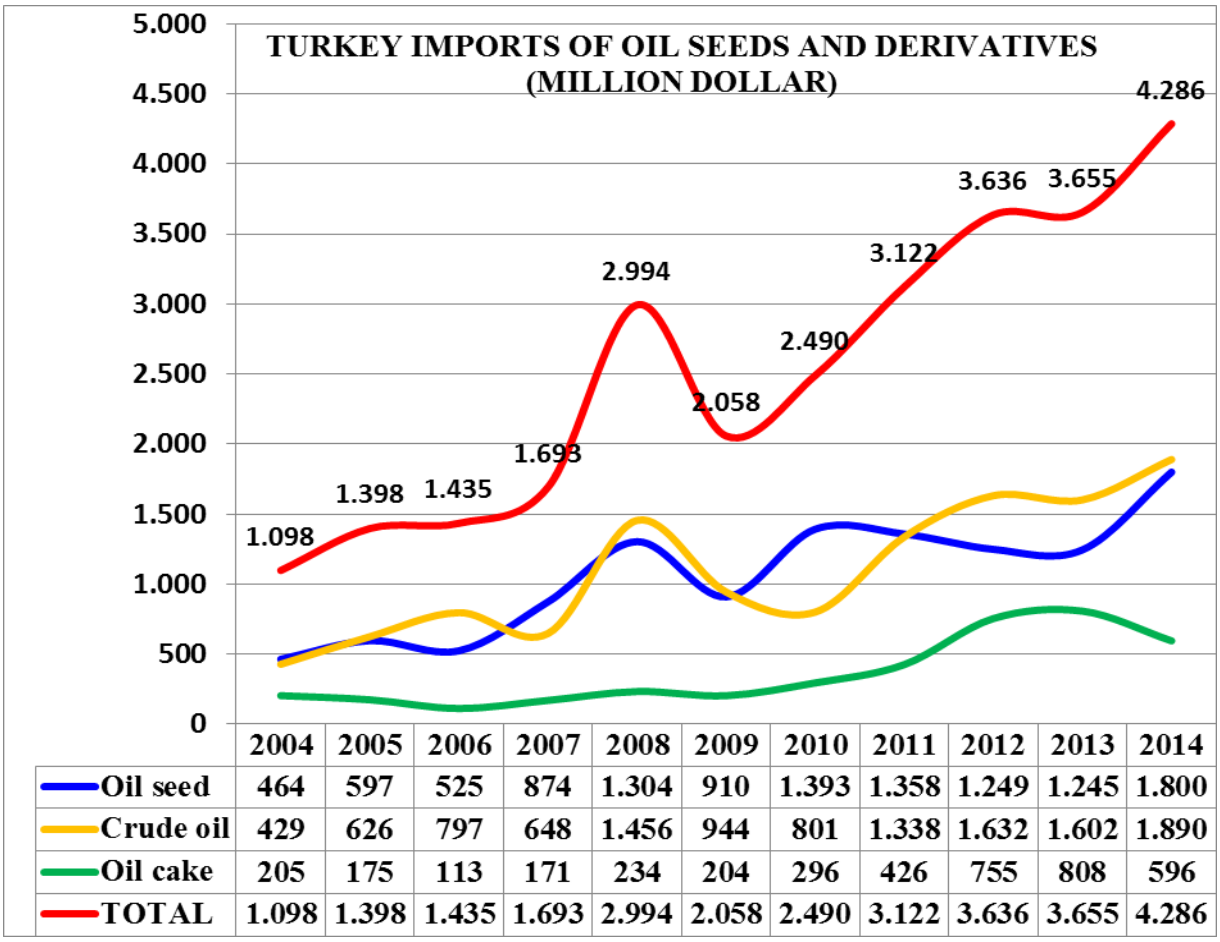
Year	Area(da)	Product(t)	Yield(kg/da)
2006	5,100,000	1,010,000	198
2007	4,857,000	770,000	159
2008	5,100,000	900,387	177
2009	5,150,000	960,300	186
2010	5,514,000	1,170,000	212
2011	5,560,000	1,170,000	210
2012	5,046,160	1,200,000	238
2013	5,202,600	1,380,000	265
2014	5,524,651	1,480,000	269
2015	5,689,013	1,500,000	264

During the last decade of sunflower cultivation (2006-2015), an increase of approximately 12% in the harvested areas, % 48.5 in the amount of production and 33% in the yield has been achieved. Turkey continuously increases the supply for increased consumption of crude vegetable oil production but it is not enough to cover domestic demand. The most important raw material in crude oil production industry has been provided from the production of sunflower oil in Turkey. But a serious vulnerability exists due to domestic consumption and exports vegetable oil in our country. Therefore, every year, vegetable oil imports are made.

Table 2: Crude sunflower oil balance of supply-demand for the season 2015/2016

Sunflower oil production from the domestic harvest	**430 thousand tons**
Sunflower oil consumption in Turkey	900 thousand tons
Turkey sunflower oil exports	500 thousand tons
Total sunflower oil demand	1,400 thousand tons
Deficient of based on sunflower	970 thousand tons

According to the Trakya Birlik sources, we have a deficient of approximately 970 thousand tons of sunflower oil on an annual basis (Tekçe, 2015). The main strategy of our country is to produce demanded oily seeds and to become a self-sufficient state by reducing imports as much as possible (Kolsarıcı et al, 2015).



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Oil seed	464	597	525	874	1.304	910	1.393	1.358	1.249	1.245	1.800
Crude oil	429	626	797	648	1.456	944	801	1.338	1.632	1.602	1.890
Oil cake	205	175	113	171	234	204	296	426	755	808	596
TOTAL	1.098	1.398	1.435	1.693	2.994	2.058	2.490	3.122	3.636	3.655	4.286

Figure 20: Turkey imports of oil seeds and derivatives (million dollar)

According to the Association of Vegetable Oil Industries report; imports of oilseeds and their derivatives were 4,286 million dollars in 2014. Due to increasing demand, the rate of imports has been increasing every year.

Sunflower (*Helianthus annus* L.) has a large natural habitat in various parts of our country. At sunflower cultivation (for oil) dry farming is applied extensively in our country. In regions where irrigation opportunities are available, a significant increase in yield can be achieved. Sunflower can grow in all geographies and adapt to different climatic conditions, however it also can be affected by changing climate conditions. Today, especially last quarter of the 20th century, climate change has become an important problem worldwide. The rising in global mean temperatures since 1850s is the most important indicator of climate change.

According to the World Meteorological Organization (WMO) resource; the global average surface temperature in 2015 broke all previous records by a strikingly wide margin, at 0.76±0.1° Celsius above the 1961-1990 average. Fifteen of the 16 hottest years on record have all been this century, with 2015 being significantly warmer than the record-level temperatures seen in 2014. Underlining the long-term trend, 2011-15 is the warmest five-year period on record. The record temperatures over both land and the ocean surface in 2015 were accompanied by many extreme weather events such as heatwaves, flooding and severe drought (WMO, 2016).

According to "State Of The Climate in Turkey in 2015" report; Turkey annual mean temperature in 2015 has been 14.3°C. This value is 0.8°C above from 1981-2010 normal (13.5°C). This makes 2015 the fifth warmest year since 1971 (TSMS, 2016).

TSMS published as a report which name is "Climate Projections with New Scenarios for Turkey and Climate Change (TR2015-CC)" in 2015. This report includes temperature and precipitation projections of three Global Climate Models (GCM) based on scenarios of RCP4.5 and RCP8.5.

In this study, the results of HadGEM2-ES, which is one of the three GCMs mentioned above, will be shared. HadGEM2-ES projections based on scenarios of RCP8.5 shows that;

- Average temperature of overall Turkey is expected to increase between 0.9-7.1°C and an average of 3.6 °C in the period of 2016-2099.
- Positive anomalies are expected at the amount of precipitation based on RCP8.5 by the end of 2035 but it is also estimated that decreases may occur in subsequent periods (Akçakaya et all., 2015).

This study was carried out in order to determine the possible effects of the future climate changes on the sunflower yield.

MATERIAL and METHODS

Material

Sunflower Production Data

10 provinces, with intensive production of sunflower in the Marmara region, were evaluated (TSI, 2015). Evaluated 10 provinces are: Balıkesir, Bilecik, Bursa, Çanakkale, Edirne, İstanbul, Kırklareli, Kocaeli, Sakarya ve Tekirdağ. In order to confirm the relationship between climate factors and the yield the period of 1985-2014 (30 years) have been analyzed.

The Meteorological Parameters

Climate, sometimes understood as the "average weather," is defined as the measurement of the mean and variability of relevant quantities of certain variables (such as temperature, precipitation or wind) over a period of time, ranging from months to thousands or millions of years. The classical period is 30 years, as defined by the World Meteorological Organization (WMO). Therefore, 30 years of meteorological parameters are preferred in order to determine relationship between crop yield and meteorological parameters. In the study, the parameters that are thought to have an effect on the yield of sunflower were selected. The data of selected meteorological parameters were obtained from TSMS. In order to determine the relationship between yield – climatic factors;

- The number of days daily minimum temperature < -5 ° C,
- Monthly Average Temperature (° C)
- The number of days daily maximum temperature > 35 ° C,
- Monthly average relative humidity (%)
- The number of days daily average relative humidity > %70,
- Monthly Total Sunshine Duration (hour)
- Monthly Total Precipitation (mm) parameters are preferred.

Projection Data of HadGEM2-ES Global Climate Model

Climate modeling is the most important work for predicting the future climate (Demircan et all., 2014). Nowadays climate modeling studies are performed in order to determine the possible effect of climate change in future periods. Turkey is located in the eastern Mediterranean basin, one of the most vulnerable regions to climate change as stated in the IPCC report (Gürkan H. et all., 2015). In Turkey, climate modeling studies have been conducted within TSMS and the final results have been shared in 2015.

HadGEM2-ES GCM projection data based on RCP8.5 for the chosen meteorological parameters' has been published as a report titled "Climate Projections with New Scenarios for Turkey and Climate Change". In this study, these projection data is used.

According to sources at TSMS; HadGEM2-ES is the second generation Global Climate Model which is developed by the Hadley Centre that is related with UK Meteorological Service-Met Office (TSMS 2013).

Methods

Correlation Analysis

In the first section of study, the relationship between meteorological parameters that occurred between the years 1985-2014 and the sunflower yield values between these years were determined by the method of multiple correlation analysis.

r: Correlation Coefficient
X: Independence Variable
Y: Dependence Variable

$$R_{Y.X_1X_2} = \sqrt{\frac{r^2_{YX_1} + r^2_{YX_2} - 2r_{YX_1}.r_{YX_2}.r_{X_1X_2}}{1 - r^2_{X_1X_2}}}$$

Regression Analysis

In the second part of the study, first of all, provinces based regression equations were established using selected seven climate parameters and sunflower yield values between the years 1985-2014 with the method of least squares (LSM) for 10 provinces. Secondly, the potential impact of climate changes that are projected for the future periods (2016-2040, 2041-

2070 and 2071-2099), on yield of sunflower have been put forward with using the generated high-rate regression equations and climate projection data.

In the study, analysis of the regression equation generated by LSM as follows:

$y = As + Bp+Ch+Dk+Et+Fm+Gv+H$

Dependence Variable;
y = Yield

Independence Variables;
s = Monthly Total Sunshine Duration (hour)
p = Monthly Total Precipitation (mm)
h = Monthly average relative humidity (%)
k = The number of days daily average relative humidity > %70
t = Monthly Average Temperature (° C)
m = The number of days daily maximum temperature > 35 ° C
v = The number of days daily minimum temperature < -5 ° C

A, B, C, D, E, F, G, H = Coefficients

The coefficients of the linear multiple regression equation generated on a provincial basis obtained by solving the following matrix formed by the method of least squares.

$Z*X = W$
$Z^{-1}*W = X$ X matrix: Coefficients

Table 3: Parameter matrix used in the least squares method

Z matrix									W matrix		X matrix
$\sum s_i^2$	$\sum p_i s_i$	$\sum h_i s_i$	$\sum k_i s_i$	$\sum t_i s_i$	$\sum m_i s_i$	$\sum v_i s_i$	$\sum s_i$		$\sum s_i$		A
$\sum s_i p_i$	$\sum p_i^2$	$\sum h_i p_i$	$\sum k_i p_i$	$\sum t_i p_i$	$\sum m_i p_i$	$\sum v_i p_i$	$\sum p_i$		$\sum p_i$		B
$\sum s_i h_i$	$\sum p_i h_i$	$\sum h_i^2$	$\sum k_i h_i$	$\sum t_i h_i$	$\sum m_i h_i$	$\sum v_i h_i$	$\sum h_i$		$\sum h_i$		C
$\sum s_i k_i$	$\sum p_i k_i$	$\sum h_i k_i$	$\sum k_i^2$	$\sum t_i k_i$	$\sum m_i k_i$	$\sum v_i k_i$	$\sum k_i$		$\sum k_i$		D
$\sum s_i t_i$	$\sum p_i t_i$	$\sum h_i t_i$	$\sum k_i t_i$	$\sum t_i^2$	$\sum m_i t_i$	$\sum v_i t_i$	$\sum t_i$		$\sum t_i$		E
$\sum s_i m_i$	$\sum p_i m_i$	$\sum h_i m_i$	$\sum k_i m_i$	$\sum t_i m_i$	$\sum m_i^2$	$\sum v_i m_i$	$\sum m_i$		$\sum m_i$		F
$\sum s_i v_i$	$\sum p_i v_i$	$\sum h_i v_i$	$\sum k_i v_i$	$\sum t_i v_i$	$\sum m_i v_i$	$\sum v_i^2$	$\sum v_i$		$\sum v_i$		G
$\sum s_i$	$\sum p_i$	$\sum h_i$	$\sum k_i$	$\sum t_i$	$\sum m_i$	$\sum v_i$	n*		$\sum v_i$		H

*****Number of the years

RESULTS AND DISCUSSION

In the first part of the research, multiple correlation analyses were conducted between the yield and meteorological parameters, due to the effect of meteorological parameters on yield as a whole. According to the results of multiple correlation analysis; the highest correlation between yield - meteorological parameters in Bilecik (0.62) and the lowest correlation have been identified in the province of Sakarya (0.36).

In the second part, it is aimed to assess the quality of the relationship between variables with using the method of regression analysis. Regression analysis that were performed with LSM; the highest value in Kırklareli (0.80) and the lowest value is determined in Sakarya (0.60). This situation is an indicator that the relationship between yields - climate factors can be modeled and can be converted to the equation.

Table 4: Provincial-based multiple correlation and multiple regression analysis

Provinces	Multiple Correlation	Multiple Regression
Balıkesir	0.55	0.74
Bilecik	0.62	0.79
Bursa	0.49	0.70
Çanakkale	0.56	0.75
Edirne	0.55	0.74
İstanbul	0.39	0.62
Kırklareli	0.65	0.80
Kocaeli	0.38	0.62
Sakarya	0.36	0.60
Tekirdağ	0.51	0.65

In the last part of the research, the potential impact of climate changes, that are projected for the future periods (2016-2040, 2041-2070 and 2071-2099), on yield of sunflower have been put forward with by using the generated high-rate regression equations and climate projection data.

As climate projection data, HadGEM2-ES Global Climate Model data (20 km resolution) based on RCP8.5 were used which is belongs to named “Climate Projections with New Scenarios for Turkey and Climate Change” released by TSMS.

Table 5: Provincial based sunflower (for oil) yield predictions

SUNFLOWER (FOR OIL) YIELD PREDICTIONS				
Provinces	Yield of Reference Period (kg/da)	Yield Change in Future Periods (%)		
	1985-2014	2016-2040	2041-2070	2071-2099
Balıkesir	111	15	-5	-28
Bilecik	107	15	19	-41
Bursa	147	-24	-14	-23
Çanakkale	171	-22	-9	19
Edirne	170	26	55	85
İstanbul	171	53	32	-33
Kırklareli	164	0	6	26
Kocaeli	129	-16	-9	-7
Sakarya	136	-21	9	31
Tekirdağ	179	-26	-41	-51

CONCLUSION

There is a high correlation between climatic factors and yield. Due to lack of individual determining factor, single correlation analysis is not very meaningful between climatic factors-yield. Therefore, level of relationship between climate factors-yield can be determined in a healthy way with multiple correlation analysis (Bulut et all., 2016).

In Marmara region; According to the assessment results of the researched 10 provinces; the region is expected to be affected adversely by climate changes in the future periods. Correspondingly, a decrease in the average yield of sunflower is estimated in the future. Increase in the number of days daily maximum temperature > 35 ° C is estimated to have a negative effect on yield by adversely affecting the pollination period of plants.

According to compared results of the yield prediction analysis performed on a regular basis with average yield values of the period of 1985-2014;

- In the period 2016-2040; decrease in 5 provinces, increase in 4 provinces and there will be any change in 1 province,
- In the period 2041-2070; decrease in 5 provinces, increase in 5 provinces,
- In the period 2071-2099; decrease in 6 provinces and increase in 4 provinces expected.

The province of Edirne is expected to be positively affected by possible climate changes in the future periods. Besides, the province of Tekirdağ is expected to be negatively affected by possible climate changes in the future periods.

As a result, it has been revealed that climate factors although they are not the sole determining factor, have significant effects on yield of sunflower. According to the results of this analysis, it is concluded that particularly temperature and humidity parameters have serious impact on the yield of sunflower.

According to yield estimates that using HadGEM2-ES global climate model projections which is based on RCP8.5 scenario; sunflower farming regions will be affected by climate changes likely to occur in future periods.

The results of this research can be used as a substrate in studies to determine the relationship by taking all the factors affecting the yield of sunflower. Also the results of this research can be used in future product planning across the country or on a regional basis and in the determination of regions that can be encouraged.

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NEW SEED TREATMENT SOLUTIONS FOR PLASMOSPORA RESISTANCE MANAGEMENT IN SUNFLOWER

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ABSTRACT

Seed treatment products help to assure a good crop establishment and reduce primary systemic infections from soil born zoospores of *Plasmopara halstedii* which result in plant loss or stunted plants. Downy mildew fungi are considered as high risk pathogens in term of becoming resistant to fungicides and therefore special attention has to be paid to minimize risks using different control methods like resistant sunflower varieties, reducing disease risk via crop rotation sunflowers and using seed treatments. Currently there are molecules from the chemical classes of phenylamides and strobilurins used as seed treatment in sunflowers. As the pathogen and as well as the chemicals are known to be at high risk to develop resistance there is a need to find new chemicals with different mode of actions to maintain high level of downy mildew control under field conditions. Field trials were set up in a number of countries in Europe, LATAM and USA to evaluate the level of activity in randomized complete block small plot trials. While Mefenoxam good protection of the young plants against downy mildew in field where *P. halstedii* was sensitive to phenylamides, Mefenoxam did not provide sufficient control in fields where *P. halstedii* was resistant to phenylamides. Two new molecules, each one with a new mode of action, were tested in the field under conditions of either sensitive or resistance *P. halstedii* races. In both cases a significant higher level of control *P. halstedii* over Mefenoxam alone was observed in all geographies. A combination of the new mode of actions with resistant sunflower traits will provide the best level of *P. halstedii* control while ensuring a sustainable approach to *P. halstedii* resistance management.

Key Words : Plasmospara seed treatment resistance management

MODELING SUNFLOWER FUNGAL COMPLEX TO HELP DESIGN INTEGRATED PEST MANAGEMENT STRATEGIES

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ABSTRACT

Sunflower is submitted to several major pathogens. Modeling is a key tool to help design Integrated Pest Management strategies to control them. A new qualitative modelling approach is currently under progress using the IPSIM platform (Aubertot and Robin, 2013) for sunflower. It aims at predicting injury profiles on sunflower as a function of cropping practices, soil, weather, and the surroundings of the considered field. Based on a literature review and expert knowledge, hierarchical deterministic bayesian networks were developed. Independent datasets were used to assess their quality of prediction. This communication will present: i) a first draft of IPSIM-Sunflower; ii) the evaluation of its predictive quality; iii) examples of simulation to help design IPM strategies to control the disease; iv) a discussion on the limits and benefits of the approach, along with perspectives.

Key Words *: Helianthus annuus, Phoma macdonaldii, Phomopsis helianthi, Plasmopara halstedii, Sclerotinia sclerotiorum*, cultural control

APPROPRIATE NITROGEN (N) AND PHOSPHORUS (P) FERTILIZER REGIME FOR SUNFLOWER (*HELIANTHUS ANNUUS* L.) IN THE HUMID TROPICS

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ABSTRACT

Application of fertilizer at the appropriate rate and time is very germane to sustainable production of crops. Two field trials were conducted on the Research Farm of the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Nigeria during the late cropping seasons (June – Nov.) of 2014 and 2015 to evaluate the agronomic performance of four recently released sunflower varieties (SAMSUN-1, SMASUN-2, SAMSUN-3 and SAMSUN-4) to three fertilizer regimes: Control, Split application of 30 kg N + 28 kg P_2O_5 at 3 week after sowing, WAS and at anthesis and Single application of 60 kg N and 56 kg P_2O_5 at 3WAS. The experiment was laid out in a randomized complete block design using a 3× 4 factorial arrangement and replicated three times. Data were collected on phenology, height at flowering (R5) and physiological maturity (R9), grain yield and yield attributes. Varietal effect was only significant in 2015 with SAMSUN-2 recording significantly (*P<0.05*) higher head weight than the other varieties. Application of N and P fertilizer either as split or single significantly (*P<0.05; F - test*) affected plant height at R5 and R9, 100 achene weight, achene weight per head and grain yield in both years. Single application resulted in significantly (*P<0.05*) higher grain yield in 2014 than the split and control and was on par with split, and superior to control in 2015. Therefore, single application of N and P fertilizers at 21 WAS is recommended for adoption in the humid tropics for the newly released four sunflower varieties.

Keywords: grain yield, nitrogen (N), phosphorus (P), regime, sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a major contributor to edible vegetable oil in the world market (Thavaprakash *et al.*, 2002). As at January, 2016, the total world area under sunflower was 24.7 m ha with an average yield of 1.67 tonnes per ha (NSA, 2016). The three leading world sunflower producers were Ukraine, Russia and European Union. However, sunflower grain yield had been static at 1.68 tonnes/ha between 2013/14 and

2014/15 and the area under sunflower cultivation also reduced by 6% during the same period (NSA 2016). The non-significant increase in grain yield despite the use of different inputs (improved seeds, inorganic and organic fertilizers e.t.c) could be partly attributed to inappropriate use of some of the inputs. Sunflower is not a common oilseed crop in the tropics even though it is a very rustic crop that can produce optimally under diverse agro-ecological conditions. Its production potential had however, been confirmed in the forest – savanna transition zone of the humid tropics (Olowe *et al.*, 2005a; Olowe and Adeyemo, 2009). Commercial fertilizers are usually used to boost the yield output of sunflower. According to Heffer (2013), the global application of fertilizers to oilseed crops was estimated at 19.0 Mt or 11.0% of the world consumption. The breakdown of this consumption stood at 7.3%, 14.7% and 19.8% of the world's total consumption of nitrogen, phosphorus and potassium fertilizers, respectively. However, it is very important that farmers apply the fertilizers at the appropriate rate and time in order to get the maximum output from the commodity being produced. The utilization of nutrients by sunflower varies depending on the stage of development of the crop. Sunflower utilizes the bulk of applied nitrogen from beginning of terminal bud appearance (R1 – R2) to end of anthesis (R6), phosphorus from emergence (VE) to R6 and potassium from R1 to ripening (Ustimenko-Bakumovski, 1980).

Research results have demonstrated increased productivity of sunflower through the application of mineral fertilizers that contain the major plant nutrients (nitrogen, phosphorus and potassium) in balanced quantities (Singh *et al.,* 1977; Noor-Mohammed and Ehdaie, 1979 and Ogunremi, 1984 and 1986; Nassim *et al.,* 2012a, 2012b) and organic fertilizers (Rasool *et al.,* 2013; Oshudiya *et al.*, 2014). The optimum rates of the major nutrients especially nitrogen vary across different ecological zones such as 90kg N/ha in the lowland areas of Nigeria (Ogunremi, 2000) and 60 kg N/ha in the derived savanna zone of Nigeria (Olowe *et al.*, 2005), 80 kg N/ha in India (Faisul-ur-aRasool *et el.*, 2013), 150 kg N/ha at Islamabad, Pakistan (Bakht *et al.*, 2010), and 180 kgN/ha at Faisalabad, Pakistan (Nasim *et al.*, 2012b). The application of different fertilizers (inorganic and organic) to sunflower either as single or split vary depending on agro-ecology and farming system being practiced. From literature, fertilizers have been applied to sunflower at planting (Zubriski and Zimmermann, 1974; Ogunremi, 1984), as basal application before planting (Bahl *et al.*, 1997), basal application of phosphorus and potassium and nitrogen at four weeks after sowing (Ogunremi, 2000), three weeks after planting to coincide with first weeding (Ogunremi, 1984 & 1986; Olowe *et al.*, 2005; Oshundiya *et al.,* 2014) or at advanced vegetative stages (Yousaf *et al.*, 1986). Earlier study on the appropriate timing of nitrogen and phosphorus fertilizers to sunflower in the forest – savanna zone revealed that single application at 21 days after sowing was optimal for local and two exotic varieties of sunflower. This study was carried out to evaluate the agronomic response of four newly released sunflower varieties (SAMSUN-1, SAMSUN-2, SAMSUN-3 and SAMSUN-4) by NASC (2013) to single and split application of nitrogen and phosphorus fertilizers in the forest-savanna transition zone of the humid tropics.

MATERIALS AND METHODS

Two field trials were carried out at the Institute of Food Security Environmental Resources and Agricultural Research (IFSERAR) Farm of the Federal University of Agriculture, Abeokuta (7º 23´ N, 3º 39´ E, altitude 139 m above sea level) in south western Nigeria on a loamy sand soil between June and November, 2014 and 2015. The soils belonged to the loamy sand textural class and were low in nitrogen, medium in phosphorus and potassium based on the rating of Anon (1989). The months of September and October were the two wettest months in both years. The coolest and hottest months were August (25.3 & 26.3ºC) and November (27.5 & 28.6ºC) in 2014 and 2015, respectively. Relative humidity was slightly above 70% during the wettest months (September and October) of both years, except 2015.

The trials were a 4 × 3 factorial arrangement laid out in randomized complete block design and replicated three times. The factors were variety: SAMSUN-1, SAMSUN-2, SAMSUN-3 and SAMSUN-4 and fertilizer regime: control, split application (30 kgN/ha + 28 kgP_2O_5/ha at 21 days after sowing, DAS and at anthesis, and single application of 60 kg N/ha + 56 kgP_2O_5/ha at 21 DAS. Each plot measured 4m x 1.8m (7.2m2) and consisted of four rows.

In each year of experimentation, the site of the experiment was ploughed twice and harrowed once. Sunflower seeds were sown at a spacing of 60 cm x 30 cm giving 56,000 plants/ha. Sowing was done on June 27, 2014 and August 7, 2015 during the late cropping seasons. Thinning to one plant per stand was done at two weeks after sowing (WAS). The sources of fertilizers used in the study were urea fertilizer (46%N), single superphosphate (18.5% P_2O_5) and muriate of potash (62%K_2O). The recommended rate of 100 kgK_2O (Ogunemi, 2000) was applied on all the fertilized plots along with N and P fertilizers at 21 DAS. Weeds were controlled manually at 3 and 6 WAS and no herbicides were sprayed in order to simulate the growing conditions of the resource-constrained farmers.

After the first weeding at 3 WAS, five randomly selected plants were tagged in the two middle rows for plant height and yield attributes measurement at maturity. Parameters measured on plot basis were number of phonological days to flowering (R5) and physiological maturity (R9) as described by Schnieter and Miller (1981), plant height (cm) at R5 and R9, head diameter (cm), head weight (g), number and weight (g) of seeds per head, 100 seed weight (g), Shelling percent (5) and seed yield (kg/ha).

All data collected on plot basis were analysed using the MASTAC package (Freed *et al.*, 1989). The treatment means of the main effects and interactions that were found significant were then separated using the least significant difference method (LSD) at 5% probability level.

RESULTS

Effect of nitrogen (N) and phosphorus (P) fertilizer regime on phenology and height characteristics of four sunflower varieties

Fertilizer regime significantly ($P \leq 0.05$; *F*-test) affected plant height of sunflower at flowering and physiological maturity in 2014 and 2015. Application of N and P fertilizers either as split or single dose significantly ($P \leq 0.05$) increased sunflower plant height at flowering and physiological maturity relative to the control treatment in both years. However, fertilizer regime had no significant effect on number of phonological days to flowering and physiological maturity of sunflower in both years. Similarly, variety and Variety × Fertilizer regime effects were not significant on number of phonological days to flowering and physiological maturity and height at R5 and R9 in both years (Table 3).

Effect of nitrogen (N) and phosphorus (P) fertilizer regime on seed yield and yield attributes of four sunflower varieties

Fertilizer regime significantly ($P \leq 0.05$; *F*-test) number of seeds per head, seed weight per head, 100 seed weight and seed yield of sunflower in both years and average head diameter and head weight, and threshing percent of sunflower in 2015 (Table 4 and 5). Split and single application of N and P fertilizers to sunflower resulted in significantly ($P \leq 0.05$) higher values for the parameters relative to the control treatment, except seed yield in 2014. Fertilizer regime had no significant effect on head diameter, average head weight and threshing percent of sunflower in 2014. Variety effect was only significant ($P \leq 0.05$; *F*-test) on average head weight, number of seeds per head and 100 seed weight of sunflower in 2015. However, in 2014, variety effect was not significant on any trait measured. Similarly, Variety × Fertilizer regime effect did not affect seed yield and any yield attribute of sunflower significantly in both years.

DISCUSSION

Adoption of the appropriate fertilizer regime is very crucial for successful sunflower cultivation and the performance of the crop depends largely on the prevailing weather conditions. The late cropping season of 2014 was wetter (610.2 mm) than that of 2015 (370.0 mm). This scenario apparently contributed to the better overall performance of sunflower in 2014 than 2015. Application of N and P fertilizers either as split or single regime significantly ($P \leq 0.05$; *F*-test) enhanced plant height of sunflower relative to the control at R5 and R9. The availability of N on the fertilized plots apparently boosted plant growth. No significant variety effect was recorded in 2014 for sunflower on seed yield and yield attributes. All the four new varieties were able to express themselves very well under the wetter growth conditions of 2014 than 2015. In an earlier trial where two exotic varieties (Record and Isaanka) and Funtua (locally adapted variety) were subjected to similar fertilizer regimes, Funtua grew taller than the exotic varieties (Olowe *et al*., 2005b). However, during the hotter and drier late cropping season of 2015, the varieties were significantly ($P \leq 0.05$; *F*-test) different for head weight , number of seeds per head and 100 seed weight. SAMSUN-1 and SAMSUN-2 recorded significantly ($P < 0.05$) higher head weight and number of seeds per head than SAMSUN-4. However, these differences did not translate to significant seed yield among the varieties.

Among the yield attributes evaluated in our study, split and single application of N and P significantly ($P \leq 0.05$; *F*-test) enhanced only 100 seed weight relative to the control in

2014. However, in 2015 all the yield attributes were significantly ($P \leq 0.05$; *F*-test) enhanced by split and single application of N and P fertilizers with the split application regime resulting in higher values for most traits. According to earlier reports, application of N up to 60 kg N/ha either as split or single significantly ($P < 0.05$) increased head diameter, seed weight per head and 1000 seed weight (Olowe *et al.*, 2005b) and 112 kg N/ha head diameter (Yousaf *et al*., 1986).

According to Robinson (1978), the seed yield of sunflower is highly dependent on number of heads per hectare, number of seeds (achene) and weight per head. The fertilizer regimes evaluated in our study significantly affected these traits in both years, except number of heads per hectare which was not calculated in our study. The seed yield values (1246 – 1994.2 kg/ha) recorded in our study during the wetter and more favourable 2014 compared very well with Nigerian (1000 kg/ha), African (812 kg/ha) averages (Olowe *et al.*, 2013) and world average (1520 kg/ha) according to USDA (2012), and the more recent forecast (1410 kg/ha) for 2012/2013 by NSA (2016). However, the imposed fertilizer regimes under drier and hotter 2015 conditions resulted in seed yield values at par with only the African average (812 kg/ha). The seed yield values recorded under split and single application of N and P fertilizers were at par in both years with the split regime resulting in slightly higher value in 2015. This trend also corroborated the results of earlier experiments on sunflower (Singh *et al*., 1977, Noor-Mohammed and Ehdaie, 1979, Ogunremi, 1984 and 1986, Olowe *et al*., 2005b).

On average, the superior performance of the sunflower varieties grown on the fertilized plots over those on the control plots suggest that they had access to the three major macronutrients (N,P and K) and these nutrients apparently contributed to their growth and development on the relatively fertile experimental soils. Lack of significant Variety × Fertilizer regime interaction in this two year study suggest that the two factors were independent of each other.

CONCLUSION

The results of this two year study indicate that the growth and seed yield responses of four newly released sunflower varieties were at par when N and P fertilizers were applied as split or single regime, and were superior to plants on the control plots. Consequently, it is recommended that single regime application of fertilizers at three weeks after sowing be adopted in the forest – savanna transition zone of the humid tropics.

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Table 1: Number of phonological days to flowering (R5) and physiological maturity (R9), and plant height of sunflower as influenced by variety and fertilizer regime in 2014 and 2015

Treatment	2014				2015			
	Days to		Height (cm) at		Days to		Height (cm) at	
	R5	R9	R5	R9	R5	R9	R5	R9
Variety (V)								
SAMSUN-1	60.7	161.6	103.0	174.8	99.6	148.6	70.6	109.7
SAMSUN-2	60.9	148.9	103.2	161.8	107.3	147.2	71.1	109.4
SAMSUN-3	60.2	155.3	103.0	173.7	114.2	164.9	71.0	110.1
SAMSUN-4	60.3	157.2	102.7	174.3	106.4	158.9	70.2	110.8
LSD 5%	ns		ns		ns	ns	ns	ns
Fertilizer Regime (FR)								
Control	59.8	141.7	102.2	156.6	98.6	144.0	71.0	109.9
Split	60.9	164.8	103.1	180.7	115.6	168.9	70.4	109.8
Single	60.9	160.7	103.5	176.2	106.3	151.7	70.7	110.3
LSD 5%	ns	8.16**	ns	10.79**	13.31*	20.32*	ns	ns
Interaction V × FR	ns	ns	ns	ns	ns	ns	ns	ns

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 2: Sunflower seed yield and yield attributes as influenced by variety and fertilizer regime in 2014

Treatment	Head diameter (cm)	Head weight (g)	No. of seeds per head	Seed weight per head (g)	100 seed weight (g)	Threshing percent (%)	Seed yield (kg/ha)
Variety (V)							
SAMSUN-1	12.4	326.8	442.4	39.4	6.2	16.2	1746.9
SAMSUN-2	11.4	365.5	410.9	34.2	4.9	9.0	1527.4
SAMSUN-3	12.0	347.8	341.9	36.8	4.7	8.7	1652.2
SAMSUN-4	11.1	357.4	423.4	36.3	4.5	10.6	1595.5
LSD 5%	ns	ns	ns	ns	ns	ns	ns
Fertilizer Regime (FR)							
Control	11.1	296.5	352.4	27.8	3.9	10.3	1246.9
Split	12.3	361.8	411.8	36.7	5.8	10.2	1650.4
Single	11.9	389.9	449.8	45.5	5.6	12.9	1994.2
LSD 5%	ns	ns	79.19*	9.97**	1.54*	ns	425.94
Interaction							
V × FR	ns	ns	ns	ns	ns	ns	ns

Notes: **, * Significant at P $\leq$ 0.001 and 0.05, respectively, ns – non-significant

Table 3: Sunflower seed yield and yield attributes as influenced by variety and fertilizer regime in 2015

Treatment	Head diameter (cm)	Head weight (g)	No. of seeds per head	Seed weight per head (g)	100 seed weight (g)	Threshing percent (%)	Seed yield (kg/ha)
Variety (V)							
SAMSUN-1	8.4	22.3	440.2	10.8	2.5	49.5	506.1
SAMSUN-2	7.8	22.9	413.6	12.0	3.0	50.2	506.7
SAMSUN-3	7.5	16.5	442.5	11.3	3.1	63.9	606.5
SAMSUN-4	6.8	17.1	192.6	8.0	4.2	46.2	599.3
LSD 5%	ns	4.80*	163.99*	ns	1.19*	ns	ns
Fertilizer Regime (FR)							
Control	6.6	13.5	227.3	6.7	2.8	41.4	361.9
Split	8.5	26.3	463.3	14.2	4.0	60.9	704.9
Single	7.8	19.2	426.0	10.6	2.8	55.1	597.0
LSD 5%	1.04**	4.16**	142.0**	2.71**	1.04*	13.09*	126.67**
Interaction							
V × FR	ns	ns	ns	ns	ns	ns	ns

Notes: **, * Significant at P $\leq$ 0.001 and 0.05, respectively, ns – non-significant

INTERACTIVE EFFECTS OF DIFFERENT INTRA-ROW SPACING AND NITROGEN LEVELS ON YIELD AND YIELD COMPONENTS OF CONFECTIONERY SUNFLOWER (*HELIANTHUS ANNUUS* L.) GENOTYPE (ALACA) UNDER ANKARA CONDITIONS

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ABSTRACT

This research was conducted at the experimental field of Department of Field Crops, Faculty of Agriculture, University of Ankara in 2007 and 2008. Experiment was carreied out to study the effects of different intra-row spacing and N (nitrogen) levels on yield performence of confectionery sunflower genotype. Seeds of Alaca genotype and ammonium sulfate (%21 N) were used as materials. The aim of the research was to determine the effects of different intra-row spacing (20, 30 and 40 cm) and N levels (0, 40, 80 and 120 kg ha^{-1}) on yield and yield components of confectionery sunflower genotype (Alaca). The experiment was laid on "Randomized Complete Block Design" as split plots with three replications. The highest seed yield obtained from 20 cm intra-row spacing and 120 kg ha^{-1} and the score were 243.3 kg da^{-1} and 315.3 kg da^{-1} in 2007 and 2008. N level increased the 1000 seed weight. In the year with irregular rainfall (2007) in the vegetation stage the highest 1000 seed weight score was obtained as 129.6 g in 40 kg ha^{-1} N treatment whereas in the year with normal rainfall distrubution the highest 1000 seed weight score was obtained as 113.4 in 80 kg ha^{-1}. Results revealed that; decreasing intra-row spacing led to decrease in seed yield per head but increase of the seed yield. However, increasing plant population caused to small seeds and this feature is not required for confectionery varieties. For this reason, 30 cm intra-row spacing and 80 kg ha^{-1} N is appropriate for Alaca genotype.

Key words: Confectionery sunflower, plant population, N doses

INTRODUCTION

Confectionery sunflower is one of the most common crop cultivated in Cenral Anatolia that has a larger head size compared to oilseed sunflower. The total cultivation area of confectionery sunflower is 104.992 ha in Turkey and 35.783 ha of this cultivation area is in Central Anatolia. It is an important cash crop due to its used for birdfeed and human consumption. The impact of plant density and N supply on sunflower yield and yield component have been studied previously with the aim to determine appropriate plant population and N levels for sunflower production (Zubriski and Zimmerman 1974, Kıllı 2004). Several researchers observed that plant spacing has an importance to enhance the yield of sunflower (Robinson et al. 1980, Redy et al. 1997, Jahangir et al. 2006). N one of the limiting factor in sunflower growth play an important role on yield (Montemurro and De Giorgio 2005). Inadequate N supply in the soil has an negative impact on vegetative and generative growth and induces premature senescence which leads yield loss (Narwal and Malik 1985, Khokani et al. 1993, Legha and Giri 1999, Tomar et al. 1999). Contrarily, high N

supply may delay crop maturity and reduce seed yield (Farah et al. 1981, Hocking et al. 1987, Özer et al. 2004). Also overfertilization with N is one of the main contamination reason of groundwater with nitrates (Magdoff et al. 1997; Strong, 1995, Scheiner et al. 2002). It is important to adjust N for different varieties and species to avoid excessive N fertilization.

In Central Anatolia, confectionery sunflower is grown under irrigated or rainfed conditions. Confectionery sunflower yield (83 kg da^{-1}) under rainfed condition is very low (Day and Kolsarıcı, 2014) compare to average yield (109 kg da^{-1}) of confectionery sunflower under irrigated conditions. However agro-techniques used in the region under irrigated conditions are comparatively poor. Plant density and proper fertilizer application to soil are necessary for optimum yield of crops. There is very limited information on confectionery sunflower (*Helianthus annuus* L.) response to different intra-row spacing and N in comparison to oil type sunflower. However, there is no published data available on plant population and N rate response on yield of confectionery sunflower in Central Anatolian region. Keeping in view, the present study was designed with objectives to investigate the role of different intra-row spacing and N levels on yield and yield components of confectionery sunflower genotype Alaca.

MATERIALS AND METHODS

The experiment was carried out at the experimental field of Department of Field Crops, Faculty of Agriculture, University of Ankara in 2007 and 2008, in Turkey. Long term average precipitation for this area was 337.6 mm. The field soil in the experiment was clay loam (25% sand, 40% clay, 35% silt), alkaline, low in organic matter, moderately calcareous (Table 2). Confectionery sunflower seeds of Alaca genotype were procured from Trakya Agricultural Research Institute. Three intra-row spacing (R) (R_1=20, R_2=30 and R_3=40 cm) and four different levels of nitrogen (N) (0 (N_0), 40 (N_1), 80 (N_2) and 120 kg ha^{-1} (N_3.)) were used in field experiment. N was applied at two different stages in equal amount by using Amonium sulfate. First application of N was made at the time of land preparation and second was applied to soil at the R-1growth stage of (The terminal bud forms a miniature floral head rather than a cluster of leaves) described by Schneiter and Miller (1981). The planting was done manually in both 2007 and 2008. The experiment was laid on randomized complete block design as split plots with three replicates. An individual plot size were 21.84 m^2 (5.2 × 4.2 m).

At the harvest time, 10 plants were selected at random from each plot. Plant height, head diameter, 1000 seed weight, harvest index, hull ratio, seed yield per plant, protein content, oil content and seed yield per decare of confectionery sunflower genotype Alaca was measured and recorded. 1000 seed weight calculated according to ISTA (8 replications of 100 weighed seperately, calculated average weight of 100 seeds and multiplied by 10). Protein content was determined with kjeldahl method (Akyıldız 1968), Oil content determination was done with hexane extraction (Akyıldız 1968). Harvest index was calculated by using this equation HI=Ye/Yb.100 (Gholinezhad et al. 2009).

HI = Harvest index
Ye = Economical yield
Yb = Biological yield

Data pertaining plant height and different yield contributing characters were analyzed using statistical analysis by MSTAT-C and Duncan's Multiple Range Test was used for post hoc tests. All data transformed into percentages were subjected to arcsine transformation (Snedecor and Cocharan, 1967).

Table 1. Average precipitaition, temperature and relative humidity of the 2007 and 2008.

Months	Precipitation(mm)			Temperature (°C)			Relative Humidity (%)		
	Long term average	2007	2008	Long term average	2007	2008	Long term average	2007	2008
January	33.1	39.0	20.1	0.7	1.2	-4.0	76.5	76.0	76.3
February	38.1	16.4	6.5	0.7	2.5	0.1	73.1	68.5	68.9
March	24.5	37.5	54.9	6.4	7.2	10.1	63.0	59.5	57.6
April	39.8	23.8	32.7	12.6	9.1	13.7	57.8	53.7	54.8
May	47.9	17.9	45.4	16.1	20.4	15.5	56.6	41.1	50.9
June	20.5	31.7	10.3	20.1	22.6	22.0	50.5	45.0	41.0
July	8.8	3.9	0.0	23.5	26.7	24.9	45.9	29.8	35.7
August	6.3	9.8	0.7	23.4	26.3	26.6	46.5	37.1	34.5
September	6.8	0	61.6	20.4	20.9	19.9	46.4	35.0	50.3
October	29.0	14.1	18.6	14.9	16.7	13.3	59.1	49.4	63.8
November	49.6	66.7	43.6	5.7	6.7	8.7	72.1	66.6	72.1
December	33.2	44.4	28.8	0.9	2.0	2.0	78.0	75.7	78.6
Tot./Ave.	337.6	305.2	323.2	12.2	13.5	12.7	60.3	53.1	57.0

*Turkish State Meteorological Service. Ankara 2009.

Table 2. Physical and chemical characteristics of soil where the experiment was conducted

Years	Depth (cm)	Soil Texture	Saturation (%)	Salinity (%)	pH	$CaCO_3$ (%)	Available P_2O_5 (kg da^{-1})	Available K_2O (kg da^{-1})	Total N (%)	Organic Compound (%)
2007	0-20	Clay-loam	50	0.085	8.0	10.34	8.65	245	0.08	1.01
	20-40	Clay-loam	53	0.087	8.0	8.31	11.02	190	0.17	1.14
2008	0-20	Clay-loam	54	0.084	7.8	9.00	7.85	160	0.06	1.25
	20-40	Clay-loam	60	0.088	8.0	10.00	6.45	125	0.15	1.02

RESULTS AND DISCUSSION

In this study, plant height, head diameter, 1000 seed weight, harvest index, hull ratio, seed yield per plant, protein raito, oil ratio and seed yield per decare of confectionery sunflower genotype Alaca was investigated under different intra-row spacing and N doses.

The data of the 2-year experiment was subjected to statistical analysis. The average precipitation for 2008 (323.2 mm) was higher than that observed in 2007 (305.2 mm). Interestingly, rainfall in May of 2007 (17.9 mm) was below than the 2008 and was least for the last 50 years (Table 1).

Table 3. Summary of the analysis of variance for several variables of confectionery sunflower genotype grown under different intra-row spacing and N doses

	Plant Height	Head diameter	1000 seed weight	Harvest Index	Hull ratio	Seed yield per plant	Seed Protein ratio	Seed Oil ratio	Seed yield
Year	**		**		**	**		**	**
R					*	*		*	**
N	**		**	**		**		**	**
R×N					*			**	
R×Y						*			**
N×Y						*	*	**	*
R×N×Y		*							**

*, **:Significant at the 0.05, 0.01level. (R: Intra row spacing, N: Nitrogen doses, Y: Year)

PLANT HEIGHT

Results on plant height clearly showed the non significant effects of intra-row spacing. Average plant height of two years ranged from 153.5 to 162.1 cm. However significant effects of N doses were observed. Average plant height of two years ranged from 148.4 to 163.6 cm with maximum plant height obtained from N1. Further levels of N resulted in slight decline in plant height but that was non significant. Results further revealed that increase in plant height during 2008 compared to 2007 irrespective of row spacing and N doses. Moreover, interactive effect of R × N on plant height was non significant. Average plant height for two years ranged from 144.0 to 168.5 cm. The increase in plant height due to the N application was also reported by Tahir (1996), Ali et al. (2004) and Özer et al. (2004).

HEAD DIAMETER

Head diameter is one of the major yield components in sunflower and the size of confectionery type sunflower is of immense importance. Results clearly indicated that impact of intra-row spacing was non significant with the scores ranged from 18.4 cm to 19.6 cm. Impact of N levels on head diameter was also non significant and average head diameter of two years ranged from 18.5 cm to 20.1 cm. Response of head diameter R × N was non significant and the average of two year differed between 17.1 cm and 20.4 cm. Apart from our results Kıllı (2004) determined that the lowest plant density resulted in increase of head diameter and significantly increase in head diameter with the high level of N. Robinson et al. (1985) also stated that head diameter significantly decreased with the increasing plant density.

Results of combined ANOVA also showed that head diameter scored at different treatments did not influenced by year and the scores were 19.1 and 19.0 for 2007 and 2008 respectively. Results further indicated that interactive effect of Y × R × N was found significant ($P < 0.05$). Head diameter ranged 15.5-21.5 cm the highest head diameter was obtained from R2 × N3 in 2007.

1000 SEED WEIGHT

1000 seed weight is one of the most important yield component in sunflower and environmental factors have an important impact on this character. Results of combined ANOVA showed that 1000 seed weight scored at different treatment influenced by N doses and years significantly.

N doses showed the positive effects on 1000 seed weight compared to control. Average 1000 seed weight ranged from 102.6 g to 121.0 g. Maximum 1000 seed weight was recorded from N2 and further increase of N doses resulted in slight decline in 1000 seed weight.

Average results of two years differed and 1000 seed weight obtained in 2007 was higher compared to 2008. Whereas, interactive effect of R × N was found non significant. Average 1000 seed weight for two years ranged from 98.4 g to 127.7 g. Increase in 1000 seed weight due to the more access to absorb nutrients, supported by Zaman and Das (1991), Fathi et al. (1997) and Gholinezhad et al. (2009) who concluded that increasing level of N led to significant proliferation in 1000 seed weight.

Table 4. Impact of different intra-row spacing and N doses on plant height, head diameter and 1000 seed weight of the confectionery sunflower.

Intra-row	Plant height (cm)			Head diameter (cm)			1000 seed weight (g)		
Spacing (R)	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean
R1	141.8	165.3	153.5	18.6	18.3	18.4	122.8	98.7	110.8
R2	150.4	173.9	162.1	20.2	19.0	19.6	128.7	110.4	119.5
R3	144.7	174.6	159.6	19.2	19.9	19.2	124.5	107.2	115.8
N doses									
N0	135.7	161.2	148.4b**	20.7	19.4	20.1	114.8	90.4	102.6b**
N1	147.8	179.3	163.6a	19.1	18.8	18.5	129.6	105.9	117.7a
N2	151.4	171.2	161.3a	18.9	18.7	18.8	128.5	113.4	121.0a
N3	147.7	173.2	160.0a	18.6	19.3	18.9	128.3	112.0	120.2a
Years	145.4b	171.3a**	158.4	19.1	19.0	19.1	125.3a	105.4b**	115.4
R × N									
R1 × N0	137.3	150.7	144.0	20.6a	19.0a-c*	19.8	115.0	81.7	98.4
R1 × N1	144.3	165.3	154.8	20.1ab	16.8b-d	18.5	127.3	100.0	113.7
R1 × N2	141.7	167.3	154.5	18.3a-d	18.5a-d	18.4	126.0	113.3	119.7
R1 × N3	143.7	177.7	160.7	15.5cd	18.8a-d	17.1	122.7	100.0	111.3
R2 × N0	138.7	163.0	150.8	20.9a	19.1a-c	20.0	117.7	95.3	106.5
R2 × N1	149.0	188.0	168.5	19.3ab	19.8ab	19.6	133.7	115.7	124.7
R2 x N2	156.7	174.7	165.7	18.9a-c	18.6a-d	18.7	136.3	114.7	125.5
R2 × N3	157.3	170.0	162.3	21.5a	18.5a-d	20.0	127.0	116.0	121.5
R3 × N0	131.0	170.0	150.5	20.6a	20.2ab	20.4	111.7	94.3	103.0
R3 × N1	150.0	184.7	167.3	15.2d	19.8ab	17.5	127.7	102.0	114.8
R3 × N2	155.7	171.7	163.7	19.5ab	18.9a-c	19.2	123.3	112.3	117.8
R3 × N3	142.0	172.0	157.0	18.5a-d	20.5ab	19.5	135.3	120.0	127.7

*, **:Significant at the 0.05, 0.01 level. There were no significant differences between the mean values shown with the same letters in 0.05 and 0.01 level. R1: 20 cm, R2: 30 cm, R3: 40 cm; N0: 0 kg N da^{-1}, N1: 4 kg N da^{-1}, N2: 8 kg N da^{-1}, N3: 12 kg N da^{-1}

HARVEST INDEX

Harvest index indicated the relative distribution of photosynthesis yield between economical yield and the biological yield of the plant. The effect of intra row spacing on harvest index was non significant and the scores varied between 39.4 % and 39.5 %. Harvest index was significantly influenced by N levels and the avarage of two years were in the range of 36.2 % to 44.3 %. The maximum harvest index was obtained from N3 and the minimum was recorded from N0. In our research increased level of N had positive impact on harvest index and the results of this study showed contradiction with the Singh et al. (1996), Gholinezhad et al. (2009).

HULL RATIO

The data of hull ratio subjected to statistical analysis was significantly influenced by intra row spacing, year and the R × N interaction. But the hull ratio did not influenced by N doses. Average hull ratio of two years ranged from 44.5 % to 47.3 % with maximum hull ratio was obtained from R3.

Results on impact of N doses on hull ratio had non significant variation and the average of two year was in the range of 45.3 % to 47.2 %. Results also revealed that more hull ratio in 2007 compared to 2008 irrespective of intra row spacing and N doses. However results related the impact of the interaction between R × N found significant on hull ratio and the values ranged from 42.8 % to 49.2 %. The minimum hull ratio was recorded from R1 × N1 and the maximum was obtained from R3 × N1. In contrast to our data, Baldini and Vannozzi (1996) reported that increasing of hullability with the N.

SEED YIELD PER PLANT

Seed yield per plant significantly showed the significant effects of intra-row spacing. Average seed yield per plant of two years ranged from 84.9 to 99.1 g plant^{-1} with minimum seed yield per plant which was recorded from 20 cm intra row space.

Results on N doses showed the positive effects on seed yield per plant and the average was in the range of 80.1 - 99.6 g plant^{-1}. The maximum seed yield per plant was observed in N2 and the minimum was obtained from control.

Effects of different N doses on the seed yield per plant was significantly differed during experiment years. Increasing N levels led to increase in seed yield per plant in both years but in 2008 N levels impact on seed yield per plant showed higher results compared to 2007. The seed yield per plant was 79.2 and 103.8 g plant^{-1} in 2007 and 2008 respectively.

The interactive effect of R × N was not statistically significant and the average of two year was in the range of 68.7-105.5 g plant^{-1}. In previous studies similar results were reported by Aless et al. (1997) and Gholinezhad et al. (2009). Also Marinkovic (1999) observed that decrease in single plant grain yield of sunflower due to decreasing nutrient space of every plant with the increasing plant density.

Table 5. Impact of different intra-row spacing and N doses on harvest index, hull ratio and seed yield per head of the confectionery sunflower.

Intra-row	Harvest index (%)			Hull ratio (%)			Seed yield per plant (g plant $^{-1}$)		
Spacing (R)	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean
R1	39.8	39.3	39.5	47.9	43.1	45.5b**	80.2bc	89.6b*	84.9b*
R2	39.5	39.3	39.4	44.7	44.3	44.5b	73.6c	108.8a	91.2ab
R3	39.1	39.9	39.5	48.9	45.8	47.3a	85.3bc	113.0a	99.1a
N doses									
N0	37.1	35.3	36.2c**	48.2	46.3	47.2	70.8d	89.5bc*	80.1b**
N1	37.3	37.2	37.3c	46.1	44.4	45.3	79.7cd	109.0a	94.3a
N2	39.8	40.7	40.2b	46.3	44.3	45.3	92.2b	106.9a	99.6a
N3	43.7	44.9	44.3a	48.1	42.5	45.3	76.0d	109.8a	92.9a
Years	39.5	39.5	39.5	47.2a	44.4b**	45.8	79.2b	103.8a**	91.5
R × N									
R1 × N0	34.1	34.0	34.1	50.0	46.7	48.4ab*	69.3	68.0	68.7
R1 × N1	36.7	36.2	36.5	45.1	40.5	42.8d	79.3	86.0	82.7
R1 × N2	41.9	41.2	41.6	45.2	43.0	44.1b-d	99.0	95.3	97.2
R1 × N3	46.4	45.8	46.1	51.5	42.2	46.9a-c	73.0	109.0	91.0
R2 × N0	38.0	34.5	36.2	46.4	45.9	46.2a-d	68.7	97.7	83.2
R2 × N1	37.7	37.8	37.8	45.0	42.6	43.8cd	73.7	120.3	97.0
R2 × N2	40.3	40.5	40.4	44.3	44.7	44.5b-d	78.3	113.7	96.0
R2 × N3	41.9	44.3	43.1	43.2	44.0	43.6cd	73.7	103.3	88.5
R3 × N0	39.1	37.3	38.2	48.1	46.4	47.3a-c	74.3	102.7	88.5
R3 × N1	37.4	37.7	37.6	48.2	50.2	49.2a	86.0	120.7	103.3
R3 × N2	37.1	40.4	38.7	49.6	45.2	47.4a-c	99.3	111.7	105.5
R3 × N3	42.8	44.5	43.7	49.6	41.4	45.5a-d	81.3	117.0	99.2

*, **:Significant at the 0.05, 0.01 level. There were no significant differences between the mean values shown with the same letters in 0.05 and 0.01 level. R1: 20 cm, R2: 30 cm, R3: 40 cm; N_0: 0 kg N da^{-1}, N_1: 4 kg N da^{-1}, N_2: 8 kg N da^{-1}, N_3: 12 kg N da^{-1}

SEED OIL RATIO

Results on seed oil ratio clearly showed the significant effects of intra row spacing ($P < 0.05$) N doses and interactive effect of R × N ($P < 0.01$). Average oil ratio of two years ranged from 43.4 % to 46.0 % with maximum oil ratio obtained from 20 cm space.

The positive effect of N doses was observed on seed oil ratio compared to control. Average seed oil ratio ranged from 40.9 % to 47.4 %. Maximum oil ratio was recorded from N3 and further increase of N doses resulted in slight incline in seed oil ratio.

Results also revealed higher seed oil ratio during 2008 compared to 2007 irrespective of row spacing or N dose. The low oil ratio probably caused by the diversity of precipitation between two years. Özer et al. 2004 also reported that low rainfall during the vegetation stage of sunflower may lead to decline in oil concentration. Interactive effect of R × N was found significant and average seed oil ratio for two years ranged from 39. 7 % to 48.6 %. Maximum oil ratio was observed in the combination of R3 × N3. Contrarily, minimum oil was recorded from R3 × N0. Our results showed contradiction with Zubriski and Zimmerman (1974), Özer et al. (2004) and Al thabet (2006) who implied that negative effect of N on oil ratio. However efficiency use of N by sunflower plant is possible with the N uptake from the soil and

remobilization of stored vegetative N accumulated before the flowering stage. Steer et al. (1984) and Ruffo et al. (2003) also reported that when N uptake of sunflower is after the flowering stage, N negatively impacts the oil ratio. Therefore in this research application of N before sowing and at R-1 growth stage might led to increase in oil ratio comparing to control.

SEED PROTEIN RATIO

Protein content one of the most important compenent of confectionery sunflower seeds. Results on seed protein ratio clearly showed the significant effect of N and Y × R × N. But the impact of intra-row spacing was statistically non significant.

Results of two years average about intra-row spacing on seed protein ratio was in the range of 28.3 % to 30.0 %. The minimum and the maximum scores were obtained from 20 cm and 40 cm respectively.

Results on N doses revealed that the significant effect of N doses on protein ratio. The scores were in the range of 27.9 % to 29.5 %.

Results further revealed that non significant effect of R × N on seed protein ratio and scores varied between 26.6 % and 31.2 %. The results are in line with the previous results of Blamey and Chapman (1981), Steer et al. (1986) and Özer et al. (2004).

SEED YIELD

Results of combined ANOVA showed that seed yield significantly affected by intra row spacing and N doses. Seed yield differed significantly within the years ($P < 0.01$). In 2008 when the precipitation was higher, the average seed yield was higher than 2007. The scored seed yield for 2007 and 2008, was 204.5 and 247.4 g da^{-1} respectively.

Increasing number of plant with the 20 cm intra-row spacing led to considerable increase in seed yield. The average of two year observed was in the range of 198.6 kg da^{-1} to 263.0 kg da^{-1}. N had a significantly effect on seed yield and the average of two year varied between 202.7 and 244.0 kg da^{-1}.

Interactive effects of R × N × Y had significant effects on seed yield. Every increasing level of N caused increase in all intra-row spacing (20, 30 and 40 cm). The highest seed yield within years obtained from R1 intra-row spacing and N3 treatment (Table 6). By plant density and N increase, seed yield per area became more due to the increase in the number of plants and N level per area. The results obtained from our research are in line with Ruffo et al. (2003), Jahangir et al. (2006), Beg et al. (2007) and Day and Kolsarıcı (2014) who reported that seed yield of sunflower influenced by plant density.

Table 6. Impact of different intra-row spacing and N doses on protein ratio, oil ratio and seed yield of the confectionery sunflower.

Intra-row Spacing (R)	Protein ratio (%)			Oil ratio (%)			Seed yield (kg da^{-1})		
	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean
R1	28.6	28.0	28.3	43.7	48.4	46.0a*	224.9b	301.0a**	263.0a**
R2	30.8	29.2	30.0	43.2	46.6	44.9ab	198.6cd	234.0b	216.3b
R3	29.2	28.6	28.9	40.6	46.2	43.4b	190.0d	207.2c	198.6c
N doses									
N0	30.3	28.7	29.5a*	39.7d	42.0cd**	40.9c**	187.2e	218.2d*	202.7d**
N1	30.6	28.4	29.5a	40.7cd	48.4ab	44.6b	197.1e	245.1c	221.1c
N2	28.0	27.8	27.9b	43.0c	49.8a	46.4ab	214.7d	257.2b	235.9b
N3	29.2	29.5	29.4a	46.6b	48.1ab	47.4a	219.0d	269.0a	244.0a
Years	29.5	28.6	29.1	42.5b	47.1a**	44.8	204.5b	247.4a**	226.0
R × N									
R1 × N0	31.0a-c	27.5d-g*	29.3	41.9	44.5	43.2cd**	202.3f-h	274.7b**	238.5
R1 × N1	29.6a-g	27.5c-g	28.6	43.3	52.6	48.0ab	212.3e-g	313.3a	262.8
R1 × N2	27.0e-g	28.6b-g	27.8	42.9	51.1	47.0a-c	241.7cd	300.7a	271.2
R1 × N3	26.6fg	28.4b-g	27.5	46.7	45.4	46.1a-c	243.3cd	315.3a	279.3
R1 × N0	29.8a-g	29.3a-g	29.6	41.2	38.4	39.8d	182.0ij	215.3ef	198.7
R2 × N1	32.3a	28.0b-g	30.2	41.2	48.6	44.9a-c	194.7g-i	229.0de	211.8
R2 × N2	30.5a-d	27.9c-g	29.2	44.8	50.3	47.6ab	205.7fg	242.7cd	224.2
R2 × N3	30.7a-d	31.7ab	31.2	45.6	49.1	47.4ab	212.0e-g	249.0c	230.5
R3 × N0	30.1a-e	29.2a-g	29.7	36.1	43.2	39.7d	177.3ij	164.7j	171.0
R3 × N1	29.8a-f	29.8a-g	29.8	37.5	44.1	40.8d	184.3hi	193.0g-i	188.7
R3 × N2	26.4g	26.8e-g	26.6	41.2	47.9	44.6bc	196.7f-i	228.3de	212.5
R3 × N3	30.3a-e	28.5b-g	29.4	47.5	49.7	48.6a	201.7f-h	242.7cd	222.2

*, **:Significant at the 0.05, 0.01 level. There were no significant differences between the mean values shown with the same letters in 0.05 and 0.01 level. R1: 20 cm, R2: 30 cm, R3: 40 cm; N_0: 0 kg N da^{-1}, N_1: 4 kg N da^{-1}, N_2: 8 kg N da^{-1}, N_3: 12 kg N da^{-1}

CONCLUSION

Different level of intra row spacing and N had different impacts on plant. Among the most important results obtained about these treatments significant increase in yield at 20 cm intra-row spacing × 120 kg ha^{-1} observed. With plant density increase, decrease in 1000 seed yield was observed which means that small sized seeds were more. These results suggests that 30 cm intra row spacing and 80 kg ha^{-1} N treatment is suitable for Alaca genotype under Ankara conditions.

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EFFECTS OF DIFFERENT ORGANOMINERAL AND INORGANIC COMPOUND FERTILIZERS ON SEED YIELD AND SOME YIELD COMPONENTS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

This research was carried out to determine the effects of different organomineral and inorganic compound fertilizers on seed yield and some yield components of sunflower (*Helianthus annuus* L.) in 2013. The experiments were conducted using Tunca MR LG 5580 oil type sunflower hybrid in Randomized Complete Block Design with 4 replications at Trakya Agricultural Research Institute in Edirne, Turkey. In the research, 5 different treatments; 1) Check 0 kg/ha no fertilizer, 2) 250 kgs/ha organomineral fertilizer of Hexaferm® 8.21.0 3) 250 kgs/ha organomineral fertilizer of Hexaferm® 6N.10P.10K 4) 250 kgs/ha inorganic compound granule fertilizer of 15N.15P.15K (farmers apply) and 5) 250 kgs/ha inorganic compound granule fertilizer of 20N.20P.0K kgs/ha (farmers apply) were evaluated. Before sunflower planting, the fertilizers were applied by hand spreading in each plot's surface uniformly and mixed well with the soil. In this research beside seed yield, oil content, oil yield, 1000 seed weight, test weight, plant height, head diameter, time to flowering, and time to physiological maturity were evaluated. The seed yield of sunflower was significantly affected by the different organomineral and inorganic compound fertilizers under natural rainfed conditions. Based on statistical analyses results; the highest sunflower seed yield with mean of 3282 kgs/ha was obtained at 250 kgs/ha the organomineral fertilizer application of Hexaferm® 6N.10P.10K.

Key words: sunflower, organomineral, fertilizer, yield, oil content

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the important edible vegetable oilseed crop in Turkey and the world. It has strong demand as a healthy vegetable oil due to their low level of saturated fats, making it popular as a cooking vegetable oil and for use in processed foods (Schneiter, 1997; Süzer, 2014).

World sunflower planted area accounts for 25.590.104 ha, production 44.753.264 ton, and yield is about 1750 kg/ha. However, Turkey sunflower planted area accounts for 609.784 ha, production 1.523.000 ton, and yield is about 2500 kg/ha (FAO STAT 2013). Turkey has around 3.4 % ratio of sunflower production comparing to world (Süzer, 2015).

Sunflower is one of the main crops in the rotation system in Trakya region of Turkey and it provides to around 46% of edible vegetable oil. The majority of Turkey's sunflower production area is located in Trakya-Marmara (%75) region and it is also grown in central Anatolia (7 %), the black sea cost (7 %), the Çukurova (7 %), the Aegean coast (3%), and the South East Anatolia (1%) regions of Turkey (Süzer, 2015).

An annual plant, sunflower need to grow such as fertile soil, water, air, light and temperature. Fertile agricultural soils usually have more than 3% organic matter for high yielding sunflower production. In fertile soils, sunflower plants require at least 16 elements

for normal growth and to get high seed and oil yield from per hectare. Therefore some essential plant nutrients need to grow high yielding sunflower such as carbon, oxygen, hydrogen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, chlorine, iron, manganese, zinc, copper and molybdenum (Hocking and Steer, 1983; Danke et al., 1992; Schneiter, 1997; Süzer, 1998; Kacar ve Katkat, 1999; Süzer, 2010 a; Süzer, 2012; Süzer, 2013).

These elements used in the largest amounts for growing sunflower crop, carbon, hydrogen and oxygen, are non-mineral elements supplied by air and water and the other 13 elements are taken up by plants only in mineral form from the soil. The sunflower plants need large amounts of nitrogen, phosphorus, potassium and referred to as primary nutrients. Those nutrients are the ones most frequently supplied to grow sunflower plants in fertilizers. The three secondary elements, calcium, magnesium, and sulfur, are required in small amounts than the primary nutrients for growing sunflower. The micronutrients are boron, copper, chlorine, iron, manganese, molybdenum, zinc that occur in very small amounts in both soils and plants, but their roles are equally as important as the primary or secondary nutrients. A deficiency of one or more of the micronutrients on sunflower plant can lead to severe depression in growth, and seed and oil yield (Robinson, 1978; Blamey et al., 1987; Schneiter 1997; Süzer, 2015)

However in sunflower production areas, some soils do not contain sufficient amounts of these nutrients to meet the plant's requirements for good growth, seed and oil yield from per hectare. In such cases, supplemental nutrient applications in the form of organomineral and inorganic compound fertilizers applications must be made to get high seed and oil yield from per hectare. But, it's continuous using of inorganic fertilizers can cause nutrient in-balance and soil pH level. Only using of either organic or inorganic fertilizers cannot met the expected in increasing crop yield. In this aspect, the combined use of in organic chemical fertilizers and organomineral fertilizer can be suggested in sunflower production in order to increase seed and oil yield from per hectare. Therefore it is essential that the combined use of organic and inorganic fertilizers (organomineral fertilizer) should be encouraged and introduced to farmers in order to achieve their yield expectancy of their sunflower crop production (Schneiter, 1997; Makinde et al., 2010; Olaniyi et al., 2010; Süzer, 2010a, 2010b; Süzer, 2012; Süzer, 2013; Süzer, 2014; Süzer 2015)

Because of the importance of sunflower as one of the major oilseed crop in Turkey, the objective of this research was to determine the responses of sunflower to different organomineral and inorganic compound fertilizers.

MATERIALS AND METHODS

This experiment was carried out in rotation with wheat crop under rain fed conditions during the 2013 sunflower growing season. The experiment field is located on latitude 41.68° N and longitude 26.56° at elevation 62 meters above sea level in Edirne, Turkey. Main properties of soil used in the field experiments are presented in Table 1. As seen in Table 1, soil texture was silty clay and organic matter was low. Edirne's climate is classified as warm and temperate, slightly continental. The average annual temperature of Edirne is 13.5 °C and the rainfall averages 597 mm. Climate data of Edirne during sunflower growing season in 2013 are presented in Table 2.

The experiments were conducted using Tunca MR LG 5580 oil type sunflower hybrid in Randomized Complete Block Design with 4 replications at Trakya Agricultural Research Institute in Edirne. In the research, 5 different treatments; **1)** Check 0 kg/ha no fertilizer, **2)** 250 kgs/ha organomineral fertilizer of Hexaferm® 8N.21P.0K **3)** 250 kgs/ha organomineral fertilizer of Hexaferm® 6N.10P.10K **4)** 250 kgs/ha inorganic compound fertilizer of

15N.15P.15K (farmers apply) and **5)** 250 kgs/ha inorganic compound fertilizer of 20N.20P.0K (farmers apply) were evaluated.

Table 1. Soil analyses data of experiment field Edirne in 2013.

Year	Depth (cm)	PH	CaCO3 (%)	P2O5 (kg/Ha)	K2O Available (kg/ha)	Total Salt (%)	Sand (%)	Silt (%)	Clay (%)	Organic Matter (%)	Texture Class
2013	0-20	6.30	1.62	320.0	674.0	0.040	36.2	36.6	27.2	1.4	Silty
	20-40	6.25	1.44	270.0	586.0	0.035	32.5	38.4	29.1	1.2	clay

*: Soil tests are done by Edirne commodity exchange.

Table 2. Climate data of Edirne during the 2013 sunflower growing season.

Month	Rain (mm)	Rainy days	Humidity level (%)	Temperature (°C)		
				Minimum	Maximum	Mean
April 2013	51,0	9	73,2	4,0	32,0	12,7
May 2013	11,0	3	66,7	4,9	32,9	20,8
June 2013	26,6	7	70,1	11,4	36,2	23,3
July 2013	14,4	4	53,6	15,0	36,1	25,5
August 2013	0,0	0	48,7	16,6	36,9	26,7
Total	103.0	23	62.46	4,0	36,9	21,8

*: Climate data is received from Edirne's meteorological station.

Plot size in planting 7 .5 x 2.8 m = 21.0 m^2, and plot size in harvest 1.4 x 4.4 m = 6.1 m^2. The intro-row spacing was 30 cm, in rows spaced 70 cm apart. Before sunflower planting, the fertilizers were applied by hand spreading in each plot's surface uniformly and mixed well with the soil. The experiments were sown in the first week of May in three years. The seeds were over planted per hill and thinned to one plant per hill three weeks after sowing. Weed control was accomplished by using both chemicals and cultural practices.

In this research beside seed yield, oil content, oil yield, 1000 seed weight, test weight, plant height, head diameter, time to flowering, and time to physiological maturity were evaluated and analyzed using ANAVO. All statistical analyses of data were performed using the JMP 5.0.1 statistical software package (SAS Institute, 2002), and the differences between means were compared using a least significant difference (LSD) test at the 0.05 probability level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The effects of different organomineral and inorganic compound fertilizers on seed yield and some yield components of sunflower are presented in Table 3. As seen in Table 3, all different organomineral and inorganic compound fertilizers affected significantly sunflower seed yield. In the experiment, Hexaferm® 6N.10P.10K organomineral fertilizer of 250 kgs/ha application gave the first rank highest seed yield (3282 kgs/ha) and oil yield (1438 kgs/ha) comparing the other four different organomineral and inorganic compound fertilizers. The other Hexaferm® 8N.21P.0K organomineral fertilizer of 250 kgs/ha application gave the second rank highest seed yield (3145 kgs/ha) and oil yield (1358 kgs/ha) in the experiment.

On the other hand compound 15N.15P.15K inorganic fertilizer of 250 kgs/ha farmers apply gave the third rank highest seed yield (3034 kgs/ha) and oil yield (1314 kgs/ha) comparing the other four different organomineral and inorganic compound fertilizers. Compound 20.20.0 inorganic granule fertilizer of 250 kgs/ha application also gave the fourth

rank highest seed yield (3004 kgs/ha) and oil yield (1328 kgs/ha) comparing the other four different organomineral and inorganic compound fertilizers. The last check 0 kg/ha no fertilizer gave the fifth rank seed yield (2609 kgs/ha) and oil yield (1124 kgs/ha) comparing the other four different organomineral and inorganic compound fertilizers.

Table 3. Mean seed yield and yield components of sunflower as affected by five different organomineral and inorganic compound fertilizers.

Entry No	Aplications	Seed Yield (kg/ha)	Oil In Seed (%)	Oil Yield (kg/ha)	Plant Height (cm)	Head Dia-meter (cm)	Time To Flowe-Ring	Time To Phsiolo-gical Maturity	1000 Seed Weight (g)	Volume Weight (kg/hl)
1	Kontrol (No fertilizer)	2609 B	43.1	1124	155	16	8.07.2013	15.08.2013	48.9	39.6
2	Hexaferm 8.21.0 250 kgs/ha	3145 A	43.2	1358	160	19	8.07.2013	15.08.2013	50.1	39.9
3	Hexaferm 6.10.10 250 kgs/ha	3282 A	43.8	1438	160	19	8.07.2013	15.08.2013	50.0	39.8
4	15.15.15 250 kgs/ha	3034 A	43.3	1314	160	17	8.07.2013	15.08.2013	49.3	39.0
5	20.20.0 250 kgs/ha	3004 A	44.2	1328	160	17	8.07.2013	15.08.2013	50.3	38.7
	LSD (0.05)	309.4**								
	C.V. (%)	6.66								

**: 0.01 different significantly at 1 % probability level.

The result of the seed and oil yield of sunflower as affected by the inorganic and organomineral fertilizer applications are presented in Table 3. The response of sunflower to the four different organomineral and inorganic compound fertilizers varied slightly for the seed and oil yield. However, the sole application of 250 kgs/ha Hexaferm® 6N.10P.10K organomineral fertilizer performed favorably well in terms of seed and oil yield of sunflower.

Despite the environmental and other yield constraints encountered by the crop during the growth production period, the overall assessment showed that it is essential to considered the main commercial fraction like the seed yield performance of sunflower in choosing the level of organomineral and inorganic fertilizers for use in sunflower production. But, it's continuous using of inorganic fertilizers can cause nutrient in-balance and soil pH level. Only using of either organic or inorganic fertilizers cannot met the expected in increasing crop yield. In this aspect, the combined use of in organic chemical fertilizers and organomineral fertilizer can be suggested in sunflower production in order to increase seed and oil yield from per hectare (Schneiter, 1997; Makinde et al., 2010; Olaniyi et al., 2010; Süzer, 2010a; Süzer, 2012; Süzer, 2013; Süzer, 2014).

CONCLUSIONS

Sunflower is the important edible vegetable oilseed crop in Turkey and the world. In the current drive by Turkey to achieve vegetable oil sufficiency in sunflower production in near future, improved yield could be achieved by the use of organomineral and inorganic fertilizers. The results of this research showed a positive influence of organomineral and inorganic fertilizers on growth and yield components of sunflower plant over the control. Yield parameters in terms of seed yield and 1000 seed weight were found to be higher at all

organomineral and inorganic fertilizers' treatment application than the control. Consequently, considerable increase in term of grain yield was brought about by applying before planting the sole application of 250 kgs Hexaferm® 6N.10P.10K organomineral fertilizer performed favorably well in terms of seed and oil yield of sunflower in dry land conditions in Edirne-Turkey. On the other hand, for balanced fertilization in sunflower the combined use of in organic chemical fertilizers and organomineral fertilizer can be suggested in sunflower production in order to increase seed and oil yield from per hectare.

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EFFECTS OF MICRO NUTRIENTS (FE, ZN, B AND MN) ON YIELD AND YIELD COMPONENTS OF TWO SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVARS IN URMIA CONDITION

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ABSTRACT

Appropriate soil fertility is necessary to obtain higher seed yield and quality of the crop. Application of chemical fertilizers as N, P and K in Iran is very high and often more than crop needs. One of the most important issues about increase of crop yield and improving the quality of agricultural products is balanced plant nutrition. Many researches indicated a positive influence of micronutrient application in increase of yield and quantitative parameters of crops. As well as, identification and accessing to cultivars which have high yield should be implemented. The aim of the study was to determine the effect of micronutrients on yield and yield components of two sunflower cultivars in Urmia condition. The trial was carried out at Nakhjavani Agricultural Research Center in Urmia, West Azerbaijan Province, Iran, using a randomized complete block design with split-plot arrangement in four replications, with cultivars in two levels (Golshid and Record) as main plot and application of fertilizer treatments in five levels (T_1: N+P+K+Mg; T_2: N+P+K+Mg+Fe; T_3: N+P+K+Mg+Fe+B; T_4: N+P+K+Mg+Fe+B+Mn; T_5: N+P+K+Mg+Fe+B+Mn+Zn) as subplot. The results showed that there were significant differences between two cultivars for all traits as: head diameter, seed yield, biologic yield, harvest index and thousand seed weight. Seed yield, harvest index and thousand seed weight, were significantly affected by fertilizers application. Cultivar and fertilizer application interactions have significant effects on seed yield, biologic yield, head diameter and thousand seed weight.

Keywords: Micronutrient, Cultivar, Sunflower, Urmia.

INTRODUCTION

Plants, like all other living things, need food for their growth and development. All green plants have the ability to manufacture their own food by using energy derived from the sun to combine chemical elements, taken up in the inorganic ion form, into a multitude of organic compounds. Lack of an important chemical element may slow a plant's growth or make the plant more susceptible to disease or even death. Seventeen elements are considered essential for plant growth. Some of these nutrients combine to form compounds which compose cells and enzymes. Others must be present in order for certain plant chemical processes to occur. Each type of plant is unique and has an optimum nutrient range as well as a minimum requirement level. Below this minimum level, plants start to show nutrient deficiency symptoms. Excessive nutrient uptake can also cause poor growth because of toxicity. Therefore, the proper amount of application and the placement of nutrients are important (Silva and Uchida, 2000; Rahimi, 2002).

Sunflower (*Helianthus annuus* L.) is quite responsive to micronutrients. The significant effect of micronutrient application on the growth of sunflower, in terms of plant height, number of

leaves and dry matter production per plant can be interpreted in terms of enhanced metabolic function of micronutrients in the plant (Siddiqui et al., 2009). Application of high analysis NPK fertilizers and very limited use of farmyard manure cause micronutrient depletion in soils, which appears to have special role in influencing the test weight and seed filling (Tufail *et al*. 1990). The functions of B: necessary in the synthesis of one of the bases for RNA formation and in cellular activities, to promote root growth, essential for pollen germination and growth of the pollen tube, associated with lignin synthesis, activities of certain enzymes, seed and cell wall formation, and sugar transport. The functions of Fe: essential in the heme enzyme system in plant metabolism, part of protein ferredoxin and required in nitrate and sulfate reductions, essential in the synthesis and maintenance of chlorophyll, and strongly associated with protein metabolism. The functions of Mn: part of the plant enzyme system, activating several metabolic functions, a constituent of pyruvate carboxylase, oxidation-reduction process in photosynthesis, necessary in Photosystem II, and activates indole acetic acid oxidase. The functions of Zn: required in the synthesis of tryptophan, an essential component of several metallo-enzymes in plants, the enzyme carbonic anhydrase is specifically activated by Zn, and a role in RNA and protein synthesis.

(Silva and Uchida, 2000).

Positive effects of micronutrients on sunflower were reported in several studies by Rahimizadeh et al. (2010), El-Fouly et al., (2001), El-Fouly *et al.* (1990) and Abdalla and Mobarak (1992). Sunflower is also one of the most sensitive crops to low boron supply and deficiency and first symptoms appear on the younger leaves, which develop a bronze color and become hardened, malformed and necrotic, the stem becomes corky, the capitulum deformed, and poor seed set results (Blamey et al., 1987). Application of adequate fertilizers led to increase the crop yields, improved the nutrient element concentrations in plant tissue and soil macro and micronutrient status (Adediran et al., 2004). Keeping in view the key role played by Fe, Zn, B and Mn nutrition in plant growth, this study was designed to enhance the productivity of sunflower, by using micronutrients like B, Zn, Mn and Fe, under soil and environmental conditions of West Azerbaijan Province, Iran.

Materials and Methods

The trial was carried out at Nakhjavani Agricultural Research Center in Urmia, West Azerbaijan Province, Iran, using a randomized complete block design (RCBD) with split-plot arrangement in four replications, with cultivars in two levels (Golshid and Record) as main plot and application of fertilizer treatments in five levels as subplot is an follows:

T_1: N+P+K+Mg

T_2: N+P+K+Mg+Fe

T_3: N+P+K+Mg+Fe+B

T_4: N+P+K+Mg+Fe+B+Mn

T_5: N+P+K+Mg+Fe+B+Mn+Zn

The soil of the experimental field was loam sand in texture. It was low in organic carbon (0.63%), pH=7.7, the percentage of T.N.V=3.3%, Pava.=7.9 ppm, Kava.=130 ppm, Feava.=4.24ppm, Mnava.=3.9ppm, Znava.=1.32ppm, Bava.=1.16ppm. A uniform application of 350 kg ha^{-1} urea-N, 100 kg ha^{-1} P_2O_5 as triple super phosphate (TSP), 200 kg ha^{-1} K_2SO_4 and 100 kg ha^{-1} MgSO4 was given to all the plots. Fe as Iron sulphate (200 kg ha^{-1}), Zn as Zinc sulphate (40kg ha^{-1}), Mn as Manganese sulphate (30kg ha^{-1}) and B as Boric acid (30kg ha^{-1}), were were supplied as per treatment.the seeds were cultivated on 20 May and harvested

on 23 September. Weather was satisfactory during the experiment for normal growth of the crop.

RESULTS AND DISCUSSION

The results showed that there were significant differences between two cultivars for all traits as: head diameter, seed yield, biologic yield, harvest index and thousand seed weight. In terms of all characteristics the Golshid (an Iranian hybrid) is better than Rcord (Table 1.). Seed yield, harvest index and thousand seed weight, were significantly affected by fertilizers application. In terms of seed yield, harvest index, and thousand seed weight, T_4 (41.79 g/plant), T_4 (25), and T_5 (48.64 g) are the best respectively (Table 2.). Interactions among cultivars and fertilizer applications have significant effects on seed yield, biologic yield, head diameter and thousand seed weight. In terms of seed yield, biologic yield, thousand seed weight, and head diameter, a_1b_4 (50.68 g/plant), a_1b_3 (188.13 g/plant), a_1b_3 (58.22 g/plant), and a_1b_4 (16.21 cm) are the best respectively (Table 3.). Rahimizadeh *et al.*, (2010) showed that micronutrient treatments increased the head diameter, seed per head, seed yield and oil percentage. El-Fouly *et al.,* (2001), showed that the number of leaves and the leaf area were increased by addition of Fe, Mn and Zn. Root size was increased by addition of Fe and Mn. Stem and root lengths were increased by Mn only.

Table 1. Effect of cultivars on seed yield, biologic yield, HI, 1000-seed weight and head diameter

Cultivars	Seed Yield/PL (g)	Biologic Yield/PL (g)	HI	1000-Seed Weight (g)	Head Diameter (cm)
Golshid	42.94 a	172.55 a	24.64 a	52.28 a	15.81 a
Record	29.74 b	150.27 b	19.96 b	38.50 b	12.27 b

Table 2. Effect of different nutritional elements on seed yield, biologic yield, HI, 1000-seed weight and head diameter

Fertilizers	Seed Yield/PL(g)	Biologic Yield/PL (g)	HI	1000-Seed Weight (g)	Head Diameter (cm)
T_1	28.89 c	158.92	18.47 c	42.41 c	14.63
T_2	33.55 bc	150.82	22.07 b	43.44 c	14.49
T_3	38.56 ab	163.71	22.87 ab	48.10 ab	14.12
T_4	41.79 a	164.50	25.00 a	44.36 bc	14.68
T_5	38.90 ab	169.08	23.07 ab	48.64 a	14.77

Table 3. Effect of different cultivar (A) and fertilizer (B) interactions on seed yield, biologic yield, HI, 1000-seed weight and head diameter

Interactions	Seed Yield/PL (g)	Biological Yield/PL(g)	HI	1000-Seed Weight (g)	Head Diameter (cm)
a_1b_1	31.61 de	148.65 ab	21.25	45.86 b	15.05 ab
a_1b_2	40.58 bc	173.98 a	23.27	48.21 b	16.02 a
a_1b_3	49.91 a	188.13 a	26.24	58.22 a	16.07 a
a_1b_4	50.68 a	185.67 a	27.32	54.86 a	16.21 a
a_1b_5	41.89 b	166.28 ab	25.10	54.36 a	15.67 a
a_2b_1	26.18 e	169.19 ab	15.69	38.96 cd	14.22 bc
a_2b_2	26.53 e	127.66 c	20.86	38.66 cd	12.96 de
a_2b_3	27.22 e	139.29 c	19.50	37.98 cd	12.17 e
a_2b_4	32.89 cde	143.33 c	22.69	33.87 d	13.14 cde
a_2b_5	35.90 bcd	171.87 a	21.03	43.03 bc	13.87 cd

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GLOBAL CHANGE ADAPTATION: WHAT FUTURE FOR SUNFLOWER CROPS AND PRODUCTS? A FORESIGHT STUDY FOR OILSEED CHAINS AT 2030 HORIZON

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ABSTRACT :

A foresight study was carried out to 2030 horizon (15 years), to shed light on the opportunities that will draw the oilcrops and grain legumes, and the areas of growth for the vegetable oil and protein sector. The thinking was organized in four different scenarios, illustrating different logical evolutions of the context and related key issues, under the pressure of demographic, economic and socio-political constraints. The economic value of the protein fraction is a key aspect of the future of oilseeds such as sunflower. However, a saturated market for oils falls within the trend but is not a certainty.

Keywords: Sunflower, oil and protein markets, vegetable oils, Vegetable proteins, oil crops, grain legumes, food demand, foresight, scenarios.

INTRODUCTION

What will be the outlets that pull the oil and protein crops in 2030? What will be the areas of growth for the oilseed sector? The reflection was launched by the French sector of oilseed crops and grain legumes, wondering about the recent evolutions in the relative economic value of oil and protein fractions in oilseeds, rebalancing the economic interest of the two co-products, oil and oilseed cakes.

Questioning "vegetable oils and proteins" brings back to the fundamental historical development of oil and protein crops in Europe, which is still very dependent on imported vegetable protein (Visser, 2013). But with a major change: when soy is still the market leader in plant proteins, now palm is leader on oils. Given this dynamic competitor from Asia, which produces more than 4 tons of oil per hectare per year when a sunflower crop produces about 1t/ha, the protein fraction is obviously part of the future of the oilseed crops. But the question needs to be reformulated in view of the demand both in quantities and qualities. Oils and vegetable proteins (and their origin commodities) are among the most traded agricultural commodities in the world: over 50% of global oilseed production is exchanged, when only a third of the sugar and 10% of cereals are (Mittaine and Mielke, 2013). The question must therefore be addressed, for part at global scale and at regional scale, since "regional" policies can strongly direct productions, as seen in the case of biofuels.

A working methodology based on the analysis, and a synthesis in the form of scenarios

The reflection was organized along the lines of strategic foresight methods (Godet M. 2007). A first step was to describe the “prospective system”, by situating the heart of the vegetable oil and protein system in its context. The experts group developed a representation of the flow diagram of vegetable oils and proteins and their products, linking 21 elements of the agro-industrial system of oils and proteins, in a surrounding context described through 13 environment factors, or "regulators". Then, the various elements of this system were the

subject of an information review. The analysis of past and present dynamics led the working group to make assumptions (145 in total) for 2030, in the trend or in rupture, for each factor or key variables.

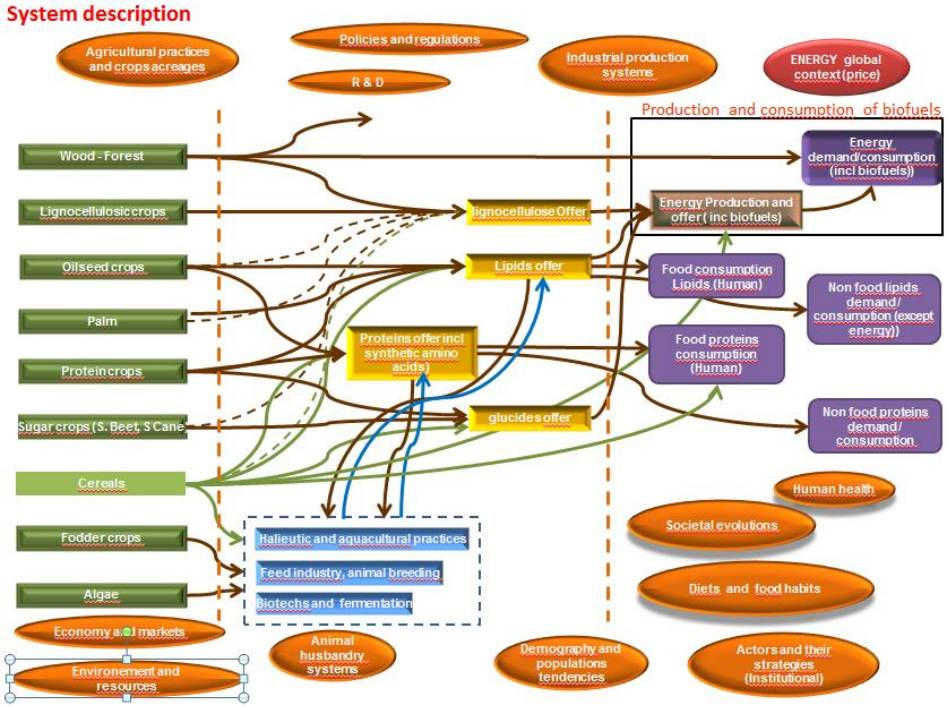

Figure 1: the protein-oil system and its context

Table 1 driving and main secondary assumptions

MAIN ASSUMPTIONS	2013	SC1 Chaos	SC2 Blocks	SC3 Trust	SC4 Climate rupt
Population	7,2	8,9	8,4	7,9	8,9
agricultural yields progress		stagnation	weak	moderate	stagnation
Per capita animal proteins consumption		low	moderate	high	low
Political behaviours regarding global change		passivity, everyone for himself	blocks policies	cooperation	cooperation
Economic growth		weak	moderate	high	moderate
petroleum price $/ barel // $/ton	105 //770	50-70 // 365-511	60-80 // 450-610	135/150 // 950-1100. contrasts between regions	135/150 // 950-1100 . contrasts between regions
animal protein consumption g/cap/day					
China	37	39	46	46	41
India	14	20	21	36	18
Africa	14	16	26	26	18
Europe	67	49	54	65	36
USA	70	55	61	66	43
World	31	29	34	39	29
Palm oil for food consumption MT/yr	41	"200MT"	42 à50MT	50MT	
nutritional recommendations are taken into account	0	no or poorly	yes	yes --> oméga3	yes --> oméga3
EU policies on biofuels	0	biofuels not mandatory	energy directive maintained/ biofuels mandatory	energy directive maintained/ biofuels not mandatory	
reinforced regulations regarding sustainability	0	no	carbon tax in Europe	yes, standardization	carbon tax and standardization
GMO regulations		deregulated	no crops no use in Europe	constraints lifted	constrainsts partially lifted
antibiotics uses in feed		free	prohibited	prohibited	?
green chemistry, biorefinery	0	stagnating	strong develpt	strong developt	strong developt

The construction of the structure of the scenarios was done using "morphological analysis," by assembling assumptions about different dimensions or variables by empirical judgment of the consistency, each scenario being based on one or more driving assumptions on key factors for the demand evolution. Table 1 shows notably the first 5 levels of assumptions assembly,

which determine the overall level of demand and the socioeconomic tone, giving the thread of each scenario. The scenarios were then written in text form, as a first step of consistency check. A quantitative assessment was then carried out to check the consistency of the assemblies of assumptions made on variable estimates. These four scenarios, which illustrate different logics of context evolution and major issues, are summarized in the box below.

FORESIGHT STUDY 2030

VEGETABLE OILS AND PROTEINS

4 scenarios as thinking framework

Scenario 1: "Towards Chaos": Economic and political crisis, food tensions, competitions and growing inequalities

Facing the strong world population and the tangible effects of climate change, the situation is tense to meet food demand. The economic crisis, generating a climate of tension, insecurity and general impoverishment, does not allow the adoption of international agreements on long-term policies (resource conservation, environment, renewable energy, health ...). Animal proteins are a luxury: their consumption is declining in Europe (where the vegetable proteins are better accepted) and tops out elsewhere. With depressed energy prices, biofuels of first generation remain only to sell the oil surplus from palm and soy. The rising cost of food leads to increased use of plant proteins. Regarding vegetable oils, palm oil, abundant and inexpensive, dominates in the food business.

Scenario 2: "The might of the blocks": Regional Policies and bilateralism

Climate change leads to contrasting effects between regions: due to divergent interests, international negotiations are blocked. Europe and the most affected countries implement unilateral policies against climate change, in a latent protectionism atmosphere. Yields - and global production – increase slowly, for a population that is growing by 15% between 2013 and 2030. The economy keeps pace with the population and the standard of living is maintained on average. In Europe, society's demands on sustainability and health led States to develop renewable energy (including biofuels), to better exploit the potential of territories and to regulate: restrictions on greenhouse gas emissions and carbon tax. Biorefinery at regional scale and non-food uses of agricultural resources are essential. Veganism rises in Europe while consumption of animal protein progresses in China and Africa. Palm oil is used in chemistry, especially in Southeast Asia, and for biofuels.

Scenario 3: "Trust": International cooperation in preventing climate change

The Nations sense the threat of climate change and integrate it in their policies, creating an international cooperation to address climate, food and energy challenges, while maintaining free trade. Green Growth is launched, based on advanced R & D and the establishment of trading standards (on products, processes and production conditions) and accompanying measures, where Europe plays leader. European consumers are aware of the nutritional recommendations and of the carbon impact of production and consumption modes, and are turning to a low-impact diet. Quite the opposite, the improvement of living conditions linked to moderate population growth and economic affluence leads to increased consumption of animal protein in many countries (Africa, China, but also India) blowing the demand for animal feed. Soy is widely grown for feeding livestock. The oleochemistry and biofuels, driven by R & D and high energy prices, are growing strongly worldwide, especially in Asia on palm oil basis.

Scenario 4: "Climate rupture": Measures for savings and forced cooperation because of climate and food tensions

The strong growth of the world population and the effects of climate change put agricultural production under pressure and cap yields. This leads states to negotiate and cooperate in emergency as on environmental policies and at the level of technology transfer and of the distribution of economic efforts between the South and the North. Constraining measures target carbon balances and GHG, playing both on production processes through standardization and on consumption through a generalized carbon tax. Energy is taxed to finance the development of alternative energies. Advances in R & D are put forward to meet the constraints and generate green growth, opening applications to GMOs, green chemistry, the development of a circular economy with local bio-refinery. Initially driven by economic growth, the development of animal protein consumption proves unsustainable and quickly reaches limits in Africa and Asia. European consumers, made aware of issues of sustainability and health by the authorities, opt for a diet less rich in animal protein.

What developments for vegetable oils and plant proteins outlets?

In terms of vegetable oils, the increase in food demand remains relatively limited, from 133 Mt (million tons) in 2013 to 156-165 Mt in 2030, an absolute increase of 23 to 32Mt, relatively limited compared to the growth capacity of the palm oil production. We can therefore expect strong competition in the vegetable oil market with two heavyweights: the

palm oil supported by its strong competitiveness in production cost per ton, and soybean oil, driven by the strong demand for protein for animal feed or food.

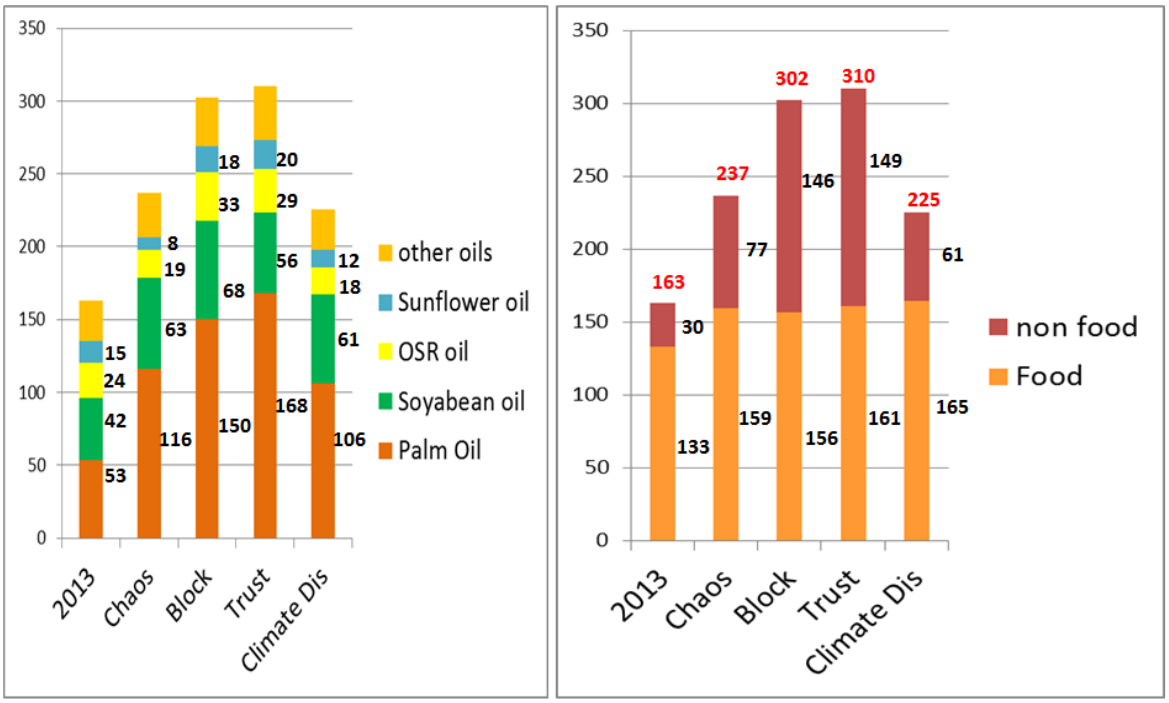

Figure 2: Global production and use of vegetable oils

The conditions for the development of non-food uses of oils are very different between scenarios: excess oil may be real, with need to dispose of surpluses for cheap non-food outlets (biofuels in Sc. 1 " chaos "), or artificial in case of real economic dynamics of non-food uses, either from a high energy market (Sc. 3" Trust "), or from technological innovation and / or incentives policies (Sc. 2 "Blocks"). In the case of Sc. 4 "climate rupture" excess oil over the food needs is relatively limited in a context where the resources scarcity, actual or of regulatory origin, makes nonfood uses economically attractive. This shows that if the strong growth of oil production seems to be a trend, the excess of the production is obvious in Sc. 1 only, where uses at low price only are possible, due economic depression. In other scenarios, noble non-food uses ensure alternatives for consumption and growth in an unbalanced world between scarce resources and the needs of an active economy. However, in the context of Sc. 1 depressed market, the development of palm could be challenged by soybean oil, as co-product from the production of protein for livestock. In scenarios with high oil prices and encouraging biobased products, palm development could be based on green chemistry, provided production is compatible with constraints related to climate change, at least in regulated scenarios (Sc.2, 3 and 4).

That said, the various oils are not completely substitutable and their technical and nutritional characteristics must also be taken into account. For example, the needs in short chains omega 3 fatty acids (alpha linolenic acid) are probably not covered today by soyabean and rapeseed production, or just covered taking into account other sources. In future, covering alpha-linolenic needs seems to be no difficulty only in scenario 3, with the lowest population.

The overall growth of food proteins of animal origin is estimated between 11 and 32 million tons of (pure) protein depending on the scenario, 26 to 39% more than in 2013. The multiplier effect of the consumption of animal products appears very clearly because of the conversion of animal proteins into vegetable proteins needed to produce them: from +52 to +141Mt

(including fodder) or +16 to +41% depending on the scenario. Whatever the scenario, the growth of plant proteins for animal feed is high and exceeds the human food destination, but much more pronounced in Sc.2 and 3, more demanding in animal protein, than in Sc. 1 and 4, where the increase in materials rich in protein (fodder excepted) is almost evenly distributed between feed and food.

Table 2: Quantities of vegetable protein to meet demand in the four scenarios

	2013	SC1	SC2	SC3	SC4
Million tons of protein	**Present**	**Chaos**	**Blocks**	**Trust**	**Climate rupt**
Feed fodder	112	153	148	162	129
Feed "rich in proteins"	**227**	**264**	**315**	**316**	**261**
Food	**139**	**174**	**159**	**146**	**168**
ratio Food/(Feed rich. + Food)	**0,38**	**0,40**	**0,33**	**0,32**	**0,39**

Regarding vegetable protein for human nutrition, Sc. 3, which combines economic growth, low population growth and "meat" dietary habits shows the lowest increase (+5.2 %), followed by Sc. 2 (+ 14%). In Sc. 1 and 4, the increase exceeds 20%. The ratio "Food" proteins/ Protein concentrated "Feed + Food" proteins shows a discrepancy between scenarios 1 and 4 on the one hand, where the relative weight of vegetable protein for human consumption increases, and scenarios 2 and 3, where it decreases.

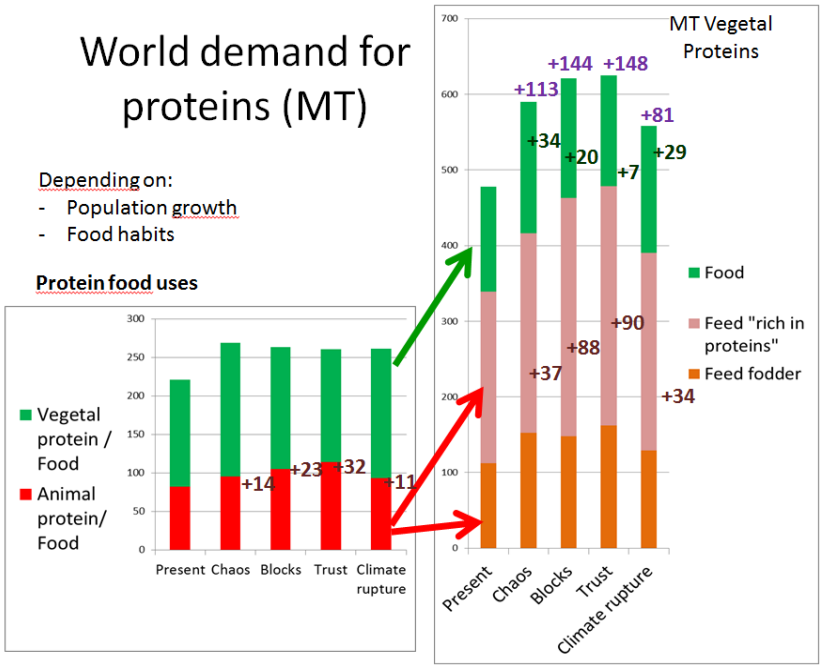

Figure 3: Evolution of demand for proteins in the 4 scenarios

The vegetable proteins that would be provided in the various scenarios are not targeted to the same uses or in the same proportions, and in rather different market conditions: Sc. 3 "Trust" demands masses of proteins for animal feed, without differentiation on the GMO origin regarding Europe, but with more regular qualities, notably because of the disappearance of antibiotics in animal production. The growth of plant proteins for food is the lowest, but also

more qualitative from a nutritional point of view, and more "technological" with an R & D driven by the needs of wealthy clients and / or with special needs (elderly, athletes). The vegetable protein is desired for nutritional and technological properties and image. Sc. 2 "blocks" demands for livestock protein masses too, but this time with differentiation of Europe, on the GMO origin and on the nutritional quality through technically more restricted feed system. The growth of plant protein for food is higher than in scenario 3 but with the same "nutritional and technological" profile globally. Sc. 1 "Chaos" demands protein masses for breeding, but for animal feeding systems with few constraints on technical practices, guided mainly by production costs reduction. As for vegetable protein for food, their development is driven by the preoccupation to offer cheap alternatives to animal proteins to impoverished populations, either as raw or minimally processed vegetable products, either as vegetable industrial products or combining animal and vegetable proteins. The role of plant protein is primarily economic. In Sc. 4 "climate rupture," the technical constraints on animal feeding are at an intermediate level and above all guided by the carbon balance sheets. Regarding food, the quality / price ratio is more strongly driven by the dietary and nutritional considerations and health concerns, and respect for the environment (image). At last, the development of aquaculture, with specific protein needs, is a constant of the scenarios.

Will the production be able to follow the demand?

The assessment of the demand in different contexts is not enough: we must ensure that supply can follow, taking into account the constraints on production. The calculations allowed us to assess the needed acreage to meet the demand for our scenarios in 2030(table 3).

Table 3 *orders of magnitude* of cultivated acreage development required in the 4 scenarios

Acreages in million ha	2013	SC1	SC2	SC3	SC4
world cultivated acreage, foddder crops excluded	1287	1461	1472	1362	1457
world cultivated acreage, fodder crops included	1534	1767	1764	1656	1731
world cultivated acreage variation, fodder crops excluded		**174**	**185**	**75**	**170**
cultivated acreage variation fodder crops included		**233**	**229**	**122**	**197**

Given the assumptions, between 75 and 185 million more hectares would be needed to meet the needs for "monogastric" quality feed, and from 122 to 233 including forage crops. The continuation of the pace for land reclamation observed between 1961 and 2000 (rate of 3.75Mha/yr on average) would make 67.5 million hectares by 2030 (18 years). The reverse calculation shows that the land reclamation rate required for the consistency of each scenario would be respectively 3.5, 3.4, 1.7 and 2.9 times the rate of past decades. The likelihood of those figures is subject to discussion, especially with depressed economy. The limit of these extensions of cultivated acreage also lies in the deforestation and greenhouse effects, even if part of the effort can be done without deforestation (INRA CIRAD, 2009). Pursuing further the reflection shows that balancing supply and demand always questions the development dynamics and sometimes asks to reconsider some assumptions, particularly in terms of ambition on improving the diets protein content. The strategic aspect of improving yields at world level (particularly protein yields) is highlighted, and the importance of climate risk in terms of global average yields evolution.

Meeting the needs will be much harder for vegetable proteins than for oils and fats, and will require to play on all levers: food consumption level, waste reduction, productivity.

So it is very clear that protein production is an important part of the future competitiveness of oilseed crops, among them sunflower.

How changing context might affect the competitiveness of different oil & protein crops?

The scenarios highlight issues and technical and scientific challenges that the different crops and agro-industrial sectors can meet more or less easily. We suggest in table 5 an interpretation of the specific characteristics of sunflower as strengths or weaknesses in the different scenarios, i.e. as existing advantages or areas of progress through R&D or value chain organization.

Table 4: composition, protein and oil yields for major crops

Composition, yields, oil and protein yields for main crops/ yields						World (FAO)			Europe (FAO)		
Crop	%	% on product for use				Average yield 2009-2013	aver. oil yield	aver. protein yield	Average yield 2009-2013	aver. oil yield	aver. protein yield
	DM	starch	oil	protein	others	t/ha					
soya	89%	5,7%	18,9%	35,2%	29%	2,5	0,5	0,9	2,65	0,50	0,93
sunflower	93%	1,2%	44,5%	15,4%	32%	1,2	0,5	0,2	2,33	1,04	0,36
rape	92%	3,1%	42,6%	19,3%	27%	1,9	0,8	0,4	3,56	1,51	0,69
pea	87%	44,4%	1,0%	20,7%	22%	1,6		0,3	3,82		0,79
maize	87%	63,7%	3,6%	7,9%	12%	5,2	0,2	0,4	9,36	0,33	0,74
wheat	86%	59,3%	0,0%	10,8%	15%	3,1		0,3	7,15		0,77
Forage and Silage alfalfa	20%	0,0%	0,0%	4,0%	16%	22,0		0,9	42,50		1,70
Forage and silage, maize	23%	3,5%	0,6%	1,9%	17%	34,6	0,2	0,7	39,60	0,24	0,75
Forage and silage, sorghum	28%	0,0%	0,5%	2,3%	25%	20,4	0,1	0,5	35,20	0,19	0,81
Forage and silage, rye grass	17%	0,0%	0,0%	2,5%	14%	10,3		0,3	9,60		0,24
Forage and silage, clover	17%	0,0%	0,5%	4,2%	12%	34,1	0,2	1,4	23,20	0,11	0,97

Table 5: Sunflower characteristics, Strenghts (S) and Weaknesses (W) in scenarios (N = Neutral)

Sunflower characteristics	*level*	SC1 Chaos	SC2 Blocks	SC3 Trust	SC4 Climate rupt
Dependance on nitrogen	medium	**N**	**S**	**S**	**S**
Dependance on pesticides	medium	**N**	**S**	**N**	**S**
Rusticity	high	**S**	**S**	**N**	**S**
Compensation capacities	weak	**W**	**N**	**N**	**W**
Protein digestibility/ feed	high (dehulled)	**S**	**S**	**S**	**S**
protein digest & quality/ food	???	**S**	**N**	**N**	**S**
ability to dehulling	good	**S**	**S**	**S**	**S**
miw oil and protein specy	44%oil/15%Pi	**W**	**N**	**S**	**S**
of fatty acids profiles	yes	**S**	**S**	**N**	**N**
yield level	relatively low	**W**	**W**	**W**	**W**

Some issues are common to all scenarios: it is the case for the protein economy, i.e. both protein productivity and efficiency of use of the produced proteins. The protein yield per hectare is a fairly discriminating criterion between species, which will play depending on the relative performance of crops in the different parts of the world. Only forage legumes such as clover or alfalfa today exceed a ton of protein per hectare. If these species are now mostly devolved to ruminant animals, protein extraction can change things and forage legumes competitiveness could increase, both in the field of conventional feed and in the area of concentrates and protein isolates. Then come pulses and soybeans, which reach protein yields

of around one ton/ha, followed by rapeseed (0.4 to 0.7 t/ha) and sunflower (0.2 to 0.4). Their global competitiveness will also come from their co-product, oil or starch. In this regard, sunflower is not very competitive: global yields are the first priority, before a specific focus on protein yields.

In the scenarios that show a significant development of the biorefinery (Sc. 3 and 4), the different species ability to easy extraction of vegetable protein may be an advantage. From this point of view, the pulses already fall in well-established industrial processes, while the extraction of protein from oil crops species will require changes in industrial processes, to avoid the protein fraction degradation in the present crushing-oil extraction process.

The efficiency of proteins use in animal feed is already a strong competitiveness criterion whose importance is expected to increase, both for economic and environmental reasons. Sunflower progressed in this aspect by including dehulling in the process and developing cultivars more adapted to dehulling. Alternative biorefinery processes for soft and direct oil and protein extraction would be an area of progress for sunflower.

The functional and technological properties of proteins from different species will be a determining criterion, especially as uses will be advanced in nutrition (health food: Sc.3 and 4) and / or technology (use of vegetable protein in food industries for their functional qualities and as substitute for animal protein: Sc.1, 2 and 4). Today the characterization of plant proteins of species grown in Europe, including sunflower, is still limited. These qualitative aspects also play in the ability of different species to meet the needs of aquaculture.

As for oil crops, fatty acids profiles could contribute to differentiate species on the edible oils market. Sunflower remains an oil crop first, and the past and current works for cultivars with specific fatty acids profiles, like High Oleic, or High Stearic High Oleic, or low saturated profiles, etc… will certainly play significantly in its future competitiveness. Regarding human nutrition, minor compounds like tocopherols are also a way to develop the sunflower attractiveness as edible oil.

Other issues and challenges relate to some of the scenarios only. Thus, the production of vegetable protein for human consumption is much higher in Sc. 1 and 4, and should quite naturally benefit pulses and soya, which are already current in the eating habits of many countries, like India, which may well become a structural importer. The fact that sunflower seeds do not content anti-nutritive or toxic compounds gives good opportunities for food uses, even if its amino-acid profile is low in the essential amino-acid lysine. Perhaps more attention could be given to proteic and amino-acids profiles, like for amino-acids until now.

Sc. 2 "blocks" presents a huge challenge for supplying Europe with non-GMO protein, which would lead to make better use of the European territory with the most suitable species and production systems to this objective. Sunflower could well contribute to this challenge, due to a large set of non GMO cultivars and to its rusticity or ability for cropping in contrasted conditions.

The synthetic nitrogen fertilizer requirements for agricultural production would penalize rapeseed, and sunflower too, to a lesser extent, and would favour grain legumes, forage legumes and soybeans in scenarios setting either carbon taxes (Sc. 2 and 4) or restrictive production standards on nitrogen and carbon energy (Sc. 3). Sunflower is relatively nitrogen efficient, and developing this characteristic would be advantageous in 3 scenarios at least.

Developments related to climate change will play in contrasting ways in different parts of the world. In Europe, more stressful conditions are expected. Heat peaks and drier prolonged periods in spring and summer would disadvantage spring crops in rainfed conditions.

Sunflower has the relative advantage of some rusticity, but its compensation capabilities are limited by the nature of its yield components.

The future competitiveness of different crop species depends on many factors (economic, political, climatic, social ...), who will play differently depending on the regions of the world, and whose the result is difficult to imagine. We venture to offer a synthesis of the outlook for different species according to their current characteristics and without assuming the future success of any current or future research and development measures (Table 5).

Table 6: Crops potential progression by 2030, based on their current features.

Crops potential progression by 2030	SC1 Chaos	SC2 Blocks	SC3 Trust	SC4 Climate rupture
WORLD				
Rapeseed	-	++	+	-
sunflower	-	+	+	-
Soyabean	++	+++	++	++
Grain legumes	++	+++	++	+
EUROPE				
Rapeseed	-	-	=	--
sunflower	-	--	-	--
Soyabean	+++	+++	++	+++
Grain legumes	+	+++	+++	+++

Table 7: Challenges for Sunflower crop chain	SC1 Chaos	SC2 Blocks	SC3 Trust	SC4 Climate rupt
Crop adaptation to climate change				
Yield T/ha & economic competitiveness				
Protein yield/ha				
Nutritional quality of oil				
Soft process for protein and oil extraction / biorefinery				
Uses of proteins for human nutrition and food industries				
priority level	*secondary*	*medium*	*high*	

This outlook may appear as pessimistic for sunflower; it shows the importance of the challenges. Compared to other sources of oil and/or proteins, sunflower is actually disadvantaged by its yield level. But we may consider that it has two main categories of assets, which are on one hand its rusticity, which makes it suitable to make profit of very diverse conditions (relatively dry, short seasons, double seasons, etc…), where other species are not suitable or not more profitable, and on the other hand specific characteristics regarding quality for oil and probably for proteins. We may attempt to propose priorities between the main challenges for sunflower in the 4 scenarios, as a preparatory step for further reflections for elaborating R&D strategies (table 7).

Conclusions: Is the protein fraction the future of oilseeds?

It seems clear that the economic value of the protein fraction is a key aspect of the future of oilseeds such as sunflower. The future tension for proteins at world scale appears quite certain. At the first level of approach, the yields and protein yields of the different crops will

determine their competitiveness for mass uses, including animal feed. At the second level, the issue of nutritional and functional properties of proteins from oilseeds must be considered, with a vast field of exploration to enhance uses with higher added value, in human nutrition, feed, and technology. From this point of view, soy is much more advanced than rapeseed and sunflower and grain legumes. The effective use of proteins from sunflower will also require technological research and adaptations of industrial extraction processes.

That said, the scenario of the oil flood is a trend ... but not absolute certainty, especially if oleochemistry shows economic developments in Asia. Vegetable oils are noble natural materials which may be used in many ways, depending on their fatty acids profiles, the first one remaining food for which sunflower oil has well-established qualities.

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ESCAPE TO TINY BUG (*NYSIUS SIMULANS* STÅL) ATTACK ACROSS PLANTING DATE ADJUSTMENT IN SUNFLOWER HYBRID SEED CROPS FROM SOUTHERN BUENOS AIRES PROVINCE, ARGENTINE.

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ABSTRACT

During 2014-15 growing season, tiny bug (*Nysius simulans* Stal.) became a serious plague in the Southern Buenos Aires Province, Argentine. The insect reach an incidence level >900 individuals per head (i/head) and seriously decreased the yield of sunflower hybrid seed. Insecticides could reduce pollinator activity during R4-R6 stage, and did not reach the floral involucres at R7-R9 stage. A sample of 32 commercial plots (≈1000 ha, 5 female lines) with early (EP = October) or late (LP = November) planting dates was studied. At the peak of plague attack, during last week of January, the EP crops showed R7 stage and incidence was > 75 i/head. At the same time, the LP crops showed R4 stage, incidence was < 50 i/head and never overcome this level. In spite of average 2-3 insecticide applications, hybrid seed yield of EP crops was (**) less than a half of LP (1.1 t/ha). This fact was associated with increase (> 10%) of seed set (*) and seed biomass (**). In spite the bug herbivory did not affect the tetrazolium test (> 94%), it produces small holes which decreases germination across a reduction of normal seedlings. Germination of EP seed (88%) was 9% less (**) with respect to LP. At planting time, the field emergence of hybrid seed obtained by EP was 17% less (**) than the obtained with LP (87%). Under plague attack risk, it is recommended to escape the tiny bug peck by means of planting date adjustment.

Key words: *Nysius* sp., Seed quality, Pest escape.

INTRODUCTION

The valley of Colorado River, in southern Buenos Aires province (VBRC), is a healthy area which constitutes the main sunflower seed production area from Argentina (Cantamutto et al. 2008). The valley comprises ca. 90,000 ha with gravitational watering, devoted to onion and forage crops (Lucanera et al. 2014). Sunflower hybrid seed production comprises around 10% of the irrigated acreage.

In South America, tiny bug, *N. simulans* Stål (Hemiptera: *Lygaeidae*) is distributed in Paraguay, Uruguay, Peru, Brazil and Argentina. This polyphagous species was registered feeding several crops and broadleaf weeds in Argentina (Molinari and Gamundi 2010). While the tiny bug had been present in the VBRC for more than a decade ago, no history was documented about its direct impact on crops (Dughetti et al. 2015).

Nysius simulans is a polyphagous suctorial insect that draws water and nutrients. Their saliva transmits toxins and spreads pathogens (Dalazen et al. 2014). Usually, the plague invades crops after host senesce at the end of summer (Demirel and Cranshaw 2006,

Charleston 2013), as had been observed on the *Conyza* sp./soybean complex in Brazil (Dalazen et al. 2014). At northern and central regions of Argentina, tiny bug attacks generally account during early crops stages of soybean or sunflower (Molinari and Gamundi 2010). The *Nysius* sp. attacks at advanced growth stages of sunflower had been registered in Australia (Forrester and Saini 1982) and Pakistan (Kakakhel and Amjad 1997).

During December 2014, the VBRC was suddenly affected by this new biotic threat to regional crops. Although the tiny bug attack was first observed feeding horticultural crops, soon it became extremely notorious on sunflower crops. This paper examines the seasonal effect of *N. simulans* on sunflower hybrid seed production and seed quality during the spring-summer cycle 2014-15.

MATERIALS AND METHODS

The population dynamic of *N. simulans* was monitored on two sunflower crop planted at early (EP = October 12 2014, 39°24´S, 62°38´W) or late (LP = November 15 2014, 39°18´S, 62°32´W) dates. Both crops were sampled at weekly intervals since pre-flowering (R4, Schneiter and Miller 1981) until physiological maturity (R9), during January to April 2015. Sampling was done on thirty plants, randomly selected in each crop. Adults and nymphs of *N. simulans* were separated from the heads by hand and the number recorded as individuals per head (i/head).

The impact of plague feeding on sunflower hybrid seed production and quality was measured on 32 commercial lots during 2014-15 growing season. The sunflower hybrid seeds lots were produced under controlled conditions for private companies. The geographical distribution of the studied lots was representative of the VBRC agroecological conditions (Fig. 1). Planting date, insecticide applications and unitary yield of each lot (kg ha^{-1}) were registered. The study comprises 5 female lines, with 20 to 100 ha per lot surface, totalizing ≈1000 ha. After physiological maturity a samples of heads were random hand harvested (n = 8), air dried and threshed in the laboratory. Each head were evaluated for 1000-seeds weight (14% moisture basis) (ISTA, 2013). Achenes were manually separated into empty and nonempty (filled). Seed setting efficiency (seed set) were calculated according eq. 1.

Seed set = (nonempty achenes / (nonempty + empty achenes)) x 100 (eq. 1)

Germination was evaluated immediately after harvest, during February to March of 2015. Nonempty (filled) seeds were submitted to method for dormancy breaking. Achenes were dried at ambient condition (28°C±2°C; 72 h) and soaked 24 h between towels wetted with a gibberellic acid (GA_3) solution at 0.05% in water (Seiler 1998). Germination test was carried out in sand in groups of 100 seeds (n = 3) in a climate-controlled chamber at 20°C during the 12 h dark period and 30°C during the 12 h light period (ISTA, 2013). Final value of germination was measured at 10 days.

At the same time, seed viability was estimated by tetrazolium test on samples of 50 achenes (n = 3). Embryos were separated from achenes after 17 h of water hydration. Embryos were placed in a 0.5% tetrazolium solution during 4 h and classified as completely dyed (viable), partly dyed, or not dyed (inviable) seeds. Location, size of undyed areas, and intensity of dyeing was considered according to ISTA (2013). Small undyed spots of some tissues due by *Nysius* damage were registered.

The seed were stored at 10±2°C (≈ 50% H°) since harvest up to planting season during seven months (October 29 2015). Thereafter, field emergence was assessed in a sandy loam soil (pH = 7.5, soil organic matter = 1.2 %, available P Bray & Kurtz = 24 mg kg^{-1}) at field capacity (21%) located in Hilario Ascasubi Experimental Station. One hundred seeds were manually sown at 30 mm deep in single row plots, 1.5 m long and 0.2 m apart, under a randomized block design with three replicates. Emergence (V2 stage) was counted at 8 and 14 days after sowing.

Analysis of Variance (ANOVA) considering early (EP = October) and late (LP = November) planting dates factors was performed using InfoStat software (2014). The relationship between valuable parameters was analyzed by regression analysis (GraphPad 6.0, San Diego, California, USA).

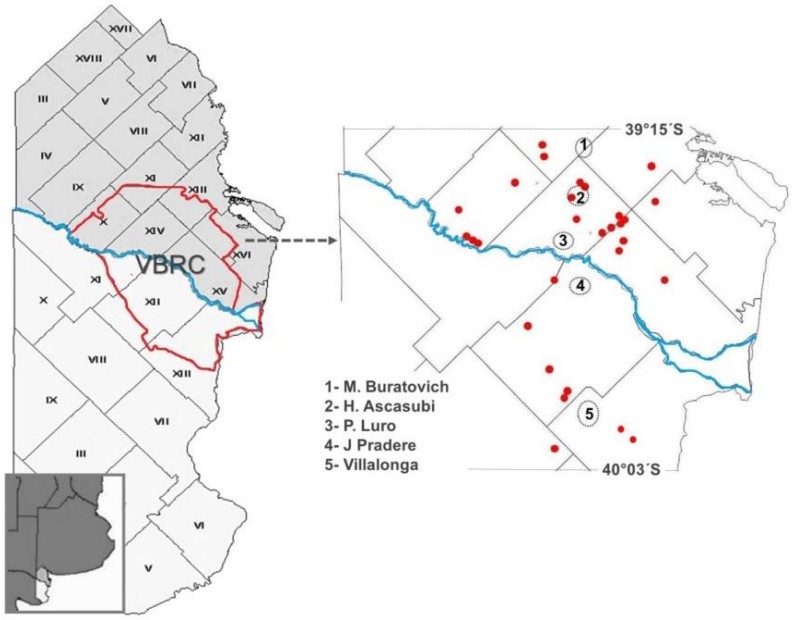

Figure 1. Geographical distribution of the sunflower hybrid seed production lots studied in the VBRC during 2014-15 growing season.

RESULTS AND DISCUSSION

During 2014 winter only 17 frosts at ground level were recorded (Renzi et al. 2015). This value was equivalent to less than half of the observed in the previous two winters, which not tiny bug attacks was happened. The fewer frost number could have reduced the field mortality of winter resistant individuals. Moreover, the conditions for winter growth of annual weeds, with 28% more rainfall in relation to the historical average (1960-2013) were excellent. Thus, an initial high population size at the end of the winter could have been possible the extreme values observed and outbreak of the pest. *Nysius* sp. is highly mobile, and can migrate from alternative crops or no-cultivated habitats to sunflower and congregate upon them causing significant injury (Demirel and Cranshaw 2006).

The tiny bug was detected on sunflower during the first half of December 2014 and the highest incidence of pest was recorded during January and early February 2015. The highest density of pest individuals was concentrated in the head, reaching extremes values over 900 i/head. Individuals mainly refuge between paleas of disk flowers. To a lesser extent, individuals were located between head bracts, upper leaves and in the stem segment near the head.

The infestation pattern dynamic of *N. simulans* on the sunflowers in the VBRC was consistent with those described by Smith and McDonald (1982). According Charleston (2013) the damage threshold ranks between 10-50 adult bugs per plant, depending of growth stage. In the VBRC, during 2014-15 season adults invaded the sunflower at the flowering stage and suddenly overcome these values in EP crops. During seed filling period (R6-R9) of EP crop the plague reached incidence levels over 75 i/ head, but it was less than 25 i/head at LP (Figure 2). As the plants senesce and dry off, there was a general incidence declination. Presumably adults began to disperse away searching for overwintering refuges, in response to the photoperiod and temperature decreases (Smith and McDonald 1982). Understanding the lifecycle of *Nysius* sp. is extremely necessary to adjust the chemical control decisions (Charleston 2013).

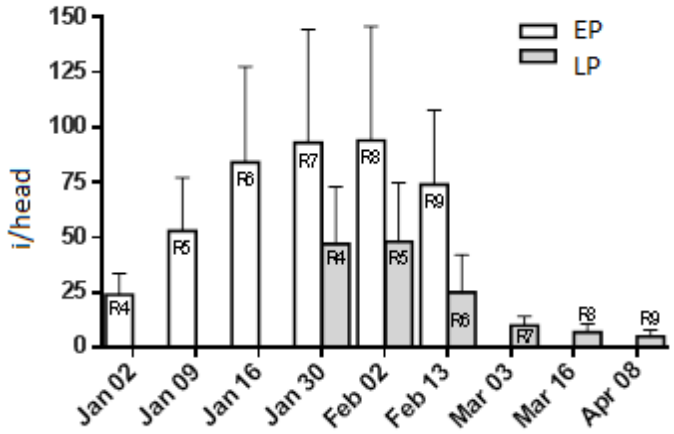

Figure 2. Incidence of *Nysius simulans* (Stål) on sunflower heads (i/head) under EP (October 12 2014) or LP (November 16 2014) planting dates. Rn means sunflower growth stages (Schneiter and Miller 1981)

Given the re-infestation of the plague, it was recorded cases with more than three insecticides applications during the R5-R6 stages, with a higher number of applications under EP (Table 1). The insecticide applications slightly reduced the set efficiency (R^2 = 0.04 *), probably due for the insecticide effect on pollinators activity. It was observed a general improvement of hybrid seed yield with LP dates (November). This improvement was associated with a declination of pest density during the flowering and fruit filling stages (Figure 3). With the delay of planting date (November), the yield improvement was associated with an increase of seed set and 1000-seed weight (Table 1).

The tiny bug feeding reduced the development of the embryo and achene, affecting germination (Kakakhel et al. 2000). However in this evaluation, bug herbivory did not affect the tetrazolium test (> 94%), but produces small holes which decreases germination value due a reduction of normal seedlings. Germination of EP seed (88%) was 9% less (**) with respect to hybrid seed harvest in plots planted during November (Table 1). Also the field emergence at 8 and 14 days after planting was higher when hybrid seed was harvest on LP crops.

Due climate change, tiny bug might challenge sunflower hybrid seed production activity in the near future. It was observed a general improvement of yield and seed quality at late planting date (November), possibly because the flowering occurred when the population pest density decreased. Under plague attack risk, it could be recommended to escape the tiny bug population peck by means of planting date adjustment.

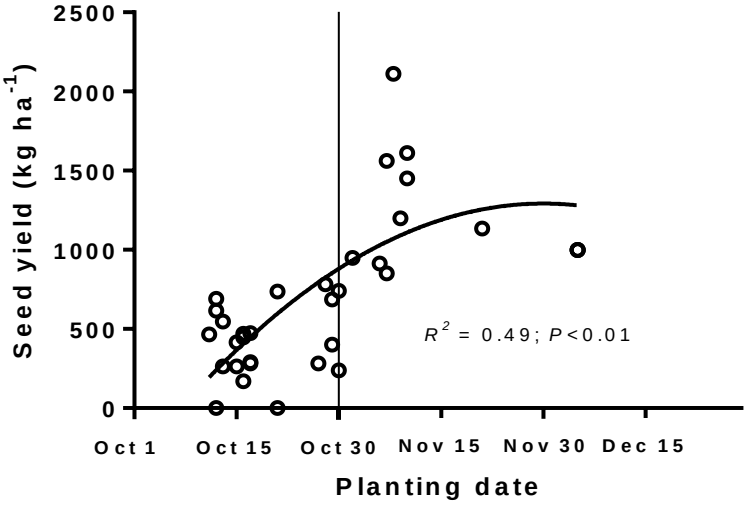

Figure 3. Effect of planting date on the yield of a sample of hybrid sunflower seed lots harvested in the VBRC during 2015. Vertical line (—) show the limit between early (= October) and late (= November) planting date.

Table 1. Effect of planting date on the seed yield, yield components and seed quality of sunflower hybrid seed

Traits	Planting date		
	EP (October)	LP (November)	
Seed production lot			
Insecticides applications (n)	3.3	2.5	**
Seed set (%)	38	47	*
1000-seeds weight (g), at 14% H°	65	79	**
Seed yield (kg ha^{-1})	460	1147	**
Produced seed			
Viability - tetrazolium test (%)	94	99	ns
Germination (%)	88	97	**
Field emergence, 8 days after sowing (%)	70	87	**
Field emergence, 14 days after sowing (%)	79	91	**

*, ** significant differences between planting dates at $P < 0.05$ and 0.01; ns not significant

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SUSTAINABILITY OF SUNFLOWER PRODUCTION FROM THE POINT OF PRODUCERS

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ABSTRACT

Vegetable oil consumption has constantly increased due to both rapid population growth and increased consumption per capita in Turkey. However, considering the charecteristics of climate and soil, though the production of oil seeds has a big potential, sufficient increase in sowing area and production has not occured. Because of insufficient production to meet consumption, the gap that has gradually increased in the important amounts of vegetable oil has met by importation. The most produced oil seed in Turkey is the sunflower because of its 85% pay in the consumption of vegetable oil and its high oil content. However, although the support that closes the gap in sunflower production and provides sustainability of production, the production increase desired is not provided since the parity between sunflower and wheat is in favor of wheat and the sunflower costs are much more than those of alternative crops. Thus, only 34% of the total supply of sunflower oil is produced from the sunflower seed domestically. Therefore, sunflower seed for oil is the most important crop to decrease the gap of vegetable oil. In this study, the factors that sunflower producers consider to increase/sustain their production and the necessary factors for them in a support policy are analysed by Best-Worst method. The results of the face to face surveys of 264 producers in 5 provinces who produce 69.3% of sunflower seed for oil in Turkey are used in the study.

Key Words : Sunflower oil, Sustainability, Support, Best-Worst Analysis

EVALUATION OF APPLICATIONS OF THE SUPERVISION PRICE AND CUSTOMS DUTY IN SUNFLOWER FOREIGN TRADE

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ABSTRACT

Oilseeds and vegetable oils have the biggest foreign agicultural trade gap in Turkey. Insufficient production to meet consumption is the main cause of this situation. Moreover, the increasing need of vegetable oil increases this gap further. To decrease this so-called gap and dependence on foreign trade, the oilseeds production that is source of raw materials in vegetable oil production should be increased. Sunflower for oil that is the most produced crop and have the big share in oil consumption in Turkey is the most privileged crop to decrease the gap of vegetable oil. However, the sunflower seed produced domestically meets only18% of the total vegetable oil demand. The rest of it is met by the importation of seed and raw oil. This situation cause Turkey to be among importing countries, processing industry to be depenedent on imported products. Therefore, the applications of supervısıon prıce and custom duty ın raw sunflower oil foreign trade are important to protect the domestic production from lower prices and sustain the production of it. In this study, the aim is to evaluate the impacts of the applications of supervısıon price and custom duty to protect producers on sunflower foreign trade and other sectors and to develop suggestions.

Presenting author : Dr. Kemalettin TAŞDAN

Key Words : Vegetable Oils, Sunflower, Supervision Price, Customs Duty

DETERMINATION OF THE YIELD AND YIELD COMPONENTS PERFORMANCE OF SOME SUNFLOWERS (*HELIANTHUS ANNUUS* L.) UNDER RAINFED CONDITIONS

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ABSTRACT

The objective of this study was to determine the yield and yield components of oilseeds sunflower cultivars in semi-arid conditions. The study was conducted at the experimental field of Ahi Evran University in a randomized complete blocks design with three replications during the years of 2012 and 2013 in Kırşehir. Ten sunflower cultivars grown in semi-arid conditions (Bosfora, Hornet, Sanbro, Sanay, Tunca, Transol, Oliva, Tarsan, Sirena, ve Reyna), were used in the experiment. The characters investigated were days to flowering, physiological maturity, plant height, head diameter, thousand seeds weight, oil content, seed yield, hull-kernel ratio, protein rate and crude oil yield. According to the results of this study plant height, physiological maturity, plant seed yield per plant of the varieties changed between 107.6 - 137.7 cm, 130 - 134 days and 35.9 - 52.8gr/plant respectively. Tunca had the highest seed yield with 152.8 kg/da. The highest crude oil ratio (53.7%) was observed from cultivar Sirena, while the lowest one (49.3%) was observed from cultivar Transol. Crude oil yield ranged from 60.2 kg/da (Sirena) to 82.0 kg/da (Bosfora). In considering with the seed and crude oil yield, cultivar Transol, Tunca and Bosfora can be recommended for the semi-arid conditions.

Key words: Sunflower, Helianthus annuus L., yield, crude oil rate

INTRODUCTION

Sunflower is the most widely cultivated oilseed plant in Turkey. To meet the rapidly growing demand for vegetable oil, the production of oilseed plants, and especially of sunflower, has been increasing countrywide. In 2015, sunflower seed production in Turkey was approximately 1.5 million tons, with 570,000 hectares of land dedicated to the cultivation of this crop. Owing to its high adaptability to arid conditions, sunflower cultivation is fairly widespread in the Central Anatolian region of Turkey, with 292,960 tons of sunflower seeds being produced over 71,890 hectares of land within this region in 2015 (Anonymous, 2016). Although sunflower is a highly adaptable plant, varieties might exhibit different responses in different ecologies, leading to considerable variability in terms of both yield and yield characteristics (Baydar, 2000).

Nearly all of the sunflowers cultivated in Turkey for oilseed production are hybrid varieties. As hybrid sunflowers varieties show greater stability than non-hybrid varieties, they also exhibit a lower degree of genotype-environment interactions, thus ensuring higher and more stable yields. The use of hybrids consequently allows higher levels of production by increasing yield per unit area (Göksoy, 1999). However, despite the availability of varieties with high yield stability, it is also necessary and important to conduct studies investigating regional varieties with high seed and oil yield as well as high pest and disease resistance that are well adapted to local environmental conditions (Karaaslan, 2001; Tunçtürk et al., 2005; Yılmaz and Kınay, 2015). As with the cultivation of other plants, the use of sunflower varieties suitable to a particular region is an important factor that affects yield and quality during sunflower cultivation. Various studies performed in different regions and ecologies of Turkey have shown that sunflower yield levels vary between 76 to 650 kg/da, while seed oil content varies between 33 to 48% (Arslan et al, 2003; Karaaslan et al., 2010; Kara and

Başalma, 2011; Çil et al., 2011; Karakaş and Arslanoğlu, 2013). The fact that sunflower is considerably affected by regional conditions during cultivation leads to significant variability between different varieties in terms of yield and yield characteristics.

As the oilseed plant species with the highest level of adaptability, sunflower can easily adapt to many different environmental conditions. The climate and soil characteristics of the Kırşehir province in Turkey are particularly suitable for sunflower cultivation. The mechanization of maintenance and harvesting activities, as well as the implementation of methods for ensuring adequate yield for per unit area, have helped promote sunflower cultivation in the region, and enabled sunflower agriculture to grow and expand rapidly. Recent incentive programs for oilseed plants have rendered the cultivation of these plants more attractive, while the use of hybrid varieties have enabled producers to obtain higher yields per unit area. In 2015, sunflower was planted in nearly 21,384 decares of land in Kırşehir, and 3,874 tons of sunflower seeds were produced, corresponding to a yield of 181 kg/da (Anonymous, 2016).

Private companies supply numerous different types of commercially produced hybrid sunflower varieties to market. Various regional studies are conducted to determine which varieties provide superior characteristics, and the results of such studies are routinely shared with producers and the industry. This study aimed to determine the performance under semi-arid conditions (rainfed) of hybrid varieties that are recommended for agricultural areas lacking adequate irrigation potential.

MATERIALS AND METHODS

The study was conducted at the experimental field of Ahi Evran University in a randomized complete blocks design with three replications during the years of 2012 and 2013 in Kırşehir. Trials were performed using 10 different hybrid sunflower varieties developed by private companies that are suitable for the region's arid conditions. As fertilizer, 8 kg/da of nitrogen, 6 kg/da of potassium and 6 kg/da phosphorus was applied to all trial parcels. Seeds were planted to the 4.2 m to 3.5 m parcels in five rows at 70 x 30 cm intervals on the second week of April (13 April 2012 and 15 April 2013).

The following parameters were evaluated within the scope of the study: days to flowering (day), physiological maturity (day), plant height (cm), head diameter (cm), thousand seeds weight (gr), oil content (%), seed yield (kg/da), hull-kernel ratio(%),protein rate (%) and crude oil yield (kg/da). Variance analysis was performed on the obtained data according to the random blocks method (Düzgüneş, 1987). The Duncan test was employed to determine the significance of the differences between the trials. All statistical calculations and the variance of data was analyzed by MSTATC software and the means were compared by Duncan's Test. Soil characteristics were generally argillaceous loamy soil with moderate salinity, moderate calcareousness and low organic content (Table 1).

Table 1. Soil properties of the experimental field at 0-30 cm depth

Saturation%	pH	EC (mmhos/cm)	Tuz (%)	Absorbable P (%)	CaCO3 (%)	Absorbable K (ppm)	Organic matter (%)
55	7,59	0,58	0,021	0,19	21,8	63,78	1,39

Table 2. Meteorological data for the study period of 2012-2013 and long term mean.

	Mean monthly								
	Relative humidity (%)			Precipitation (mm)			Temperature (°C)		
	1970-2013	2012	2013	1970-2013	2012	2013	1970-2013	2012	2013
January	83.70	78.00	83.70	43.60	97.10	29.10	-0.20	-2.20	1.30
February	79.80	74.50	74.40	34.60	30.90	39.40	1.10	-2.70	4.70
March	68.40	67.60	63.00	35.90	36.20	14.20	5.40	2.40	7.10
April	50.30	63.80	63.20	45.60	20.10	46.20	10.60	13.30	11.90
May	66.50	61.00	50.70	43.90	109.50	15.10	15.30	15.40	18.00
June	47.70	54.30	41.10	34.50	11.90	1.00	19.60	21.60	20.40
July	38.80	48.40	41.20	6.70	1.40	6.60	23.10	25.30	22.70
August	42.00	48.70	39.70	5.00	0.00	0.20	22.80	23.00	23.10
September	39.40	53.20	50.00	11.80	1.20	32.00	18.20	20.60	16.80
October	63.00	63.70	52.90	29.20	59.30	20.50	12.40	14.70	10.50
November	82.50	73.00	67.10	37.90	41.70	40.00	6.20	7.40	7.60
December	84.60	78.60	75.70	48.60	90.10	10.40	2.00	3.30	-2.31
Total				377.30	499.40	254.70			
Mean	62.23	63.73	58.56				11.38	11.84	11.82

Relative humidity between the months of April and September (the period the study was conducted) was close to the long-term annual averages in 2012, and slightly below the long-term annual average in 2013. Annual precipitation was 499.40 kg/m^2 in 2012, which was above the long-term average precipitation values. On the other hand, annual precipitation was 254.70 kg/m^2 in 2013, which was considerably below the long-term average precipitation values. Total monthly precipitation was observed to be irregular during the months of sunflower cultivation. Precipitation was 109.5 kg/m^2 in April 2012; although this level of precipitation might appear to have a positive impact on the total level of annual precipitation, irregular precipitation in the other months have the potential to adversely affect yield. Temperature values during the cultivation period were above the long-term average for the region. In sunflower cultivation, climatic factors have an important effect on yield and yield characteristics, especially in rainfed agricultural areas.

RESULTS AND DISCUSSION

Data analysis showed that observed parameters during the two years were significantly ($p<0.01$) difference between years. In arid regions, climatic condition during the cultivation period have a significantly effect on yield and the yield components. Differences between the varieties in terms of the time to flowering, time to physiological maturation, plant height and head diameter were significant ($p<0.001$), while differences between the varieties with respect to BDA were not significant (Table 3).

Table 3. Analysis of variance for sunflower cultivars under rainfed conditions.

Source	DF	Time to flowering (day)	Time to physiological maturation (day)	Plant height (cm)	Head diameter (cm)	Thousand seed Weight (gr)
Replication	2	0.117ns	0.017ns	0.113ns	0.329ns	75.819ns
Year	1	749.067**	984.150**	3,523.601**	45.763**	3,412.695**
Cultivars	9	3.748**	14.350**	481.318**	3.224**	25.587ns
Year*Cultivars	9	0.215ns	0.780ns	51.495ns	0.415ns	47.689ns
Error	38	0.731	2.859		0.435	42.148
CV (%)		1.27	1.28	5.06	4.89	13.58

* Significant at 5% level; ** significant at 1% level; ns: not significant.

The date of flowering, date of physiological maturation, plant height and head diameter ranged between 66.33 to 68.83 days, 129.5 to 134.3 days, 107.60 to 137.60 cm, 12.52 to 14.85 cm and 44.48 to 50.90 gr, respectively (Table 4).

The longest time to flowering (68.83 days) was observed with the Hornet variety, while the shortest time to flowering (66.33 days) was observed with the Tunca variety. Under arid conditions, late flowering (i.e. a longer time to flowering) enhances the negative effects of warmer and drier conditions. Although significant differences were noted between the studied varieties in terms of their times to flowering, all varieties actually flowered within a period of two days. In parallel to the time to flowering, the Hornet variety exhibited the longest time to maturation (134.30 days), while the Tunca and Transol varieties exhibited the shortest time to maturation (129.50 days). In studies performed across different regions, researchers have reported physiological maturation periods ranging from 90 to 137 days (Kaya et al., 2009; Evci et al., 2011; Kara, 1991). The highest plant height was observed with the Bosfora variety (137.60), while the lowest was observed with the Reyna variety (107.60 cm). Higher plant height negatively affects conventional and mechanical harvesting procedures. In this study where irrigation practices were not used, it was observed that precipitation was an important factor affecting growth, and that the growth of sunflower varieties were limited by insufficient water. Varieties with longer vegetation period were generally taller than varieties with shorter vegetation periods. The head diameter of the varieties displayed a pattern similar to one observed with plant height, with the Bosfora variety having the largest head diameter (14.58 cm), and the Reyna variety having the smallest head diameter (12.50 cm).

Table 4. Mean comparisons for time to flowering, time to physiological maturation, plant height, head diameter and thousand seed weight

Cultivars	Time to flowering (day)	Day to physiological maturation (day)	Plant height (cm)	Head diameter (cm)	Thousand seed Weight (gr)
Bosfora	67.83abcd	132.20abc	137.60a	14.85a	50.46
Hornet	68.83a	134.30a	130.60ab	13.53 bcd	49.23
Oliva	68.00abc	132.70ab	123.10 bc	13.53 bcd	44.48
Reyna	67.50abcd	131.50abc	107.60 e	12.50 d	46.25
Sanbro	68.50ab	133.30ab	118.00 cd	14.32ab	47.82
Sirena	67.17 bcd	130.80 bc	119.70 c	13.33 bcd	50.90
Sonay	67.33abcd	131.20 bc	118.40 cd	13.60 bcd	47.82
Tarsan	67.67abcd	131.80abc	117.40 de	12.52 d	47.59
Transol	66.50 cd	129.50 c	119.70 c	13.85abc	45.32
Tunca	66.33 d	129.50 c	109.00 de	13.03 cd	48.11
LSD	1.34	2.647	9.523	1.033	2.650

It was observed that head dimeter and plant yield increased in parallel to the increase in Plant height and vegetation period. In sunflowers, head diameter varies according to various different parameters such as the characteristics of the variety, ecological conditions, growing techniques, soil structure, and irrigation (Gürbüz et al., 2003; Arıoğlu, 2007; Gürbüz and Kınay, 2015). The difference between the varieties in terms of 1000 seed weight was not statistically significant. Weight for 1000 seeds ranged between 44.48 g (Oliva) and 50.90 g (Sirena). For sunflower varieties, weight for 1000 seeds generally ranges between 35 to 120 g, and varies considerably depending on variety and cultivation conditions (İlbaş et al.,1996; Özer et al., 2004).

Significant differences ($P<0.01$) were observed between the years with respect to the kernel:hull ratio, protein ratio, crude oil ratio, yield and crude oil yield. Significant differences were identified between the varieties at a $P<0.01$ level in terms of their kernel:hull and oil yield, and at a $P<0.05$ level in terms of their protein ratio. No significant differences were identified between the varieties with respect to crude oil ratio. Kernel/Hull ratio ($P<0.01$) interacted significantly with year and variety (Table 5).

The highest kernel/hull ratio was observed with Oliva variety (74.72%), which is high in oleic acid, while the lowest kernel/hull ratio was observed with the Bosfora variety (%68.71) (Table 6). For sunflower varieties cultivated for their oil, a high kernel/hull ratio is particularly important for the oilseed industry. It was observed that under arid conditions, the kernel/hull ratio increased despite the decrease in seed size. It is reported that kernel/hull ratio varies between 55.47% to 77.30% depending on variety, cultivation conditions and cultivation practices (Karaaslan et al., 2007;, 1996; Karakaş and Arslanoğlu, 2010).

Table 5. Analysis of variance for sunflower cultivars under rainfed conditions.

Source	DF	Kernel:hull ratio (%)	Protein ratio (%)	Crude Oil ratio (%)	Yield (kg/da)	Crude oil yield (kg/da)
Replication	2	0.408ns	1.259ns	2.202ns	88.541ns	18.042ns
Year	1	30.025**	82.537**	405.179**	18,079.119**	1,731.116**
Cultivars	9	17.848**	8.260*	9.860ns	2,298.985**	507.808**
Year*Cultivars	9	11.322**	5.605ns	8.267ns	106.282ns	71.940ns
Error	38	2.918	3.197	7.584	241.046	67.896
CV (%)		2.39	9.58	5.40	11.51	12.10

* Significant at 5% level; ** significant at 1% level; ns: not significant.

Table 6. Means comparisons for Kernel:hull ratio (%), Protein ratio (%) , Crude Oil ratio (%), Yield (kg/da) and Crude oil yield (kg/da)

Cultivars	Kernel:hull ratio (%)	Protein ratio (%)	Crude Oil ratio (%)	Yield (kg/da)	Crude oil yield (kg/da)
Bosfora	68.71 c	18.68 b	51.53	146.30ab	82.00a
Hornet	71.93 ab	17.80 b	50.71	144.80ab	75.70abc
Oliva	74.72 a	19.31ab	50.27	132.80abc	71.20abcd
Reyna	71.17 bc	21.11a	51.75	93.61 d	60.20 d
Sanbro	70.09 bc	19.80ab	50.31	147.70ab	80.90ab
Sirena	73.03 ab	17.60 b	53.66	131.10abc	74.90abc
Sonay	70.44 bc	19.18ab	51.78	114.00 cd	67.30abcd
Tarsan	72.74 ab	17.86 b	50.78	127.40 bc	64.80 cd
Transol	71.05 bc	17.62 b	49.29	157.70a	80.60ab
Tunca	70.43 bc	17.76 b	49.50	152.80ab	77.00abc
LSD	2.674	2.090		24.31	12.90

The highest seed protein ratio was observed with the Reyna variety (21.11%), while the lowest ratios were observed in the Bosfora (18.68%), Tarsan (17.86%), Hornet (17.80%), Tunca (17.76%), Sirena (17.60%) and Transol (17.62%) varieties. The highest plant seed yield was observed with the Transol variety (157.7 kg/da), while the lowest was observed with the Reyna variety (93.61 kg/da). When exposed to arid conditions, sunflower varieties tend to respond differently to other environmental conditions. In this context, while yield was higher than the regional average, it is was fairly below the levels reported in other studies. The highest oil yield was obtained from the Bosfora variety (83.00 kg/da), while the lowest

oil yield was obtained from the Reyna variety (60.20 kg/da) (Table 6). For sunflowers, the crude oil ratio and, by extension, the oil yield can vary depending on the characteristics of the variety, the cultivation techniques employed, and ecological factors (Çil et al., 2011).

The study results demonstrated the importance of variety selection in sunflower cultivation, and highlighted the necessity of choosing varieties according to regional conditions. Furthermore, the study also illustrated the need to perform yield trials when selecting varieties suitable for the climatic and environmental conditions of a particular region, as well as the need to conduct such trials over a long period and in different areas of the relevant region. Yearly variations in climatic factors, and especially the increase in overall temperature and the irregularities in precipitation caused by climate change, present a significant problem for the future.

CONCLUSION

In conclusion, this study – which was performed under rainfed conditions – demonstrated that among 10 different varieties of sunflower cultivated for their oilseeds, the Transol variety had the highest yield with 157.70 kg/da, and that the Tunca (152.8 kg/da), Sanbro (147.7 kg/da), Bosfora (146.3 kg/da) and Hornet (144.8 kg/da) were also high-yield varieties. On the other hand, the lowest yield of 93.61 kg/da was obtained with the Reyna variety. Oil yield, a parameter that is particularly important for the oilseed industry, was the highest in the Bosfora variety (82 kg/da), and the lowest in the Reyna variety (60.20 kg/da). Therefore, for regions with irregular and insufficient precipitation or that lack irrigated conditions; the Transol, Tunca, Sanbro, Bosfora and Hornet hybrid sunflower varieties present better options with respect to yield performance, since they exhibit greater tolerance to negative environmental conditions and stresses.

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MICROBIAL DRESSING OF SUNFLOWER SEEDS WITH TRICHODERMA HARZIANUM KUEN 1585

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For extensive use by arable crops, seed dressing (coating) may be the most convenient concept for supplying a biological agent. Seed coating is used for commercial seed dressings. However, seed coating with PGPR's is often challenging, requiring a long shelf life, and compatibility with other seed dressings. *Trichoderma harzianum* KUEN 1585 (commercial brand name Sim Derma) formulations for wet and dry seed coating are patented (TR/2007/09242, 31.12.2007; EP8866322,4, 13.11.2008; TR/2009/08397, 05.11.2009). Although *Trichoderma harzianum* is commonly known as a fungus with bio-control effects, the strain KUEN 1585 has strong root growth promoting effects. *Trichoderma harzianum* KUEN 1585 colonize roots of the sunflower. The result is longer, stronger, more capillary roots and higher chlorophyll content. In addition *Trichoderma harzianum* KUEN 1585 makes soil micro elements like phosphorus available for the plant. There are two application methods, dry and wet seed coating. By dry coating 1 kg seeds and 10 g *Trichoderma harzianum* Powder are mixed properly in box or bag; by wet seed coating 500 g of Concentrated *Trichoderma harzianum* Powder is solved in 10 L water together with fungicides and insecticides and sprayed homogenously on 400 kg seeds. Field results between 2006 and 2014 in Turkey and Ukraine show that *Trichoderma harzianum* KUEN 1585 application results in strong and early sprouting, stronger and more capillary roots, increased leaf chlorophyll content up to 10% and yield gain up to 15%.

Key Words : microbial seed dressing, Trichoderma harzianum, sunflower

CURRENT SITUATION, PROBLEMS AND SOLUTIONS OF SUNFLOWER IN THE CENTRAL ANATOLIAN REGION

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ABSTRACT

Sunflower, with holding the first position in terms of cultivation area and production of oilseed crops in our country, is the primary oil plant in Central Anatolia Region. It constitutes the 46.7% of the total oil crops production in Turkey. The seed yield is 196.8 kg.da^{-1} in The Central Anatolia Region, where owns 30,3% of cultivation area and 19,5% of sunflower production in Turkey. Konya has the first rank in terms of cultivation area and as well as production amount in the sunflower with 36.8% of cultivation area and 58.7% production amount. Diverse cultivation of sunflower in fallow fields will make great profits to both farmers in the region and country's economy in Central Anatolia Region of Turkey, which has the 30,3% of arable land.

Key words: Sunflower, Oilseed, Confectionary sunflower, Central Anatolia

INTRODUCTION

World annual sunflower production is about 23 million tonnes and of, 1 million production have accomplished each year in Turkey which places it among top ten important sunflower producing country (Anonymous, 2014). There are two major uses of sunflower: oilseed and confection sunflower consumption. Oilseed having black and thin sheath around kernel and which is abundant for linoleic and oleic fatty acid content is a major source for oil production while confection sunflower which is comparably big and has a very thick sheath is mostly used for food consumption and animal feeding (Anonymous, 2007). Sunflower production in Turkey differs in planting region with varying oilseed and confectionary sunflower purposes. Trakya and Marmara regions are the biggest oilseed production areas, however confectionary sunflower production areas mostly localize in Middle Anatolia region of Turkey. Several studies have showed that even under drought conditions, sunflower production can be maintained but with low yield however after even one time irrigation of water may be enough to get high yield of sunflower (Anonymous). Besides, less demand on labour force with very suitability to mechanization makes sunflower a promising crop to increase its production rates in Turkey for near future (Anonymous, 2014).

AGRICULTURAL SITUATION OF MIDDLE ANATOLIA REGION AND PRODUCTION OF THE COMMON OIL CROPS IN THE REGION

Making 20% of whole country land, Middle Anatolia region composes 30.3% of arable area of Turkey and around half of this area has access to proper irrigation (TÜİK,

2015). Precipitation of this region is about 300-400 mm and the least precipitation falls at summer days. Aksaray, Ankara, Cankiri, Eskisehir, Karaman, Kayseri, Kirikkale, Kirsehir, Konya, Nevsehir, Nigde, Sivas and Yozgat- with diverse cultivation areas and crops like potato, apple, bean and sunflower- are the provinces of Middle Anatolia region. About 60% of planting area includes the area for crop cultivation, and nearly 30% is fallow area (Gultekin et al. 2013). Figure 1 shows the main crops produced in Middle Anatolia region and different cultivation areas in terms of only Middle Anatolia and in respect M. A. to Turkey.

a)

	Total area (m da)	**Crop (m da)**	**Fallow (m da)**	**Vegetable (m da)**	**Fruit (m da)**	**Ornamental plants (m da)**
2015 Middle Anotolia	72.602	47.399	21.672	1.532	2.007	1.324
2015 Turkey	239.486	157.377	41.139	8.085	32.838	45.972
Within Middle Anotolia		*65,29*	*29,85*	*2,11*	*2,76*	*1,82*
Middle Anotolia/Turkey	*30,32*	*30,12*	*52,68*	*18,95*	*6,11*	*2,88*

b)

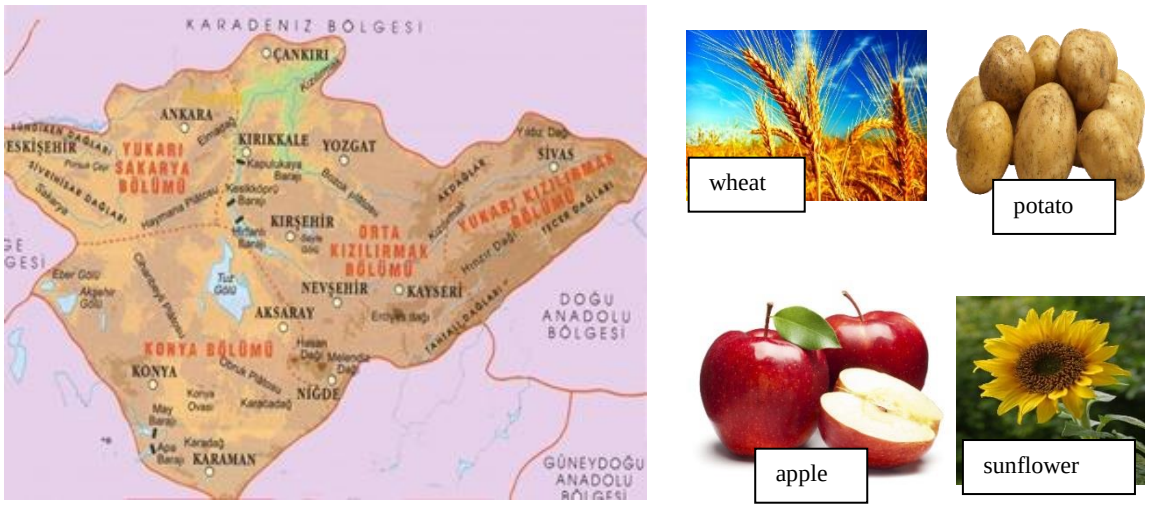

Figure 1.a) Shows the major cultivation areas of Middle Anatolia and Turkey **b)** Shows map of Middle Anatolia Region and its main cultivated crops. M.A.: Middle Anatolia

Besides canola, soybean, cotton seed, oil production from oilseed sunflower constitutes about 70% of total oil production in Turkey with fluctuating planting areas by year however (Caliskan, 2015) Turkey has about 530-690 thousand hectare for oilseed sunflower planting overall with 2.169 kg ha^{-1} yield for 2014. Annual oilseed sunflower production in Turkey is about 900.000 tonnes and mostly Trakya region meets the demand for oilseed sunflower production (Anonymous, 2015).

Sunflower is the second crop after safflower in terms of oil production in Middle Anatolia region as shown in Table1. This region constitute almost 80% of safflower production along with arable area of Turkey. Oil production values from soybean, rapeseed and cotton seed are very low even not forming 0.1% of total production.

Table1. Production of major oil crops in Middle Anatolia region

	Soybean		Safflower		Rapeseed		Sunflower	
	M.A.	Turkey	M.A.	Turkey	M.A.	Turkey	M.A.	Turkey
Planting Area (da)	357	367.323	341.539	431.071	6.194	350.817	719.245	5.689.650
Production (t)	147	161.000	56.201	70.000	1.998	120.000	291.951	1.500.000
Yield (kg da^{-1})	412	440	156	164	218	344	386	264
M.A./Turkey	Area	*0,10*		*79,23*		*1,77*		*12,64*
M.A./Turkey	Production	*0,09*		*80,29*		*1,67*		*19,46*
M.A./Turkey	Yield	*-28*		*-8*		*-126*		*122*

*M.A.= Middle Anatolia

SUNFLOWER PRODUCTION IN CENTRAL ANATOLIA

To increase arable areas for sunflower production in Turkey, attentions to other parts of Turkey have captured specifically Middle Anatolia region with provinces like Konya, Aksaray and Karaman however these regions are partly suitable for confectionary sunflower production (Anonymous, 2015). However, there are also government supported initiatives especially for Middle Anatolia to increase oilseed production to reverse decreasing planting areas, specifically when the climate and soil conditions of Middle Anatolia region are considered, it is thought that this area will be a great opportunity not only for oilseed sunflower production also for safflower and rapeseed (Anonymous, 2015).

By time, both the planting area and production percentages have increased from 2005 to 2015 with small fluctuations between the years as listed in Table 2. The production rates have decreased for two last years, 2014 and 2015 for oilseed while the rates for confection sunflower has increased from 2005 to 2015 gradually in Middle Anatolia. The fluctuation between land and production were consistent with each other for example, from 2012 to 2015, positive or negative change in arable area also reflected the change in production rates. Although, confectionary sunflower production still makes more than half of Turkey production as of 2015, both the production and arable area of sunflower production with respect to confectionary sunflower rates have increased dramatically 44% for area, 41% for production and 36% for area, 10% for production, respectively. The highest production amount for confectionary production recorded at 2015 with 53% while the highest record for oilseed sunflower production happened at 2013 with 19%.

Oilseed sunflower production for Middle Anatolia region was determined as 316.131 tonnes which made up 21.08% of total production in Turkey for 2015. Konya along with the biggest arable area, Aksaray, Eskisehir, Karaman and Ankara were top producers of oilseed production while Nigde with very few production even did not show any statistical information which was followed by Cankiri and Kayseri with comparably very low production value. However, the total sown area over total area in Turkey is too meager with 13.86 %, thus, it promises several advantages in terms of production values. Figure 2 shows oilseed production in Central Anatolia region as of 2015.

Table2. Shows the sunflower production Middle Anatolia region respect to Turkey

	Middle Anatolia		Middle Anatolia		Turkey		Turkey		M.A/Turkey	M.A/Turkey
	Oilseed sunflower		Confectionary sunflower		Oilseed sunflower		Confectionary sunflower		Confectionary	Oilseed sunflower
	Area (da)	Production (t)	Area (da)	Production (t)	Area (da)	Production (t)	Area (da)	Production (t)	Production (t)	Production (t)
2005	259.480	30.988	351.380	40.346	4.900.000	865.000	760.000	110.000	36,68	5,30
2006	266.270	40.809	359.044	41.117	5.100.000	1.010.000	754.000	108.000	38,07	5,22
2007	382.259	65.457	337.812	33.142	4.857.000	770.000	689.778	84.407	39,26	7,87
2008	370.746	66.281	327.022	32.645	5.100.000	900.387	700.000	91.613	35,63	7,27
2009	390.457	79.681	328.030	36.137	5.150.000	960.300	690.000	96.825	37,32	7,58
2010	422.656	83.473	508.532	68.515	5.514.000	1.170.000	900.000	150.000	45,68	7,67
2011	538.819	148.254	612.413	81.715	5.560.000	1.170.000	997.000	165.000	49,52	9,69
2012	865.443	282.713	609.655	79.317	5.046.160	1.200.000	1.000.000	170.000	46,66	17,15
2013	1.031.228	365.494	540.893	64.849	5.202.600	1.380.000	895.239	143.000	45,35	19,82
2014	932.526	353.908	622.900	67.677	5.524.651	1.480.000	1.049.925	157.900	42,86	16,88
2015	719.245	291.951	784.924	97.101	5.689.950	1.500.000	1.163.224	180.700	53,74	12,64

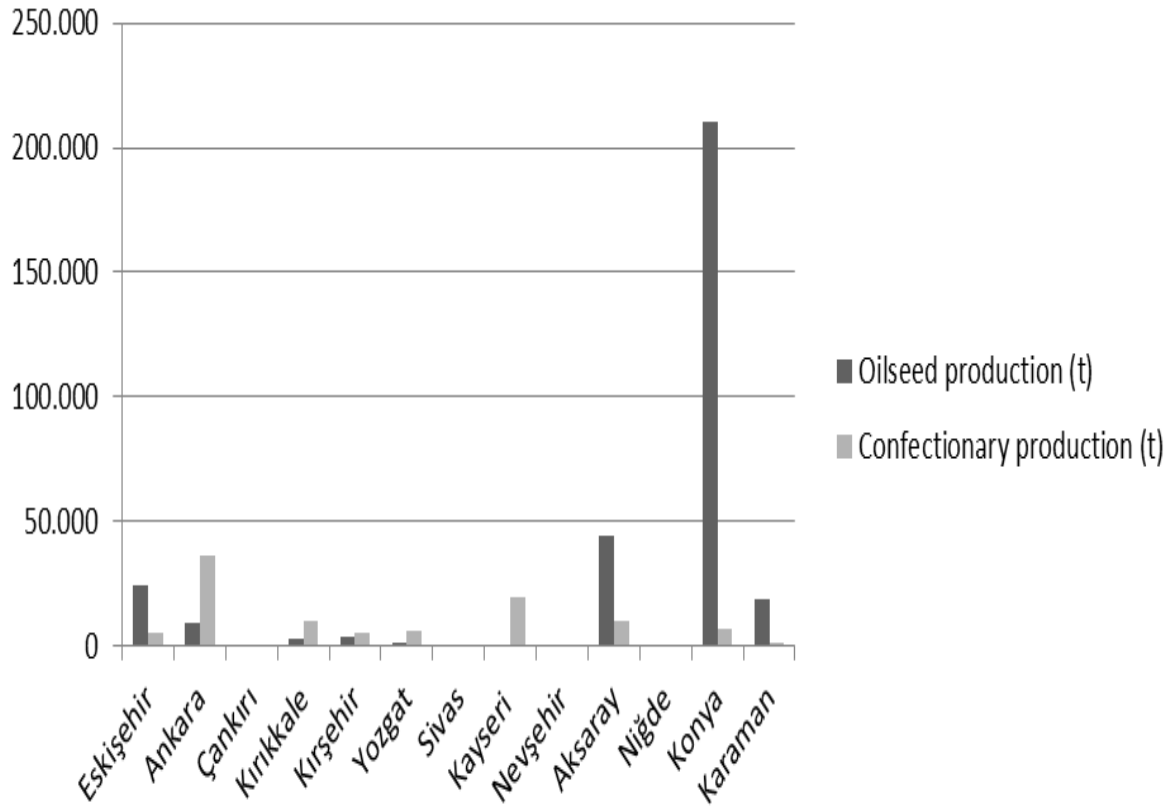

Figure 2. Shows the oilseed and confectionary sunflower production in provinces of Central Anatolia in 2015

Although it is actually, following an increasing trend in sunflower production in Central Anatolia region, not all provinces contribute equally to this percent, because Konya, Aksaray, Eskisehir, Ankara, and Karaman are the most important producers of sunflower production. Konya showed a great increase in sunflower production which makes 68.4% of overall Central Anatolia region and 26.03% of Turkey as of 2014. This makes Konya fourth largest sunflower producer after Tekirdag, Edirne, and Kırklareli. However, there were also notable increase in Aksaray, Karaman, Eskisehir, Ankara and Kırsehir sunflower production through the years. The other cities still need some actions to manage the sunflower production even there are some bare problems having confronted like few irrigation, wide row spacing and not regular appliement of intensive cultivation (Kolsarici et al. 2005).

Despite of relatively important contribution of Middle Anatolia region for oilseed production, this region can produce more than half of confectionary sunflower of Turkey for 2015 as shown in Table 3.

Ankara, Kırıkkale, Kayseri, Kirsehir and Yozgat are having the largest confectionary sunflower production areas in Central Anatolia region, but yield values are not well correlated for sown areas and production like Kırıkkale, which has second largest area, only produced 9.800 tonnes in 130.475 decares when compared with Kayseri with 19.828 tonnes in 97.850 decares. Central Anatolia made up 69.72% and 56.79% of sown areas and confectionary sunflower production of Turkey, respectively. Cankiri and Nevsehir were the least confectionary sunflower producers while Nigde showed no visible production as also happened for oilseed production.

Table3. Shows confectionary sunflower production in Middle Anatolia region

Provinces	Sown areas (decares)	Production (tonnes)	Yield (kg/da^{-1})
Aksaray	47.175	10.288	218
Ankara	344.915	36.295	105
Çankırı	3.230	406	126
Eskişehir	22.886	5.114	223
Karaman	13.714	1.484	108
Kayseri	97.850	19.828	203
Kırıkkale	130.475	9.800	75
Kırşehir	59.929	4.973	83
Konya	26.060	7.327	281
Nevşehir	1.672	204	122
Niğde	-	-	-
Sivas	3.400	514	151
Yozgat	59.734	6.388	107
Total	811.040	102.621	150
Turkey total production	1.163.224	180.700	155
Total/Turkey total production (%)	69,72	56,79	-5

COMMON PROBLEMS AND THEIR POSSIBLE SOLUTIONS FOR SUNFLOWER PRODUCTION IN CENTRAL ANATOLIA REGION

Unlike Trakya and Marmara region especially named after great sunflower production in Turkey, it needs some new areas to eliminate production fluctuation throughtout the years and spesifically Konya and Eskisehir, Ankara, Aksaray and Karaman are promising cities to create new arable areas. It was actually known that Central Anatolia region was really good source of confectionary sunflower production however when it is concerned that Konya ranks fourth in oilseed production, Middle Anatolia region promises a lot for sunflower production for future.

Although the conditions and production for sunflower gets better each year in Central Anatolia, this area is thought to have more potential, thus, some precautions should be taken to improve production in this region:

Problems

- First, it is needed to be clearly understood that the sunflower production is made in dry areas and if irrigation is possible, other crops, like potato, sugar beet and bean, are preferred for that lands,
- Farmers are not well informed both for sunflower, government support, and marketing.
- Yield rates for sunflower production are too low in Middle Anatolia

Solutions

Solutions to these problems begins with;

- teaching farmers the value of sunflower by bringing the awareness.

- mechanization supply should be done properly for each farmer.
- Farmers should be encouraged to plant sunflower to fallow and dry areas.
- The studies in this area should be supported.
- The competency in price rates should be balanced to promote the sunflower cultivation (Caliskan, 2013).

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NITROGEN ECONOMY THROUGH BIO-FERTILIZER IN SUNFLOWER (HELIANTHUS ANNUUS L.)

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ABSTRACT

Field experiment was carried out at G B Pant University of Agriculture & Technology, Pantnagar (India) during spring season of 2013 and 2014 to study the response of biofertilizers on productivity, profitability and nitrogen economy of sunflower in Indo-Gangetic plains of India. The experimental site was loamy in texture with 0.625% organic carbon, 269, 24.6 and 227 kg/ha available nitrogen, phosphorus and potassium, respectively and neutral in soil reaction with 6.85 soil pH. The experiment was laid out in completely randomized block design with 11 treatments i.e. No N (T1), 50% N (T2), 100% N (T3), *Azospirillum (Azos)* seed treatment(T4), *Azotobacter (Azot)* seed treatment (T5), *Azos+Azot* seed treatment (T6), 50% N+*Azos* seed treatment (T7), 50% N *Azot* seed treatment (T8), 50% N+*Azos+Azot* seed treatment (T9), 100% N+*Azos+Azot* seed treatment(T10) and 75% N + *Azos+Azot* seed treatment (T11) in three replication during spring season (February to May). The recommended dose of fertilizers were 120, 60 and 40 kg/ha nitrogen (N), phosphorus (P205) and potassium (K20), respectively. The nitrogen was applied as per treatments including 50% at sowing and 50% at budding stage but total P and K were applied at the sowing time. The crop was grown under recommended agronomy except the treatment variations. The growth and yield attributes, seed yield and yield reduction over 100% N at harvest were affected significantly by bio fertilizer application. The sunflower seed yield was recorded significantly highest at 100% N+ seed treatment with Azot+Azos and was significantly similar to 100% N application during both years and average value was only 4% greater than 100% N application. The seed treatment with Azot was found better than Azos with 6.7% higher average seed yield. Similarly the combined treatment with Azot+Azos gave 6.2% higher seed yield than seed treatment with only Azot. The seed yield was increased when N application was combined with seed treatment either of Azot and Aozs or both. The average seed yield under 100% N+ seed treatment with Azos+Azot gave 6.8 and 2.4% higher yield than 100% N during 2013 and 2014, respectively. The seed yield at 75% N+seed treatment with Azos+Azot was recorded significantly equal to 100% N and 100% N+seed treatment with Azos+Azot in 2013 but was significantly lower in 2014. However the average seed yield under 75% N + seed treatment with Azos+Azot was 7.4 and 11.0% lower than 100%N and 100% N+seed treatment with Azost+Azot. The biofertilizers did not influence the oil content. The gross,net returns and B:C ratio were found significantly higher at 100% N+seed treatment of Azos+Azot but remained significantly equal to 100% N during both years. Similarly the average gross, net returns and B:C ratio were found almost equal at both 100% N and 75% N+ seed treatment with Azos + Azot. It is therefore recommended that 25% N can be saved with seed treatment with Azotobacter only. Hence, 75% N+seed treatment with Azotobacter may be recommended for higher productivity, profitability and N economy of sunflower production in Indo-Gangetic plains of India.

Key Words : Azotobacter, *Azospirillum*, Bio fertilizer, Nitrogen economy, Indo-Gangetic plains

THE EVALUATION OF SUNFLOWER HARVEST WASTE AS SILAGE FEED

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ABSTRACT

This project was carried out to investigate the possibility of sunflower remaining after harvest crops as animal feed (silage) made from the residue left in the field. Sunflower stalks wastes are chopped 3 to 5 cm long grass shredding machine before ensiling process, and then sunflower stalks wastes were ensiled for 60 days in 1 kg vacuum bags. In the study, the pH of the silage dry matter (DM), crude protein (CP), crude ash (CA), acid detergent fiber (ADF), neutral detergent fiber (NDF) content and Fleig points were determined. Offered in silage dry matter content of 16-20%, pH 3.8-3.9, HP 9-10%, NDF 27-28% ADF 22-23%, CA 16-17% ranged. On the other hand, silage Fleig point which is an important parameter in determining the silage quality, this value ranges from 85-90 is located in “very good quality" class. As a result, after the harvesting of sunflower plants can be ensiled alone, to be ensiled with many other high-value crops, especially in terms of energy in order to increase dry matter seems to be appropriate. In this way, it is concluded that the waste product can be effectively used as silage material.

Key Words : Sunflower, harvest waste, Silage, Animal Feed

PATH ANALYSES OF YIELD IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) PARENTAL LINES

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ABSTRACT

Seed yield is very complex trait; it depends on genotype, environmental conditions, on various plant traits etc. Eighteen sunflower commercial parental lines were evaluated for various parameters under field conditions to estimate genetic parameters and path analyses. The ten female and eight restorer line were chosen for the experiment. Observed parameters were seed yield, 1000 seed, seed germination, oil and protein content. Objective of this study was determination of direct and indirect effects by path analysis; to compare given results of path analysis from female lines with results from restorer lines in order to identify research priorities in sunflower breeding. Path coefficient analysis, in observed female lines, indicates that 1000 seed weight has maximum positive and seed germination maximum negative direct effect on yield. In restorer lines, path coefficient analysis indicates that seed germination and 1000 seed weight have negative direct effect on yield, but effect of seed germination was highly significant.

Key words: Sunflower parent lines, Seed and yield components, Path coefficient analysis

INTRODUCTION

Seed yield is a very complex trait, has low heritability and it is very dependent on environmental conditions. It depends on various plant traits and it is very important for plant breeders to find out the association between the traits themselves and with the seed yield (Škorić, 1974; 2012). The main goal in sunflower breeding is to develop hybrids with high seed yield and high oil content and therefore to improve productivity of this important oil crop (Jocković at al., 2015).

As the aproach proved to be ineffective, numerous researchers (Shankar et al., 2006; Darvishzadeh et al., 2011; Radić at al., 2013) concluded that path-coefficient analyses provided information about direct and indirect effetcs of the examined characters on seed yield per plant. Yasin and Singh (2010) also concluded that path-coefficient is helpful in partitioning the correlation into direct and indirect effects. In this way, relative contribution of each component character to the yield can be assessed. In other words, path analysis measures direct and indirect contribution of various independent characters to a dependent character. Using path-coefficient analysis, it is easy to determine which yield component influences the yield substantially. These researchers also concluded that with this information, selection can be based on that criterion in limited time (Farhatullah et al., 2006).

This study was conducted in order to obtain information about interrelationship (direct and indirect effects by path analyses) between seed yield and other observed seed characters as well as to identify research priorities in sunflower breeding.

MATERIAL AND METHODS

Experiment was carried out in field conditions throughout three years on plots where seed production of sunflower parental lines was established. Ten genotypes were examined which represent lines that were based on cytoplasmic mail sterility (CMS). All examined genotypes represent parental components of the best-selling sunflower hybrids of the Institute of Field and Vegetable Crops, Novi Sad, Serbia.

The following parameters were studied:

Seed yield – upon maturity, 10 plants were picked manually, from different locations on the plot, and seed yield per plant was determined. By the application of previously determined plant density *(50.000 plants ha^{-1}*), obtained seed yield per plant was redetermined in kg ha^{-1} with 9% of moisture.

Upon seed drying, specimens were purified and cleaned. Seed for determining the remaining observed parameters were picked from the given specimens:

Seed germination- Standard laboratory method for seed germination testing was used (ISTA, 2014). Examination of seed germination was repeated 4 times. Each time 100 seeds were used. Germination was determined after 10 days. Only naturally formed germed seeds were used for determination of this parameter. Germination was expressed in relative values.

1000 seed weight- Examination of 1000 seed weight was repeated 4 times. Each time 100 seeds were used. Obtained value was applied to 1000 seed weight and was specified in grams.

Oil content- Determined by nuclear - magnetic resonance (NMR) according to Granlund and Zimmerman (1975) and expressed in relative value.

Protein content- Determined by standard Kjeldahl method with the help of VAP-50-Gerhardt apparatus. This parameter is also expressed in relative value.

Analysis of variance of two-factorial experiment, simple correlation coefficient and path-coefficient analysis for examined characters were done using GENSTAT computer program.

RESULTS AND DISCUSSION

The data were processed by the path-coefficient analysis which enabled the partitioning of direct and indirect effects of individual yield components and identification of yield components applicable as selection criteria in sunflower breeding (Table 1 and 2).

Relatively low coefficient of determination (R^2) at trait of sterile lines (0.330) and restorer lines (0.211) level give rise to high residual effects (0.818 and 0.888) meaning that besides parametrs used in this study other causal variables are also responsible for seed yield.

Seed germination, in both traits, had the highest negative direct effect on seed yield (-0,354). Only differences between this two effects was that in restorer traits this effect was more significant (-0,485). These results are in agreement with the studies of Radić et al. (2013). In the study of indirect effects, the existence of negative indirect effects was determined in sterile lines (seed germination *via* 1000 seed weight) while in restorer lines this effect was determined as positive. In both traits this indirect effects were not significant.

The study of direct effects on seed yield showed that the 1000 seed weight had high positive direct effect (0.339) in sterile lines, while in restorer lines this parameter had also high direct effect on seed yield, but this effect was negative. In the study of indirect effects, the existence of positive not significant indirect effects on seed yield was determined. Škorić

(1974) and Joksimović et al. (1999) concluded that is necessary that 1000 seed weight has negative direct effect on restorer lines, since this restorer plant has a lot of branches (purpose of exciting of restorer is to have a lot of polen for polination). These results are in agreement with the studies of Merrien et al. (1982), Marinković (1992) and Dušanić (1998, 2004). These researchers also concluded that 1000 seed weight has higher effect on seed yield than number of filled seed per head and other yield components. Vanishree et al. (1988) and Tahir et al. (2002) concluded that increasing 1000 seed weight may result in higher yield. As opposed to this, Alba and Greco (1978) and Lakshmanrao et al. (1985) reported that 1000 seed weight has significant direct effect on seed yield, but this is, based on their research, a negative effect.

Table 1. Analysis of direct and indirect effects of observed characters on seed yield in sterile lines

Character	Direct effect	Indirect effect:				Total
		Seed germination	1000 seed weight	Oil content	Protein content	
Seed germination	-0.354	-	-0.010	0.015	0.036	-0.313
1000 seed weight	0.339	0.010	-	0.037	0.009	0.395
Oil content	0.221	-0.024	0.057	-	0.010	0.265
Protein content	0.185	-0.068	0.016	0.012	-	0.146

Coefficient of determination R^2=0.330

Oil content had positive direct effect on seed yield and negative indirect effect *via* seed germination on seed yield at both traits. Other indirect effects were positive and also not significant, except indirect effect of oil content *via* 1000 seed weight on seed yield. This effect was negative. Punia and Gill (1994), Husain et al. (1995) and Chikkadevaiah et al. (2002) concluded in their research that oil content had maximum direct effect on seed yield. On the other side, Habib et al. (2007) confirmed positive direct effect of oil content on seed yield. Arshad et al. (2007) and Kaya et al. (2009) found that oil content had negative direct effect on seed yield as well as negative indirect effect *via* plant height.

Protein content had positive direct effect in sterile line traits but also had negative direct effect in restorer line traits. Both effects are not significant. In both traits two negative indirect effects on seed yield were determined. One of them is in sterile line trait *via* seed germination and other one is *via* oil content in restorer line trait. All other indirect effects are positive. All indirect effects are not significant. Jocković at al. (2015) in their research concluded the same.

Table 2. Analysis of direct and indirect effects of observed characters on seed yield in restorer lines

Character	Direct effect	Indirect effect:				Total
		Seed germination	1000 seed weight	Oil content	Protein content	
Seed germination	-0,485*	-	0,112	0,018	0,005	-0,321
1000 seed weight	-0,337	0,162	-	0,006	0,005	-0,164
Oil content	0,153	-0,150	-0,014	-	0,008	-0,003
Protein content	-0,065	0,035	0,026	-0,018	-	-0,022

Coefficient of determination R^2=0.211

CONCLUSION

Bringing these observed characters into optimal balance with seed yield is one of main principal for succsesfull sunflower breeding program. In this report, path coefficient analysis revealed that the greatest improvement in sunflower seed yield can be achieved through selection on seed germination and 1000 seed weight, because they have the highest direct effect on seed yield. Difference between observed parametrs is that effect of seed germination is negative while in 1000 seed weight is positive in sterile line trait and negative in restorer line trait.

Further research should be aimed at observation of the relationship between certain characters of seed quality, with the intention of obtaining high quality sunflower seed.

ACKNOWLEDGEMENTS

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EFFECT OF THE PLANT DENSITY AND FOLIAR FERTILIZATION ON THE YIELD FROM NEW BULGARIAN HUBRIDS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

During 2014–2015 a field experiment was conducted with three new sunflower hybrids (Veleka, Vokil and Sava) in the trial field of Dobrudzha Agricultural Institute. The aim was to determine the effect of the plant density and of a set of foliar fertilizers on the quantitative and qualitative indices of seed yield as a part of determining the elements forming the optimum agronomy practices for growing of the above hybrids. The variants of the experiment were the following: 1) check (untreated); 2) mineral fertilization at norm $N_{60}P_{120}K_{80}$ (active matter/ha); foliar fertilization with: 3) Root; 4) Siapton; 5. Lebosol B; 6) Lebosol Mg-S; 7) Lebosol Mn; 8) Yara Vita Brassitrel pro. The foliar treatment was done by sprinkling the leaf mass at stage 6-7th pair of leaves. Each hybrid was sown at four densities – 35 000, 45 000, 55 000 and 65000 plants/ha. The soil in the experimental field was leached chernozem (*Luvic phaeosem*) with 3.30% humus content classified as very suitable for sunflower growing. Based on the data obtained, it was found that the factor sowing norm had greater effect on the seed yield than foliar fertilization. This factor enhanced seed yield with 5 to 10 %, 1000 seed weight decreased with the higher sowing norm with 12 to 19 %, while oil content in seed did not change. The factor fertilization did not affect significantly the three followed indices. The reason for this result was the amount of rainfalls. During the first year they were abundant and during the second – scare; as a result from these deviations from the precipitation norm of the region the differences between the individual variants were leveled up.

Key words: sunflower, sowing norms, foliar fertilization, yield, oil content

INTRODUCTION

Investigations on the topic of this presentation are being permanently carried out in Bulgaria and abroad since sunflower is an important major oil seed crop in many agro ecological regions worldwide. New hybrids and promising lines of sunflower are being constantly introduced in practice, which have various peculiarities and growing requirements (Georgiev et al., 2006; Georgiev et al., 2013; Nenova et al., 2013).

Therefore constant studies on the agro technology of this crop are needed. Such studies would give an answer to the question what are the values of certain factors under which the tested hybrid can express to a maximum degree its biological potential. These particular factors are the parameters of the sowing norm (Amjed et al. 2011; Petcu et al. 2000; Sin & Partal, 2011; Yankov et al., 2009), the mineral macro and micro fertilization (Nankova and Tonev, 2004; Tonev, 2005,a; Tonev, 2005 b, Suzes, 2010), the use of bio stimulants (Ebrahimian et al., 2011; Milev, 2015), etc.

The topicality and significance of this problem consists in searching for the optimal combination of the agronomy practices related to the introduction of new sunflower hybrids developed at DAI – General Toshevo in production, with regard to both yield and the quality indices of the produce.

The aim of this investigation was to determine the effect of some main agronomy factors such as the plant density, the macro and micro fertilization and the use of bio stimulants on the quality and quantity of production from new Bulgarian oil seed sunflower hybrids.

MATERIAL AND METHODS

During 2014 – 2015, a field experiment was carried out with oil seed sunflower in the trial field of Dobrudzha Agricultural Institute – General Toshevo (DAI), Bulgaria. The experiment was designed according to the split plot method, in four replications of the variants, the size of the harvest plot being 12.6 m. The three hybrids were planted in the first-order plots, using 4 plant densities for each hybrid – 35 000, 45 000, 55 000 and 65 000 plants/ha. The following variants of treatment were positioned across the first-order plots: 1) check (untreated); 2) mineral soil fertilization with norm $N_{60}P_{120}K_{80}$, active matter per ha; foliar treatment with: 3) Root; 4) Siapton; 5) Lebosol B (boron); 6) Lebosol Mg-S (magnesium - sulfur); 7) Lebosol Mn (manganese); 8) Yara Vita Brassitrel pro (YVB). The foliar treatment was done by sprinkling the leaf mass at stage 3rd pair of leaves with the bio stimulant Root, and at stage 6th - 7th pair of leaves with the rest of the products. The applied doses were in accordance with the recommendations of the producers. Brief description of the tested products is given in Table 1.

Table 1. Characteristics of the tested products

Name	Type	Active substance	Action
Root	Bio stimulant for foliar application	Molecular complex similar to chlorophyll	Alters the energy balance in favor of yield. Accelerates rooting.
Siapton	Bio stimulant for foliar application	Natural hydrolyzed proteins	Accelerates the formation of amino acids and the uptake of nitrate nitrogen from soil.
Lebosol B	Foliar one-component fertilizer	11% B	Uniform flowering and maturation.
Lebosol MgS	Foliar combined fertilizer	24.1% MgO и 16.6% S	Higher plant vigor.
Lebososl Mn	Foliar one-component fertilizer	6% Mn	Enhances immunity
YVB (Yara Vita Brassitrel Pro)	Foliar combined fertilizer	6.9% amide nitrogen, MgO 11.6%, B 6%, Mn 7%, Mo 0.4%, CaO 8.9%	Higher yield and quality of production

Three new oil seed sunflower hybrids developed through interlinear hybridization were tested in the field experiment carried out. In the breeding field of DAI, these hybrids

underwent three-year testing according to a scheme approved for this crop. The first hybrid **Veleka** is medium early, with vegetation period 120-123 days. Plants are medium high, 155 – 160 cm, with head diameter 18-22 cm, absolute weight of seeds 55 – 65 g, oil seed content 48 – 49 %, and the percent of kernel is within 72 – 76 %. The number of seeds per head is between 1060 and 1530, and the weight of seeds per plant is 61.5 – 89.3 g. The duration of flowering is 11-13 days. **Veleka** is shorter than the other Bulgarian hybrids distributed up to now. It gives stable yields in climatically unfavorable years. This hybrid exceeded the mean standard by seed and oil yield during the preliminary testing in the experimental fields of DAI. The exceeding by seed yield was within the range 12.7 – 21.1 %, and by oil yield – from 14.5 to 25.8 %. The maximum seed yield obtained from the new hybrid in the experimental fields was 4837 kg/ha and the oil yield was 2370 kg/ha, oil percent in seed reaching up to 49.0 %. Hybrid Veleka was acknowledged as clearly distinct from all other varieties, sufficiently uniform and stable with a technical testing report of the National Executive Agency of Variety Testing, Field Inspection and Seed Control of Bulgaria. Following three-year official testing, hybrid Veleka was registered also in Romania and was included in the European catalog of field and vegetable crop varieties.

The second hybrid **Vokil** is medium early, with vegetation period of 122 – 125 days, plant height 150 – 160 cm and head diameter 18 – 21 cm. Oil of seeds is of linoleic type, and its content is 49 – 51 %. Thousand seed weight is 53 – 58 g, number of seeds per head is 1180 – 1360, and their weight per plant is 81 – 91 cm. The percent of the kernel in the seed is 72 – 75 %. The duration of flowering is 11 – 13 days. During the three years of testing the hybrid exceeded the mean standard with 4.9 – 14.9 %, the maximum obtained seed yield being 4570 kg/ha, and the maximum oil yield – 2344 kg/ha. Oil content in seed reached up to 51.7 %.

Hybrid Vokil was officially registered in Romania in 2013 and was enlisted in the European catalog of field and vegetable crop varieties.

The last tested hybrid **Sava** is early. Its vegetation period is shorter in comparison to the other two hybrids with 10 to 12 days. The height of plants is 140 – 150 cm. Head diameter is 24 – 27 cm. The vegetation period is 109 – 112 days. Oil content in seeds is 49 – 50 %. It is resistant to downy mildew race 731 and to *Orobanche* races from A to F.

In 2012 hybrid Sava was presented for official testing within the structures of the Romanian varietal commission at 10 locations. During the three years of official testing the new hybrid exceeded the Romanian standard with averagely 2.2 % by the index seed yield. The mean seed yield per hectar for the three years of testing was 3273 кg/ha. Hybrid Sava was officially registered in Romania in 2015 and was enlisted in the European catalog of field and vegetable crop varieties under the name Sevar.

The hybrids were sown in mid-April, within the optimal dates for this agro-ecological region. Sowing was manual, and the number of plants per unit area was in accordance with the methodological requirements. All other elements of the agro technology of the hybrids in this experiment, which were not the aim of this investigation, were performed according to the traditional agronomy practices applied to sunflower production in the region (Klochkov et al., 1988).

The soil in the trial field was leached chernozem (*Luvic phaeozem*) with humus content 3.30 %, with neutral reaction, and can be classified as very suitable for growing of sunflower. The vegetation conditions for growth and development of sunflower in 2014 can be described as very favorable. The autumn-and-winter rainfalls of 323.5 mm exceeded their referential values and were a prerequisite for excellent moisture reserves in soil. The amount

of vegetation rainfalls (370.5 mm) and their distribution during the respective growth stages were also entirely sufficient and suitable to meet the demands of the crop.

Highest vegetation rainfalls were registered in June - 192.5 mm, i.e. during the month when sunflower undergoes intensive vegetation growth. These rainfalls were accompanied by stormy winds and caused lodging of the plants to various degrees. Hybrid Veleka was affected most, and hybrid Sava – least. The degree of lodging was determined by the growth stage of the respective hybrids at the moment of this unfavorable occurrence. Lodging lead to formation of a non-uniform crop (deviations from the row, the height, instances of curved stems, etc.).

The vegetation conditions for growth and development of sunflower during the second year can be described as relatively unfavorable. The rainfalls at the beginning of the growth season were scarce, significantly below the mean monthly referential values (Table 2). The August rainfalls were too late and did not have significant economic effect on yield, especially on the earlier hybrid Sava.

Comparatively, the total amount of vegetation rainfalls during this growth season (158.9 mm) was 2.3 times lower than the rainfalls in 2014 (370.5 mm). The autumn-and-winter rainfalls, however, were abundant - 372.9 mm, considerably exceeding their referential values, and created excellent moisture reserves in soil.

Table 2. Vegetation and autumn-and-winter rainfalls during the investigated period, mm

Month	Years		Averaged for 2 years	Averaged for 60 years
	2014	2015		
April	29.6	46.3	37.9	49.1
May	78.2	12.9	45.5	50.6
June	192.5	31.3	111.9	65.4
July	50.9	27.2	39.5	49.2.
August	19.3	41.2	30.2	40.3
Amount for Apr-Aug	370.5	158.9	264.7	254.0
Autumn-and-winter rainfalls for Oct-Mar	323.5	372.9	348.2	294.0

With regard to air temperatures, the growth season of sunflower in 2014 was not interrupted by any disturbances. The growth season of 2015 occurred under recurrently high air temperatures exceeding the norm (above 32-35ºC). Such temperatures were registered during 25th – 30th July and 12th – 15th August. The combination of scarce rainfalls and extreme high temperatures caused severe soil drought during May – July. Under these meteorological conditions seed yield was formed mainly at the expense of the autumn-and-winter moisture reserves in soil.

RESULTS AND DISCUTION

The results presented in Table 3 show that the variants of treatment with the set of micro fertilizers, bio stimulants and mineral fertilization had zero effect on seed yield. The

better and more vigorous vegetative growth observed during the growth season was not expressed on seed yield. What is more – the seed yield from the check variant was higher than the treated variants. This phenomenon can be explained by the greater lodging and the more severe stress which, however, the plants with better supply of nutrients suffered in these variants.

Table 3. Seed yield according to the major action of the factors for two years, kg/ha

Hybrid	Variant of treatment		Crop density		Year	
Veleka	Check	3363	35000	2930	2014	2711
	$N_{60}P_{120}K_{80}$	2996*	45000	3185*	2015	3307**
	Root	2911*	55000	3005 *NS*		
	Siapton	3000*	65000	2928 *NS*		
	Lebosol B	3000*				
	Lebososl Mg-S	2891**				
	YVB#	2897**				
	Lebososl Mn	3029*				
Vokil	Check	3465	35000	3250	2014	3398
	$N_{60}P_{120}K_{80}$	3344 *NS*	45000	3427 *NS*	2015	3393 *NS*
	Root	3274 *NS*	55000	3445*		
	Siapton	3496 *NS*	65000	3461*		
	Lebososl B	3482 *NS*				
	Lebososl Mg-S	3244NS				
	YVB	3497 *NS*				
	Lebososl Mn	3367 *NS*				
Sava	Check	3414	35000	3211	2014	3482
	$N_{60}P_{120}K_{80}$	3387 *NS*	45000	3490**	2015	3214*
	Root	3331 *NS*	55000	3382*		
	Siapton	3380 *NS*	65000	3289 *NS*		
	Lebososl B	3364 *NS*				
	Lebososl Mg-S	3283 *NS*				
	YVB	3392 *NS*				
	Lebososl Mn	3271 *NS*				

,,*** - Significance of differences at p<0.05, p<0.01, p<0.001 respectively;*
NS – not significant; # - Yara Vita Brassitrel

The factor sowing norm had significantly higher effect on the seed yield in comparison to the variants with fertilization. The variation from the lowest to highest yield caused by this factor by hybrids was the following: hybrid Sava – 279 kg/ha, hybrid Veleka – 257 kg/ha and hybrid Vokil – 211 kg/ha. Hybrids Veleka and Sava realized highest yield at crop density of 45000 plants/ha. In hybrid Vokil, the higher sowing norms gave higher seed yield than the check variant, without significant differences being observed between themselves.

The respective year conditions had more significant effect on the size of the seed yield from hybrids Veleka (a difference of 59 kg/ha) and Sava (a difference of 27 kg/ha), and did not have any effect on hybrid Vokil. In hybrid Veleka, the lodging of the crop in 2014 played a considerable role for this high variation in yield and lead to its logical decrease.

Hybrid Veleka realized highest 1000 seed weight in the variants with Lebosol Mg-S, Yara Vita Brasitrel and Lebososl Mn, respectively. The exceeding of the values of the index in these variants was from 3.2 to 5.4 g, respectively (Table 4).

In hybrid Vokil, only the treatment with Lebosol Mn significantly exceeded the check variant. The value of the index for the variants with $N_{60}P_{120}K_{80}$, Root, Siapton, Lebosol Mg-S and Lebosol B was even lower than the check variant with about 2.5 g.

Table 4. 1000 seed weight (g) according to the variants of the experiment averaged for two years,

Hybrid	Variant of treatment		Crop density		Year	
Veleka	Check variant	60.5	35000	69.0	2014	65.0
	$N_{60}P_{120}K_{80}$	59.1 *NS*	45000	62.8***	2015	58.0**
	Root	59.1 *NS*	55000	58.5***		
	Siapton	59.8 *NS*	65000	55.7***		
	Lebosol B	58.9 *NS*				
	Lebososl Mg-S	63.7*				
	YVB#	64.6*				
	Lebososl Mn	65.9**				
Vokil	Check variant	64.9	35000	58.0	2014	62.8
	$N_{60}P_{120}K_{80}$	62.5*	45000	55.8**	2015	64.3 *NS*
	Root	61.9*	55000	52.1***		
	Siapton	63.6 *NS*	65000	50.9***		
	Lebososl B	62.1*				
	Lebosol Mg-S	62.5*				
	YVB	64.6 *NS*				
	Lebosol Mn	66.6*				
Sava	Check variant	53.6	35000	58.0	2014	54.9
	$N_{60}P_{120}K_{80}$	53.1 *NS*	45000	55.8*	2015	53.2 *NS*
	Root	52.2 *NS*	55000	52.1***		
	Siapton	53.6 *NS*	65000	50.9***		
	Lebososl B	53.6 *NS*				
	Lebososl Mg-S	52.6 *NS*				
	YVB	55.7**				
	Lebsosl Mn	58.2***				

,,*** - Significance of differences at p<0.05, p<0.01, p<0.001 respectively; NS – not significant; # - Yara Vita Brassitrel*

Highest 1000 seed weight in the third hybrid Sava was registered for the variants Lebosol Mn and Yara Vita Brasitrel, the exceeding being with about 2-5 g.

The higher crop density gradually and definitely decreased 1000 seed weight, i.e. the correlation between them was inversely proportional.

The conditions of the respective year did not significantly change the value of 1000 seed weight of hybrids Vokil and Sava. In hybrid Veleka, 1000 seed weight was significantly higher during the first year of the experiment. The reason for this could be the thinning of the crop as a result from its lodging; at the expense of this, however, larger and more plums seeds were formed.

The results for the index oil content in seed are given in Table 5. The data clearly show that this index is a strong genetic peculiarity of the hybrid hardly affected by the factors of the trial. Variation was within extremely range even during the individual years and was not statistically significant.

Table 5. Oil content in seed averaged for two years, g

Hybrid	Variant of treatment		Crop density		Year	
Veleka	Check variant	51.50	35000	50.23	2014	50.98
	$N_{60}P_{120}K_{80}$	51.14 *NS*	45000	51.00 *NS*	2015	51.25 *NS*
	Root	51.04 *NS*	55000	51.42 *NS*		
	Siapton	50.89 *NS*	65000	51.30 *NS*		
	Lebososl B	51.35 *NS*				
	Lebososl Mg-S	51.18 *NS*				
	YVB#	51.02 *NS*				
	Lebososl Mn	51.74 *NS*				
Vokil	Check variant	52.59	35000	51.66	2014	52.35
	$N_{60}P_{120}K_{80}$	52.47 *NS*	45000	52.20 *NS*	2015	52.65 *NS*
	Root	52.01 *NS*	55000	52.87 *NS*		
	Siapton	52.22 *NS*	65000	52.68 *NS*		
	Lebososl B	52.58 *NS*				
	Lebosol Mg-S	52.75 *NS*				
	YVB	52.45 *NS*				
	Lebososl Mn	52.75 *NS*				
Sava	Check variant	50.53	35000	49.85	2014	50.38
	$N_{60}P_{120}K_{80}$	49.96 *NS*	45000	50.60 *NS*	2015	50.95 *NS*
	Root	50.83 *NS*	55000	50.57 *NS*		
	Siapton	49.94 *NS*	65000	50.50 *NS*		
	Lebososl B	49.90 *NS*				
	Lebososl Mg-S	50.40 *NS*				
	YVB	50.73 *NS*				
	Lebososl Mn					

,,*** - Significance of differences at p<0.05, p<0.01, p<0.001 respectively; NS – not significant; # - Yara Vita Brassitrel*

CONCLUSION

Based on the obtained data, it was found that the factor crop density of the plants had higher strength of effect on seed yield than the foliar fertilization. The first factor increased seed yield with 5 to 10 %, 1000 seed weight decreased with 12 to 19 % with the higher sowing norms, while oil percent in seed did not change. The factor fertilization did not have a significant effect on the three followed indices.

The significant deviation from the precipitation norm during the growth season of sunflower played a major role for the low effect of the investigated factors, fertilization in particular. During the first year the rainfalls were abundant, and during the second – scarce; as a result these deviations in both years of investigation neutralized the differences between the individual variants.

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EFFECT OF SOWING DATE ON HEAD DIAMETER IN SUNFLOWER

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ABSTRACT

Head diameter is very important trait in the sunflower seed yield structure. The size of head diameter influences the number of flowers and seeds per head which directly influence the seed yield per plant. In order to evaluate the effect of sowing date on head diameter, 3 sunflower hybrids, sown in 4 sowing dates during three growing seasons, were carried out. Head diameter was evaluated in the stage of flowering and physiological maturity. Head diameter at the stage of flowering was largely influenced by the year (Y) of investigation (46.6%), although other sources of variation (H-hybrid, SD-sowing date) showed also significance, except interaction H × SD. The largest head diameter is manifested in the hybrid Pobednik (11.9 cm) in the three-year average. In SD4 (20th of May) head diameter had the highest value (11.8 cm). In physiological maturity head diameter varied significantly depending on the sowing date (18.6%). Years, as well as hybrids had no significant influence on this trait. All interactions (Y × SD, H × SD, Y × H × SD), except year × hybrid (Y × H), were highly significant. Regarding sowing dates significantly higher mean value for head diameter can be noted in SD4, compared with earlier sowing dates in the three-year average. Values of head diameter, are doubled in the stage of physiological maturity in relation to the flowering stage. Coefficient of variation in the stage of physiological maturity was rather low (4,7%). The results of this study could be of importance in sunflower production.

Key Words : sunflower, sowing date, head diameter, flowering and physiological maturity

EFFICACY OF *TRICHODERMA* SPP. ISOLATES AGAINST *SCLEROTINIA SCLEROTIORUM* ON SUNFLOWER SEEDLINGS

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ABSTRACT

Trichoderma species are well known as effective antagonists to a variety of soil fungal pathogens. The aim of this research was to test the ability of *Trichoderma* spp. isolates, which previously indicated antagonistic activity (Tančić et al. 2012), to protect sunflower seedlings from *Sclerotinia sclerotiorum*. Ten *Trichoderma* spp. isolates obtained from different soil types and localities in Vojvodina province and one *S. sclerotiorum* isolate from sunflower grown at Rimski Šančevi (Serbia) were used in research. Biological efficacy was tested on 100 sunflower seeds treated with *Trichoderma* spp. suspensions ($1x10^6$) in two different treatments: T-30 (modified Mukhtar et al., 2012) and T-1.2 (Maslienko, 2005). *Trichoderma*-coated seeds were placed in four replicates on wet filter paper in Petri dishes. Next to each *Trichoderma*-coated seed the 5 mm^2 plug of *S. sclerotiorum* mycelia was placed, and incubated under the optimal laboratory conditions. Seeds treated with sterile distilled water with pathogen and without it were used as a positive and negative control, respectively. After seven days, biological efficacy of *Trichoderma* spp. isolates was assessed and calculated according to Liu et al. (2009). According to obtained results, biological efficacy of all tested *Trichoderma* isolates was statistically significant as compared to the positive control in both treatments. Good antagonism with over 50% of biological efficacy was registered in 8 isolates in T-30, and 3 isolates in treatment T-1.2. Three *Trichoderma* isolates which showed biological efficacy over 50% in both treatments can be considered as potential biocontrol agents which should be included in further more comprehensive research.

Key words: *Trichoderma*, *Sclerotinia sclerotiorum*, Antagonism, Sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important annual species grown in Serbia mostly for its edible oil. Areas grown under sunflower were around 170 000 hectares with the expected yield of 422 000 tones and sunflower oil production of 139 000 tones in economical year 2015/2016 (Chamber of Commerce and Industry of Serbia, 2016).

Farmers around the world are familiar with *S. sclerotiorum* (Lib.) de Bary as a threat to numerous crops such as sunflower, soybean, oilseed rape, edible dry bean, chickpea, peanut, dry pea, lentils and various vegetables as well. Occurrence of diseases caused by *S. sclerotiorum* on sunflower is influenced by genotype and weather conditions. Moist and cold weather conditions prevailing in temperate climate regions favours *S. sclerotiorum* development. In Serbia, weather conditions favoured economically important Sclerotinia development on sunflower head in 1999 and 2005 with diseased plants even over 60% (Maširević and Forgić, 2000; Maširević and Dedić, 2006). Diseases caused by this fungus can

appear during the whole sunflower growing season, and yield loss depends on the sunflower development stage in which the disease occurs. Sunflower plants infected at the beginning of flowering stage can lose up to 98% of their potential yield, while plants infected eight weeks after flowering can lose not more than 12% of their potential yield (Maširević & Gulya 1992). The major control method for *Sclerotinia* diseases has been fungicide application in combination with host resistance.

Considering growing demand for organic food production, biocontrol of such cosmopolitan and devastating pathogen is a big challenge. The use of biofertilizers and biopesticides is an alternative for sustaining high eco-friendly production. Integrated control is facilitated by the fact that *Trichoderma* species are resistant to most chemical pesticides (Harman, 2011; FRAC, 2016). *Trichoderma* species have been known since 1930s (Weindling, 1932), but since 1990s their usage in commercial agriculture has been increased (Harman 2004, 2011). So far, *Trichoderma* species have been known as effective on nutrient utilization with high reproductive potential which allow them to survive under unfavourable conditions and makes them very competitive. Presence of great variety of lytic enzymes (cell-wall degrading enzymes) and secondary metabolites (gliotoxin, gliovirin, viridin, viridiol etc.) makes *Trichoderma* strongly aggressive to broad range of phytopathogenic fungi (Vinale et al., 2008). The main biocontrol mechanisms of *Trichoderma* species, when direct confrontation with pathogen occurs, are mycoparasitism and antibiosis (Howel, 2003). Another mechanism which is quite effective as well, but do not consider direct confrontation with the pathogen, is competition for soil nutrients and space. Additionally, *Trichoderma* species are known as well as plant growth promoter agents and promoters of plant defense mechanisms (Shoresh et al., 2010; Harman, 2011). *Trichoderma* colonizes roots and provides at least season-long benefits to plants, although it can be even for life because the best strains fully colonize roots as they grow (Harman 2000). So far it is recorded that *Trichoderma* species improve growth of lettuce, tomato, pepper, wheat, maize, soybean, chilli (Vinale et al., 2004; Tucci et al., 2011; Sukla et al., 2015; Maisuria and Patel, 2009; Asaduzzaman et al., 2010). Also, *Trichoderma* spp. is stimulating defense responses in its host plants and is known as one of the best induced systemic resistance (ISR) agents (Shoresh et al., 2010; Shoresh, 2005).

Trichoderma species are mainly soil fungi found in agricultural soils, native prairie, forests, salt marsh, desert soils of all climatic zones, but also in dead plant material, living roots of various plant species, seeds, lake water and air (Monte, 2001). World-wide distribution, fast growth and high spore production make those species easy to find and isolate. After all, one should bear in mind that not all *Trichoderma* strains are effective, most of them are not, and some may even be phytotoxic or pathogenic (Menzies, 1993), so strain selection is of crucial importance. Given that, the aim of this study was to test ability of native Serbian *Trichoderma* strains to protect sunflower seedlings in early stage from pathogen *S. sclerotiorum*.

MATERIAL AND METHODS

Plant and fungal material used: Fungal material was obtained from soil samples originated from different soil types and localities in Serbia, mainly from Vojvodina province. All *Trichoderma* spp. isolates were refined to single-spore according to Leslie and Summerell (2006). Ten isolates which previously indicated good antagonistic activity in dual culture test (Tančić et al., 2012) were selected for this research. Pathogen *S. sclerotiorum* was isolated from diseased sunflower plant at Rimski Šančevi.

Trichoderma isolates' efficacy against *S. sclerotiorum* was tested on sunflower seeds of sterile parental line VL-A-8A.

Preparation of conidia suspension: A conidia suspensions of ten tested *Trichoderma* isolates were prepared from 7-days old isolates by flooding method. Such suspensions were filtered through cheesecloth, and conidial concentrations were adjusted to 10^6 conidia/ml by Neubauer's haemocytometer. Additionally, suspensions were amended according to Mukhtar et al. (2012) method.

Treatment T-1.2 considered that seeds were treated with 1.2 µl of *Trichoderma* suspension which was equally distributed per g of seeds and air dried on filter paper in Petri plates for 24 hours at room temperature (Маслиенко, 2005). Control was treated with 1.2 µl of sterile distilled water per g of seeds.

Treatment T-30 considered that seeds were dipped in seed-coating suspensions for 30 minutes and air dried on filter paper in Petri plates for 24 hours at room temperature, while sterile distilled water was used as a control.

Biological efficacy test: was done on 100 sunflower seeds treated with *Trichoderma* suspensions of different intensities (T-1.2 and T-30). Treated seeds were germinated in four replicates on double wet filter paper. Next to each sunflower seed, the 5 mm^2 plug of potato dextrose agar (PDA) with 7-day old micelia of *S. sclerotiorum*, was placed. Seeds treated with sterile distilled water without presence of pathogen *S. sclerotiorum* plugs were used as negative control, while seeds treated with sterile distilled water with presence of *S. sclerotiorum* plugs were used as positive control. Seeds were germinated in growth chamber with 12h photoperiod at 25±1ºC. After seven days diseased seedlings and seeds were counted, and biological efficacy of the *Trichoderma* isolate was calculated according to formula (Liu et al. 2009):

$$C\ (\%) = 100 * (a - b) / a$$

where C is biological efficacy in %, a – number of diseased seeds and seedlings in positive control, and b – number of diseased seeds and seedlings in treatment.

Beside biological efficacy, germination (G) was calculated as well on 7th day of the experiment.

Statistical analyses: All obtained data were analyzed in Statistica 12 using Duncan's test (percentages were previously transformed in ArcSin√%).

RESULTS AND DISCUSSION

Formation of rhizosphere microflora occurs usually in first three days after germination, and its progress in the deeper soil layers follows root growing and stimulates plant exometabolites at the same time (Асатурова, 2009). This is very important in biocontrol especially because young seedlings are often infected by pathogens in early stage of their development. Due to above mentioned, biological efficacy was estimated in the first days of sunflower germination and expressed as a percentage of protected seeds and seedlings comparing positive control (seeds without *Trichoderma* treatment grown in presence of pathogen *S. sclerotiorum*).

Germination was calculated on 7th day of incubation. Lower germination rates were registered in treatments with lower biological efficacy. Biological efficacy of all tested isolates was statistically significant as compared to the positive control in both treatments. According to obtained results, biological efficacy of tested *Trichoderma* isolates varied from 36 – 68% and 23.8 – 60.6% for treatments T-30 and T-1.2 respectively (Table 1). Excellent

antagonism with over 50% of biological efficacy was registered in 8 isolates in T-30, and 3 isolates in treatment T-1.2 (bold values in Table 1). Three *Trichoderma* isolates – K150, K173 and K174 showed biological efficacy over 50% in both treatments. These are promising results considering that some authors with bacterial antagonist reached biological efficacy against *Fusarium* spp. on sunflower seedlings from 0-36% (Асатурова, 2009), while biological efficacy of fungal and bacterial antagonist against *S. sclerotiorum* on sunflower stem under the field conditions was much higher - 54.5-100% (Фирсов et al., 2009). Besides on sunflower, the antagonistic activity of *Trichoderma* spp. against *S. sclerotiorum* was proven on other crops as well. Thus, the application of *T. harzianum* as alignate capsules increased the survival of soybean plants more than 100% and 40% in greenhouse and in the field, respectively (Menendez and Godeas, 1998). Isolates of *T. harzianum* also protected over 80% of tomato, squash and eggplant seedlings inoculated with *S. sclerotiorum* in the greenhouse experiments (Abdullah et al., 2008). Further, *T. virens* significantly reduces the percentage of viable sclerotia and number of apothecia produced (Huang and Erickson, 2008) which can be used for bioregulation of pathogen density in soil.

Table 1. Biological efficacy of two different treatments with *Trichoderma* spp. isolates against *S. sclerotiorum* on sunflower seedlings

	T-30		T-1.2	
Isolate No	C (%)	G (%)	C (%)	G (%)
K114	**50.0^{ab}**	86^{ab}	26.2^{bc}	78^{a}
K132	36.0^{b}	74^{a}	45.2^{a}	84^{ab}
K150	**58.0^{ac}**	94^{ab}	**58.3^{a}**	80^{ab}
K160	**50.0^{ab}**	90^{ab}	40.5^{abc}	77^{a}
K173	**56.0^{ac}**	76^{a}	**54.8^{a}**	85^{ab}
K174	**58.0^{ac}**	86^{ab}	**60.6^{a}**	85^{ab}
K175	**68.0^{c}**	74^{a}	23.8^{b}	91^{b}
K176	**59.5^{a}**	88^{ab}	48.0^{ab}	87^{ab}
K178	40.0^{b}	90^{ab}	42.9^{ac}	80^{ab}
K179	**53.6^{a}**	98^{b}	44.0^{ab}	87^{ab}
- Control	100^{d}	88^{ab}	100^{d}	96^{ab}
+ Control	0.00^{e}	80^{ab}	0.00^{e}	82^{ab}

Legend: Values in the columns followed by the same letters are not significantly different ($p<0.05$) by Duncan's test; Values are average of four replicates;

Beside *S. sclerotiorum*, it has been proven that *Trichoderma* spp. are aggressive to broad range of phytopathogenic fungi – *Rhizoctonia, Fusarium, Alternaria, Ustilago, Venturia, Colletotrichum, Pythium, Phytophthora, Thielaviopsis, Sclerotium cepivorum, Sclerotinia minor* etc. (Vinale et al., 2008; Thomas et al., 2004; McLean et al., 2012).

All mentioned above is leading to a conclusion that those perspective isolates from our research could also be good antagonists for some other important sunflower pathogens which should be tested in some further research.

CONCLUSION

Three out of ten tested *Trichoderma* isolates originating from Serbia expressed excellent ability to protect sunflower seedlings from pathogen *S. sclerotiorum* in both treatments. Those isolates can be considered as potential biocontrol agents and should be included in further, more comprehensive, research.

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EFFECT OF BIOSTIMULATORS ON SEED QUALITY, YIELD AND OIL CONTENT IN SUNFLOWER

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ABSTRACT

The effect of five biostimulators on seed quality, yield and oil content in sunflower was tested in this study. Seed was treated with biostimulators Amalgerol, Slavol, Иммуноцитофит,ТАБ, Raykat Start and НИКФАН, ж, as well as with fungicide Apron XL 350 ES and insecticide Cruiser 350 FS with added polymer Sepiret. Seed treatment with particular biostimulators had significant effect on the germination energy and germination. This effect was especially visible in the second trial year, when seeds lost their germination due to a long storage period. Treatments with pure Slavol and НИКФАН, ж showed the most significant positive effect. Positive effect was completely reduced when fungicide and insecticide were used with biostimulators. The largest average seed yield was achieved in treatment with НИКФАН,ж+insecticide and fungicide (4467 kg/ha), while the highest average oil content was achieved in treatment with pure НИКФАН,ж (53.34%). However, the effect of all treatments on seed yield and oil content was weak.

Key words: Sunflower, Biostimulators, Germination Energy, Germination, Seed Yield, Oil Content

INTRODUCTION

The most important target of sunflower production is high seed yield and high oil yield. In order to achieve this, it is necessary to grow high-yielding hybrids using optimal cultivation practices. Apart from these standard measures, various biostimulators are more often used via seed treatment or foliar treatment, with various degrees of success. Biostimulators are substances that can enhance the immunity of cultivated crops, benefit their metabolism (Kolomaznik et al., 2012), and decrease the effects of stress. The type of the biostimulator, its application, genotype and environmental conditions all affect its performance.

Using different foliar biostimulators based on 2-(1-Naphthyl) acetic acid, naphthalene derivatives, etc., Tahsin and Kolev (2005) found significant increase of sunflower seed yield with treatment in the flowering phase, but not with treatment in the budding phase; additionally there was no significant effect on the oil content. Beltrano et al. (1994) used gibberellic acid and benzyladenine and recorded yield increase of 25% through the increase of 1000-seed weight and pollination in the middle part of the sunflower head. Using various biostimulators on different oil crops, Ghosh et al. (1991) reached yield increase of 10-40%, but it was inconsistent throughout the trial years. Foliar application of amino acids had positive effect on the head diameter and pollination in sunflower in drought conditions (Kheybari et al., 2013). With foliar application of Fertileader Gold (patented extract of sea algae with addition of nitrogen, boron and molybdenum), Glijin et al. (2013) found significant increase in plant height and head diameter. By treating the seed with BION (active matter BTH), Buschmann and Sauerborn (2002) achieved induced resistance of sunflower to broomrape infection.

Jakienė and Liakas (2013) treated the soil with Azofit and Amalgerol and recorded significant increase in sugar beet root yield (7.26-9.67%), sugar content and sugar yield. Boteva (2014) found that fertilization with bioproducts Biofa and Amalgerol on background Biosol resulted in increased number of fruits in pepper – on average 3.1 fruits per plant. The increase of pepper yield was recorded from 6.2% (background Lumbrikal) to 16.9% (background Biosol+Amalgerol). The foliar products Amalgerol+Cereal mix, Foliar extra and KTS were the most effective for wheat grain yield, and the increase of yield compared to untreated control was 39.3%, 38.1% and 36.2%, respectively (Kostadinova et al., 2015). Šimunić et al. (2011) reported that foliar application of Amalgerol caused increased sunflower oil yield per hectare by 7.26% and soybean grain yield by 2.56%. On the other hand, under the conditions of extremely high temperatures during the growing season and severe soil drought in the region of Dobrudzha, Milev and Todorova (2014) found that foliar application of Almagerol on soybean did not have a significant positive effect neither on seed yield nor on 1000-seed weight. Treatment of growth stimulator Amalgerol premium with herbicides Goal, Raft, Wing, Pledge and Modown as tank mixtures increased the selectivity of these herbicides (Delchev, 2013).

The seed of Nadine F1 lettuce, treated with Slavol before sowing, sprung up two days earlier than the seed that was not treated at all (Kaliđerović and Mirecki, 2013). Treating sunflower seed with Slavol (indole-3-acetic acid) and Bioplant Flora (mixture of humic and fulvic acids, amino acids, macro and micro elements) Miladinov et al. (2014a) recorded increased length of sprout root (but not sprout shoot) in individual sunflower genotypes, but also found negative effects in certain treatments. Miladinov et al. (2014b) applied the same products while testing germination energy and germination, and found positive effect in certain treatments, higher on filter paper than on sterile sand.

Чухланцев (2010) reported that sunflower seed treatment with Vermikulen ŽK (3 l/t) + Иммуноцитофит, ТАБ (0.5 g/t) VDB (0.2 l/t) provided biological efficiency in the management of root white rot and fusariosis. Treatment of sunflower seed with a mixture of Иммуноцитофит and several other formulations increased yield, 1000-seed weight and number of seeds per head in sunflower (Высоцкая, 2013), as well as assimilating leaf area by 8.9-9.1% and seed yield by 320-360 kg/ha (Фирсов et al., 2010). Иммуноцитофит stimulated the mass germination of the sunflower seeds and increased their germination ability. The yield obtained from such plants (treated seeds + threefold treatment during the vegetation: in bud formation phase + two fold treatments every 15 days with addition of 0.5% Kristalon 18, 18, 18) was on average higher by 23.54% than control (Masheva et al., 2012).

Maize seed was treated with НИКФАН and germination increased by 20-40%, fresh weight yield increased by 22-32%, quality of fresh weight also increased (Маркелова et al., 2011). Петров and Шершнев (2007) found that maize seed treated with Agat – 25K and НИКФАН had better plant development and shorter growing season (by 7-8 days) and significant increase in seed yield.

Савенкова (2011) found that treatment of *Galega officinalis* seed with Raykat Start enhanced germination and root growth. Агафонов and Шабалдас (2013) reported increased yield of soybean seed treated with Raykat Start and several other products. However, this was not in agreement with Гракова (2011).

The aim of this study was to assess the effects of treating sunflower seed with biostimulators Amalgerol, Slavol, Иммуноцитофит, ТАБ, Raykat Start and НИКФАН, ж on seed yield, oil content and seed quality parameters.

MATERIAL AND METHODS

The trial was set up as split-plot design at the experimental field Rimski šančevi of the Institute of Field and Vegetable Crops in Novi Sad, Serbia in 2012 and 2013. The seed of sunflower hybrid Baća produced in 2011 was used in the trial and regular cultivation practices were performed.

The seed was treated with the systemic insecticide Cruiser 350 FS (1 l per 100 kg seed) and fungicide Apron XL 350–EC (300 ml per 100 kg seed), with addition of polymer and colorant Sepiret (300 ml per 100 kg seed), according to the regular sunflower seed processing procedure at the Institute of Field and Vegetable Crops. Additionally, the seed was treated with biostimulators in doses recommended by the manufacturers: Amalgerol at a concentration of 2%, Slavol at a concentration of 25%, Иммуноцитофит,ТАБ - one tablet in 10-15 ml of water per 5 g seed, Raykat Start - 0.5 l per 1000 kg seed, НИКФАН,ж- 0.6 l in 10 l of water per 1000 kg seed.

Amalgerol is an organic stimulator and soil enhancer. It contains essential oils, plant extracts and plant oils, marine algae extracts and mineral oil distillates. Slavol is a liquid microbiological fertilizer and growth stimulator certified for organic and traditional agricultural crop production. This product contains no chemical additives and has beneficial effect on the crops, soil and the environment. Иммуноцитофит, ТАБ is a plant growth regulator with active matter arachidonic acid ethyl ester. Raykat Start is a special fertilizer for the initial plant growth, used as seed / tuber dressing (free amino acids 4%, polysaccharides 15%, cytokine 0.05%, nitrogen (N) a single 4%, phosphorus pentoxide (P_2O_5) water-soluble 8%, potassium (K_2O) soluble in water 3%, iron (Fe) chelate EDDHA 0.1%, zinc (Zn) chelate of EDTA - 0.02%, boron (B) water-soluble 0.03%). НИКФАН, ж is an environmentally-friendly fertilizer, a product of microbiological synthesis mushroom-producing properties with strong stimulator for plant growth and development.

Oil content in clean seed was determined by nuclear magnetic resonance (NMR) method, according to Granlund and Zimmerman (1975). Sunflower seed yield was calculated to t/ha and corrected to 11% moisture. Laboratory analyses were performed in 2012 and 2013 at the Laboratory for Seed Testing of the Institute of Field and Vegetable Crops according to randomized block design in four repetitions, and the tested parameters were determined by standard laboratory methods. The data were processed in GENSTAT, and two-factorial analysis of variance was used for assessing results.

RESULTS AND DISCUSSION

The treatment of sunflower seed with the tested biostimulators did not show significant effect on the oil content in seed (Table 1). The highest oil content on average for both trial years was found in seed treated with pure НИКФАН, ж (53.34%), and the lowest in seed treated with the combination of Raykat Start with Apron and Cruiser (51.78%). Between these two treatments the differences were significant, but not highly significant, and in individual years there were no significant differences among the treatments (Table 2). No significant differences were found between the control and the treatments. In 2013 there was a higher average oil content than in 2012, but the differences were not significant.

Sunflower seed treatment with the tested biostimulators did not significantly affect the seed yield (Table 3). The highest seed yield was found in seed treated with НИКФАН, ж + Apron and Cruiser (4467 kg/ha), and the lowest in seed treated with Slavol + Apron and Cruiser (3846 kg/ha). The differences between these two treatments were significant, but not highly significant (Table 4). There were no significant differences in relation to the control, and there were no significant differences among all treatments in individual trial years. The

highest average seed yield was achieved in 2013 (4431 kg/ha), and the lowest in 2012 (3853 kg/ha), there were no significant differences between the trial years.

Table 1. ANOVA for oil content in hybrid Baća seed

Source of variation	df	SS	MS	F	P
Year of study (Y)	1	74.64	74.64	6.71	0.122^{ns}
Error Y	2	22.25	11.13	10.14	-
Treatment (T)	15	18.56	1.24	1.13	0.353^{ns}
Y x T	15	7.60	0.51	0.46	0.950^{ns}
Error T	60	65.84	1.10	-	-
Total	93	188.89	-	-	-

**significant at 1% level; *significant at 5% level; ^{ns}not significant

Table 2. Effect of year and biostimulators on oil content (%) in hybrid Baća seed

Treatments (T)	Trial year (Y)		Mean (T)
	2012	2013	
Control	53.64	51.64	52.64
Amalgerol	53.18	50.98	52.08
Amalgerol+Apron XL 350 ES	54.35	52.08	53.21
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	54.50	52.11	53.30
Slavol	53.09	51.80	52.45
Slavol+Apron XL 350 ES	54.00	51.28	52.64
Slavol+Apron XL 350 ES+Cruiser 350 FS	53.61	51.63	52.62
Иммуноцитофит,ТАБ	53.70	51.72	52.71
Иммуноцитофит,ТАБ+Apron XL 350 ES	54.09	52.04	53.06
Иммуноцитофит,ТАБ+Apron XL 350 ES+Cruiser 350 FS	53.55	51.38	52.46
Raykat Start	53.27	52.17	52.72
Raykat Start+Apron XL 350 ES	53.90	52.68	53.29
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	52.30	51.26	51.78
НИКФАН,ж	53.66	53.02	53.34
НИКФАН,ж+Apron XL 350 ES	53.62	51.83	52.72
НИКФАН,ж+Apron XL 350 ES+Cruiser 350 FS	52.92	51.57	52.25
Mean (Y)	53.59	51.82	

	Y	T	Y x T
$LSD_{0.05}$	2.93	1.21	2.35
$LSD_{0.01}$	6.76	1.61	3.30

Both seed treatment and trial year showed highly significant effect on the germination energy of sunflower seed (Table 5). The highest average of germination energy was found in seed treated with pure Slavol (90.62%), and the lowest in seed treated with the combination Amalgerol + Apron and Cruiser (80.12%). The differences between these two treatments were highly significant and the treatment with Slavol gave significantly higher germination energy than the control (Table 6). In 2012 there was highly significantly higher average of germination energy (91.89%) than in 2013 (78.48%). In 2012 no treatment showed significantly higher germination energy than the control, but there were several combinations with highly significant differences. Namely, highly significantly higher germination energy

was found in seed treated with НИКФАН, ж + Apron than in Raykat Start + Apron and Cruiser. In 2013 the differences among treatments were much higher – treatments with only Slavol and НИКФАН, ж were highly significantly higher or significantly higher than the control, but there were also significant reductions in some treatments, mostly in Amalgerol + Apron and Cruiser. These results imply that seed treatment with biostimulators showed more effect on the seed with lower average of germination energy, as was the case in 2013. It was discovered that in treatments with certain biostimulators which showed positive effect, the positive effect was lacking in combinations of biostimulator with fungicide and insecticide. Since seed treatment with fungicides (and insecticides as well) is a mandatory measure in seed processing, the practical possibility of biostimulator application is questionable.

Table 3. ANOVA for seed yield of hybrid Baća

Source of variation	**df**	**SS**	**MS**	**F**	**P**
Year of study (Y)	1	8015126	8015126	5.21	0.150^{ns}
Error Y	2	3076814	1538407	7.92	-
Treatment (T)	15	3328390	221893	1.14	0.341^{ns}
Y x T	15	1022172	68145	0.35	0.986^{ns}
Error T	60	11649289	194155	-	-
Total	93	39227240	-	-	-

**significant at 1% level; *significant at 5% level; ^{ns}not significant

Table 4. Effect of year and biotimulators on seed yield (kg/ha) of hybrid Baća

Treatments (T)	Trial year (Y)		Mean
	2012	2013	(T)
Control	3817	4317	4067
Amalgerol	3817	4692	4255
Amalgerol+Apron XL 350 ES	3994	4725	4360
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	4053	4279	4166
Slavol	3661	4237	3949
Slavol+Apron XL 350 ES	3918	4139	4028
Slavol+Apron XL 350 ES+Cruiser 350 FS	3718	3975	3846
Иммуноцитофит,ТАБ	4013	4585	4299
Иммуноцитофит,ТАБ+Apron XL 350 ES	3764	4630	4197
Иммуноцитофит,ТАБ+Apron XL 350 ES+Cruiser 350 FS	3530	4221	3875
Raykat Start	3868	4554	4211
Raykat Start+Apron XL 350 ES	3901	4740	4320
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	4081	4518	4299
НИКФАН,ж	3826	4319	4072
НИКФАН,ж+Apron XL 350 ES	3531	4186	3858
НИКФАН,ж+Apron XL 350 ES+Cruiser 350 FS	4156	4778	4467
Mean (Y)	3853	4431	

	Y	T	Y x T
$LSD_{0.05}$	1089	509	918
$LSD_{0.01}$	2513	677	1270

Seed treatment and trial year showed highly significant effect on the seed germination (Table 7). The highest mean seed germination was achieved in seed treated with pure Slavol

(91.12%), and the lowest in seed treated with Amalgerol + Apron and Cruiser (81.12%). The difference was highly significant and the treatment with Slavol showed significantly higher germination than the control (Table 8). In 2012 mean seed germination (93.03%) was highly significantly higher than in 2013 (79.80%). In individual years, the trends were similar to the germination energy, i.e. the treatment was more effective in a year with lower mean germination. In 2012 no treatment showed significant difference in relation to the control, but there were highly significant differences among individual treatments. In 2013 the treatments with Slavol and НИКФАН, ж showed highly significant increase in seed germination than the control. In 2013 the treatment with pure biostimulators showed better results than the treatments with added fungicides and insecticides, while in 2012 this was not the case.

Table 5. ANOVA for the germination energy of hybrid Baća seed

Source of variation	df	SS	MS	F	P
Year of study (Y)	1	5751.28	5751.28	446.68	<.001**
Error Y	15	1016.50	67.77	5.26	<.001**
Treatment (T)	15	675.72	45.05	3.50	<.001**
Y x T	93	1197.44	12.88	-	-
Total	124	8855.50	-	-	-

**significant at 1% level; *significant at 5% level; nsnot significant

Table 6. Effect of year and biostimulators on the germination energy (%) of hybrid Baća seed

Treatments (T)	Trial year (Y)		Mean
	2012	2013	(T)
Control	93.75	80.00	86.88
Amalgerol	90.75	79.25	85.00
Amalgerol+Apron XL 350 ES	94.25	79.25	86.75
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	90.75	69.50	80.12
Slavol	92.75	88.50	90.62
Slavol+Apron XL 350 ES	90.25	73.25	81.75
Slavol+Apron XL 350 ES+Cruiser 350 FS	90.00	74.25	82.12
Иммуноцитофит,ТАБ	91.75	85.00	88.38
Иммуноцитофит,ТАБ+Apron XL 350 ES	91.50	78.50	85.00
Иммуноцитофит,ТАБ+Apron XL 350 ES+Cruiser 350 FS	94.00	76.50	85.25
Raykat Start	91.75	78.50	85.12
Raykat Start+Apron XL 350 ES	92.25	77.25	84.75
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	88.50	76.75	82.62
НИКФАН,ж	91.50	86.75	89.12
НИКФАН,ж+Apron XL 350 ES	95.50	79.00	87.25
НИКФАН,ж+Apron XL 350 ES+Cruiser 350 FS	91.00	73.50	82.25
Mean (Y)	91.89	78.48	

	Y	T	Y x T
$LSD_{0.05}$	1.26	3.56	5.04
$LSD_{0.01}$	1.67	4.72	6.67

It is evident that the sunflower seed treatment with the tested biostimulators did not generally result in a significant increase of oil content in seed and seed yield, which is contrary to the results on sunflower reported by Šimunić et al. (2011), Высоцкая (2013),

Фирсов et al. (2010), on maize by Маркелова et al. (2011), and on soybean by Агафонов and Шабалдас (2013). Lack of biostimulator effect on soybean yield was reported by Milev and Todorova (2014). There was a certain effect in individual treatments, but it was difficult to deduce any regularity which could justify commercially viable recommendations for general use.

Table 7. ANOVA for seed germination of hybrid Baća

Source of variation	df	SS	MS	F	P
Year of study (Y)	1	5604.76	5604.76	504.45	<.001**
Error Y	15	784.18	52.28	4.71	<.001**
Treatment (T)	15	702.37	46.82	4.21	<.001**
Y x T	93	1033.29	11.11	-	-
Total	124	8269.05	-	-	-

**significant at 1% level; *significant at 5% level; nsnot significant

None the less, the seed quality parameters showed different results. The effect of the treatment was much higher, especially in years with low mean values of germination and germination energy. The best effect was achieved with Slavol and НИКФАН, ж. The positive effects of individual biostimulators on the sunflower seed quality parameters were previously reported by Miladinov et al. (2014b), Masheva et al. (2012), and on other crops by Маркелова et al. (2011) and Савенкова (2011). The problem is that the combination of biostimulators with fungicides or fungicides and insecticides did not show any positive effects as pure biostimulators did, which greatly impedes the practical use of biostimulators.

Table8. Effect of year and biostimulators on seed germination (%) of hybrid Baća

Treatments (T)	Trial year (Y)		Mean
	2012	2013	(T)
Control	94.00	81.00	87.50
Amalgerol	92.00	79.50	85.75
Amalgerol+Apron XL 350 ES	95.25	79.50	87.38
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	91.50	70.75	81.12
Slavol	93.75	88.50	91.12
Slavol+Apron XL 350 ES	90.75	74.25	82.50
Slavol+Apron XL 350 ES+Cruiser 350 FS	91.50	76.75	84.12
Иммуноцитофит,ТАБ	92.75	85.25	89.00
Иммуноцитофит,ТАБ+Apron XL 350 ES	93.50	79.00	86.25
Иммуноцитофит,ТАБ+Apron XL 350 ES+Cruiser 350 FS	97.50	78.00	87.75
Raykat Start	93.00	79.25	86.12
Raykat Start+Apron XL 350 ES	93.75	78.50	86.12
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	89.25	82.50	85.88
НИКФАН,ж	92.00	87.50	89.75
НИКФАН,ж+Apron XL 350 ES	96.00	79.25	87.62
НИКФАН,ж+Apron XL 350 ES+Cruiser 350 FS	92.00	77.25	84.62
Mean (Y)	93.03	79.80	

	Y	T	Y x T
$LSD_{0.05}$	1.17	3.31	4.68
$LSD_{0.01}$	1.55	4.38	6.20

In the current situation of slow increase of genetic yield and quality potential in new cultivars of many crops and the level of cultivation practices that cannot easily be revolutionized nor quickly improved, various biostimulators are more often being used. The results show that the positive effects were not as spectacular as marketed or reported in different studies. Individual biostimulators certainly hold their place in the improvement of individual crops cultivation, so investments into biostimulators application must be economically viable, which is only possible through detailed and objective studies in different agricultural environments using different genotypes.

CONCLUSIONS

Sunflower seed treatment with the tested biostimulators did not show significant effect on oil content in seed nor the seed yield. The highest oil content on average for both trial years was found in seed treated with pure НИКФАН, ж (53.34%), and the lowest in seed treated with Raykat Start in combination with Apron and Cruiser (51.78%). Between these two treatments the differences were significant, but not highly significant, and in individual years there were no differences between the treatments. The highest seed yield was found in seed treated with НИКФАН, ж + Apron and Cruiser (4467 kg/ha), and the lowest in seed treated with Slavol + Apron and Cruiser (3846 kg/ha). The differences between these two treatments were significant, but not highly significant.

Sunflower seed treatment with the tested biostimulators showed highly significant effect on the seed quality parameters. The highest mean first count was found in seed treated with pure Slavol (90.62%), and the lowest in seed treated with Amalgerol + Apron and Cruiser (80.12%). The differences between these two treatments were highly significant, and the treatment with Slavol showed significantly higher germination energy than the control. The highest mean seed germination was found in the seed treated only with Slavol (91.12%), and the lowest in seed treated with Amalgerol + Apron and Cruiser (81.12%). The difference was highly significant and treatment with Slavol showed significantly higher germination than the control.

The conclusion is that the tested biostimulators could be more applicable in seed production than in commercial (mercantile) production. Practical application of individual biostimulators for enhancement of seed quality parameters is restricted by the fact that the positive effects drastically drop when biostimulators are combined with fungicides and insecticides, which should further be studied. This indicates that biostimulators can be used more successfully in organic production.

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INSECT MONITORING IN SUNFLOWER CROPS (HELIANTHUS ANNUUS) IN NORTHERN GREECE (2010-2015)

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ABSTRACT

Twenty-eight insect species were recorded in sunflower crops in Northern Greece, during the 2010-2015 period. The recorded species were classified into three categories: a) pests, b) beneficial and c) insects that were merely observed in sunflower fields. In the first category, the recorded insect species in sunflower crops were green peach aphids (*Myzus persicae*, Hem.: Aphididae), thrips (Thys.: Thripidae), ground beetle grubs (Col.: Scarabaeidae), meadow froghoppers (*Philaenus spumarius,* Hem.: Aphrophoridae), common meadow bugs (*Lygus pratensis,* Hem.: Miridae), click beetle larvae (*Agriotes* spp., Col.: Elateridae), grasshoppers (Orth.: Acrididae), cutworms (*Agrotis* spp.), long horn beetle larvae (*Agapanthia cynarae*, Col.: Cerambycidae), sugarbeet weevils (*Bothynoderes punctiventris,* Col.: Curculionidae), leafhoppers (Hem.: Cicadellidae), whiteflies (*Bemisia tabaci,* Hem.: Aleyrodidae), vine chafer beetles (*Anomala vitis,* Col.: Scarabaeidae), flea beetles (*Chaetocnema tibialis,* Col.: Chrysomelidae), bordered straw larvae (*Heliothis peltigera,* Lep.: Noctuidae), black bean aphids (*Aphis fabae,* Hem.: Aphididae), two-spotted spider mites (*Tetranychus urticae,* Tetranychidae) and nematodes (*Meloidogyne hispanica,* Tylenchida). In the beneficial insects category, ladybirds (*Coccinella septempunctata,* Col.: Coccinellidae), damsel bugs (*Nabis* spp., Hem.: Nabidae) and lacewings (*Chrysoperla carnea,* Neur.: Chrysopidae) were recorded. In the third category, sloe bugs (*Dolycoris baccarum, Hem.: Pentatomidae*), red shield bugs (*Carpocoris mediterraneus,* Hem.: Pentatomidae), lucerne bugs (*Adelphocoris lineolatus,* Hem.: Miridae), clearwing flies (*Terellia* spp., Dip.: Tephritidae), green stink bugs (*Nezara viridula,*Hem.: Pentatomidae), the harmless pollen-feeding beetles (Col.: Oedemeridae) and painted-lady adults (*Vanessa cardui,* Lep.: Nymphalidae) were recorded.

Key Words : sunflower

INFLUENCE OF SEED SIZE GRADE ON SUNFLOWER PLANT HIGH

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ABSTRACT

The seed size is one of the seed quality components which affect the performance of the crop. In order to equalize the quality, the seeds are processed and packed by grade where seeds are separate by size and specific weight. The objective of this study was to examine the effect of seed processing and grading on sunflower plant height at bud stage. The field experiment was carried out during the growing seasons of 2010 and 2011, with six seed grade of hybrid Sremac at the experimental fields of Institute of Field and Vegetable Crops at Rimski šančevi and Zrenjanin Agricultural Advisory Services. Data were analyzed using three-way ANOVA for a split-split-plot design. Based on obtained results it can be concluded that the effect of all three observed factors - locality, growing seasons and seed grade on sunflower plant height at bud stage was statistically highly significant as well as interaction year x locality, while other interactions did not show statistical significance. Also, significant differences were observed between bigger and smaller grades, where the plant height proportionally decreased with seed size.

Key words: Plant Height, Seed Size, Sunflower

INTRODUCTION

Seed quality is a collection of properties that are considered to have a significant impact on the seed value which is used for sowing (FAO, 1999). The seed size is one of the seed quality components which affect the performance of the crop, and therefore on the yield (Singh et al., 2010; Adebisi et al., 2013).

In the sunflower seed production the most important is to get a high quality seed as a final product. The quality, and therefore the seed size, primarily is determined by growing conditions and it is necessary to cultivate crop in optimal plant density, the more fertile soils, with timely and quality execution of all other agro-technical measures (Crnobarac, 1992). On the other hand, seed quality depends not only on field conditions and cultural practices applied but also on seed processing (Miklič et al., 2012). During processing, unwanted ingredients are removed from the natural seeds by applying various technological processes, based on differences in the seed characteristics (Prole et al., 2011).

Commercially seed are rarely uniform in size, and in one lot can be found the seeds of different sizes and different quality (Komba et al., 2007). The goal of seed processing is to prepare seed material in form and condition suitable for sowing, by separation seeds by size

(Štatkić et al., 2007). Pucarić (1992) considered it necessary make seed calibration because it affects the uniform germination, emergence and early growth of plants, and therefore provides a higher yield. Similar attitudes present Pucarić and Ujević (1986) and conclude that the corn seed calibration can accomplish 15% higher yields compared to commonly seed processing. Calibration, i.e. equalizing of seed by size and weight, affect seed quality, but also visual appearance on the increasingly demanding market and provides uniform sowing. Global standards do not prescribe calibration parameters, and calibration of sunflower seeds is done by experience (Prole et al., 2011).

Processing can have various and very large impact on seed quality in different sunflower genotypes, and it is necessary to know the specific characteristics of each genotype, especially in the case of seeds with lower quality (Miklič et al., 2012).

The objective of this study was to examine the effect of seed processing and grading on sunflower plant height at bud stage.

MATERIAL AND METHODS

The research was carried out on seed of hybrids Sremac, conventional oil hybrid created in the Institute of Field and Vegetable Crops from Novi Sad. The seed is produced within the seed production by applying agro-technical measures required by the technology of sunflower hybrid seed production.

Natural seed was pre-cleaned, and after processing separated into six grades. First, seed was graded with a *Cimbria Heid* type *ZS 500* cylinder grader, with the screens set to make two grades, small seeds of 2.8 -3.5 mm and large seeds of 3.5-5.0 mm. After that, both grades were run through a *Cimbria Heid* type *GA 200* gravity table to separate seeds by specific weight. 1000-seed weight was determined for each grade (Table 1).

Table 1. Seed grade of hybrid Sremac

Seed grade	Diameter (mm)	1000-seed weight (g)
I	3.0 – 5.0	66.4
II	3.0 – 5.0	71.8
III	3.0 – 5.0	57.1
IV	3.0 – 5.0	54.1
V	2.8 – 3.0	50.5
VI	2.8 – 3.0	47.7

After processing seed was chemically treated with Cimbria *Heid* type *CC 50* centrifugal duster with fungicide *Apron XL 350 EC* (300 ml per 100 kg seed) and insecticide *Cruiser 350 FS* (1 l per 100 kg seed), with addition of polymer Sepiret (500 ml per 100 kg seed), that allows a better adhesion of pesticides for seed. Seed processing and treatment, as well as sample preparation was conducted in the Oil Crops Department of Institute of Field and Vegetable Crops in Novi Sad.

The field experiment was carried out during the growing seasons of 2010 and 2011, at the experimental fields of Institute of Field and Vegetable Crops at Rimski šančevi and Zrenjanin Agricultural Advisory Services according to the *split-plot* model design with three replications.

Plant height was measured by a graduated stick, and parameter values are expressed in cm. The measurement was carried out in the bud stage (R2), according to Schneiter and Miller (1981).

Statistical analysis of data was performed by analysis of variance (ANOVA) of the tri-factorial trial using the statistical package *STATISTIKA 10.0* for *split-split-plot* design model. Table of analysis of variance shows the probability of significance of differences by F-test, and based on the participation in the treatment sum of squares, percentage ratio of each factor was calculated in the total variability. LSD values at 1% and 5% were computed to compare differences between treatments of the observed factor.

RESULTS AND DISCUSSION

The results of ANOVA showed that the interaction year x locality had the greatest influence on plant height in bud stage, with a participation of 65% in the total variation of these properties. Also, a highly significant influence had all the examined individual factors although their participation was a smaller percentage, at seed grade only 7% (Table 2).

Table 2. ANOVA for hybrid Sremac plant height at bud stage

Source of variation	df	SS	% in SS	MS	F	P
Y x L x Rep.	8	1291.00	-	161.38	3.72	0.002**
Year (Y)	1	3500.06	13	3500.06	80.79	<.001**
Locality (L)	1	3253.56	12	3253.56	75.10	<.001**
Seed grade (G)	5	1997.17	7	399.43	9.22	<.001**
Y x L	1	17734.72	65	17734.72	409.34	<.001**
Y x G	5	331.78	1	66.36	1.53	0.202ns
L x G	5	304.28	1	60.86	1.40	0.243ns
Y x L x G	5	184.44	1	36.89	0.85	0.522ns
Error	40	1733.00	-	43.33	-	-
Total	71	30330.00	100	-	-	-

**significant at the 1% level of probability; *significant at 5% level of probability; ns not significant

On average, highly significantly higher plant height was measured in 2010 (130.31 cm), and at the locality Rimski šančevi (130.06 cm). The reason for these results we can see on the interaction year x location, where is in the locality of Zrenjanin in 2011 the highly significantly lowest plant height was measured (93.94 cm). Also, highly significantly lower height had plants at Rimski šančevi in 2010 in relation to Zrenjanin in 2010 and Rimski šančevi in 2011, where no statistically significant difference was found (Figure 1).

Looking at the seed grades, we can see that the plant height, on average, decreased proportionally the seed size (Figure 2). Therefore, the largest plant height was measured in I grade, and the lowest in VI, and this difference was highly significant. Plant height of I grade was significantly higher compared to other tested grades, except in relation to II grade, where the difference was not statistically significant. Also, significant differences were observed between II grade and other tested grades, among which no statistically significant difference was found. Almost identical results can be noted for the first order interactions - year x seed grade and location x seed grade, for both investigation years and both localities.

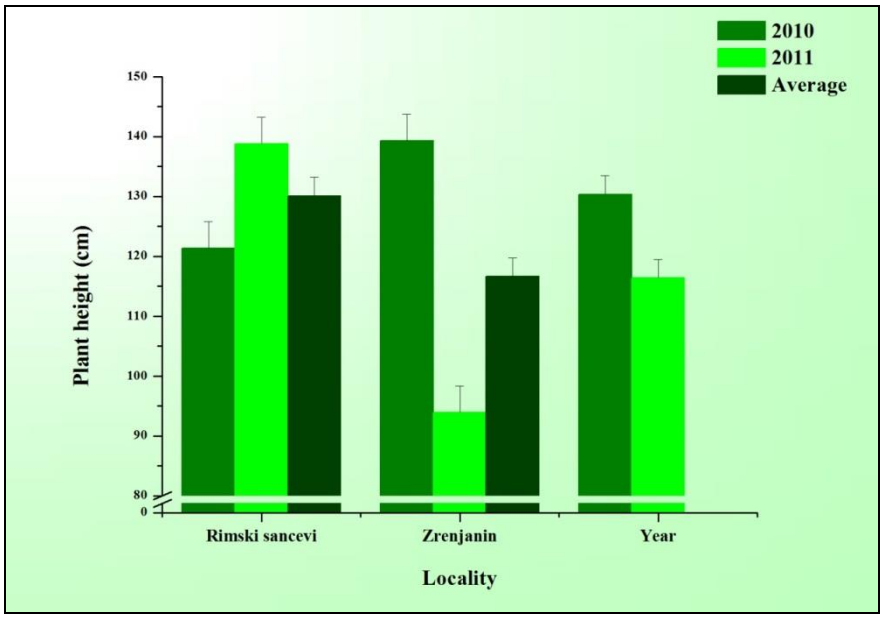

Figure 1. The effect of locality and production year on hybrid Sremac plant height at bud stage

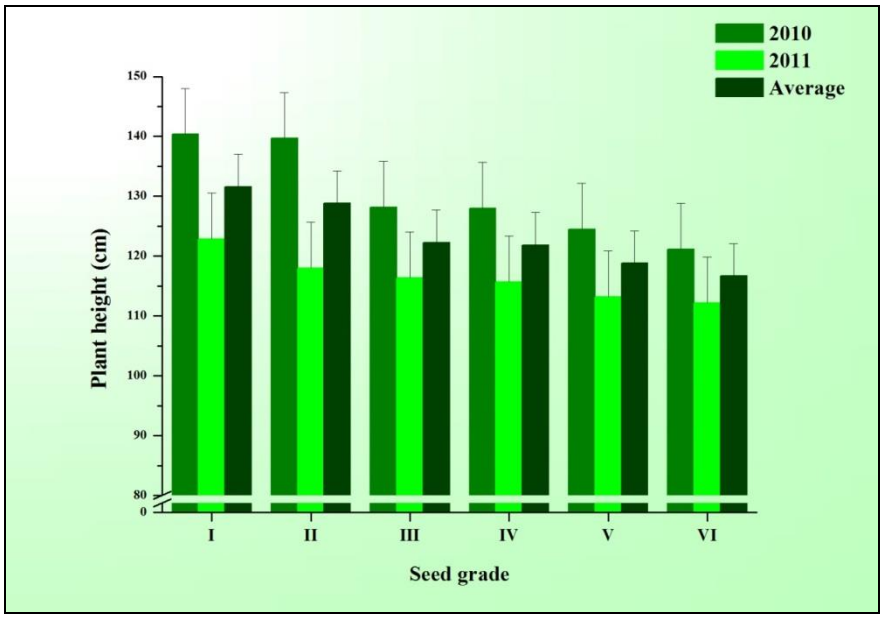

Figure 2. The effect of seed grade and production year on hybrid Sremac plant height at bud stage

Plant height mostly depends on genotype (Velasco et al., 2003; Mijić et al, 2005) and cropping practices and especially soil moisture content (Human et al., 1990; Iqbal et al., 2013) and growing spaces (Hall et al., 2010; Sposaro et al., 2010). Results from our research are in compliance with previous statement, plant height is significantly dependent on locality and year of trial. For hybrid Sremac year and locality had similar part in total variation, with difference of 1%. The third factor, seed grade, had significantly influenced plant height although it had only 7% in total variation. Rogers and Lomman (1988) obtained similar results. Usage of seed smaller size resulted in delay of the initial plant growth and

development in comparison with plants grown using larger seed. In this research plant height was negatively correlated to seed size.

Results from other researchers are confirming that seed size influence yield and plant development. In the research of Haskins and Gorz (1975) plants of same variety but growing using large seed were much stronger. They confirm statement made by Kaufmann (1958) that plants from large seed were stronger and pointed that seed size as source of variation. Fenner (1983) proved that seedlings develop from large seed easily penetrates deeper in soil, which helps early plant growth.

Mishra et al. (2008) research gave conclusion that usage of seed larger in size is much better considering field emergence well as plant performance then use of middle size seed. Same authors recommended avoidance of use of seed small in size. Explanation for these results, according to Jevtić (1981), is that large seed have larger endosperm, higher auxin content which positively influence development of young seedlings root and enhance plant growth.

CONCLUSION

Based on obtained results it can be concluded that the effect of all three observed factors - locality, growing seasons and seed grade on sunflower plant height at bud stage was statistically highly significant as well as interaction year x locality, while other interactions did not show statistical significance. Also, significant differences were observed between bigger and smaller grades, where the plant height proportionally decreased with seed size.

ACKNOWLEDGEMENTS

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AGRONOMIC PERFORMANCE OF SUNFLOWER CULTIVARS IN CAMPO NOVO DO PARECIS - MT, BRAZIL

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ABSTRACT

Sunflower seeds are a promising future crop in Brazil, especially as a catch crop in soy bean production. Sunflower seeds are processed mainly for their oil leaving press cakes or meals as by-products. Sunflower is an annual cycle plant of rapid growth, less needs of water and resistance to high and low temperatures comparing to most common explored oil plants in Brazil. Sunflower is cultivated in Brazil mainly in the winter season in the central west regions of Brazil. This study aimed to evaluate the agronomic characteristics of different genotypes of sunflower sown as a second crop in the year 2014. The work was implemented in the experimental field of the Instituto Federal de Educação Ciência e Tecnologia de Mato Grosso - Campus Campo Novo do Parecis - Brazil. The experimental design was a randomized block design with 16 treatments (16 genotypes) and four replications. The experimental plots consisted of four rows 6.5 m long with row spacing of 0.45 m, containing area of 11.7 m², totaling an area of 748 m². A population of 45,000 plants per hectare is used. The genotypes performance were evaluated according to following parameters: plant height (PH; cm), date to the early flowering (DEF), days until physiological maturity (DPM), stem curvature (SC), capitulum size (CS; cm), number of broken plants (NBP), harvest index (HI), achenes productivity (PR; kg ha^{-1}) and mass of thousand achenes (MTA, g). All variables showed significant differences ($p<0.05$) in the analysis of variance, especially the SYN 045 (PH and SC), ADV 5504 (DEF and DPM), PARAISO 20 (CS), AGUARÁ 06 (NBP) and AGUARÁ 04 (HI). For the mass of a thousand achenes, genotypes that stood out were BRS 323, MG 360 and M734 while the most productive were the genotypes MG 360, AGUARÁ 06, MG 305, AGUARÁ 04, CF 101, SYN 045, GNZ NEON, HELIO 251 and SYN 3950HO.

Keywords: Brazilian savannah, *Helianthus annuus* L., oilseed, genotypes performance, sunflower meal.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an annual cycle plant and its characteristics rapid growth, straight stem, sub woody and little branched at the apex, with characteristics of resistance to drought, cold and heat, more than most cultivated species of economic interest in Brazil. Sunflower fractions like presscake and hulls can be used for various purposes such as the for the production of food ingredients, animal feed and high quality oil as well. Furthermore, sunflower can be used as ornamental plant and to feed birds (Leite et al., 2005).

The cultivated sunflower area in Brazil in the harverst year of 2013/2014 was 145,700 hectares, totalizing 244,100 tons. The national average yield in the mentioned period was 1,599 kg ha^{-1}. In the state of Mato Grosso, sunflower planted area represented 87.2% compared to the total planted area (126,200 hectares) and with the highest production (202,300 tons) with an average yield of 1,611 kg ha^{-1}. The Region of Campo Novo do Parecis is Brazil´s largest sunflower producer, with a planted area of over 100,000 hectares (CONAB, 2014).

Among the various technologies developed for sunflower production, the proper choice of cultivar and the planting season, which has high grain yield is important to ensure the success of culture as one of the production system components (Porto et al., 2007). In the region of Campo Novo do Parecis, the sunflower is cultivated as second crop from February / March, due to the occurrence of rainfall conditions (500-700 mm evenly distributed throughout its cycle) and appropriate temperatures (20 at 28 °C) for its cultivation (Castro and Farias, 2005). Despite being the main growing region in the country, little information is available on adaptation and other agronomic characteristics of genotypes that facilitate the cultivation, reducing risk and increasing profitability.

In this sense, this study aimed to evaluate the agronomic characteristics of different genotypes of sunflower as a second crop in the region of Campo Novo do Parecis – MT. In the frame of this study, results will be used to identify best performance materials and a Sunflower meal for human consumption will be developed in collaboration with german researchers

MATERIAL AND METHODS

The work was carried out at the experimental fields and facilities of the Instituto Federal de Educação Ciência e Tecnologia de Mato Grosso - Campo Novo do Parecis in second-crop system in succession to soybeans in the agricultural year 2013/2014. The soil, according to the American System of Soil Classification (USDA, 1960) is the Typic Tropudox. The initial characterization of fertility, for the first layer of 0-0.20 m, presented the following values: pH ($CaCl_2$) = 5,7; MO = 26 g dm^{-3}; P (resina) = 5,9 mg dm^{-3}; K, Ca, Mg e H+Al = 1,5; 32; 11 e 40 $mmol_c$ dm^{-3}, respectively; with V = 54,8%.

Average temperatures occurred during the experimental period were: 30.3; 23.2 and 18,9 °C for maximum temperature, medium and minimum, respectively, and 570 mm rainfall, meeting the water demands required by sunflower between 500 and 700 mm distributed along its growing cycle (Castro and Farias, 2005).

The experimental design was a randomized complete block design with 16 treatments (genotypes) and four replications, as follows: ADV 5504, AGUARÁ 04, AGUARÁ 06, BRS 323, BRS G42, CF 101, GNZ NEON, HELIO 250, HELIO 251, HLA 2012, M734, MG 305, MG 360, PARAISO 20, SYN 045 and SYN 3950HO. The experimental plots consisted of 4 rows with 6.5 m long, with row spacing of 0.45 m, containing area of 11.7 m² (1.8 x 6.5 m). Only the two 5 meters central rows of each genotype were considered for data collection. The plotted area comprises 4.5 m².

The plot of the rows, was done on March 7, 2014, and the previous application of fertilizers was carried out with the aid of a sowing machine and was distributed at a depth of 0.10 m, 45 kg ha^{-1} Potassium Chloride + 267 kg ha^{-1} NPK 10-30-20, totalizing: 26.7 kg ha^{-1} N; 80 kg ha^{-1} P_2O_5; 80 kg ha^{-1} K_2O, according to the results of soil analysis and recommendation (EMBRAPA, 2004). Further, beside the row fertilization at 0.04 m deep, three seeds were placed in each hole, each 0.495 m, by manual planter.

The desiccation and the application of boron was performed on March 07, using trawl trailed sprayer with an application volume of 150 L ha^{-1} using glyphosate (648 g a.i. L^{-1}) at a dosage of 2 L ha^{-1} + Prometryn dosage 2 L ha^{-1} + mineral oil (0.5 L ha^{-1}) + boric acid dosage of 3 kg ha^{-1} (600 g ha^{-1} Boron). Thinning was done 10 days after emergence (DAE) with a scissor, leaving only one plant per hole, reaching a population of 45,000 plants ha^{-1}.

The following coverage fertilizations were made: 1) 32 DAE with a dosage of 50 kg ha^{-1} N (urea); 2) foliar application of boron, with knapsack sprayer at 35 DAE using a dosage of 3 kg ha^{-1} (600 g ha^{-1} Boron), and 43 DAE with a dosage of 11 kg ha^{-1} (1.1 kg ha^{-1} of Boron). The source of Boron used was boric acid 150 L ha^{-1} according to the requirement of sunflower of 2 kg ha^{-1} B, Control of weed, pests and diseases have been carried out according to the recommendations of EMBRAPA (2004). To avoid birds attacks, the plotted sections of the central rows were protected (stage R6) by using polypropylene based bags (30 x 30 cm) and fixed with clips.

The following agronomic characteristics were evaluated: plant height (**PH**; cm), collected in ten plotted plants from the soil base to the apex of the plant, in $R_{5.5}$; date of the early flowering (**DEF**), when 50% of the plants showed yellow petals R_4; days to physiological maturity (**DPM**), when 90% of plotted plants showed color between yellow and brown; stem curvature (**SC**), visual assessment using the rating scale Castiglioni et al. (1997); capitulum size (**CS**; cm) collected in the diameter of the ten capitulums demarcated plants, R_9; number of broken plants (**NBP**), counting on the two central lines of 5 meters; harvest index (**HI**), determined by dividing the mass of achenes by the mass of capitulums collected from ten demarcated plants.

The achenes productivity (**PR**; kg ha^{-1}) was defined based on two central 5 meters rows, this being corrected for moisture condition of 11% (wet basis) by obtaining a reading of the moisture value of achenes, and calculated according to the Equation 1, proposed by Dalchiavon et al. (2011):

$$PR = P.[(100-Uob) / (100 – Ud)] \quad \text{.....Eq. (1)}$$

where: PR represented the corrected mass of achenes (kg ha^{-1}); **P** represented the field of mass (uncorrected) of achenes (kg ha^{-1}); **Uob** represented the moisture observed for each plot (%) and **Ud** representing the desired moisture as standard (11%). Mass thousand achenes (**MMA**; g) was obtained by counting and weighing samples collected from ten marked plants.

The harvest of the capitulums was performed manually in the two 5 meter central rows in R_9 with pruning shears aid. Later the capitulum inflorescence were natural dryied, cleaned and weighed. The datas collected were submitted to analysis of variance followed by the average test Scott-Knott, both 5% probability, with the help of statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

All variables showed significant differences ($p < 0.05$) in the analysis of variance (Table 1). For plant height, the genotype with the highest average was the SYN 045 (198.5 cm), and the lowest average were BRS G42, MG 360, CF 101, ADV 5504, HELIO 250 and BRS 323, with values between 142.1 and 157.4 cm (Table 2). Nobre et al. (2012), carried out tests with different genotypes in the North of Minas Gerais (Brazil) and reported averages of 170.0 and 200.0 cm, respectively, for genotypes CF 101 and M734. The smallest plant height facilitates the cultivation and reduces the loss in mechanical harvesting.

Table 1. ABSTRACT of the analysis of variance for the sunflower productivitiy parameters

Variables[1]	F[2]	CV (%)[3]	GA[4]
PH (cm)	27.4*	3.6	167.7
DEF	489.0*	0.4	57.6
DPM	15103.9*	0.1	101.4
SC	8.6*	7.0	4.7
CS (cm)	8.2*	6.8	15.4
NBP	8.4*	27.4	8.6
HI	2.2*	11.2	0.64
PR (kg ha^{-1})	6.4*	12.4	1846.9
MTA (g)	9.5*	9.2	54.1

[1] PH = plant height, DEF = date to the early flowering, DPM = days to physiological maturity, SC = stem curvature, CS = capitulum size, NBP = number of broken plants, HI = harvest index, PR = achenes productivity and MTA = mass thousand achenes; [2] * significant at 5%; [3] CV = Coefficient of variation; [4] GA = General average.

For the early flowering, the HELIO 250 and HELIO 251 were the earliest, starting flowering to 53 days after emergence (DAE), as shown in Table 2. The genotype that flourished later was the GNZ NEON, at 63 DAE, followed SYN 045, 61 DAE. According to Castro and Farias (2005), high temperature and dry weather accelerate flowering.

With regard to the days to physiological maturity, the earliest genotypes were ADV 5504 and BRS 323, both with 92 DAE (Table 2). The AGUARÁ 06, GNZ NEON and MG 305 had longer cycle (112 DAE). In the work of Backes et al. (2008), in Southern Brazil, it was observed 105 days between emergence and maturation for the M734 genotype while in the present study that moment was reached at 97 DAE (M734), confirming the influence of environmental question in the growth stages of culture. Because it is grown in second harvest in Campo Novo do Parecis, early sunflower genotypes as ADV 5504 and BRS 323 are desirable to facilitate the adjustment of sowing time within the production system of the region.

As for stem curvature, the genotypes AGUARÁ 04, AGUARÁ 06 and HLA 2012 had the lowest average (Table 2). According to Santos et al. (2011), stem larger curvature may be influenced by the wind speed, contributing to increase bedding and break of plants. The more affected genotypes were PARAISO 20, ADV 5504, BRS G42, CF 101, GNZ NEON, HELIO 250, HELIO 251, M734, MG 305, SYN 045 and BRS 323.

Regarding the capitulum size, genotypes AGUARÁ 06 (18.3 cm) and PARAISO 20 (18.5 cm) have shown the highest average. Balbinot et al. (2009), when studying performance of sunflower genotypes in the northeast of Santa Catarina, Brazil, has reached higher averages of 15.4 (AGUARÁ 04) and 18.4 cm (M734). The capitulum size can be considered an indicator for assessing the development and productivity of plants, although extreme conditions of stress can cause low productivity of achenes.

Table 2. Mean values for plant height (PH), the date for early flowering (DEF), days to physiological maturity (DPM), stem curvature (SC), capitulum size (CS) and number of broken plants (NBP) different sunflower genotypes

Genotypes	PH (cm)	DEI	DPF	SC	CS (cm)	NBP
ADV 5504	153.6 e	56 h	92 l	4.8 a	13.7 d	11.3 a
AGUARÁ 04	170.4 c	57 g	98 f	4.0 c	15.4 c	6.3 b
AGUARÁ 06	184.6 b	60 c	112 a	3.8 c	18.3 a	1.3 c
BRS 323	157.4 e	55 i	92 l	5.5 a	14.7 c	6.5 b
BRS G42	142.1 e	56 h	95 h	5.0 a	13.3 d	13.5 a
CF 101	153.3 e	56 h	94 i	5.0 a	15.1 c	10.5 a
GNZ NEON	172.5 c	63 a	112 a	5.0 a	14.6 c	2.7 c
HELIO 250	155.5 e	53 j	93 j	5.0 a	13.7 d	7.3 b
HELIO 251	163.2 d	53 j	100 e	5.0 a	16.2 b	8.5 b
HLA 2012	185.9 b	60 c	110 c	4.0 c	15.2 c	8.5 b
M734	169.8 c	59 d	97 g	5.0 a	15.5 c	9.8 a
MG 305	165.0 d	58 f	112 a	5.0 a	15.4 c	12.0 a
MG 360	148.1 e	57 g	100 e	4.5 b	16.1 b	11.3 a
PARAISO 20	188.0 b	58 e	111 b	4.7 a	18.5 a	8.3 b
SYN 045	198.5 a	61 b	104 d	5.0 a	14.3 d	7.8 b
SYN 3950HO	175.3 c	59 d	100 e	4.3 b	16.9 b	13.0 a

Different letters differ by Scott-Knott test at 5% probability.

The lowest rates of broken plants were observed for genotype AGUARÁ 06, in average 1.3 broken plants per plot(Table 2) or 2,888 plants ha^{-1} and GNZ NEON, with 2.7 broken plants per plot (6,000 plants ha^{-1}). Genotypes with the highest averages were SYN 3950HO, MG 360, MG 305, M734, CF 101, BRS G42 and ADV 5504, with values ranging between 9.8 and 13.5, ie, 21,777 and 28,889 broken plants ha^{-1}. Higher results for M734 of 33,750 plant ha^{-1} were mentioned by Backes et al. (2008) as well as lower value for the AGUARÁ 04, with an average 10,000 broken plants ha^{-1} on tests carried out in Southern Brazil.

For the harvest index, the higher the value, the higher the mass of the trade capitulum. This parameter is considered important for the processing industry, as the achenes are needed. Thus, the genotypes MG 305, SYN 045, ADV 5504, BRS 323, CF 101, PARAISO 20 and AGUARÁ 04 showed higher harvest index (Table 3), which were 0.67; 0.67; 0.67, 0.68; 0.69; 0.69 and 0.74, respectively, reaching statistical indexes equal to each other. The lowest rate observed was 0.54 (HLA 2012), indicating that 46% of the total mass of the capitulum does not have commercial value.

In respect to the achenes productivity (Table 3), the best performance genotypes were the SYN 3950HO (2,205.5 kg ha^{-1}) and HELIO 251 (2,204.1 kg ha^{-1}), but did not differ statistically from GNZ NEON, SYN 045, CF 101, AGUARÁ 04, MG 305, AGUARÁ 06 and MG 360, who had productivity averages between 1,836.8 and 2,132.5 kg ha^{-1}. It is also important to highlight, that lower productivities were observed for HLA 2012 and BRS G42 genotypes, with an average of 40% lower than the most productive genotypes. Vogt et al. (2010) worked with sunflower crop tests sown in November in Northern Santa Catarina, Brazil and reported higher productivities for AGUARÁ 04 (1,916.0 kg ha^{-1}) and M734 (1,962.0 kg ha^{-1}) and lower average for HELIO 250 (1,450.0 kg ha^{-1}).

Table 3. Mean values for harvest index (HI), achenes productivity (PR) and mass of thousand achenes (MTA) from different sunflower genotypes

Genotypes	HI	PR (kg ha^{-1})	MTA (g)
ADV 5504	0.67 a	1,446.9 c	46.4 c
AGUARÁ 04	0.74 a	2,084.1 a	47.9 c
AGUARÁ 06	0.61 b	1,859.5 a	46.0 c
BRS 323	0.68 a	1,782.0 b	63.3 a
BRS G42	0.61 b	1,425.9 c	60.3 b
CF 101	0.69 a	2,104.4 a	50.3 c
GNZ NEON	0.62 b	2,132.5 a	51.6 c
HELIO 250	0.61 b	1,694.7 b	45.7 c
HELIO 251	0.63 b	2,204.1 a	45.5 c
HLA 2012	0.54 b	1,313.0 c	50.3 c
M734	0.58 b	1,673.7 b	68.5 a
MG 305	0.67 a	1,993.8 a	55.7 b
MG 360	0.56 b	1,836.8 a	64.3 a
PARAISO 20	0.69 a	1,685.3 b	47.9 c
SYN 045	0.67 a	2,108.5 a	59.7 b
SYN 3950HO	0.64 b	2,205.5 a	60.7 b

Different letters differ by Scott-Knott test at 5% probability.

In respect to the mass of thousand achenes, the highest averages were represented by genotypes M734, MG 360 and BRS 323, whose values ranged from 63.3 (BRS 323) and 68.6 g (M734) (Table 3). MTA is the sunflower crop main component of production along with characteristic number of seeds for each capitulum, having a direct relationship with the achenes productivity. In this study, the genotype with highest MTA (MG 360) was also the most productive when these variables (PR and MTA) were analyzed by statistical grouping to which they belong.

CONCLUSIONS

The mass of thousand achenes for the higher performance genotypes are between 63.3 and 68.5 g, represented by BRS 323, MG 360 and M734. The most productive genotypes are MG 360, AGUARÁ 06, MG 305, AGUARÁ 04, CF 101, SYN 045, GNZ NEON, HELIO 251 and SYN 3950HO, whose values are between 1,836.8 and 2,205.5 kg ha^{-1}.

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OR MASTER APP, THE UNIC SMARTPHONE APPLICATION TO FIGHT AGAINST *OROBANCHE CUMANA*

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ABSTRACT

Euralis Semences, one of the European leader sunflower seeds company in Europe, has developed an innovative smartphone application allowing the farmers to fight against Orobanche Cumana, a very dangerous parasite of the sunflower crop, affecting more than 60% of the European surfaces. Launched under the name OR MASTER App, this new application is based on a cross expertise of field observation and broomrape samples analysis. First of all we evaluated the broomrape risk level for each local administrative unit (NUTS 3) in the 8 main sunflower countries: Russia, Ukraine, Turkey, Romania, Bulgaria, Spain, France, Hungary and summarized the data collected in a risk scale (0= no risk to 4= high risk). According to the field information (crop rotation, previous variety and observation) entered by the farmer in OR MASTER app, the software calculates the risk level at farmer level by pooling the regional database and the local conditions of the farmer field. In this way, OR MASTER app suggests to the farmers a list of varieties well adapted with this risk level and helps them protecting themselves against the parasite by a fully personalized solution. The user can choose among different strategies using genetically OR resistant hybrids, Clearfield varieties, or combination of both technologies inside the same hybrids in order to get the maximum protection. In any case, the application will advise a tailor made strategy fitting with the OR pressure and with the farm needs.

Key Words : Orobanche Cumana, smartphone, Euralis Semences, Broomrape, OR MASTER, sunflower

PICTOR® – A BROAD-SPECTRUM FUNGICIDE FOR SUNFLOWER

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ABSTRACT

During the growing season, a number of fungal diseases attack sunflower plants. For the effective control of yield-devastating diseases, BASF SE developed a fungicide with a broad spectrum of efficacy, PICTOR®. PICTOR® is formulated as a suspension concentrate. It combines two highly active fungicidal substances, 200 gai/l Boscalid and 200 gai/l Dimoxystrobin with different modes of action. Boscalid is a pyridine carboxamide and inhibits the enzyme succinate dehydrogenase (SDH), also known as complex II in the mitochondrial electron transport chain (Kulka and von Schmeling 1995). Dimoxystrobin belongs to the QoI group of fungicides and inhibits the mitochondrial respiration, resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c. Since 2006, BASF is running trial series to proof efficacy on the major diseases of sunflower as well as the beneficial effects on sunflower yield. Applied preventatively PICTOR® is very effective against *Phoma macdonaldii*, *Botrytis cinerea*, *Alternaria helianthi*, *Sclerotinia sclerotiorum* and *Diaporthe helianthi*. Even in years when disease occurrence was low due to unfavorable weather conditions for the fungi, yield increases have been observed following the application of PICTOR®. The yield benefits cannot be explained by disease control only. A vitalizing effect on the crop was visible across trials. The effect can be explained amongst others factors by an inhibition of ethylene synthesis, resulting in a delayed senescence and an improved stress tolerance. PICTOR® is currently registered and sold in all sunflower growing countries, all over Europe. The recommended dose rate is up to 0,5 l/ha. During the vegetation, PICTOR® can be applied up to 2 times from 8-leaf stage to the end of flowering.

Key Words : Boscalid – Dimoxystrobin – fungal disease – fungicide – PICTOR® – sunflower

PATHOGENICITY AND MOLECULAR CHARACTERIZATION OF AN INTERNATIONAL COLLECTION OF VERTICILLIUM DAHLIAE, PATHOGEN OF SUNFLOWER

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ABSTRACT

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae*, is one of the most important diseases in the world, causing high economic losses in crops such as artichoke, olive tree, cotton, sunflower, potato, tomato, etc. Since the disease is genetically controlled, knowing the virulence and genetic diversity of the pathogen are key factors to increase the effectiveness of control measures. Both have been the objectives of this study. An international collection of seven *V. dahliae* isolates from sunflower was pathogenically characterized in an experiment under greenhouse conditions. Eight sunflower genotypes were inoculated with the fungal isolates and their virulence was determined for eight weeks. The molecular characterization of 13 isolates of *V. dahliae* from sunflower and from olive tree was carried out using molecular markers generated by five microsatellite primers. Concerning the study of virulence, significant differences of disease were found for isolates, genotypes, and most interestingly, for their interaction. Our results show that, although all the isolates caused different symptoms severity in the plants, certain genotypes of sunflower were less susceptible to the disease than others. In addition, the new race of *V. dahliae* recently reported in Spain was also identified in France and in Rumania. On the other hand, molecular differences between isolates of *V. dahliae* were related to the host (sunflower or olive tree) and, within those from sunflower, to geographical origin, as reported for other pathogens of this crop.

Key Words : disease control, genetic resistance, leaf mottle, SSR, verticillium wilt

SOCIO-ECONOMIC IMPACTS OF NEW SUNFLOWER IDEOTYPES

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ABSTRACT

The study of barriers to adoption and diffusion of innovation is highly relevant when considering the agricultural sector. Even if innovative practices are technically feasible and socially beneficial - in the sense that they improve the collective welfare by reducing the use of inputs (including water) - actors may not implement them. Moreover, these inputs seem more important when they come from Nature, due to the high intensity of the relationships between agriculture, agricultural practices, and the environment. In this context, understanding the adaptation of the various agro chains agents, from farmers to industry, facing this innovation is an important issue. The SUNRISE project *(SUNflower Resources to Improve yield Stability in a changing Environment)* is an 8 years project supported by the French National Research Agency and gathering 16 public and private French partners of sunflower sector since 2012. As part of this project, societal impacts of new sunflower ideotypes are analyzed at different relevant scales (national or European...). Main objectives of this study are (i) Microeconomic farm-focused analysis of competitiveness and acceptability of new sunflower hybrids and (ii) Mesoeconomic analysis of impacts and diffusion conditions of the innovation within industrial chains and territories. To carry this study, analyses are performed at farm level, as individual unit of adoption of new practices, and at agro-chains level, in order to identify coordination aspects which can enhance competitiveness and conditions of acceptance of new hybrids. Quantitative and qualitative methods are mobilized to meet the objectives identified: field surveys and interviews, scenario-building method with multi-criteria analysis, bioeconomic modelling and econometrics of individual data, including contracts. This study will allow to measure socio-economic impact of newly developed sunflower ideotypes and to better adapt this innovation to agricultural sector, with a view to improve environmental sustainability.

Key Words : SUNRISE, New ideotypes, Social sciences, Economic sciences

SUNFLOWER YIELD RESPONSE TO CROP DENSITY UNDER CLIMATIC UNCERTAINTY: COUPLING AN EXPERIMENTAL AND A SIMULATION APPROACH.

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ABSTRACT

Crop establishment is a critical step for optimal agronomic and economic crop performances. In sunflower, average emergence rate stands between 70-85% in France, with a tendency to decrease because of increasing bird predation. Consequently, farmers have to sow again the crop in about 8% of the situations. But obtaining a successful plant stand depends not only on the crop emergence rate but also on the initial sowing density. Currently, the practical recommendation for sowing density is between 65-70k seeds per ha (target of 50-60k plants/ha) which should be increased (70-75k seeds/ha) in case of minimum tillage, because of lower crop emergence risk. Ideally sowing density should also be adapted to expected soil water resources, pathogen pressure in the growing area, and cultivar. Our study aims to refine the current sowing recommendations from *Terres Innovia* by providing site-specific decisions based on climate, soil, and cultivar. We used field experiments (multi-environment trials) to assess yield and oil concentration response to plant density, soil type, and cultivar in French growing conditions. We then extended this dataset with numerical experiments (simulations from *SUNFLO* crop model) to evaluate how climatic uncertainty and crop management were impacting or not current sowing recommendations. Datasets were analyzed with linear and quantile regressions both globally and for subsets of the studied population of crops. We found that crop yield variance was mainly explained by farm location (soil and soil x climate interactions), with a weak average impact of plant density (<= 5%); the hierarchy of factors was similar with field or simulated dataset. When refining practical recommendations to account for available soil water content (AWC), adjustment of plant density had a greater impact on crop performance. In deep (AWC > 200 mm) and intermediate (100mm < AWC < 200mm) soils, optimal density range was around 50-60k plants/ha, with important yield loss (10-30%) below this level in deep soils and above it for intermediate soils. In shallow soils (AWC < 100 mm), sparse plant stands were more adequate, given low water available for growth. From the exploration of unexperienced climatic conditions with simulation, we concluded that sowing density recommendations should be adapted to climatic conditions, to better account for soil x climate interactions. Finally, our method coupling field and simulation experiments contributed to adapt more efficiently the crop water demand (through plant population) to available soil water ressources, hence refining the scope of technical support.

Key Words : crop density, simulation, crop model, yield, sunflower

FERTILIZATION OF SUNFLOWER, ACCORDING TO DATA FROM FOUR-CROP ROTATION LONG-TERM EXPERIMENT

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ABSTRACT

The sunflower is the most important oil crop in Serbia. It can be grown on poor soils more successfully than other crops, and usually on rich soils the effect of fertilizers is low. Optimal fertilising, beside supplying plant with required nutrients in the amounts and at the times they are most needed, also should simultaneously sustain nutrient level in soil and maximizes the economic benefits of nutrients while minimizing any environmental impact. For such multi purpose approach the most appropriate are data from long term experiment (LTE). The research was carried out on LTE, established in 1966 on chernozem soil type at Institute of filed and vegetable crops in Novi Sad. It is four crop rotation (sugar beet, corn, sunflower and wheat) trial with 20 variants of different rates and quantities of single, double and triple NPK fertilizers (F). During near 50 years each plot was fertilized with same fertilizer and now there are plots with very different fertility. This paper presents the results of three year (Y) (2013-2015) for three domestic sunflower hybrids (H) (Ns–oskar, Ns–fantazija and NS-orfej). All factors(Y, F, H) and their interaction had highly significant influence on seed yield. Partitioning in total of sum squares off all treatments for yield were the highest for year (59%), while for F and H were 23% and 1%, respectively. Partitioning for oil content were the highest for hybrids (40%), for F is 29% and the lowest only 13% for Y. In three-year average the lowest seed yield and the highest oil content were in 2014, while the highest yield and the lowest oil content had hybrid Ns-orfej. Optimal seed yield reach with triple nutrients, with amounts of 50-100 $kgha^{-1}$ of each. Oil content regularly decreases for about 1% with each increasing of nitrogen for 50 $kgha^{-1}$, considering triple nutrients combinations.

Key words: Sunflower, Fertilization, LTE, Seed yield, Oil content

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oilseed crop which ranks fourth next only to soybean, groundnut and rapeseed as a source of edible oil of premier quality in the world (Malligawad et al, 2004) and for Serbia is the most important oil crop in. Sunflower could be successfully grown in a great range of climatic conditions and soils. It could also play an important role in the cultivation of the new reclaimed lands, which are suffering drought, high temperatures and salinity effects (Keshta et al, 2008) In Serbia it can be grown on poor soils more successfully than other crops since it uses less available water and nutritients from deep soil layers. It also responds positively to residual nitrogen from previous crop (Crnobarac et al, 2002). That is the reason why the effect of fertilization on good soils for sunflower is low. Among the different nutrients required, N and P are the primary limiting nutrients under most environments where it is being cultivated. Further, it is also reported that

sunflower has high N and moderate P requirements (Malligawad et al, 2004). Nitrogen deficiency reduces the leaf production, individual leaf area and total leaf area resulting in a reduced area for light interception for photosynthesis. Also, up to 75% of leaf nitrogen is found in the chloroplasts so in conditions of the nitrogen limitation, are often lower rates of photosynthesis and consequently sunflower yield decreasing (Cechin et al, 2004). At the same time it is necessary to bear in mind that it is more sensitive to diseases, which especially develop with increasing N in nutrition. N also decreases oil content, but like in other crops it is the most important for seed yield. Beyond monitoring of input costs and reduction of pollution risks due to N excess it is also necessary to increase net income avoiding yield losses due to N excess and N deficiency, as well as improving the oil content of the seeds.(Reau et al, 2004). Phosphorus and potassium are also very important for disease and drought resistance, oil content and positive interaction which increase nitrogen effect.

METHODS

The data of sunflower seed yield and oil content are from long term experiment with a four crop rotation (sugar beet, corn, sunflower and wheat) which was established on chernozem soil type in 1966. There are 20 treatments with different rates and quantities of single, double and triple NPK fertilizers (F). The amounts of NPK are in kg per hectare of N, P_2O_5 and K_2O (Fig. 2 and 3). During near 50 years each plot was fertilized with same fertilizer and now there are plots with very different nutritient content. This paper presents the results of this practice on three new NS sunflower hybrids (H) (Ns–oskar, Ns–fantazija and NS-orfej) in the period 2013-2015 (Y). Data were analyzed by ANOVA using Mstatc method 19 (two factorial randomized complete block design combined over years) and graphically presented in Origin. For data interpretation, beside F-test and LSD- test, we used partitioning of each experimental factor in the total of sum squares off all treatments.

RESULTS

According to F-test of ANOVA all factors (Y, F, H) and their interaction had highly significant influence on seed yield and oil content. Partitioning in total of sum squares off all treatments for seed yield were the highest for year (59%), while for F and H were 23% and 1%, respectively. Partitioning for oil content were the highest for hybrids (40%), for F is 29% and the lowest only 13% was for Y (Table 1). It means that the highest influence on seed yield had year and hybrids on oil content, while influence of fertilizers for both traits were almost similar. The highest interaction for seed yield was Y*F (8%) and for oil content Y*H (10%).

On the three year average significantly the highest seed yield had hybrid NS-orfej (3.80 tha^{-1}), mainly due to relatively high yield in 2014. Between other two hybrids there were no significant differences. (Fig.1). On average for all hybrids in 2014 was extremely low seed yield caused by diseases. Differences in oil content between all hybrids were significant and the highest had NS-oskar and the lower NS-orfej. This relation was similar in almost all year.

On average for three years and hybrids, nitrogen alone or in combination with P and K significantly increased seed yield, but it simultaneously decreased oil content related to control, alone P and K and double PK treatments. (Colons in Fig. 2 and 3). P and K alone or in combination had significantly higher seed yield and lower oil content only in regard to control. The effect of NK treatment was slightly better than NP for both traits.

Triple NPK combination with the lowest amount of 50 kg ha^{-1} of each nutrient had significantly higher oil content than treatments with nitrogen alone or in combination with P

and K, but there is no significant differences between them in seed yield. It is proof that balanced triple combination of NPK is better then any single or double combinations.

Table 1. Probability of F –test and partitioning of experimental factor in the total treatments of sum off squares

Source of variation	Seed yield		Oil content	
	F probabability.	Partitioning	F probabobability	Partitioning
Year	<,001	59%	<,001	13%
Fertilisers	<,001	23%	<,001	29%
Year * Fertilisers	<,001	8%	<,001	4%
Hybrids	<,001	1%	<,001	40%
Year * Hybrids	<,001	2%	<,001	10%
Fertilisers * Hybrids	<,001	2%	<,001	2%
Year * Fertilisers * Hybrids	<,001	4%	<,001	3%

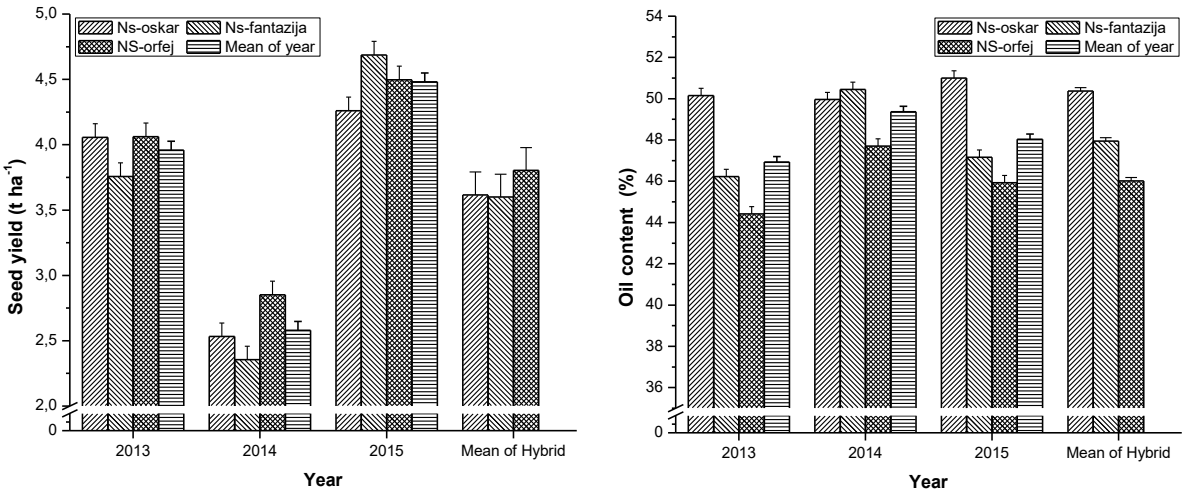

Figure 1. Seed yield and oil content, average values per year, hybrids and their interaction

Among triple NPK combinations the lowest combination with 50 kg ha^{-1} of each nutrient had significantly the highest oil content but also the lowest seed yield. Next three triple combination had similar oil content which were significantly higher then all other triple treatments. Also, after the same triple combinations, in all other triple treatments there were no significantly higher seed yields. Regarding seed yield and oil content simultaneously in average for three year and three sunflower hybrids in our environmental condition the best is balanced nutrition, with triple NPK combinations in amount between 50-100 kg ha^{-1} of each nutrient.

Considering triple nutrients combinations in average for three hybrids, oil content regularly decreases for about 1% with each increasing of nitrogen for 50 $kgha^{-1}$ (Fig. 3)

Differences in oil content between hybrids were similar in all fertilizers treatments, the highest had NS-oskar and the lowest NS-orfej (Fig 3). Contrary, hybrid NS-orfej had especially higher seed yield in treatments without N and in triple combination with highest amount of 100-150 kg ha-1 of each nutrient (Fig. 2).

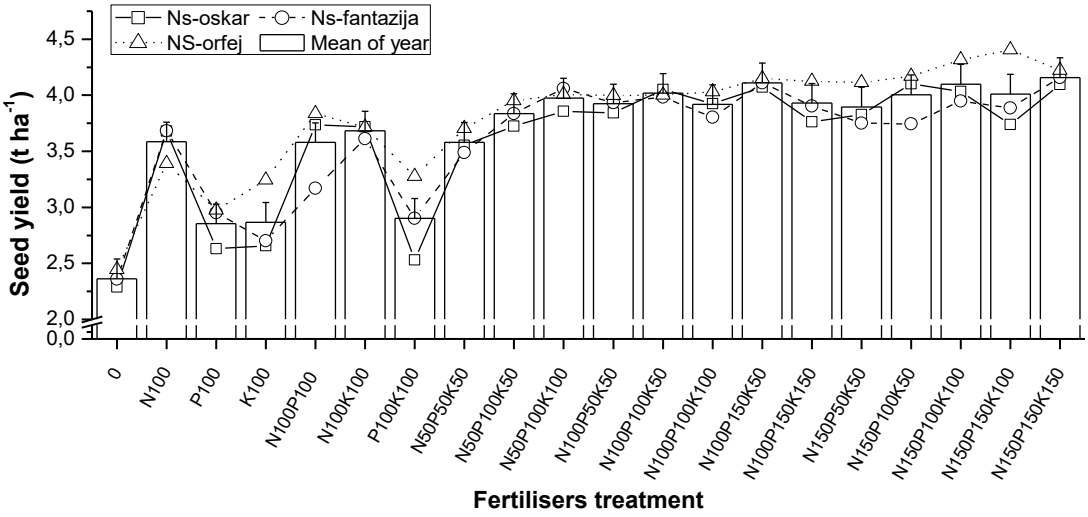

Figure 2. The effect of NPK fertilizers and hybrids on sunflower seed yield (average of 3 year)

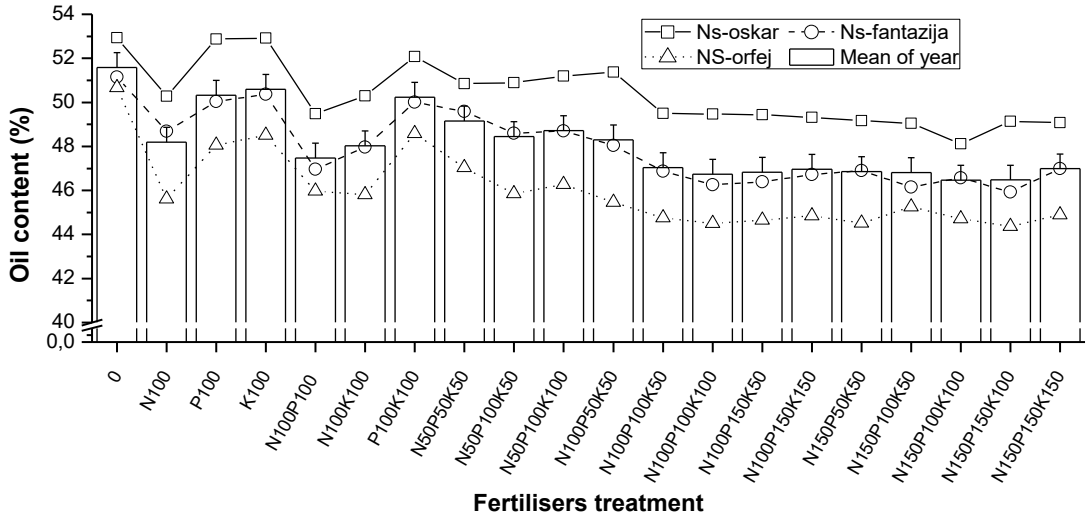

Figure 3. The effect of NPK fertilizers and hybrids on sunflower oil content (average of 3 year)

CONCLUSION

Based on obtained results it can be concluded that regarding seed yield and oil content simultaneously in environmental condition of Serbia the best is balanced nutrition, with triple NPK combinations in amount between 50-100 kg ha-1 of each nutrient.

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RELATIONSHIP BETWEEN SEED YIELD AND SOME QUALITATIVE TRAITS *OF* SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER DIFFERENT IRRIGATION REGIMES AND FERTILIZER TREATMENTS

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ABSTRACT

In order to investigate the relationships between yield and qualitative traits by correlation of sunflower in response to biofertilizers under different irrigation regimes. This study was arranged in split plot laid out in RCB design with three replications at the Kermanshah province, Iran, during 2006-2007. Three levels of irrigation regimes (irrigation at 85% field capacity, irrigation at 70% field capacity, and irrigation at 55% field capacity) set as main plot and six levels of fertilization (control, recommended N, *Azotobacter* & *Azospirillum* (AA), AA+100% recommended N , AA+75% recommended N, and AA+50% recommended N) set as subplot. The results showed that there were positive and significant correlations among seed yield and oil content and protein content. There were negative and significant correlation among seed yield and saturated fatty acids (palmitic acid and stearic acid); whereas there were positive and significant correlation among seed yield and unsaturated fatty acids (oleic acid and linolenic acid). There were negative correlation among seed yield and osmolytes content (proline and soluble carbohydrates).

Keywords: Biofertilizers, Correlation, Irrigation, Qualitative Trait, Seed Yield, Sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil-producing plants in the world because its oil exhibits a fatty acid composition that is favorable for human consumption (Jalilian *et al.,* 2012). Oil quality and yield are both dependent upon the genotype of the plant and its interaction with the environment. Irrigation (Blum, 1997) and fertilizer (Reddy *et al.,* 2003) among the various factors responsible for increasing the yield and quality of crops, are the most important. Environmental stresses restricted the growth and development, of plants. Water deficit stress is one of the most serious stresses that affect crop productivity (Boyer, 1982). The absorption of nitrogen fertilizer is heavily impacted by water deficit. Nitrogen (N) is an essential mineral nutrient for plants, which increases total biomass production and yield. In the context of plant nutrition, nitrogen deficiency is the most important limit to sunflower production, as is true for most other crops (Blamey *et al.,* 1997). Biofertilizers are products that contain living cells of different types of microorganisms, which stimulate plant growth (Vessey, 2003). Because of the high N requirement of sunflowers, application of biofertilizer can decrease potential groundwater pollution, facilitating the adoption of eco-friendly agronomic techniques (Jalilian *et al.,* 2012). Plant breeders commonly prefer yield components that indirectly increase yield. Indirect selection of yield components such as 1000-seed weight, plant height, and head diameter can increase grain yield. Therefore, it is important to know the relationships among yield traits in sunflower to get higher yields. Although relationships between seed yield and other traits were investigated in many studies using correlation, path analysis, combining ability, and similar statistical techniques (Kaya and Atakifli, 2003; Dusanic *et al.,* 2004; Hladni *et al.,*

2006), no research using correlation analysis for relationships between seed yield and quality traits in sunflower.

Due to the global economic importance of the sunflower and the limited knowledge available regarding the effects of combining biofertilizer and different levels of N fertilizer on sunflower characteristics under water stress conditions, this research was performed to determine the relationship between seed yield and some quality traits of sunflower under water deficit condition.

MATERIALS AND METHODS

Field experiments were conducted at the Eslam Abad-Gharb Agricultural Research Station (34° 8′ N and 46° 26′ E) with an altitude of 1346 m, Kermanshah province, Iran during the 2006 and 2007 growing seasons (May to September). The clay loam soil texture was determined. The field capacity and permanent wilting point, were 34 and 16.2%, respectively. The fertility status of the soil was classified as low in available and high in available and K. Nitrogen was applied as urea. Half the dose of recommended N (124 kg ha^{-1}) was applied at sowing time, and the remaining N was topdressed at the 5-6 leaf stage.

Three strains of bacteria, including Azospirillum lipoferum, Azospirillum brasilense, and Azotobacter chroccoccum were used in this study for seed inoculation of sunflowers. Nature BioTechnology Co. (NBICO), Iran, supplied these bacteria. Seed inoculation involved placement of the seeds in bacterial suspensions at 109 CFU ml-1 for 30 min before planting (Ozturk et al., 2003).

The experimental design was a randomized complete block (RCB) design with a split-plot arrangement of treatments in three replicates. The treatments were three levels of irrigation regimes [irrigation at 85% field capacity (I1), irrigation at 70% field capacity (I2) and irrigation at 55% field capacity (I3) in the main plot] and six types of fertilization [control (C), nitrogen recommended (N), Azotobacter and Azospirillum (AA), AA+100%N recommended (AA100), AA+75%N recommended (AA75) and AA+50% N recommended (AA50) in subplots].

Inoculated seeds were hand-sown on 4m×2.6 m plots (5 rows) on 12 May 2006 and 13 May 2007, respectively. Seeds of the sunflower (H. annuus L., cv. Azargol) were sown at 3 cm depth in the middle of rows, with 0.6 m between rows and 0.30 m between seed groups. This yielded a population density of about 5.7 plants m-2. Before planting, the soil surface of the cultivated area was thoroughly irrigated using a solid-set movable sprinkler system. At the 3-4 leaf stage, plants were selected for uniformity and thinned out to the recommended plant density. Weed control was performed manually without any chemical additive. All plants were irrigated equally by sprinkler, until the 8-leaf growth stage (V8) (Aliary *et al.*, 2000). A sample comprising 50 g clean seeds from each plot was isolated for measuring oil and protein content. Oil and protein content were determined using an Inframatic 8620 near-infrared spectrometer (Ludwiga et al., 2006). The fatty acid composition of the sunflower seed oils was determined according to Metcalf et al., (1966) by using a UNICAM 4600 gas chromatograph. Levels of palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids were determined using a computing integrator. Statistical analysis was carried out using the SAS software package (SAS Institute, 1997) and MSTAT-C. Differences among the treatments were evaluated with the least significant difference (LSD) method. $P<0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

Among the sunflower traits seed yield, oil content, protein and fatty acids are particularly important. Therefore, the correlation of these traits with other traits studied. In correlation results, sunflower seed yield had negative and significant relationship with saturated fatty acids (palmitic and stearic acid) and osmolytes (proline and soluble carbohydrates). Also the results showed that seed yield had significant positive correlation with oil content and protein, unsaturated fatty acids (oleic and linoleic acid), quantum yield of photosynthesis (Fv/Fm) and chlorophyll a and b (Table 1.).

Palmitic and stearic acids has negative correlation with oil content (Table 1). Among the unsaturated fatty acids (oleic and linoleic acid) only oleic acid was positively correlated with protein content (Table 1.). Proline and soluble carbohydrates correlation with sunflower oil content was negative and significant. Proline was a significant negative correlation with seed yield in sunflower. In fact, sunflower plants in response to drought stress increased proline and soluble carbohydrates in the leaves, which was consistent with the reduced yield. In other words sunflower plants in such condition to deal with stress through yield reduction to cost a lot of that with the results correspond (Fredeen *et al.*, 1991).

Table1. Sunflower seed yield and seed quality correlation affected by different irrigation regimes and fertilizer treatments

	1	2	3	4	5	6	7	8	9	10	11	12
1-seed yield	1											
2-oil content	0.6**	1										
3-protein content	0.39**	-0.16	1									
4-Palmetic acid	-0.74**	-0.82**	0.19	1								
5-stearic acid	-0.79**	-0.78	-0.11	0.84**	1							
6-Oleic acid	0.92**	0.59**	0.3*	-0.77**	-0.74**	1						
7-Linoleic acid	0.85**.	0.77**	0.1	-0.88**	-0.84**	0.82**	1					
8-Fv/Fm	0.83**	0.65**	-0.06	-0.86**	-0.73**	0.85**	0.87**	1				
9-Proline	-0.3*	-0.53**	0.69*	0.71**	0.36**	-0.37**	-0.48**	-0.69**	1			
10-Chl a	0.84**	0.7**	0.1	-0.89**	-0.81**	0.84**	0.9**	0.83**	-0.51**	1		
11-Chl b	0.77**	*0.69***	0.06	-0.81**	-0.76**	0.8**	0.84**	0.8**	-0.5**	0.82**	1	
12-Soluble Carbohydrate	-0.16	*-0.33*	0.67**	0.5**	0.17	-0.20	-0.3*	-0.54**	0.92**	-0.32*	-0.33*	1

*and **, Significant at 0.05 and 0.01 probability level, respectively.

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LONG TERM CHANGES IN GERMINATION AND VIGOUR OF SUNFLOWER HYBRID SEEDS HARVESTED AFTER CHEMICAL DESICCATION WITH PARAQUAT

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ABSTRACT

Anticipated harvest reduces the risk of losses caused by exposure to weather, pathogens and birds in sunflower seed production. Chemical desiccation with paraquat could affect physiological quality of hybrid seed during a long-term storage. This study was aimed to analyze the effect of chemical desiccation on the germination and vigour of low and high-oleic sunflower hybrid seeds, during long-term storage. Tree female parental lines where desiccated at 30% seed moisture content by means of: (i) spraying with paraquat (2 L ha-1) or (ii) mechanical cut. Control plants remained in the field until seeds reached 10% moisture content. After dried, seeds of the three treatments were stored under room (25 °C, 30 – 80 % RH) or cold chamber (10 °C, 60 % RH) conditions during 1, 5, 9, 13 and 19 months. Germination (pre-chilling, pericarp + seed coat excision), vigour (electrical conductivity without pericarp), oil and oleic acid content (by NMR) were analysed. Chemical desiccation advanced the harvest condition by one month. The seeds of low oleic hybrids showed high levels of germination (>85%) and vigour (<70 mS.m-1.g-1) across all the storage period, discarding desiccant toxic effects. The high-oleic hybrid had low germination (< 85%) and vigour (85-110mS.m-1.g-1) during all storage period. Cold chamber kept higher levels of vigour during 5-13 months, mainly in chemically desiccated seeds. Changes in seed quality were not associated with oil content or composition. Plant chemical desiccation with paraquat at 30% seed moisture did not affect the sunflower hybrid seed quality during 19 months storage.

Key Words : seed storage; oleic; electrical conductivity; physiological quality

VARIABILITY OF THE LIFE CYCLE ASSESSMENT RESULTS OF SUNFLOWER ACCORDING TO DIFFERENT AGRICULTURAL PRACTICES

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ABSTRACT

Animals' feeding contribute very significantly to the overall environmental impact of animal products (meat, milk). The French project ECOALIM aims to improve the environmental impacts of husbandries by optimizing their feed. This project defines the environmental impacts of the production of raw materials for animal feeding and optimizes the formulation of feed compounds with environmental constraints in order to improve environmental footprint of animal products. The project covers different farming systems and production areas in France and is based on Life Cycle Assessment (LCA). The ECOALIM database provides life cycle inventories and environmental impacts of feed ingredients utilized in France. It contains 150 values of which 58 national average feed ingredients (cereals, oilseeds crops, pulses and processed products like meals, etc), 27 variants (crops with improved cultural practices) and 10 foreign feed ingredients. The results obtained for French sunflower crops are discussed here. Several results were calculated: national average data representative of the country, and results with different crop managements based on case studies. The LCA methodology was applied, considering environmental burdens at the rotation system scale. Focus was made on four impact indicators (Energy demand, GHG emissions, Acidification, Eutrophication), while identifying the most contributing steps during crops life cycle. The main contributor to selected environmental impacts were field emissions. The assessment of practices such as organic fertilization, the introduction of intermediate crops or the introduction of legumes in the rotation showed some improvements on environmental impacts.

Key Words : LCA, environmental impact, sunflower, emission models, agricultural practices

STUDIES OF SOME HYBRID SUNFLOWER(HELIANTHUS ANNUUS L.) CULTIVARS FOR THEIR YIELD AND YIELD COMPONENTS IN THRACE AREA

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ABSTRACT

This research was carried out to determine yield and yield components for 6 different sunflower varieties (Tunca, NK Califa, P4223, DKF2525, C70165 and Sanbro) in Kırklareli (Lüleburgaz) ve Tekirdağ (Malkara) ecological conditions during years of 2008- 2009. In this research, characteristics such as plant height, stem circumference, head diameter, thousand grain weight and yield per hectare were investigated. The highest grain yields were obtained from variety Tunca (237.2 kg / da) and DKF2525 (224.7 kg / da) by an average of two years of research.

Key Words : sunflower, plant height, thousand grain weight, seed yield

TOWARDS DEVELOPMENT OF SUNFLOWER IN WEST AFRICA: BURKINA FASO AND MALI

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ABSTRACT

For several years, with the support of the French sector of vegetable oils and proteins, a development project on sunflower crop has emerged in West Africa, supported by FAO This program was born of the desire shared Burkinabe farmers, members of the local Unions farmers and French farmers, members of the Federation of Oilseed Producers (FOP) to test the culture, set up the technics, evaluated the potential and develop a sunflower industry (oil and cake). The emergence of such industries is indeed a strategic lever of agricultural and economic development for countries heavily dependent on imports of oil and strong deficit in plant proteins. The program aims to increase domestic oil supply and oil cakes, diversify income sources of family farming and improve the performance and sustainability of farming systems. Technical expertise benefits of support of Terres Inovia and project coordination is ensured by Agropol. After several years of experimentation, it appears possible to realize an analysis of the strengths and weaknesses of the project but also opportunities and risks.

• Strength : An important agricultural development potential for this crop and strong motivation of the actors (professional, institutional organizations and also the private sector).

• Weaknesses: Technical practices are not enough mastered by producers and lead to little motivating incomes; soils are compacted (lake of equipment's, most of the soil preparation is done by hand) and the root system (pivot) is not deep enough. Random climatology like water stress, lack of sustainable support structures for the production and marketing; the strong dependence of seed imported (hybrids) at a high cost; the lack of a sustainable mechanism for pre-financing of the crop year.

• Opportunities: a high demand for edible oil (mainly due to the quality, versus cotton oil or imported oils) and cake for animal feed; a strong national political will.

• Threats: unfair competition from uncontrolled imports; soil fertility problems and an emerging parasitic context (Alternaria helianthii).

Key words : Sunflower, limiting factors, Africa

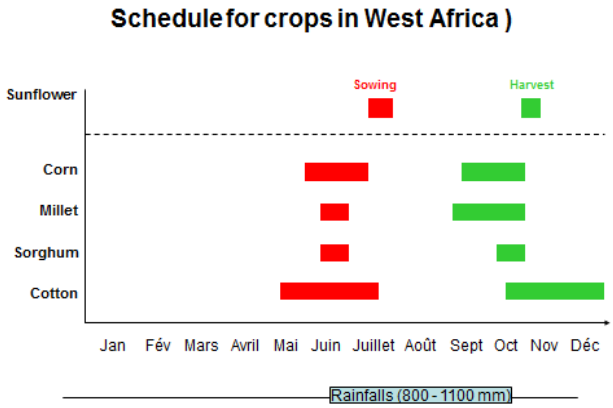
INTRODUCTION

In West Africa, most of the oil came from cotton (local production). Nevertheless, the quality of this oil is bad. According to the climatic windows during the rainy season, we identified some opportunity to grow sunflower during the rainy season. The interest of this crop is not only for the oil (sunflower oils is available on the market, coming from South Africa or from Turkey). The meal is also very well appreciated to feeds cattle's, especially during the dry season.

The sowing date needs to be adjusted as soon as the first rains are well established. This for at least two reasons:

- The soil needs to be wet enough to be plough (by hand or animal traction)
- To prevent from water stress at germination stage

The graph bellows show the best opportunity for the cycle of sunflower. Due to the high level of temperature and the heat units, the growing cycle take place is less than 100 days. The crops yield are subjected to a strong variation mainly due to the water stress, to the poor quality of the soils and a lots of problems with the root growth due to a bad soil preparation and a high level of compaction.



A progress is nevertheless obtained, and the yield ranks from 0.5 t/ha to 2t/ha, improving each year. The economic threshold is around 1 t/ha.

	2011	2012	2013	2014	2015
Average Yield (kg/ha)	267	489	545	450	620
% of farmers that succeeded with more than 1000 kg/ha	3,1	6,1	8,9	8.1	10.2
Average yield > 1000 kg/ha	1267	1222	1305	1150	1300
Records yield (kg/ha)	1884	1860	1904	1900	1920

Following the project for 5 years now, a SWOT analysis could be done.

1. **Strength :**
   - An opportunity of diversification sought by farmers and compared to cotton (crops – often GMO, that required a lot of labor, still pesticide interventions, and delay in the payment ...).

- A crop adapted to the work schedules, especially with the food crops (corn, millet ...)
- An crop “simple”, compare to cotton
- An oil meeting more and more a consumer audience aware of his qualities.
- New opportunities for honey
- The ability to have access to meal for animal feed, the capital in the areas of short rainy season.
- Small mechanization projects (seed driller, harvest)
- A new crop promoted today by the authorities and a well-structured project
- News interests for some breeders (local or international)

2. Weaknesses :

- Yields leveled are too often around 1 t / ha, sometimes less, which does not match the viable economic threshold for crop. The establishment of the crop is often there, but the end of the growing cycle and takes place under high water stress. Seed abortions on capitulates are detected and seed filling defects.
- Logistics for routing inputs remains a critical issue in the absence of reliable local circuits. This needs to be improve (seeds distribution, nitrogen availability, seed collected at harvest)
- It now offers an alternative to genetic seed: continue work on improving populations of Peredovik and / or development of local production of hybrids.
- Soil quality and soil tillage undoubtedly remains one of the major obstacles
- Although culture is considered less restrictive than cotton, some improvements in mechanization are expected by producers
- A sunflower fertilizer at the same price as the cotton fertilizer (that includes subsidies) would undoubtedly an important leverage effect on the development of culture and profitability.
- Structuring the project in islands of production must remain the rule (this could also be classified as "assets" since today is the case .....)
- The industrial tool is undoubtedly an obstacle to date to the success of the project :
- Local initiatives should be well identified and keep under control.

3. Opportunities :

- There is a real desire to diversify among both authorities and producers: the main target is undoubtedly growing cotton (working time, soil depletion, GMO and pesticide use). This is undeniably a "window" favorable for growing sunflower in the heading of diversification opportunities.
- The project is emphasized by the local authorities (Ministry of Agriculture, Research / INERA, ...).
- The project led by AGROPOL for 4 years is now recognized as credible and locally, the work on the project is appreciated by all actors.

- The evidence of the potential of crop in the context of Burkina Faso and Mali, despite the identification of limiting factors well identified, is now established : 1.5 t/ha could be the average goal.
- Since the beginning of this project, there is a mobilization and support of all stakeholders of the French oilseed around this project.

4. Threats :

- The disease risk is not excluded : Alternaria Helianthii, bacterial disorder.
- Potential for lines versus hybrids (due to the cost of hybrids seeds, the returns could be a weak point).
- Up to day, bird damages remains fairly anecdotal (at sowing time as at harvest)
- The curvature of the flower head, triggered by an acceleration of the development phase (appearance of the flower head and flowering) when growth is not yet completed can induce a strong curvature of the stem under the head that could affect translocations during seed filling.

CONCLUSIONS

The potential for sunflower crops during rainy season is real in West Africa. The uses of the oil and of the meal are very well adopted by the local consumers. For sunflower oil, the price of local production could easily compete with the price of imported oil. The valorization of meal is also very good. The crushing stage needs to be securing (size and efficiency of the equipment, seed collections from the field farmers ...). An improvement in the genetic material is also expected, mainly from lines through a local selection program to produced material adapted to the local conditions.

MICROMYCETES ASSOCIATED WITH SUNFLOWER SEEDS DURING STORAGE PERIOD

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ABSTRACT

Sunflower seeds can become contaminated with fungi which attack the plants at different stages of development and subsequently during harvesting and storage. An important role for the production of healthy crops is being played by quality seeds. Although the initial seed quality and storage environment are mandatory to prolong the shelf-life of seeds, the invasion of fungal pathogens causes various abnormalities like damaged seeds, undersized, rotted seeds and reduced in germinability. Fungal organisms play a significant role in infection, altering quality and longevity of seeds during the storage. The sunflower seeds losses recorded during storage period on worldwide scale according to FAO estimations are 10-15% of total production. In developing countries, due to reduced possibilities of implementing appropriate technologies, the reported damages during storage period may increase up to 30%. The paper work presents a study regarding the occurrence and development of specific storage micromycetes of sunflower achenes, collected from a deposit in Prahova County, Romania. Analysis performed on four sunflower hybrids' seeds, regarding the fungal load, revealed the occurrence of several pathogenic and saprophytic fungi belonging to the following genera: *Aspegillus, Fusarium, Alternaria, Stemphylium, Trichoderma, Cladosporium, Rhizopus*, *Mucor*, *Pencillium*, and *Sclerotinia*. All studied hybrids were contaminated with species of *Alternaria* and *Stemphylium,* the highest incidence noticed was for NK Adagio hybrid with 56 % of *Alternaria* spp. Placing on the market safe food and feed products is first and foremost a question of good management practices at each stage of the feed and food chain from primary production to final.

Key words: sunflower, micoflora, *Alternaria* spp., stored products

INTRODUCTION

Sunflower (*Helianthus annuus*) is one of the most cultivated oilseed crops worldwide. In 2015, Romania ranked first in the European Union both in terms of area and sunflower production, with a total harvest of 1.75 million tones obtained from one million hectares (www.paginadeagricultura.ro/). Compared to 2014, sunflower production decreased in 2015 by 19.7% due to the yield per hectare which fell by 19.6%.

Storage of sunflower seeds may last for various periods of time, when this product is kept for subsequent planting, oil processing or use in confectionery industry. A series of environmental factors, when improperly managed, could make sunflower seed storage questionable, particularly when long-term keeping is foreseen. Among the microbes that

interact with the seeds during their storage, fungi play a dominant role in decreasing quality and longevity of the seeds (Mardare et al., 2015, Cristea et al., 2004, Cristea et al., 2009, Mardare, 2014) Fungi cause various abnormalities like damaged seeds, undersized, rotted seeds and reduced in germinability. Fungal organisms play a significant role in infection, altering quality and longevity of seeds during the storage. In order to develop the list of storage fungi of sunflower, sunflower seeds were screened to study the incidence of fungi which gave the occurrence of *Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme, Penicillium citrinum, Macrophomina phaseolina* and *Rhizopus nigricans* with sunflower seeds (Afzal R., et al., 2010). Same types of fungi including species of *Cladosporium* and *Drechslera* have been reported from sunflower seeds. (Kakde R. B. et al., 2012). *Absidia corymbifera, Alternaria alternata, Aspergillus flavus, A. niger, A. terreus, Chaetomium bostrychodes, C. globosum, Emericella nidulans, Fusarium pallidoroseum, F. solani, Macrophomina phaseolina, Penicillium spp., Rhizoctonia solani* and *Rhizopus stolonifer* were predominantly isolated from sunflower (Nahar, S. et al., 2005).

Sunflower seed storage needs particular care, due to its high fat content, easily cracking shell, exposing the kernel to various alterations, such as germination loss, colonization of fungi and attacks by insects and mites. Seed moisture content is critical mainly if long-term storage is intended. Proper management of equilibrium between relative air humidity and seed moisture is an essential element, dictating need for seed drying at storage start and frequent aeration during storage (Beratlief, 1997). During the stockpile, a series of fungi appear on the seeds' surface, which generally not produce damages to the crop, whereas at the moment of tilling, they disappear due to the inauspicious soil conditions. However, certain situations have been encountered when the storage funguses have had a major influence while keeping the seeds in deposits. It can ultimately get to the germination diminution, so much that it can drop under the limit allowed by the law (specifically for seeding), and, in this case, the lot is entirely compromised and the loss is total. Also, the storage fungi negatively influenced seeds in process of field germination, leading to weakling plants, more susceptible to diseases (Zala et al., 2010).

MATERIALS AND METHODS

The research aimed to identify the spectrum of pathogens present on sunflower achenes, in order to determine the yield's health status after being harvest and recommend an effective treatment.

The biological material consisted in samples of sunflower seeds, naturally infected with pathogens and collected from a storage unit Prahova County, in South-Eastern part of Romania. The achenes belonged to four sunflower cultivars, two Romanian hybrids (Performer and Favorit) and two international ones (NK Adagio and NK Meldimi), adapted to growing conditions in this part of the country.

In order to study the micoflora, sunflower contaminating fungi were isolated using Ulster method (Hulea A., 1969) and identified with optical microscope, after 12 days of growth. Using the Ulster method there can be identified the majority of seed pathogens, regardless the species and type of seed. In separate Petri dishes of 10 mm diameter, were placed 15 wheat seeds on growth solid media (water-agar, 20 g/l, autoclaved 20' at 121 ºC), with space between the achenes in order to allow the development of fungi or bacteria. The water agar media was preferred due to its low nutrients composition which allows the fungi growth, but not its abundant sporulation. This is an important step in order to be able to isolate each fungus from the Petri dish multitude of pathogens. The dishes were kept at room temperature (22-24 ºC) and 12h/12h light conditions. After 7 days there were performed

macroscopic observations regarding the mycelia growth in Petri dishes, followed by optical microscope determinations, using a Zeiss Primo Star microscope. For further isolation and purification of each fungus was used the Potato-Dextrose-Agar medium (Hulea A., 1969). The determination of sunflower seeds' germination was performed on filter paper.

Figure 1. Sunflower seeds inoculated on water-agar medium, incubated for 7 days

RESULTS AND DISCUSSIONS

Microscopic examinations revealed a spectrum of fungi belonging to *Ascomycetes* and *Deuteromycetes* classes.(Gheorghies et al, 2001, Kieffer et al., 2000, Varga et al., 2009).

The data in table 1 presents the pathogen species detected on sunflower achenes belonging to *Alternaria* spp., *Penicillium* spp., *Stemphylium* spp., *Rhizopus* spp., *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Sclerotinia* spp., *Trichoderma* spp., and *Cladosporium* spp.

Table 1. Micoflora detected on sunflower achenes

The pathogenic agent	***Hybrid***			
	PERFORMER	FAVORIT	NK ADAGIO	NK MELDIMI
Alternaria spp.	+	+	+	+
Stemphylium spp.	+	+	+	+
Penicillium spp.	+	+	-	+
Rhizopus spp.	+	+	+	+
Aspergillus spp.	+	+	+	-
Fusarium spp.	-	-	-	+
Sclerotinia sclerotiorum	-	+	-	-
Mucor spp.	+	+	+	+
Trichoderma spp.	-	+	-	-
Cladosporium spp.	+	+	+	+

From our studies, it was found that pathogens *Alternaria* spp., *Stemphylium* spp., *Rhizopus* spp., *Mucor* spp. and *Cladosporium* spp. have populated the achenes of all studied sunflower hybrids. The fungi belonging to *Penicillium* were present on the following achenes hybrids: Performer, Favorit, NK Meldimi. Species of *Fusarium* were isolated from NK Meldimi hybrid achenes. *Aspergillus* spp. has populated seeds from Performer, Favorit and NK Adagio cultivars. Species of *Trichoderma* and *Sclerotinia* were observed on achenes from Favorit hybrid.

In figures 2 - 7 are presented microscopical observations captured with the Zeiss Primo Star microscope.

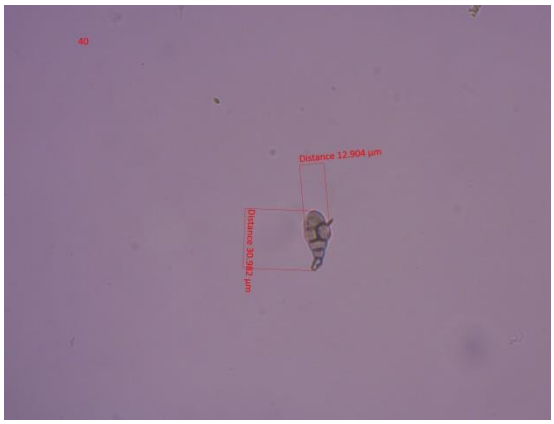

Figure 2. *Alternaria* sp. conidia

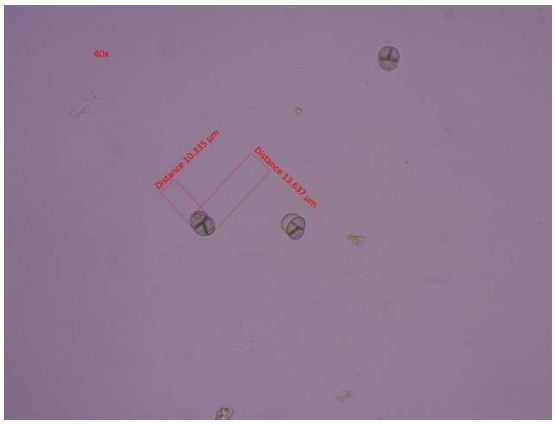

Figure 3. *Stemphylium* sp. conidia

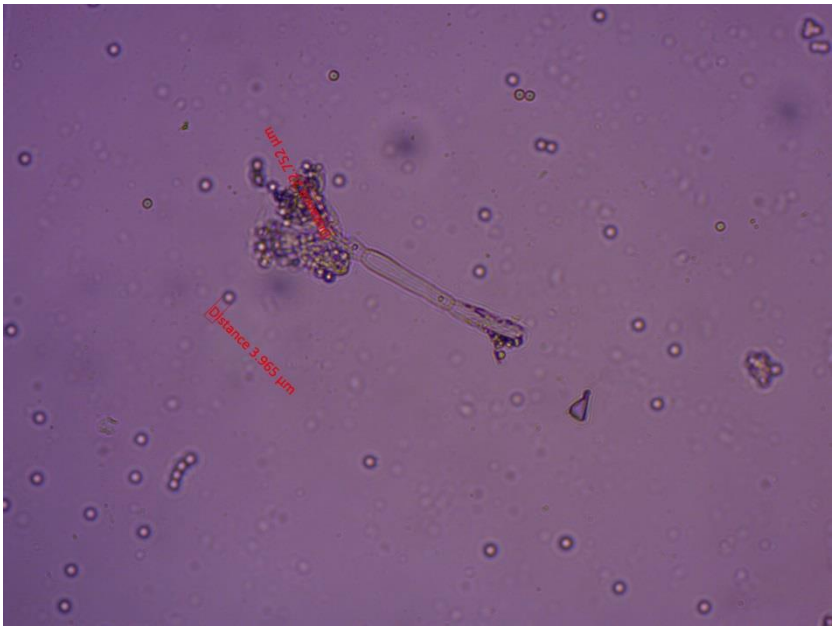

Figure 4. *Penicillium* sp. conidiophore and conidia

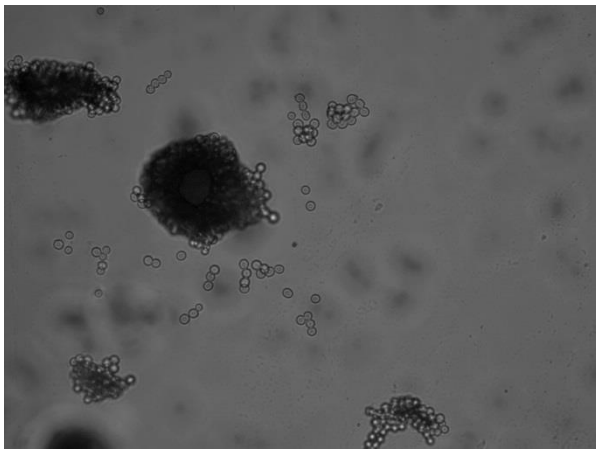

Figure 5. *Aspergillus* sp. conidias

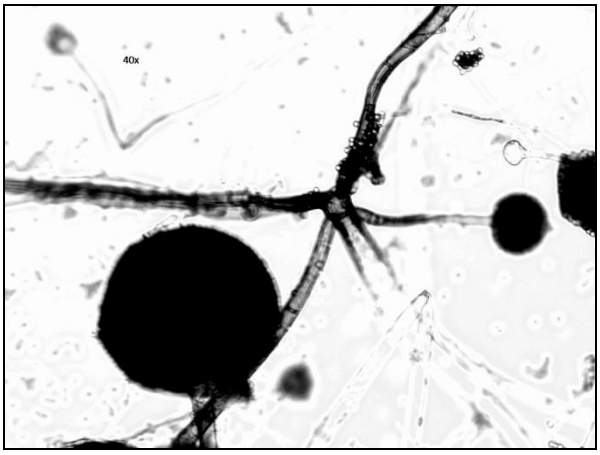

Figure 6. *Rhizopus* sp. sporangium

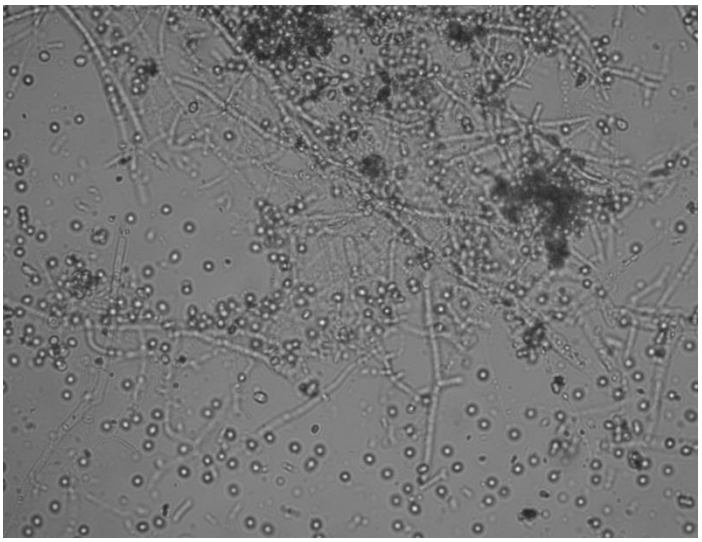

Figure 7. *Trichoderma* sp. fructifications

The incidence' rate of detected micoflora is presented in table 2. *Alternaria* spp. were present on sunflower achenes from all four hybrids, with highest values of frequency from all identified pathogens, respectively 56% for NK Adagio, 47% for NK Meldimi, 35% for Performer and 31% for Favorit hybrid.

The pathogens from *Stemphylium* spp. presented the highest values of incidence for Performer hybrid with 33% and Favorit hybrid with 30%. The other two hybrids presented 19% incidence for NK Adagio and 18% incidence for NK Meldimi.

Penicillium spp. were present in highest rate on sunflower achenes from NK Meldimi hybrid in 14%, 10% for Favorit hybrid and only 7% for Performer hybrid.

Species from *Mucor* and *Rhizopus* were detected on sunflower achenes belonging to all four hybrids, with relatively close incidence values. *Mucor* spp. was present in 3% on Performer and Favorit hybrids achenes, in 5% NK Adagio achenes and 6% the highest value for Favorit hybrid. *Rhizopus* spp. had the highest incidence value for Favorit hybrid, followed by Perfomer hybrid with 8% incidence, NK Adagio with 7% incidence and NK Meldimi with 4% incidence.

Fusarium sp. was detected with a low incidence of 5% only for NK Meldimi hybrid; Also *Trichoderma* sp. and *Sclerotina sclerotiorum* were present in 3% respectively 2% on sunflower achenes from Favorit hybrid.

Cladosporium spp. were detected with low incidence values on all sunflower hybrids achenes.

Table. 2 The micoflora's incidence detected on sunflower achenes

Fungi ***(****observations after 9 days****)***	***Pathogens' incidence on hybrid (%)***			
	PERFORMER	FAVORIT	NK ADAGIO	NK MELDIMI
Alternaria spp.	35	31	56	47
Stemphylium spp.	33	30	19	18
Penicillium spp.	7	10	0	14
Rhizopus spp.	8	10	7	4
Aspergillus spp.	3	2	3	0
Fusarium spp.	0	0	0	5
Sclerotinia sclerotiorum	0	2	0	0
Mucor spp.	3	6	5	3
Trichoderma spp.	0	3	0	0
Cladosporium spp.	4	3	3	4
Other pathogens	7	3	7	5

Table 3. Micoflora's influence on sunflower germination

Hybrid	**Germination (%)**	
	After 4 days	After 7 days
Performer	80	90
Favorit	85	95
NK Adagio	85	100
NK Meldimi	80	95

From our experiments, as it is shown in table 3, it was observed that after 7 days sunflower seeds germination was not affected for NK Adagio hybrid also was 95% for Favorit and NK Meldimi. The lowest seed germination rate was recorded for Performer hybrid seeds, respectively 90%.

CONCLUSIONS

The micoflora detected on the sunflower achenes was numerous, with pathogens belonging to *Alternaria*, *Penicillium*, *Stemphylium*, *Rhizopus*, *Aspergillus*, *Fusarium*, *Mucor*, *Sclerotinia*, *Trichoderma* and *Cladosporium* genera.

Alternaria spp. and *Stehphylium* spp. were the pathogens with the highest incidence rate on all analyzed hybrids.

Pathogen association did not affect the germination rate of seeds from the analyzed hybrids, except for Performer hybrid that presented the lowest germination rate of 90%.

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PROJECTION OF SUNFLOWER AND SUNFLOWER OIL PRODUCTION AND FOREIGN TRADE

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ABSTRACT

Turkey has a big potential for sunflower seed production, considering climate and soil charectristics. Although sunflower seed is the first in the sowing area and production of oilseeds in Turkey, its production is not enough to meet the need of sunflower oil. The production gap in sunflower oil that is the first in the vegetable oils consumpted in Turkey is met by importation because of lacking of raw material. 65% of sunflower raw oil supply is met by seed and total raw oil importation. Therefore, the oilseed crop whose production firstly should be increased is sunflower seed to meet the gap of vegetable oil, considering the consumption pay of it. In this context, making long term projections is important to present the state of the production and trade of sunflower seed and oil with regard to production planning and developing policies for the future. The aim of this study is to project the production and foreign trade, presenting the current situation of sunflower seed production in Turkey. For this aim, times series analysis method will be carried out using the data of Turkish Statistical Institute (TSI) in the study.

Key Words : Sunflower Seed, Sunflower Oil, Time Series Analysis

SUNEO: TECHNOLOGY FOR YIELD PROTECTION

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ABSTRACT

Nowadays it is not enough for seed companies to focusing only on breeding new varieties with good resistance for broomrape. The broomrape races evoluate rapidly with increasing aggressiveness, gaining surface across Europe. In this context it is necessary to be revolutionary if we want to win against this parasite. The main pillars of development durable solution:

Breeding for resistance: Finding new genetic resources is a key priority of Limagrain. Besides running field screening in the highly infected areas, it is reinforced with fundamentally different approach, by using high throughput screening platform Oroscreen ®. The platform serves our breeding since 2012 and allows promising progress in exploring genetic diversities of resistance against *Orobanche*.

Monitoring: Drawing the map of distribution of *Orobanche* races based on systematic collections of broomrape along with analysing the risk of drought across Europe is enabling us to create customer specific recommendation.

Integrating herbicide technology: Post- emergence herbicide can control the spread broomrape in new areas and to reduce the damage in infected areas. Limagrain is the first to coupling the newest herbicide tolerance with most powerful genetic resistance. This double security is giving full protection during the vegetation period from early to late attack. The new formulation of the herbicide is permitting to reduce also the residue in the soil and to give flexible protection in dry areas.

Limagrain Europe launched SUNEO® brand in 2013. This concept integrates LG 's best sunflower genetics issue from Soltis breeding program, an innovative screening platform for Orobanche resistance and the Clearfield® system

Key Words : broomrape resistance, herbicide tolerance, broomrape monitoring

RESULTS REGARDING BIOMASS YIELD AT SUNFLOWER UNDER DIFFERENT TECHNOLOGICAL CONDITIONS

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ABSTRACT

Crop biomass is an important source of renewable energy and it is used by the mankind from the ancient times. It was used mainly for producing heat, but recently it is also of interest as source of bioethanol, biodiesel, biogas, and even electricity. Nowadays, we are more and more interested in so called energy crops, including here the crops used for producing biomass. The specific crop technology for these energy crops is of great importance for producing biomass in an efficient way. Sunflower is one of the energy crops which are of interest for its above-ground biomass that could be used as substrate for producing biogas. Considering these matters, the aim of the present paper is to present the biomass yield obtained at an assortment of five sunflower hybrids under different preceding crop conditions (triticale with harrowing as soil tillage, maize with harrowing as soil tillage, and maize with ploughing as soil tillage) and row spacing conditions (70 cm, 50 cm, and twin-rows of 70/40 cm). For accomplishing this aim, a field experiment was performed in 2015 which was located in South Romania, respectively in the specific conditions from Moara Domneasca Experimental Farm belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Determinations of fresh and dry biomass were performed in the early dough - dough plant growth stage. For biomass purposes, the grower has to choose a sunflower hybrid with a high potential to produce biomass among other characteristic, but also he has to use a proper crop technology within which the preceding crop and row spacing are important elements.

Key words: Sunflower, Biomass, Yield, Preceding crop, Row spacing

INTRODUCTION

Biomass could be defined as a form of energy storage whose major peculiarity is that to be renewable. Starting from this idea, the biomass that was used by humankind as source of energy since ancient times has become of great importance in the present context of global warming and searching for alternative ways of energy.

Biomass crops can take many forms and can be converted to a number of different products (Alexopoulou and Kretschmer, 2011). Concerning the use of crop biomass for energy purposes, this is of interest either as crop residues or as energy crops especially grown to accomplish this purpose.

Among the energy crops, sunflower could be used as a source of lignocelluloses biomass (Ziebell et al., 2013; Ion et al., 2014), which could be used as substrate for biogas

production. In this respect, sunflower offers an advantage as its oil produces a higher methane content in biogas than other biogas crops (Hahn and Ganssmann, 2008). Also, sunflower is of interest because it could be characterized as being a crop tolerating the drought and succeeding under limited input conditions (Ion et al., 2015), and having the capacity to supply important yields of above-ground dry biomass, usually of 10-15 tons.ha^{-1}, reaching even 20 tons.ha^{-1} (Stefan et al., 2008).

But, one of the important conditions to produce biomass in an efficient way is to use the most appropriate cultivation techniques (Balodis et al., 2011; Basa et al., 2014; Beg et al., 2007; Ion et al., 2014). There are several technological measures with great impact on the biomass production. Thus, apart from choosing the most appropriate sunflower hybrid according to the growing and technological conditions, one can consider the preceding crop and the row spacing.

Energy crops for biogas production need to be grown in sustainable crop rotations (Amon et al., 2007; Hahn and Ganssmann, 2008). Sunflower is one of the energy crops which could be included into the crop rotations in view to assure its sustainability.

Diepenbrock et al. (2001) found in 1996 and 1998 that the above-ground biomass increased significantly with increasing row spacing, but in 1997, however, the differences between the row spacing were small. Sunflower can be manipulated over a wide range of plant populations and row spacing without seriously affecting yield (Vijayalakshmi et al., 1975). Nevertheless, the experimental results show that different planting patterns sometimes produced higher yield, but not always (Zarea et al., 2005). Also, lodging increased with wider row spacing (Holt and Zentner, 1985). All these findings reveal the fact that there are several implications of row spacing but not always the results are very clear, which means that this subject needs further investigations.

The aim of the present paper is to present the biomass yield obtained at an assortment of five sunflower hybrids under different preceding crops (triticale with harrowing as soil tillage, maize with harrowing as soil tillage, and maize with ploughing as soil tillage) and row spacing conditions (70 cm, 50 cm, and twin-rows of 70/40 cm).

MATERIALS AND METHODS

Researches were performed in a field experiment in the year 2015, which was located in South Romania, respectively in the specific conditions from Moara Domneasca Experimental Farm (44º29’ N latitude and 26º15’ E longitude) belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The specific soil for Moara Domneasca area is reddish preluvosoil, which is characterized by a humus content of 2.2-2.8%, clay loam texture, and pH of 6.2-6.6. Taking into account the growing period of sunflower in this area (April-August), this period is characterized by the multiannual average temperature of 18.5ºC and by the multiannual average rainfall of 313.2 mm. In the year 2015, the growing period of sunflower in the studying area was characterized by the average temperature of 19.2ºC and the sum of rainfall of 237 mm. This means that the year 2015 can be characterized as being warmer and drier than normal years.

Five sunflower hybrids were studied, respectively: Favorit, Performer, LG56.62, P64LE19, and Pro 144. Each hybrid was studied under three preceding crop conditions (triticale with harrowing as soil tillage, maize with harrowing as soil tillage, and maize with ploughing as soil tillage) and under three row spacing (70 cm, 50 cm, and twin-rows of 70/40 cm). The total area of the field experiment was of 1,640 m^2.

Soil tillage consisted in ploughing performed on 10th of November 2014, one harrowing work performed on 14th of April 2015, and seedbed preparation performed on 21st of April 2015 with a cultivator. The sowing was performed manually on 22nd of April 2015 and the plant density was of 70,000 plants.ha^{-1} for all the 45 experimental variants. The fertilization was performed with 80 kg.ha^{-1} of nitrogen and 40 kg.ha^{-1} of phosphorus. The weed control was performed by the help of herbicide (Dual Gold 960 EC based on the active substance S-metolaclor 960 g/l) applied at a dose of 1.2 l.ha^{-1} one day after sowing (on 23rd of April, 2015). The herbicide weed control was completed by one manual hoeing performed on 26th of May 2015.

Determinations of fresh and dry biomass were performed in the early dough - dough plant growth stage, respectively in the growth stage when the sunflower biomass is of importance to be used as substrate for biogas production. For each experimental variant the sunflower plants from one square meter were cut at soil level and were weighed immediately for determining the fresh biomass yield (as above-ground biomass). One sunflower plant for each variant was taken into the laboratory and dried in the oven at 80^{o}C for 24 hours in view to be determined the dry biomass yield. The biomass to which we are referring in this paper represents the above-ground biomass.

RESULTS AND DISCUSSIONS

Among the three studied preceding crops, the highest biomass yields as average values were obtained when sunflower followed after maize with ploughing as soil tillage, respectively 33.46 tons.ha^{-1} of fresh biomass and 8.72 tons.ha^{-1} of dry biomass (Figure 1). When the preceding crop was maize but with harrowing as soil tillage (without ploughing) the biomass yield decreased compared to the variant with ploughing as soil tillage, but the decrease was less important for fresh biomass (33.24 tons.ha^{-1}) and more important for dry biomass (8.28 tons.ha^{-1}). The smallest biomass yields were obtained after triticale with harrowing as soil tillage, respectively 32.12 tons.ha^{-1} of fresh biomass and 7.91 tons.ha^{-1} of dry biomass.

Despite the fact that maize as preceding crop with ploughing as soil tillage determined the largest variation of fresh biomass yields, the dry biomass yields registered less variations and the highest values (Figure 1).

Regarding the biomass yields obtained at different row spacing conditions, the average values of the biomass yields were not significant different (Figure 2). The differences among the average values were more visible for fresh biomass than for dry biomass. It is interesting to point out that narrow rows determined higher yields of fresh biomass compared to row spacing of 70 cm, but with larger variations of the values. Concerning the dry biomass, the yields registered the highest values at wider rows, respectively at row spacing of 70 cm (8.36 tons.ha^{-1}). Taking into account the fact that the results were obtained in a year characterized as being warmer and drier than normal years for the studying area, these results are confirming partly the previous results we have obtained in the area where the field experiment was performed, respectively when growing conditions are less favourable, the yields of fresh and dry biomass tend to be higher at narrow rows (Ion et al., 2014; Ion et al., 2015).

Despite the fact that the row spacing of 70 cm determined the smallest average value for the fresh biomass (32.44 tons.ha^{-1}), however it determined the highest value for the dry biomass (8.36 tons.ha^{-1}). Also, despite the fact that the row spacing of 70 cm determined the

smallest variation for the fresh biomass yield, it determined the largest variations for the dry biomass yield (Figure 2).

It is interesting to underline that the dry biomass obtained at row spacing of 50 cm (8.28 tons.ha^{-1}), which is quite closed to that obtained at row spacing of 70 cm (8.36 tons.ha^{-1}), it registered the smallest variations and the highest minimum values, this being quite stable, (Figure 2).

The twin-rows of 70/40 cm determined the highest average value for the fresh biomass yield (33.53 tons.ha^{-1}) but, however the smallest average value for the dry biomass yield (8.25 tons.ha^{-1}).

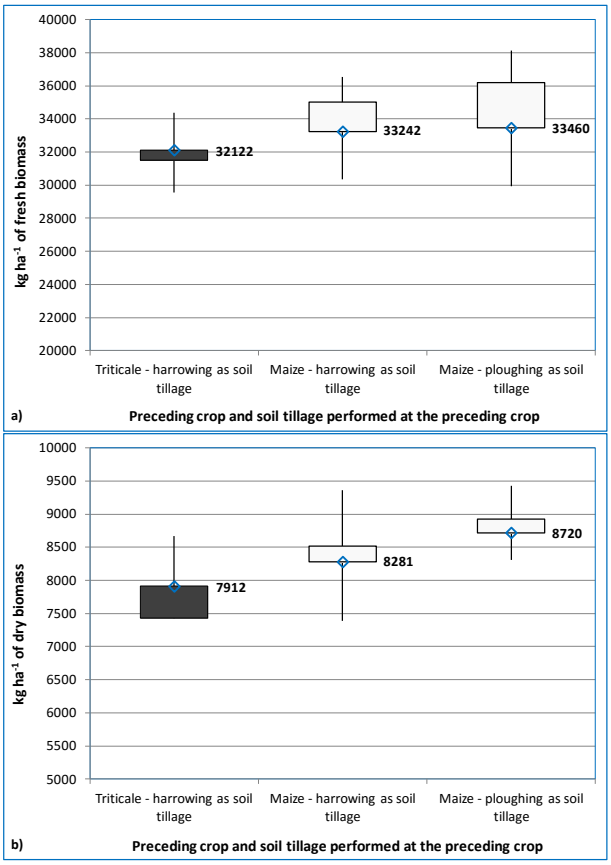

Figure 1. The fresh (a) and dry (b) biomass yields obtained at sunflower after different preceding crop conditions (Moara Domnească location, 2015)

As it was expected, there are important variations regarding the fresh and dry biomass yields according to hybrid, function of the hybrid characteristics (Figure 3). This is confirming the idea that the hybrid has to be chosen also according to the crop destination, respectively for biomass purposes has to be chosen a hybrid with a high potential to produce biomass among other characteristic.

In the year 2015, which was affected by drought, and for the studied conditions, the average dry biomass yields were less than 10 tons.ha^{-1}, while in the same area but under better climatic condition the average dry biomass yields were between 13 and 18 tons.ha^{-1} (Ion et al., 2014; Ion et al., 2015).

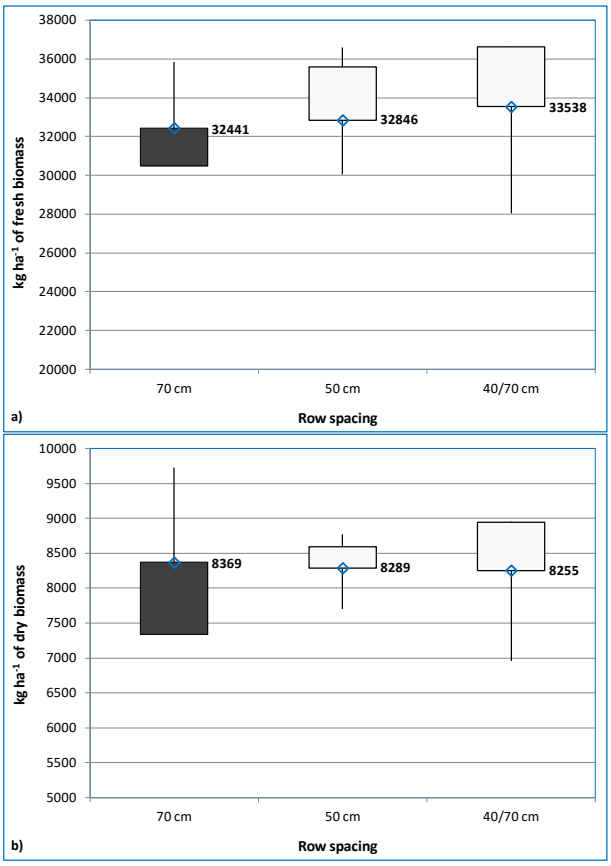

Figure 2. The fresh (a) and dry (b) biomass yields obtained at sunflower at different row spacing conditions (Moara Domnească location, 2015)

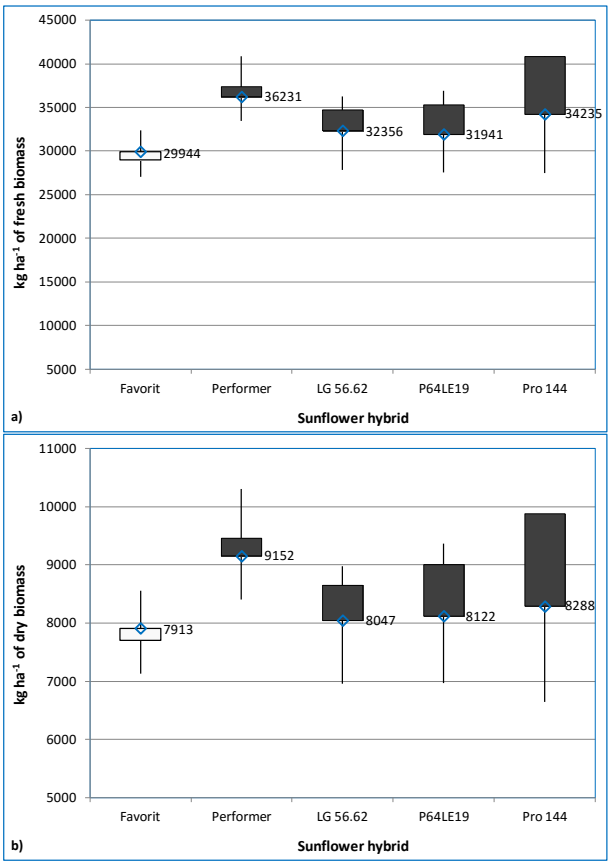

Figure 3. The fresh (a) and dry (b) biomass yields obtained at different sunflower hybrids (Moara Domnească location, 2015)

CONCLUSIONS

For biomass purposes, the grower has to choose a sunflower hybrid with a high potential to produce biomass among other characteristic, but also he has to use a proper crop technology. Thus, for producing biomass at sunflower crop and for the studied conditions, in a year affected by drought, maize was a better preceding crop compared to triticale, especially when maize had the ploughing as soil tillage. The different row spacing conditions determined dry biomass yields quite closed, but with a larger variation at 70 cm between rows and a smaller variation at 50 cm between rows.

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RESULTS REGARDING THE CORRELATION OF THE GRAIN YIELD WITH THE YIELD OF ABOVE-GROUND BIOMASS AT SUNFLOWER CROP

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ABSTRACT

Climatic and soil conditions, and the crop technology influence the correlation of the grain yield with the yield of above-ground biomass at sunflower crop. The aim of the paper is to present the results we have obtained at different hybrids of sunflower studied in the specific conditions of South Romania under different environmental and technological conditions regarding the correlation of the grain yield with the yield of above-ground biomass. The grain yields were expressed as yields at 9% moisture content of the grains, while the yields of above-ground biomass were expressed as yields of dry matter. Researches were performed in field experiments in 2013 and 2014 in two locations from South Romania (Fundulea from Calarasi County and Moara Domneasca from Ilfov County). In both experimental years, four sunflower hybrids were studied (Pro 111, Pro 953, LG 56.62 and P64LE19) at three row spacing (75 cm, 50 cm, and twin-rows of 75/45 cm) and three plant densities (50,000, 60,000, and 70,000 plants.ha^{-1}). Under the climatic conditions of 2013 and 2014 and in the two locations from South Romania with different soil conditions (chernozem and reddish preluvosoil), the grain yield did not correlated very well with the yield of above-ground biomass, whatever was the row spacing and plant density. However, there are some differences according to row spacing and plant density. Generally, the grain yields correlated negatively with the yield of above-ground biomass, except the situations registered at narrow rows under less favorable climatic conditions, especially when these were associated with less favorable soil conditions.

Key words: Sunflower, Correlation, Yield, Grains, Biomass

INTRODUCTION

Yield is the most economic character in almost all of the crops (Yasin and Singh, 2010). The yield is determined by the so called yield components. In sunflower, yield is determined by the proportions of the various components (Fetri et al., 2013). Sunflower seed yield, like other crops, is dependent of yield components which have interrelation among them and affect the seed yield directly or indirectly (Gjorgjieva et al., 2015).

Plant growth, plant biomass and plant yield are conditioned by different factors (Basa et al., 2014). The yield of achenes and the yield components of the head are specific to each sunflower hybrid, but they are influenced by the different growing factors, such as environmental factors (e.g. soil and climatic conditions) and technological factors (e.g. row

spacing and plant population) (Ion et al., 2015). Under optimal conditions it is expected that grain yield correlates positively, at least to some point with the biological yield, respectively the yield of above-ground dry biomass (Basa et al., 2015). Climatic and soil conditions, and the crop technology influence the correlation of the grain yield with the yield of above-ground biomass at sunflower crop.

The aim of the present paper is to present the results we have obtained at different hybrids of sunflower studied in the specific conditions of South Romania under different environmental and technological conditions regarding the correlation of the grain yield with the yield of above-ground biomass.

MATERIALS AND METHODS

Researches were performed in field experiments in the years 2013 and 2014, in two locations from South Romania, respectively Fundulea (Calarasi County) and Moara Domneasca (Ilfov County). These two locations represented different soil and climatic conditions. In both experimental years (2013 and 2014), researches were performed in field experiments with four sunflower hybrids, respectively: Pro 111, Pro 953, LG 56.62, and P64LE19.

The soil from Fundulea area is chernozem (cambic chernozem soil). At Fundulea area and for the growing period of sunflower, respectively period April-August, the average temperature was 20.1ºC in 2013 and 18.9ºC in 2014, while the multiannual average value for the same period is 18.6ºC. The sum of rainfall for the same period was 381.1 mm in 2013 and 399.0 mm in 2014, while the multiannual average value is 327.9 mm.

The soil from Moara Domneasca area is reddish preluvosoil. At Moara Domneasca area and for the growing period of sunflower, respectively period April-August, the average temperature was 20.5ºC in 2013 and 18.8ºC in 2014, while the multiannual average value for the same period is 18.5ºC. The sum of rainfall for the same period was 115 mm in 2013 and 408 mm in 2014, while the multiannual average value is 313.2 mm.

At Fundulea area, the rainfall was higher than the multiannual average value, the year 2014 being more humid than the year 2013. At Moara Domneasca area, the rainfall in 2013 was much less than multiannual average value, this year being characterised as a drought one, while 2014 was a humid one with more rainfall than multiannual average value.

Each sunflower hybrid was studied under three row spacing (75 cm, 50 cm, and twin-rows of 75/45 cm), and three plant densities (50,000, 60,000, and 70,000 plants.ha^{-1}).

In each location and from each variant, the sunflower plants from one square meter were cut at soil level and were weighed immediately in view to be determined the yield of fresh above-ground biomass. The seeds of sunflower heads were collected and weighed in view to be determined the yield of grains. It was determined the moisture content of the sunflower seeds to let us calculate the yield of grains in kg.ha^{-1} at moisture content of 9%. One sunflower plant for each variant was taken into the laboratory, where it was determined the dry biomass by oven drying at 80ºC for 24 hours, as to be determined the yield of dry above-ground biomass. In both experimental years, the determinations were performed at fully ripe stage. The yield of dry biomass was calculated in kg.ha^{-1} and represents the yield of above-ground biomass.

RESULTS AND DISCUSSIONS

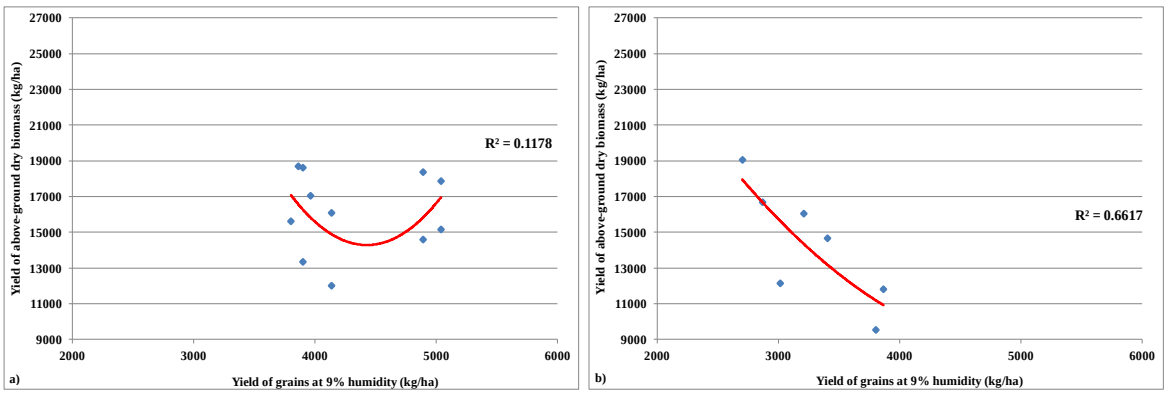
Under the climatic conditions of 2013 and 2014 and in the two locations from South Romania with different soil conditions (chernozem and reddish preluvosoil), the grain yields of the four sunflower hybrids, as average values, did not correlated very well with the yields of above-ground biomass, regardless of distance between rows and plant density (Figures 1-6). However, there are some differences according to row spacing and plant density.

At row spacing of 75 cm, the grain yields correlated negatively with the yield of above-ground biomass in both experimental years and both soil conditions (Figure 1).

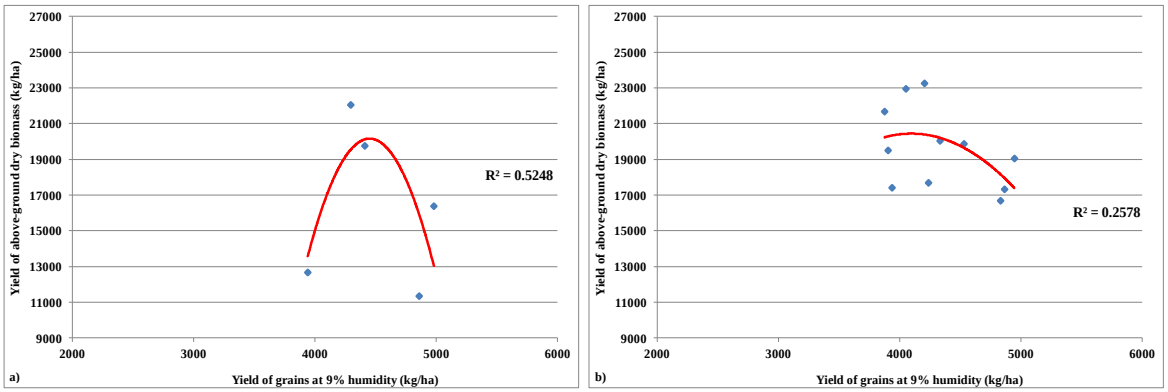
At row spacing of 50 cm, the grain yields correlated positively with the yields of above-ground biomass in the year 2013 under both soil conditions, but especially on reddish preluvosoil which was associated with less favorable climatic conditions, especially drought. In the year 2014, characterized by better climatic conditions for the two soil conditions, the grain yields correlated negatively with the yields of above-ground biomass (Figure 2). These findings can be explain by the fact that under less favorable growing conditions, the sunflower plants produce more grains once the above-ground biomass increase. But, under favorable growing conditions, the sunflower plants react by producing more vegetative biomass than reproductive one, respectively despite the fact that the above-ground biomass increases the weight of seeds decrease. This means that at row spacing of 50 cm and under favorable growing conditions the harvest index decrease.

At twin-rows of 75/45 cm, as in the case of row spacing of 50 cm, the grain yields correlated positively with the yields of above-ground biomass in the year 2013 under both soil conditions, but especially on reddish preluvosoil, which was associated with drought. Also as in the case of row spacing of 50 cm, in the year 2014, respectively under better climatic conditions, the grain yields correlated slightly negatively with the yields of above-ground biomass, especially on reddish preluvosoil (Figure 3).

Generally, at different plant densities, the grain yields correlated negatively with the yields of above-ground biomass (Figures 4-6), except the situation registered at plant density of 50,000 plants.ha^{-1} and reddish preluvosoil (Figure 4) and the situation registered at plant density of 60,000 plants.ha^{-1} and chernozem (Figure 5).

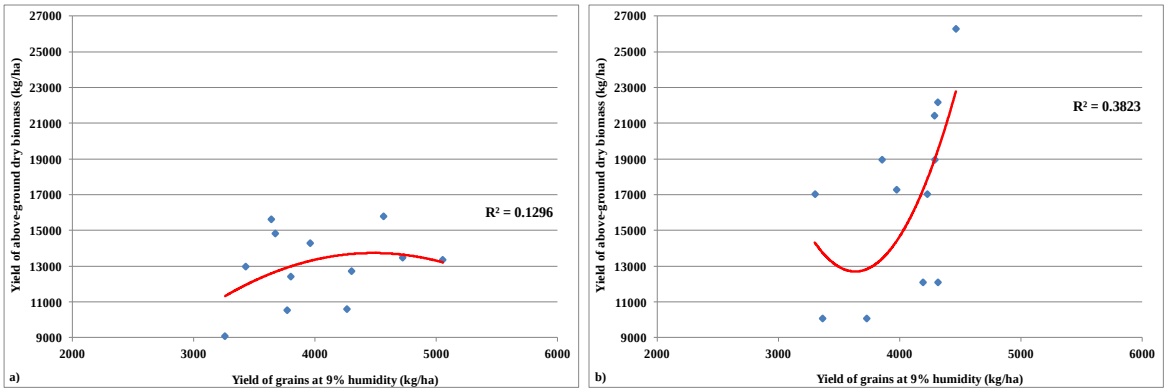


Climatic conditions of 2013 (a- chernozem soil; b- reddish preluvosoil)

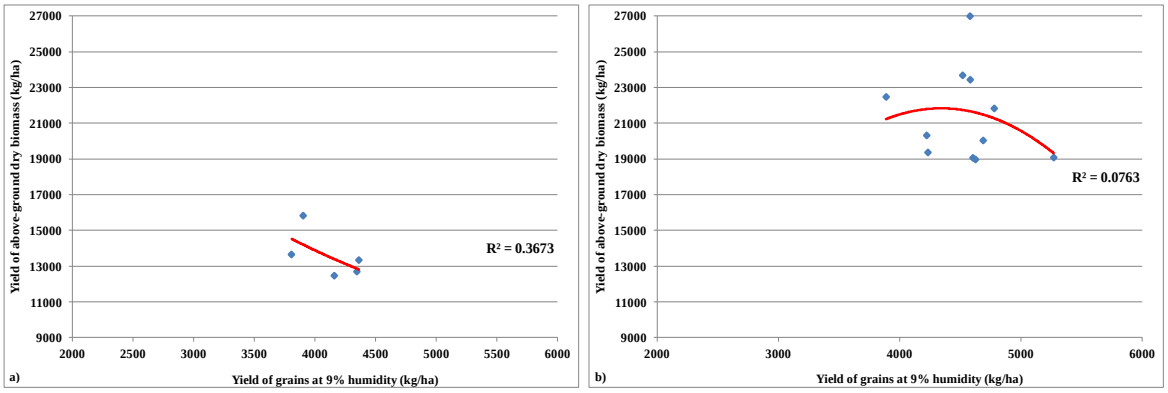


Climatic conditions of 2014 (a- chernozem soil; b- reddish preluvosoil)

Figure 1. Correlations of the yield of grains with the yield of above-ground dry biomass at sunflower at row spacing of 75 cm and under different climatic and soil conditions

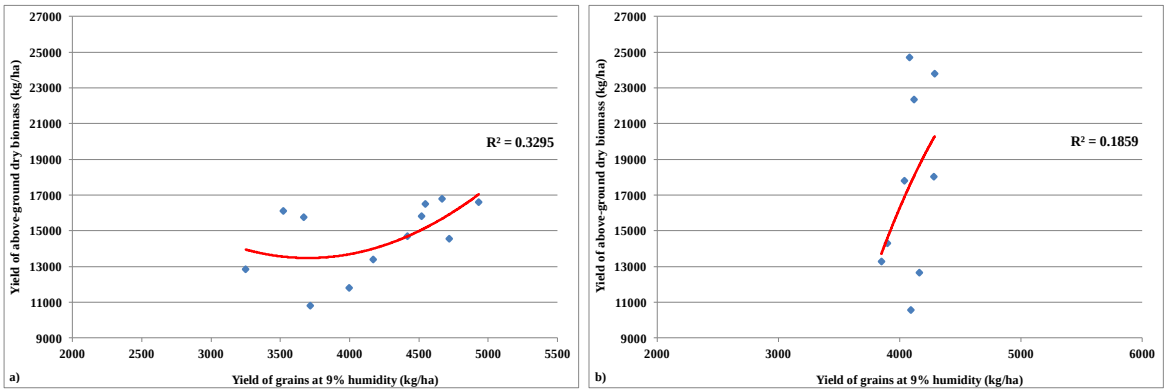


Climatic conditions of 2013 (a- chernozem soil; b- reddish preluvosoil)

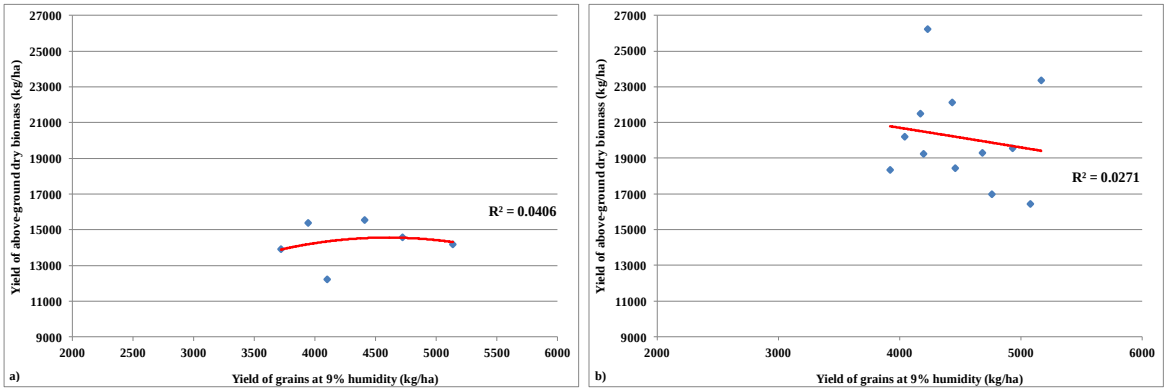


Climatic conditions of 2014 (a- chernozem soil; b- reddish preluvosoil)

Figure 2. Correlations of the yield of grains with the yield of above-ground dry biomass at sunflower at row spacing of 50 cm and under different climatic and soil conditions

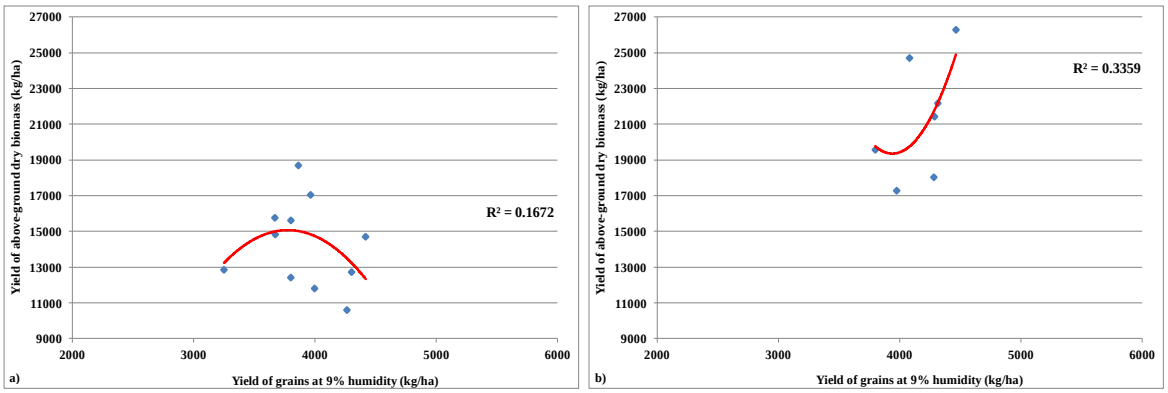


Climatic conditions of 2013 (a- chernozem soil; b- reddish preluvosoil)

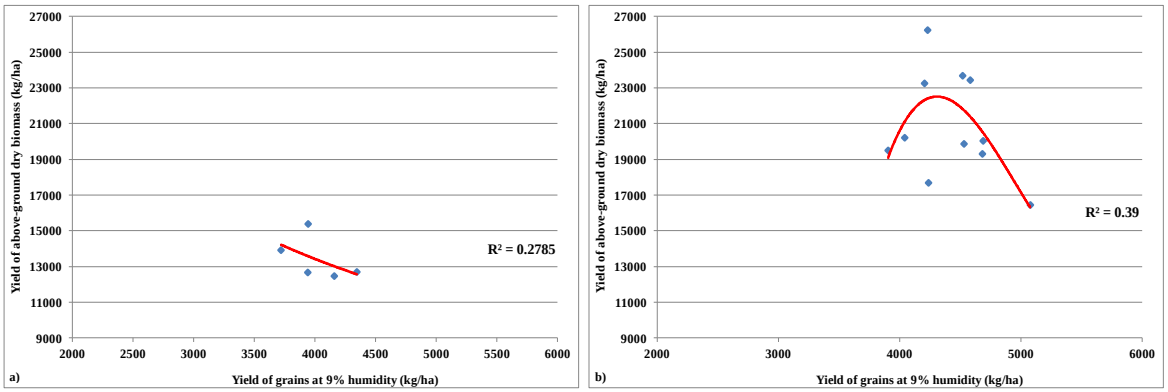


Climatic conditions of 2014 (a- chernozem soil; b- reddish preluvosoil)

Figure 3. Correlations of the yield of grains with the yield of above-ground dry biomass at sunflower at twin-rows of 75/45 cm and under different climatic and soil conditions

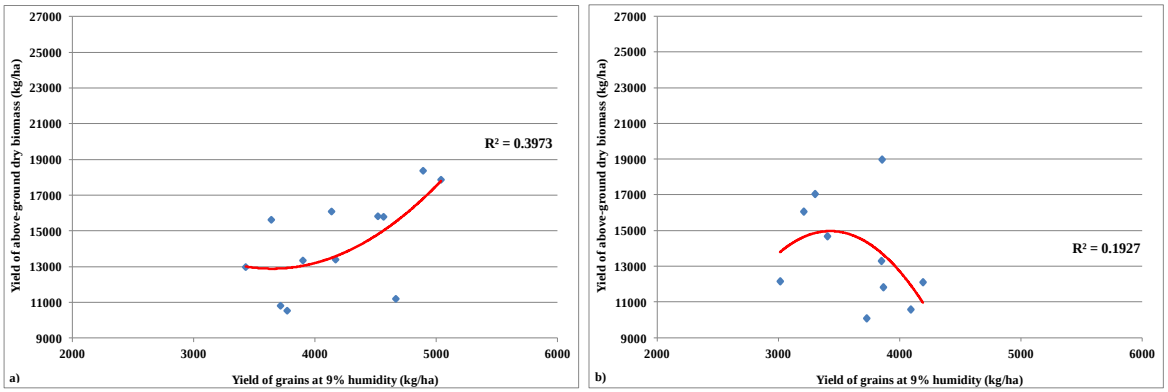


Climatic conditions of 2013 (a- chernozem soil; b- reddish preluvosoil)

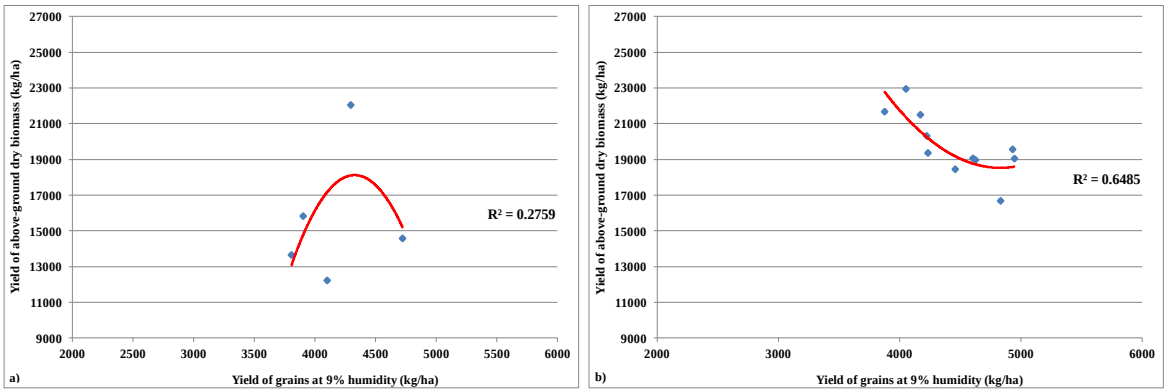


Climatic conditions of 2014 (a- chernozem soil; b- reddish preluvosoil)

Figure 4. Correlations of the yield of grains with the yield of above-ground dry biomass at sunflower at plant density of 50,000 plants.ha^{-1}, under different climatic and soil conditions

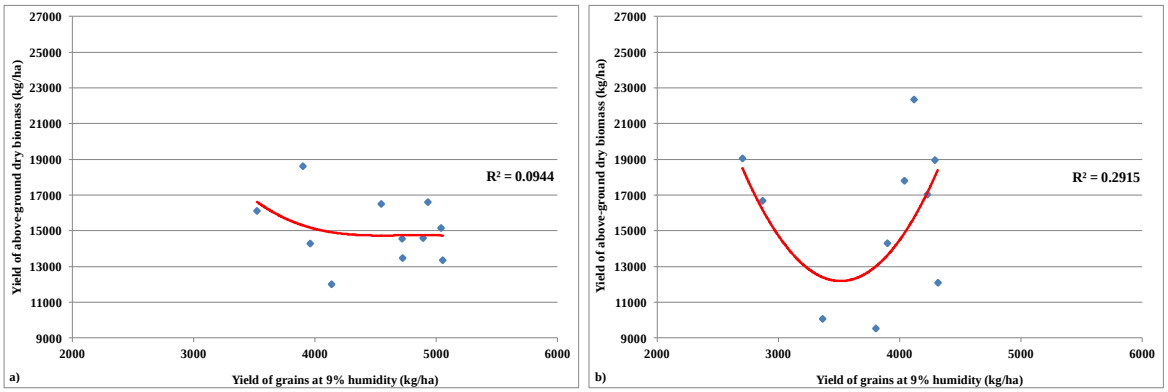


Climatic conditions of 2013 (a- chernozem soil; b- reddish preluvosoil)

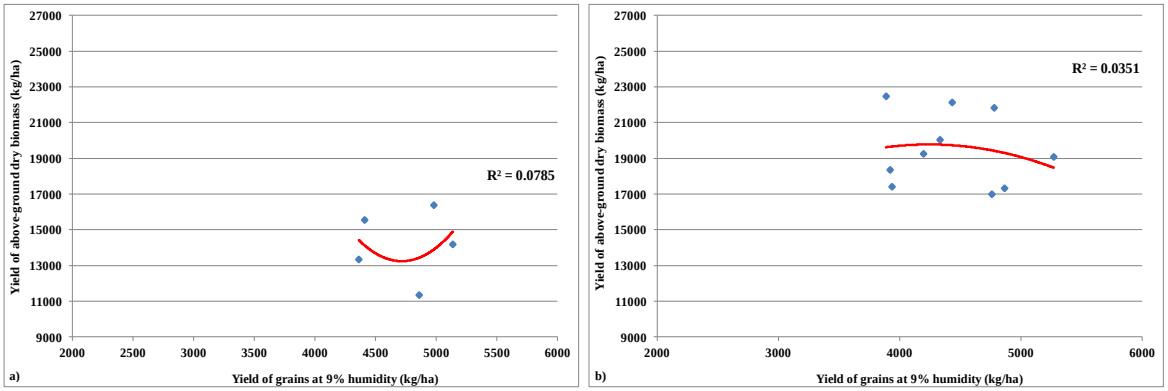


Climatic conditions of 2014 (a- chernozem soil; b- reddish preluvosoil)

Figure 5. Correlations of the yield of grains with the yield of above-ground dry biomass at sunflower at plant density of 60,000 plants.ha^{-1}, under different climatic and soil conditions



Climatic conditions of 2013 (a- chernozem soil; b- reddish preluvosoil)



Climatic conditions of 2014 (a- chernozem soil; b- reddish preluvosoil)

Figure 6. Correlations of the yield of grains with the yield of above-ground dry biomass at sunflower at plant density of 70,000 plants.ha^{-1}, under different climatic and soil conditions

CONCLUSIONS

Under the climatic conditions of 2013 and 2014 and in the two locations from South Romania with different soil conditions, the grain yield did not correlated very well with the yield of above-ground biomass, whatever was the row spacing and plant density. However, there are some differences according to row spacing and plant density. Generally, the grain yield correlated negatively with the yield of above-ground biomass, except the situations registered at narrow rows under less favorable climatic conditions, especially when these were associated with less favorable soil conditions, situations when the grain yield correlated positively with the yield of above-ground biomass.

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TOWARD REAL TIME INSPECTION OF QUALITY IN SUNFLOWER SEEDS: MACHINE VISION

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ABSTRACT

Quality assurance is one of the most important and challenging tasks of industries before export of food and agricultural produce. Sunflower seed and oil sector is one of the main industries in Turkey. The key quality parameters for safety evaluation of sunflower seeds includes heat damage, insect-infestation, mold growing, heterochromatic appearance and rancidity. Currently, time consuming and inconsistent conventional methods are generally utilized for inspecting the products, which are sampled individually or in large batches. Man-made visual inspection methods are generally performed by trained technicians to detect defects, colors, sizes or abnormalities, and classify the product in its appropriate category. In addition, the variety of sunflower seeds hinders their classification in terms of color, texture or different types of defects. Machine vision is an emerging technology that combines mechanics, optical instrumentation, electromagnetic sensing, digital video and image processing. Integrating mechanical, optical, electronic devices, this non-destructive method has been widely accepted in a broad range of sectors. The food industry is among the fastest growing segments of machine vision systems. Recent research has highlighted the possible application of machine vision in other areas of agriculture. Since it is of critical importance to readily acquire the quality characteristics of sunflower seeds in order to meet the demands of high-quality food processing industry, machine vision can be utilized as a promising novel tool for real time examining, monitoring and controlling quality characteristics of sun flower seeds.

Key Words : sunflower seeds; image processing; machine vision

POTENTIAL OF HYPERSPECTRAL IMAGE PROCESSING FOR CLASSIFICATION AND QUALITY EVALUATION OF SUNFLOWER SEEDS

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops grown in the world. Current methods for safety evaluation of sunflower seeds mainly depend on spot check, sampling inspection and frequent analysis, requiring destructive, expensive and time consuming treatments. Moreover, conventional imaging systems can hardly distinguish the inferior seeds due to presence of hard shells, hindering photoelectric information from the kernel. There is a growing interest in hyperspectral imaging (HSI) for being a promising, non-destructive and real-time tool for inspecting quality, safety and authenticity features of food products. Unlike conventional imaging systems, which operate at visible wavelengths in the form of monochromatic or discrete color images (red, green and blue), hyperspectral systems collect images in several contiguous and/or regularly spaced bands. Therefore, HSI produces a fuller spectrum of wavelength information for each pixel of the image. In HSI, spatial and spectral information can be simultaneously obtained. Combining visual imaging and computer based vision techniques, HSI has been successfully implemented in monitoring and prediction of physical parameters, determination of chemical composition, assessment of quality attributes, detection of microbial spoilage and inspection of contaminants. There is an urgent need for a reliable, rapid and accurate automatic detection technique for assessing the quality parameters of sunflower seeds. Up to date no research has been reported on hyperspectral imaging in sunflower seeds. However previous studies have shown that the hyperspectral imaging holds potential for future on-line and real-time monitoring of grains.

Key Words : sunflower seed, hyperspectral imaging , quality

SOME MORPHOLOGICAL CHARACTERISTICS OF CONFECTIONARY SUNFLOWER GENOTYPES OBTAINED THROUGH SELECTION BREEDING

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ABSTRACT

Sunflower is cultivated in many countries including Turkey because of high adaptation capacity and various uses. Sunflower is grown primarily to meet the cooking oil demands of households and industry, but it is also used in cookies, bakeries and various other foodstuffs. Turkey has 180 thousand tons/year confectionary sunflower production with an average yield level of 155 kg/da (Tüik, 2015). In Turkey, Ankara, Aydın, Denizli, Izmir, Kayseri, Sivas, Yozgat, Kırıkkale, Aksaray, Nigde, Nevsehir, Kırsehir, Hatay, Kahramanmaras, Osmaniye and many other provinces are the primary confectionary sunflower growers. Despite the sufficient potential and cultivation area, yield levels are quite low in Turkey due to lack of sufficient availability of certified seeds. Poor quality effects seed size and homogeneity desired by consumers. Confectionary sunflower has been ignored in most countries except for a few countries in the world. The USA is the leading country in confectionary sunflower marketing. International agricultural organizations usually consider confectionary sunflower with oilseed sunflowers. This study was conducted to purify confectionary sunflower cultivars in Turkey to meet consumer demands and to improve adaptation capacity of the cultivars. For this purpose, confectionary sunflower lines were sown and their head diameters, head shapes and self-pollination ratios were determined.

Key words: Confectionary sunflower, Selection, Self-pollination

INTRODUCTION

Today, sunflower is largely used to meet the demands for cooking oil. In some countries, beside oil seed cultivars, confectionary cultivars are also produced (Lofgren, 1978). The confectionary seeds are rich in nutrients and they are commonly used in confectionary production through mixing them with salt, butter and honey, used as seasoning over vegetable, fish and salads, they are also consumed as snack food in roasted or non-roasted type (Millete, 1974).

Sunflower production is widespread in Black Sea, Thrace-Marmara, Aegean, Central Anatolia and Çukurova regions of Turkey. It is cultivated in almost every part of the country under dry or irrigated conditions. Despite quite well adaptation capacity of sunflower, the cultivated lands are limited with 500-600 thousand hectares in Turkey. Confectionary sunflower production of Turkey is 180 thousand tons/year and the average yield level is 155 kg/da (Tüik, 2015).

Confectionary use of sunflower is quite common both in Turkey and various other parts of the world and it is most commonly consumed as snack food in several countries.

Sunflower has been used for confectionary purposes for a long time and it is used in more than a hundred foodstuffs worldwide including bakery, ice-cream, chocolate, cookies and etc.

(Lofgren, 1997). As it was in Turkey, confectionary sunflower production is a great income source for world farmers, but it is usually considered in world literature with oilseed sunflowers. It is nationally qualified separately as oilseed and confectionary in practice, confectionary statistics are not usually presented by international agricultural organizations (OECD, FAO, ISA and etc.). The USA, Hungry, Argentina, Spain, Israel, China, Turkey, Moldova and some Eastern European countries are the leading confectionary sunflower producers. Today, the USA has the greatest confectionary sunflower production. On the other hand, Germany, Denmark, the Netherlands, Canada, Mexico, The United Kingdom and Belgium are the leading shelled confectionary sunflower buyers and Spain, China, Turkey, Jordan, Canada, Mexico, Israel, Germany and Japan are the primary unshelled confectionary sunflower buyer countries.

In Turkey, commercial confectionary sunflower production activities are commonly implemented in Kahramanmaras, Elazıg, Ankara, Aksaray, Balıkesir, Bursa, Usak, Burdur, Yozgat, Kırsehir, Amasya, Corum, Erzurum, Kayseri, Igdır, Isparta, Eskisehir, Tekirdag and Edirne provinces (Tan, 2011). The seeds used in productions are mostly open-pollinating village-type populations (Tan, 2011), however, certified cultivars have recently been used. Some of these certified seeds are foreign breeds (Avesa Çrz 2012, Marker, Confeta CL and Shelly), but there are some local breeds and local confectionary sunflower cultivars (Çiğdem 1, Palancı 1, 09 TR Ç 004, Çetinbey and İnegöl alası).

Confectionary sunflower is commonly cultivated in Central and Eastern Anatolia provinces and village populations called based on physical appearance like Alaca, Kıbrıs, Inegöl and etc are used. Unit area yield levels are quite lower under dry conditions than the yield levels obtained from hybrid oilseed cultivars (Kaya, 2004). Previous researches revealed that confectionary types have low oil content, but high protein content. Low shell ratio and wide seeds are desired parameters in confectionary sunflowers (Kaya et al., 2008; Hladni et al., 2011).

The primary objective of the present study was to purify confectionary sunflower cultivars with high adaptation capacities and consumer desired quality parameters. In this way, new cultivars may be developed and registered to meet the country needs and further breeding will also be possible to develop advanced cultivars. Along with these objectives, head height, head shape and self-pollination ratios were determined.

MATERIAL AND METHOD

The present study was conducted to purify 12 genotypes collected from Central and Eastern Anatolia regions in 2002 and went through selection works for 10 years. During the purification study, head of each plant of 12 confectionary sunflower genotypes planted to field (50 plants per genotype) was isolated to prevent external pollination and plants were allowed to self-pollinate. Beside purification of the lines, self-pollination which is a significant parameter for sunflowers, ratios were determined.

The 12 lines obtained through single plant selection were sown in a single row. Proper care practices were implemented throughout the experiments. Before flowering (head formation), randomly selected 6 plants were bagged with isolation bags to provide self-pollination. Bags were removed when the seed formation was completed. Heads of each line were harvested and relevant measurements and counts were performed. The results for 10 lines with successful self-pollination (7, 12, 18, 21, 25, 27, 28, 34, 37 and 38) were

assessed. The empty/full seed ratios (%), head diameter (cm), head shape and head central spot diameter (cm) are provided in Table 1.

Table 1. Results for self-pollinated confectionary sunflower genotypes

Line No	Replication	Average number seeds per head	Average number of full seeds per head	Self-pollination ratio (%)	Average head diameter, cm	Head shape	Average central spot diameter, cm
7	2	1222	10	0.8	17.5	Smooth conical	0
12	4	538.5	40	7.4	11.3	Smooth-Smooth	1.8
18	2	972	175	18.0	16.7	Smooth conical	2.7
21	5	708	59.8	8.4	13	Smooth-Smooth	4.2
25	1	232	34	14.6	9.5	Smooth	0
27	2	709.5	49	6.9	12.2	Smooth conical	0
28	10	767	62.8	8.1	14.3	Smooth-Smooth	1.4
34	4	1063.5	55	5.1	18.5	Smooth-Invert	2.8
37	2	577	35.5	6.1	13.7	Smooth conical-	1.2
38	3	547	56.6	10.3	11	Smooth-Smoot	2.2

RESULTS

Of the 12 lines used to homogenize the genotypes, 10 exhibited self-pollination and two lines were not self-pollinated. Since self-pollination is a significant parameter in breeding works, these two lines were omitted. Considering the remaining 10 lines, the greatest self-pollination ratio was observed in line 18 with 18.0%. It was followed by line 25 (14.6%) and line 38 (10.3%). Self-pollination ratios of the other lines were below 10% level. The lines with a self-pollination ratio over 10.0% (lines 18, 25 and 38) were considered as promising lines. These lines had smooth or smooth conical heads as desired. Considering the head diameters, line 18 had larger heads than the promising lines 25 and 38. With regard to non-seed formation at central spot, line 25 was full, line 18 had 2.7 cm and line 38 had 2.2 cm central spot without seeds.

CONCLUSION

The lines 18, 25 and 38, which were identified as promising lines with regard to self-pollination, should be self-pollinated for couple more years. Then, during this further self-

pollination period, the ones preserving or improving self-pollination ratios can be used in further breeding works as advanced promising genotypes.

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A PRELIMINARY STUDY ON CONTROL OF SUNFLOWER DOWNY MILDEW (PLASMOPARA HALSTEDII) WITH CULTURE FILTRATES OF ANTAGONISTIC FUNGI

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ABSTRACT

Sunflower downy mildew caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni is a serious disease on sunflower grown worldwide. Occurrence of fungicide-tolerant races of the pathogen limits chemical control and the use of resistant cultivars to overcome the disease. Biological control is one of the alternative environment-friendly methods for managing this disease. Culture filtrates of the fungal isolates, AS3 (non-aflatoxigenic *Aspergillus flavus* Link), TRIC7 and TRIC8 (*Trichoderma harzianum* Rifai) from soils (Tekirdağ/Turkey), which controlled black mold disease in onion, were tested for their effects on sporulation density caused by *P. halstedii* on cotyledon leaf of sunflower. Fungal isolates were cultured on potato dextrose broth (PDB) for 15 days at 24oC. The fungal biomass was centrifuged at 5000 rpm for 20 min and the supernatant was filtered using a sterile membrane filter (0.22 μm). The filtrates were analysed by gas chromatography/mass spectrometer (GC/MS). Sunflower seeds surface-sterilized with sodium hypochlorite (10%) for 1 min were treated with the filtrate of each potential antagonists by shaking the seeds for 6 hour. Pathogen was inoculated to the roots of pre-germinated seeds for three days. All treatments significantly reduced sporulation density of pathogen, representing the effectiveness of 87%, 66% and 60% by AS3, TRIC7 and TRIC8, respectively. GC/MS analysis showed that culture filtrates of the tested antagonist fungi contained several compounds known for their antifungal activity such as amines, amides, fatty acids and esters, ketones, phenols and, derivatives of imidazole, inden, indole and thiazole, differing to the isolates.

Key Words : *Plasmopara halstedii*, biological control, *Aspergillus flavus, Trichoderma harzianum*, antagonistic fungi, antifungal activity

SUNFLOWER OIL QUALITY SYMPOSIUM

AGRONOMIC PERFORMANCE OF SUNFLOWER (HELIANTHUS ANNUUS L.) IN AN ORGANIC CROP ROTATION SYSTEM IN THE HUMID TROPICS

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ABSTRACT

The demand for organic sunflower seeds is very high in the international market. Sunflower is a rustic plant that is cultivated under different production systems across several agro-ecological zones in the world. A locally adapted and late maturing sunflower variety ('Funtua') was sown after soybean, sesame and maize between 2008 and 2012 to assess its agronomic performance under continuous, rotational and conventional cropping systems in the forest – savanna transition zone. The field trials were carried out during the late cropping season (June – Nov.) in a randomized complete block design and replicated four times. Data were collected on plant height at maturity, seed yield and yield attributes of sunflower each year. Varying results were obtained on the effects of cropping systems on the agronomic parameters measured across the years. However, cropping system significantly (*P<0.05; F-test*) affected seed yield of sunflower in 2009, 2011 and 2012. The conventional cropping system only significantly (*P<0.05*) produced seed yield (1642.6 $kg.ha^{-1}$) higher than the continuous (778.0 $kg.ha^{-1}$) and rotational cropping (1262.0 $kg.ha^{-1}$)systems in 2009. Thereafter, as the system stabilized, the rotational cropping system recorded higher seed yield than the continuous and conventional cropping systems in 2010, 2011 and 2012. The difference was significant (*P<0.05*) in 2012 with the rotational cropping system producing seed yield higher by 7.3 and 31.3% than the conventional and continuous cropping systems, respectively. Adoption of rotational cropping system is hereby recommended for sustainable organic crop production system in the humid tropics.

Key words: crop rotation, sesame, sunflower, yield, yield characters

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oilseed crop that has a very wide range of adaptation ability, low labour requirement for its cultivation and also very suitable for mechanization (Ozer *et al.,* 2004; Kaemeini *et al.,* 2009). Consequently, sunflower can be described as a suitable crop for crop rotation scheme in the tropics where water and not temperature is the major growth limiting factor. It also exhibits erect growth habit, comparable resistance to lodging, short duration, limited ground cover and has easily harvestable heads (Robinson, 1984; Kamal and Bano, 2009). Sunflower is grown principally for its seed that contains oil (36–52%) and protein (28–32%) as reported by Rosa *et al.* (2009). According to NSA (2016), the world's average yield and total land area statistics of sunflower increased appreciably by 11.3% and 9.3% between 2007/2008 and 2012/2013, respectively.

Crop rotation is a planned order (sequence) of specific crops from different genus, species, subspecies or varieties on the same field over a given period of time (Helm, 1993). The advantages of crop rotation include: prevention of soil depletion; improvement of soil fertility,

internal resource utilization; reduction of soil erosion, reliance on synthetic chemicals, allelopathic or phytotoxic effects and environmental impact; control of diseases and pest infestation; enhancement of workload distribution and distribution of economic risks (Helm, 1993). According to Kamal and Bano (2009), over 200 natural allelopathic compounds have been discovered and isolated from different cultivars of sunflower. However, the responses of crops that follow sunflower in a sequence of as companion crops vary (Farooq *et al*., 2011; Nikneshan *et al.*, 2011). It was recently reported that the α-pinene in essential oil of sunflower head is very critical to the inhibitory effect of head extract (Kaya *et al.*, 2013). Consequently, it was suggested that removing the head of sunflower could be beneficial for alleviating the allelopathic effect. Unfortunately, this crop is rarely cultivated in rotation with other crops in the tropics. Therefore, in a bid to develop a production package for some staple and commercial food crops with high export potentials a crop rotation scheme was initiated consisting of four component crops with export potentials (soybean, sunflower, sesame and maize) in 2008. The objective of the study was to evaluate the performance of the component crops in rotation relative to continuous and conventional cropping systems.

MATERIALS AND METHODS

The mean monthly rainfall data during the late copping season of 2008 – 2009 are presented in Table 1. Year 2010 was the wettest year (791.2 mm) during the late cropping season and 2008 was the driest (328 mm). Although, the highest rainfall (288.1 mm) was recorded in 2011 during the most critical month for sunflower (October) which coincided with grain filling. The crop rotation scheme involved four component crops (soybean, sesame, sunflower and maize as shown in Table 2) and the study was carried out at the Organic plot of the Teaching and Research Farm of the University of Agriculture, Abeokuta (7º 15' N, 3º 25' E, altitude 140 m.a.s.l). The soil of the experimental field is oxic Paleudulf (Adetunji, 1991). The test variety of sunflower was Funtua (a local adapted and late maturing variety). The experimental design was randomized complete block design (RCBD) with four replicates. Treatments evaluated were continuous, rotational and conventional cropping systems. The plots of the conventional cropping system were located about 15 m away from the organic plots to avoid commingling. The row spacing adopted for sunflower under the three cropping systems was 60 x 30 cm and each plot measured 6.5m by 6.0m ($39m^2$). Sowing of sunflower seeds was done on August 15, 2008, July 2, 2009, August 15, 2010, July 18, 2011 and July 20, 2012 based on the onset of rains in the late cropping season. No herbicides or inorganic fertilizers were applied on the continuous and rotation plots. However, pre-emergence herbicides (Galex and Gramoxone) and fertilizer combination (60 kgN/ha, 56 kg P_2O_5/ha and 100 kgK_2O/ha) were applied on the conventional plots at sowing and 4 weeks after sowing (WAS), respectively. Manual weeding was done on all plots at 3 and 6 WAS. The organic fertilizer (Aleshinloye Fertilizer (Grade B) contained 1.2%N, 76 ppm P, 13.75 cmol K, 10.28 cmol Na) was applied at the rate of 25 tonnes/ha to the continuous and rotational cropping systems plots at 4 WAS. This rate was equivalent to 60 kg N ha/ha of the inorganic fertilizer recommended for sunflower in the transition zone (Olowe *et al.,* 2005). Application of organic fertilizer commenced in 2009 a year after the rotation scheme took off. Harvesting was done at physiological maturity (R8) as described by Schneiter and Milner (1981). Five randomly selected plants per plot were tagged from the net plot for plant height measurement and yield attribute analysis. Data were collected on plant height at physiological maturity, head weight and diameter, number and weight of seeds per head and seed yield on plot basis. All data collected were subjected to analysis of variance and means of significant treatment were separated using the least significant difference method as described by Steel and Torrie (1984).

RESULTS

Effect of cropping systems on plant height, seed yield and yield attributes of sunflower

Cropping system only significantly ($P \leq 0.05$; *F*-test) affected plant height in 2012 with sunflower plants on rotational and conventional plots significantly taller than plants on under continuous cropping system (Table 3). However, the pooled mean indicated that the plant height of sunflower under the conventional and rotational cropping systems were at par. Average head diameter and weight of sunflower were significantly ($P \leq 0.05$; *F*-test) affected by cropping system in 2009 and when pooled, and the plants under continuous cropping system recorded significantly lower head diameter and weight than those under rotational and conventional cropping systems (Table 4 and 5). Weight of seeds per head was significantly ($P \leq 0.05$; *F*-test) affected by cropping system in 2009, 2012 and when pooled (Table 6). Similarly, the effect of cropping system was only significant ($P \leq 0.05$; *F*-test) for number of seeds per head in 2009 and when pooled (Table 7).However, sunflower seed yield was significantly ($P \leq 0.05$; *F*-test) affected by cropping system in 2009, 2011, 2012 and when pooled. Sunflower under continuous cropping system produced lower (significant at $P \leq 0.05$) seed yield than the plants under rotational and conventional cropping systems during the three years, except when yield values were pooled and the continuous was at par with rotational system (Table 8).

DISCUSSION

Rainfall distribution which is the main growth limiting factor in tropical agriculture varied markedly during the five year period of experimentation. The total rainfall during the late cropping season of 2008 – 2012 ranged between 328.0 and 791.2 mm and these values compared favorably with the rainfall amount (500 – 750 mm) reported to be adequate for optimum performance of sunflower (Weiss, 2000). Year 2008 with the smallest amount of rainfall (328.0 mm) also recorded the lowest seed yield (540.8 kg/ha). This could be attributed to the low rainfall in October (84.5 mm) which coincided with the grain filling period. The rotational and conventional cropping systems recorded grain yields above 1,000 kg/ha between 2009 and 2012, except conventional cropping system in 2010 and 2011. These yield values must have been enhanced by the rainfall in the months of September and October, and the availability of nutrients supplied through fertilizer application and they compared favorably with Nigerian (1000 kg/ha), African (812 kg/ha) averages (Olowe *et al.*, 2013) and world average (1520 kg/ha) according to USDA (2012), and the more recent forecast (1410 kg/ha) for 2012/2013 by NSA (2016). The consistently higher seed yield recorded under rotational cropping system in 2010, 2011 and 2012 could be due to the gradual stabilization of the system following application of organic fertilizers and rotation of soybean and sesame as preceding crops to sunflower.

The main agronomic traits that critically contribute to seed yield of sunflower include number of heads per hectare, weight of seeds per head and number of seeds per head (Robinson, 1978). However, in our study, the pooled mean revealed that cropping system significantly affected grain yield with the conventional and rotational cropping systems recording higher values for weight and number of seeds per head relative to sunflower under continuous copping system. Furthermore, the relatively lower values for plant height, number and weight of seeds per head, head weight and diameter on sunflower under continuous cropping system could also be attributed to depleted nutrients in the soil and accumulation of pest and disease organisms following continuous cropping of sunflower for the fourth year on the same plot. However, no serious disease or pest problem was recorded during our study.

CONCLUSION

Based on the pooled results of this study, the agronomic performance of sunflower that received organic fertilizer under rotational cropping system confirmed the huge potential for sunflower being a crop with high adaptability and low labour requirement as a viable component in organic crop rotation system in the tropics.

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Table 1: Mean monthly rainfall (mm) during the late cropping season (July – November) of 2008 - 2012

Year	July	August	September	October	November	Total
2008	299.2	106.7	136.8	84.5	0.0	328.0
2009	160.0	162.1	151.6	180.1	64.6	718.3
2010	322.9	266.6	257.6	172.3	94.7	791.2
2011	349.5	88.7	204.1	288.1	3.6	584.5
2012	155.4	36.3	181.4	184.7	49.6	607.4

Table 2: Crop rotation scheme involving soybean, sesame, sunflower and maize (2008 -2012)

2008	2009	2010	2011	2012
Sunflower	Sesame	Maize	Soybean	Sunflower
Sesame	Soybean	Sunflower	Maize	Sesame
Maize	Sunflower	Soybean	Sesame	Maize
Soybean	Maize	Sesame	Sunflower	Soybean

Table 3: Effect of cropping systems on plant height (cm) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	184.7	125.8	206.1	218.0	183.7
Rotational	208.0	209.0	224.3	255.8	235.0	226.4
Conventional	192.0	206.4	216.7	243.7	237.8	219.3
LSD 5%	ns	ns	ns	ns	10.19	32.32

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 4: Effect of cropping systems on head diameter (cm) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	9.6	9.1	14.1	17.1	12.5
Rotational	9.8	12.1	10.5	16.2	18.0	13.3
Conventional	8.6	12.8	11.3	16.4	18.1	13.4
LSD 5%	ns	2.17	ns	ns	ns	0.69

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 5: Effect of cropping systems on head weight (g) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	32.4	39.9	28.5	112.5	53.3
Rotational	60.3	58.0	57.2	41.3	123.5	68.1
Conventional	43.4	68.0	79.1	41.6	122.5	70.9
LSD 5%	ns	26.50	ns	ns	ns	13.36

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 6: Effect of cropping systems on seed weight (g) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	20.2	21.9	19.7	41.3	25.8
Rotational	21.5	33.1	31.9	37.9	57.7	36.4
Conventional	28.3	42.2	35.1	36.2	53.4	39.1
LSD 5%	ns	9.53	ns	ns	3.02	9.93

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 7: Effect of cropping systems on number of seeds per head of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	2008	2009	2010	2011	2012	Mean
Continuous	-	319.0	580.0	385.0	580.5	466.0
Rotational	540.8	680.0	853.0	547.0	607.0	645.4
Conventional	664.9	715.0	659.7	520.0	591.5	630.2
LSD 5%	ns	257.2	ns	ns	ns	57.44

Notes: **, * Significant at P ≤ 0.001 and 0.05, respectively, ns – non-significant

Table 8: Effect of cropping systems on seed yield (kg/ha) of sunflower during the late cropping season (July – Nov.) in 2008 - 2012

Cropping systems	**2008**	**2009**	**2010**	**2011**	**2012**	**Mean**
Continuous	-	778.0	1000.0	584.7	981.1	835.9
Rotational	540.8	1262.0	1150.0	1348.5	1428.2	906.0
Conventional	664.9	1642.6	750.0	808.9	1324.0	1038.0
LSD 5%	ns	366.75	ns	579.8	75.23	145.0

Notes: **, * Significant at P $\leq$ 0.001 and 0.05, respectively, ns – non-significant

LESSONS FROM TEN YEARS OF AN INTERPROFESSIONAL SURVEY PLAN ON OILSEEDS FOOD SAFETY

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ABSTRACT

French oilseeds food chain operators are coordinated through a food safety survey plan, in order to get a realistic picture of the contamination in oilseed products (seeds, oilseed meal, and vegetable oil). Concerned crops are those cultivated or processed in France: rapeseed, sunflower and soybean. Grain storage companies, feeding industries and oil industries participate voluntarily, and send their self-data that are pooled in a database. Thirty-three companies are actively involved, providing each year about 60000 to 180000 analytical results coming from about 2000 to 3000 samples of seeds, meals and oils (note: on one sample, several contaminants can be analyzed giving several analytical results). Pesticide residues represent more than 90% of the analytical results of this database as the laboratories can determine a large number of active substances with multi-methods. Other sought contaminants are: trace elements (cadmium, lead, arsenic, mercury, fluorine), mycotoxins (mainly aflatoxin B1 and total aflatoxins), toxic organic compounds (polycyclic aromatic hydrocarbons, dioxins and PCBs), microbiological contaminants (salmonella in meals), botanical impurities (eg seeds of *Datura spp.* in sunflower seeds), mineral oils or compounds likely to be formed during refining such as esters of 3-MCPD and glycidyl esters in oils. The food safety of oilseeds survey plan allows to identify which are main concerns, for instance post-harvest insecticide residues from cross contamination during storage. Results of this monitoring plan were transmitted to the French government and the European Commission in cases of regulatory threshold revisions (eg for cadmium in oilseeds, for the revision of pirimiphos-methyl thresholds).

Key words: Oilseeds, vegetable oil, survey plan, contaminants, pesticide residues

INTRODUCTION

The French oilseed food supply chain got together with food safety issues since the early 2000s that correspond to the establishment of a set of European regulations called "Hygiene Package" (Dauguet et al, 2006). In this context, the food safety survey plan (called PSO) was implemented from the 2005 campaign, helping to control the quality of products (seeds, meal and oil) in a interprofessional framework. Since PSO was launched, more and more operators of the oilseed supply chain have become active partners. This article gives a review of the seven years of the PSO.

Today, each operator of the food chain is facing a legal obligation:

- to implement a HACCP approach, based on sound analysis of health risks inherent in its business,
- to ensure the sanitary compliance of products that it puts on the market,

- to carry out self-monitoring.

The PSO, set up by Terres Inovia, ITERG and Terres Univia since 2005, is an observatory of the sanitary quality of oilseed products in France (Lacoste et al, 2005). This survey plan is based on a shared private database on oilseed contaminants. This base is fed by self-monitoring data from industries (crushing industry and feed industry) and storage agencies that join this PSO, as well as by series of analyzes on seeds, meal and oil by Terres Inovia, ITERG and Terres Univia (figure 1).

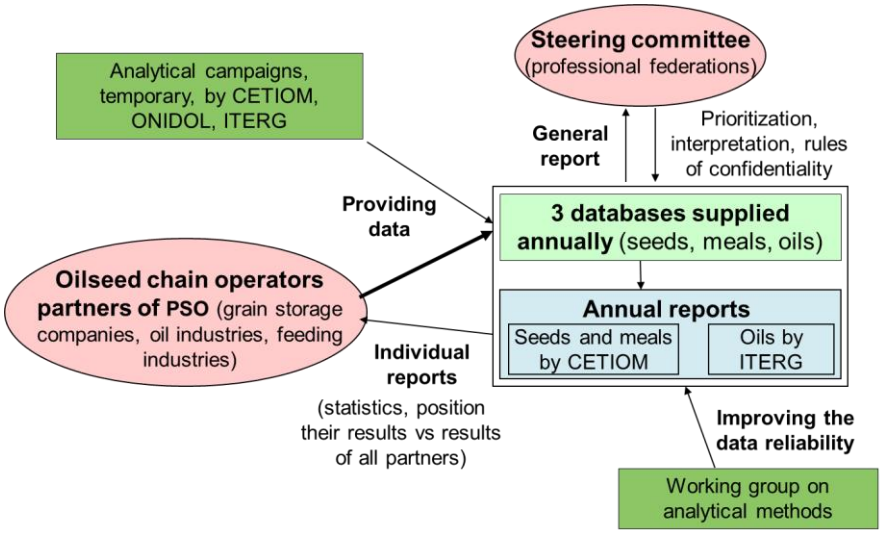

Figure 1. Organization of the Oilseed Survey Plan (PSO)

Intended for storage agencies to industrial oil processors and feed manufacturers, the PSO deals with:

- oilseeds: rapeseed, sunflower, soybean
- products: seeds, meals, crude and refined oils, byproducts of refining
- contaminants: residues of plant protection products, trace metals, mycotoxins, toxic organics, salmonella, botanical impurities ...

The confidentiality of data is guaranteed for partners, and no commercial exploitation of this database is made. The database on seeds and meals is managed by Terres Inovia, and the database on crude and refined oils is managed by ITERG.

So, the PSO is a tool of the oilseed supply chain, allowing a collective coordination on the safety aspects, highlighting progress and contributing to setting realistic regulatory thresholds. It represents also a forum for exchange of information between the operators in the sector, where are identified relevant research avenues.

A GOOD REPRESENTATIVENESS

To date, the PSO has 33 active partners: 28 grain storage agencies distributed throughout France, which represents 30-40% of the of the French oilseed harvest, 4 oil industrials (the main groups in France) and 1 partner in the feed industry, the OQUALIM association, which brings together 57 feeding companies (over 71% of the feed production). The representativeness of the PSO partners is correct. This plan is open to all interested companies and new members join it every year. Thus, each partner provides analysis data from its own self-monitoring data, and annually receives an individual report with its results compared to regulatory limits and to the overall PSO results: a moderate analytical investment gives access to a rich database, allowing refining its risk analysis.

For the last ten years, the PSO collected data annually from about 2,000 to 3,500 samples of seeds, cake, oils, and providing between 40000 and 120000 analytical results per

year (several contaminants checked in each sample). Plant protection products residues (pesticides) represent over 90% of the results. The other investigated contaminants are: metal and mineral trace elements (cadmium, lead, arsenic, mercury, fluorine), mycotoxins (aflatoxin B1 and total aflatoxins essentially), toxic organic (PAHs, dioxins and PCBs), microbiological contamination (salmonella cakes), botanical impurities (seeds of *Datura spp*. in sunflower seeds), mineral oils or compounds likely to form during refining such as the esters of 3-MCPD and glycidol esters in the oil.

THE PSO RESULTS

PSO allows us to check that almost all oilseed products comply with the regulations. Regulatory limits on oilseeds are defined in different texts: maximum limits for pesticide residues (MRLs, EC Regulation No. 396/2005 and Regulations amending it), maximum levels in feed (Directive 2002/32 / EC and texts the modifying) maximum levels in foodstuffs for human consumption (Regulation No. 1881/2006 and other regulations amending it).

However, PSO provides the observation that oil refining is necessary to remove some pesticide residues from crude oils. These are mainly insecticide residues, coming from post-harvest treatments (pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin), applied on the empty storage cells or on cereal grains stored in the same sites, and being incidentally found on oilseeds by cross-contamination (Dauguet, 2007; Dauguet, 2009). These molecules are then removed at various steps of oil refining, and therefore marketed refined oils are pesticide free.

Through the PSO, the real effort provided by crushing plant in order to control the microbiological quality of the meal could be checked: today salmonella nearly disappeared in rapeseed and sunflower meals produced in France.

The PSO suggests that mycotoxins are a danger almost inexistent for oilseeds, considering the regulated toxins. Only aflatoxin can be detected occasionally in sunflower, but at very low levels, far below the regulatory threshold. But monitoring of aflatoxins should as a regulatory threshold for aflatoxins in human food has been fixed for oilseeds intended for direct human consumption (2 mg/kg for aflatoxin B1 and 4 mg/kg for total aflatoxins) without industrial processing (confectionery sunflower), with a much higher maximum levels in feed (20 mg/kg for aflatoxin B1). For other not yet regulated toxins such as toxins of Alternaria, EFSA recommends Member States to acquire data in food. Within PSO, analyzes of these toxins have been carried out and their presence can be seen occasionally on sunflower. However, toxicological studies are not sufficiently substantiated to date to conclude on the risk posed by the toxins of Alternaria.

The trace metals are not a family at risk as oilseeds never exceed these regulatory limits. In the case of cadmium, the concentrations found in the sunflower seeds and meal can be sometimes close to the threshold in animal feed (1 mg/kg).

A contaminant was identified recently in the PSO: *Datura spp* seeds, which are botanical impurities that can be found in sunflower seed crops. This weed is toxic, since it contains tropane alkaloids, and the presence of *Datura spp*. seeds is regulated in the raw materials for animal feed (1000 mg/kg of whole seeds of *Datura*). Indeed, the alkaloids contained in these impurities will be transferred in the meal after the oil extraction process.

Organic toxic substances, such as polycyclic aromatic hydrocarbons (PAHs) and dioxins and PCBs, are specifically monitored in crude and refined oils. The levels measured for these substances show that these substances do not pose a problem in the French oilseed sector.

Recently, the presence of esters of 3-MCPD and glycidol esters has been reported in refined vegetable oils, and in formulated food products containing vegetable fats (Zelinková, 2006). Palm oil is the oil with the highest infection rates likely related to the high temperatures used

during deodorization of physical refining, while the seed oils are generally less prone to the formation of this contaminant (Kuhlmann, 2011). The few results collected via the PSO confirm the low contamination of refined rapeseed and sunflower oils.

Following a sunflower crude oil contamination from Ukraine by mineral oils (Lacoste, 2010), manufacturers have established since 2008 a systematic verification of import sunflower oil. The data collected within the PSO showed that the contamination in 2008 was an isolated case.

PSO, A TOOL FOR THE OILSEED FOOD CHAIN

The results of PSO therefore enable operators in the sector to carry out an analysis of health hazards in oilseed products. Thus, the subject of post-harvest insecticide residues appeared important. This encouraged the operators to carry out specific actions to identify the sources of cross-contaminations of oilseeds by these pesticide residues in storage facilities. Surveys conducted in collaboration with companies have enabled the identification of these situations leading to cross-contamination (Dauguet, 2007; Dauguet, 2009), and recommendations were relayed by the federations. According to the latest PSO results, the contents of these pesticide residues tend to decline.

The PSO has also been involved to argue for re-examine the maximum residue level of pirimiphos-in oilseeds, taking into account the phenomenon of cross-contamination during storage. This data were studied by EFSA which issued an opinion (EFSA, 2011) in which European food safety authority says that an MRL of 0.5 mg/kg would be suitable for oilseeds (while the current MRL was 0.05 mg/kg).

As part of the review of the regulatory thresholds of cadmium in food, PSO partners have also mobilized to provide the public authorities with data so that future limits are not an obstacle to trade in oilseeds. This issue mainly concerns the sunflower, which accumulates cadmium in its seeds. Today, none sunflower sample exceeded the regulatory threshold for feed, but a lower threshold could be a problem. Finally, this regulation does not apply to oilseeds. PSO data were transmitted to the French authorities in the context of the European discussions on the revision of cadmium thresholds, with the agreement of all PSO's members. This also illustrates the value of reliable data to assist in setting realistic regulatory thresholds.

CONCLUSION

The PSO is now considered a sustainable action for the benefit of operators in the French oilseed sector, which has no equivalent in other countries. In 2016, new means of communication and information are available for PSO members, with a dedicated and protected website. This provides more responsiveness and flexibility: more ease for online data entry and data reading.

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THE EFFECTS OF VACUUM AND ATMOSPHERIC DEEP-FAT FRYING PROCESS ON TOTAL FRYING-USE TIME OF SUNFLOWER OIL AND ON FRENCH FRIES QUALITY

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ABSTRACT

Deep-fat frying, which is one of the oldest and popular food preparation methods, is a process of immersing food in hot oil at a high temperature. In this study a vacuum cooking equipment prototype which could work both atmospheric pressure and under vacuum was developed for deep-fat frying process. The effect of vacuum and atmospheric frying temperature and number of frying in the same sunflower oil on the quality of French fries and sunflower oil was evaluated. Potato pieces was fried in ratio 1:6 (potato:oil) at atmospheric pressure and under vacuum at 135 and 180°C, respectively, for 10 min in every frying interval for a total of 7 (atmospheric pressure) and 15 (under vacuum) times of frying in the same oil. The free fatty acid content of the frying oil at atmospheric condition was determined to be excessively high compared to that of vacuum frying oil. TPM of oil at the atmospheric frying after the 3th frying rapidly reached to TPM content of the 15th vacuum frying oil. It was observed that peroxide value of the oil at atmospheric frying was higher than that of vacuum frying oil. Viscosity of the oil at atmospheric condition increased rapidly with an increase in exposure time compared to that of vacuum frying oil. The color values of vacuum and atmospheric fried French fries were not significantly different from each other. No significant changes in texture of French fries were determined with oil utilization time in the both of frying process.

Key Words : Deep-fat frying, vacuum frying, oil utilization time, sunflower oil, oxidation

EFFECT OF CURCUMIN NANOPARTICLES ON OXIDATIVE STABILITY OF SUNFLOWER OIL-IN-WATER EMULSIONS

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ABSTRACT

Curcumin is a natural polyphenolic compound that is obtained from the root of *Curcuma longa* Linn (turmeric). Oil oxidation is an undesirable series of chemical reactions involving oxygen that degrades the quality of oil. The aim of the present study was to develop a method to nano-particularize curcumin in order to increase its antioxidant efficiency against oxidation of sunflower oil. For this purpose, curcumin was dissolved in dichloromethane, injected in heating water (60 ºC) including tween 80 and then stirred. After characterization of the particle size and distribution of the fabricated curcumin nanoparticles, they were lyophilized. In formation of the oil phase of emulsion with nanocurcumin (ENC), nanocurcumin was added into oil-in-water system in which sunflower oil was used as the oil phase. Oxidation stability of oil-in-water emulsions including curcumin nanoparticles was measured by oxidation test reactor. As a result, 98 % of the particles were in mean diameter of 9-10 nm. The formed nanoparticles were characterized by scanning electron microscope, Fourier Transform Infrared Spectroscopy and thermogravimetric analysis. Unlike curcumin, nanocurcumin was found to be freely dispersible in the presence of the surfactant. The chemical structure of nanocurcumin was the same as that of curcumin, and no remarkable change was observed during nanoparticle preparation. Thermal degradation of the nanocurcumin was similar to that of curcumin. It was found that emulsion with nanocurcumin (ENC) was more effective than those with and without curcumin against oxidation of the sunflower oil, as revealed by the longer induction periods (IP) for ENC (1 hr 20 min) than those for emulsions with and without curcumin (60 min. and 53 min.) The results demonstrated that the water solubility and antioxidant activity of curcumin was markedly improved by particle size within the nano-range.

Key Words : Sunflower oil, nanocurcumin, nanotechnology, oxidative stability, molecular and thermal characterization.

DETERMINATION OF TEXTURAL, RHEOLOGICAL PROPERTIES AND SFC, SMP VALUES OF OLEOGELS PREPARED USING SUNFLOWER OIL

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ABSTRACT

In Recent years, food products which is designed to provide development for human health and researches is to improve such products have been intensively carried out all over the World. Oils Reduced Trans and saturated fatty acids levels have come firstly. To this end, oleogels, which have a spreadable elastic structure, by adding organic or polymer gelling agents (oleogelators) to oils, have been used. In our country, sunflower seed provides about 45% our total oil seed production and sunflower oil comes first in mostly consumed edible oils. Oil obtained from sunflower seed is rich in linoleic acid. Also recently, production of high oleic sunflower oil, by reducing linoleic acid content of sunflower oil, has been started. In this study, creating of oleogels formulations include sunflower and high oleic sunflower oil, have low amount of trans and saturated fatty acids, alternate to margarines and determination of textural, rheological, SFC and SMP values of this samples was purposed. For 6 samples (1 reference and 5 new formulations) Textural properties according to Ogutcu and Yilmaz, 2015; rheological properties according to Lupi et. Al., 2013 (with some modifications); SFC values according to AOCS Official Method Cd 16b-93:2009 and SMP values according to ISO 6321:2002 have been proceeding.

Key Words : oleogels, sunflower oil, rhelogical, SFC

AFLATOXIN CONTAMINATION IN SUNFLOWER OIL

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is an annual ornamental herb grown as an oil seed crop. Because of their chemical composition and nutritional value sunflower seeds are considered to be a great source of lipids and proteins and are largely used in the production of edible oils and animal feed. The protein content of the seeds is approximately 50%–60%. Sunflower oil is the preferred oil in most of Europe, East Europe, Russia, Mexico, countries along with Mediterranean and several South American countries. Mycotoxins are poisonous organic compounds produced by several species of fungi. In studies have shown that isolates of different mold species were able to produce aflatoxins B_1,B_2,G_1 and G_2, sterigmatocystin, ochratoxin A, patulin, citrinin, penicillic acid, zearalenone and griseofulvin in sunflower. Aflatoxins are a major group of mycotoxins, which have toxic, carcinogenic and mutagenic activity, causes important health problems and economic losses. The production of oils from oilseeds requires the following steps: storage of grains, preparation, extraction of crude oil and refine (degumming, deacidification, bleaching, deodorization). Some of these steps may be harsh and lead to inactivation or elimination of important compounds, such as vitamins, antioxidants and enzymes, although the effect on undesirable compounds like aflatoxins varies markedly among methods. The high contamination of oilseeds by aflatoxins generates a concern on a global scale due to the high consumption of these products. Several reports have shown high incidences of aflatoxin contamination in plant-derived oils in regions of China, Sudan, India and Sri Lanka. Experimental studies have shown that aflatoxins present in the oleaginous material can be transferred to the final oil product. However, depending on the type processing (extraction and purification) of the crude oil, the levels of these contaminants can be reduced.

Key words: Sunflower oil, mycotoxins, aflatoxins, oil processing

INTRODUCTION

Mycotoxins are secondary metabolites mainly produced by different fungal species when they contaminate food and feed. Various fungal species like such as *Aspergillus*, *Penicillium* and *Fusarium* under different climatic conditions of temperature and humidity can contaminate cereals. Mycotoxin contamination occurs frequently in various food commodities globally, leading to animal and human health risks. More than 400 mycotoxins have been identified and reported and the key important mycotoxins that are highly prevalent in the contaminated agro-food products are aflatoxins, ochratoxin A (OTA), deoxynivalenol (DON), fumonisins (FBs), zearalenone (ZEN), citrinin (CIT) and patulin. Among the types of aflatoxins are aflatoxin B_1, B_2, G_1 and G_2, which are a group of closely related mycotoxins (Selveraj et al., 2015).

Aflatoxin B_1, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, is one of the most toxic and common contaminants in food and feed. Ingestion of aflatoxin-contaminated food leads to acute and chronic toxic effects, which may be hepatocarcinogenic, mutagenic, teratogenic or genotoxic (Samuel et al., 2014).

Aflatoxins are commonly found in nuts, peanuts, corn, cottonseed and other oil seeds which affect not only the health of humans and animals but also the economics of agriculture and food. Aflatoxins are produced under optimum temperature and moisture conditions and cannot be completely avoided, so aflatoxins have become a threat worldwide. In order to protect human safety, limits on aflatoxins have been set in many countries. In the European Commission, the current maximum levels are 2 μg/kg for AFB_1 and 4 μg/kg for total aflatoxins for groundnuts, nuts, dried fruits and cereal (Fan et al., 2013).

Sunflower (*Helianthus annuus* L.) is an annual ornamental herb grown as an oil seed crop (Nahar et al., 2005). Because of their chemical composition and nutritional value sunflower seeds are considered to be a great source of lipids and proteins and are largely used in the production of edible oils and animal feed. The protein content of the seeds is approximately 50%–60%. (Beheshti and Asadi, 2013). Sunflower oil is the preferred oil in most of Europe, East Europe, Russia, Mexico, countries along with Mediterranean and several South American countries.

Sunflower seed contain 25-32 % edible oil which is a rich source of polyunsaturated fatty acids used for human consumption. Sunflower seeds also provide a nutritious food for cattle, poultry hogs and cage birds. The seeds are also consumed roasted, salted and a coffee substitute is prepared from roasted seeds. Of the different fungi isolated from sunflower seed, *Aspergillus flavus* Link. was found to be most predominant (Dawar and Ghaffar, 1991).

The high contamination of oilseeds by aflatoxins generates a concern on a global scale due to the high consumption of these products. Several reports have shown high incidences of aflatoxin contamination in plant-derived oils in regions of China, Sudan, India and Sri Lanka. Experimental studies have shown that aflatoxins present in the oleaginous material can be transferred to the final oil product. However, depending on the type processing (extraction and purification) of the crude oil, the levels of these contaminants can be reduced (Bordin et al., 2014).

SUNFLOWER OIL PROCESSING

Oil processing involves three major conventional processes which include continuous neutralizing, bleaching and deodorisation. Neutralisation of crude oil with caustic soda is still an essential feature for a refinery required to produce a consistently high quality product and to handle a number of different oil types. A bleaching step is necessary to remove soap, trace metals, sulphurous compounds and part of the more stable pigments and pigment breakdown products which have resulted from raw materials damage or oxidation. The deodorization process involves steam distillation under vacuum. Its purpose is to remove so far as possible residual free fatty acids, aldehydes and ketones which are responsible for unacceptable oil odours and flavors and, more recently to decolourise the oil by heat decomposition (270ºC) of the pigments and distillation of the decomposition products (Banu and Muthumary, 2010).

The sunflower oil process flow diagram is shown in Fig. 1 (Pal et al., 2015).

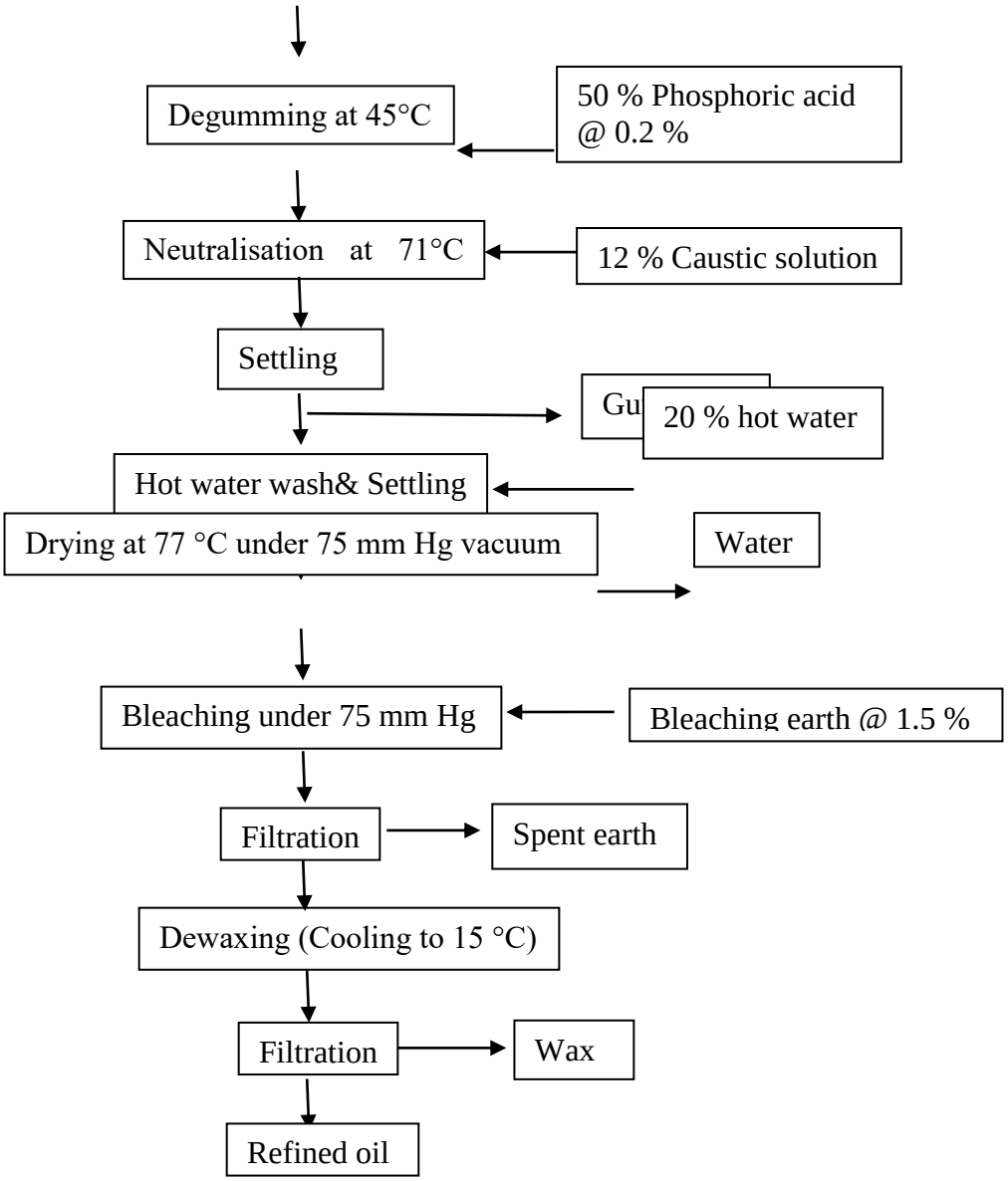

Fig. 1 Process flow diagramme for refining of sunflower oil

SUNFLOWER OIL AND AFLATOXIN

The seeds of sunflower (*Helianthus annuus),* may first have been cultivated by Indian tribes in North America, about 3000 B. C. The seeds were used as a food, medicine and as a religious symbol. The plant was brought by Spanish travelers to Europe sometime around 1500 AD. The sunflower thrives in temperate climate and is today cultivated in USA, Europe, Russia and Canada among others. For the food industry the seeds are largely cultivated for oil production, but the seeds are also used for food or bird feed (National Sunflower Association 2013). Sunflower seeds can contain up to 45% oil (Eklöf, 2013). Sunflower seeds are a good substrate for aflatoxin production. Lipids may play, an important role in the biosynthesis of aflatoxin (Chulze et al., 1990).

The mycoflora of sunflower seeds appeared to be diverse, with many toxin producing species as common contributors. The analysis of toxins did however not detect any toxins, and since

the commodity was storage stable (the highest aw detected was 0.63), there did not seem to be any risk involved with consuming these products. However, if the commodity would be exposed to moisture there would be a risk of toxin production. Abdullah et al. (2010) found common genera in sunflower seeds to be *Aspergillus, Alternaria, Fusarium,* and *Penicillum. A. niger* and *A. flavus* were the most common species, but also *Penicillium expansum* were common. Jimenez et al. (1991) found highest counts of *A. niger* and *Penicillium spp*. and Shahnaz et al. (1991) found high incidence of *A.flavus* and *A. niger.* Sharfun-Nahar et al. (2005) also found along with high incidence of *Penicillium* spp. The study also revealed *A. ochraceus* as a contributor to the mycoflora of sunflower seeds, and both studies found species of *Alternaria* and *Fusarium* (Eklöf, 2013).

The oil extraction step, both by pressing and solvent, is not capable to eliminate the aflatoxins. In fact, the toxin is shared between the phases, remaining in both the oil and defatted meal. The oil extraction with non-traditional solvents has been shown viable, and studies performed with ethanol, and isopropanol have also shown the ability to partially remove aflatoxins from oil seeds. However, a point to be elucidated is the appropriate treatment given to the solvent leading to the elimination of toxins, so that it allows its reuse in the process (Bordin et al., 2014).

There are no studies in the literature that relate the degumming with the presence of aflatoxins. However, it is known that the toxins are soluble in polar organic solvents. Thus, it is assumed that the procedures used in this step may reduce contamination of the vegetable oil (Bordin et al., 2014). Aflatoxins decompose at high temperatures ranging from 237 to 306°C. Thus, the conditions adopted in deodorizing process can be essential for complete removal of aflatoxins from vegetable oils (Bordin et al., 2014).

Parker and Melnick (1966) were the first researchers to assert that the refining process is effective in removing aflatoxins. The authors evaluated the effect of chemical refining on peanut crude oil initially infected with 812 μg/kg aflatoxin, being possible after the bleaching stage, obtaining an oil containing traces of aflatoxins in a concentration lower than 1 μg/kg.

Banu and Muthumary (2010) reported that among the 23 different crude sunflower oil samples were tested, 10 of them showed positive results to AFB_1 and the remaining 13 showed negative results to AFB_1. All the refined oil samples were free from AFB_1 contamination. This was supported by the absence of fungi in the refined oil samples. This may be due to oil processing which includes continuous neutralization, bleaching and deodorizations. During these processes, fungal propagules are probably removed from the oils. The toxic AFB_1 have been found to be heat stable up to their melting points of around 250°C. Therefore, AFB_1 was not completely destroyed by such processes and was carried along the way from seeds to oil samples. The complete conventional processes remove these compounds from the crude oil. But the quantity of contamination is very least ranging from 0.1 to 0.4 ppm. This low level was due to extraction of oil using food grade hexane. The extraction plays a role of partially removing aflatoxin from the oil samples.

CONCLUSION

Knowledge of contaminating sunflower mycoflora is important because undetectability of a mycotoxin at the time of analysis does not mean that this metabolite could not be found later if the toxigenic species is present in the sunflower, and if favorable conditions allow for fungal development and mycotoxin formation. Control of moisture and temperature levels of these commodities is necessary to prevent mould growth and mycotoxin production.

Mycotoxins are partially destroyed in refined oils by the conditions employed in the refining stages. Regardless the process for obtaining oil by pressing or solvent from a feedstock contaminated with aflatoxins, studies have showed that the toxins are partitioned between the oil and meal, requiring the application of physical, chemical or biological products for the reduction or elimination of these contaminants. There is limited evidence indicating that the process of refining crude oil was efficient for removing not only the aflatoxins, but also other mycotoxins produced by *Fusarium* spp., such as trichothecenes and zearalenone. Thus, it is possible to ensure safe edible oil for human provided it is properly processed. (Bordin et al., 2014).

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APPLICATION OF COLD NEUTRALIZATION IN SUNFLOWER OIL REFINING

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ABSTRACT

The aim of refining oils is to remove impurities from crude oils and to give them edible properties. The crude oils may be subjected to degumming, neutralization, bleaching, deodorization, and possibly winterization. In continue system of chemical refining; neutralisation and degumming processes were done with together at the same stage. Then oil bleached with adsorbents and deodorised. Finally in winterization step, oil is cooled, waxes and stearins crystallized and then filtered with helping agent perlit. Cold neutralization is a new process for chemical refining used in a few oil factories in Turkey. In cold neutralization, a part of the winterization step, neutralization and degumming is carried out together. The necessary amount of phosphoric acid is added to oil, mixed and cooled to 5 ^{o}C. Then required amount of caustic added to oil and kept in the crystallization tank at 5 ^{o}C for 12 hours and then waxes and soap-stock are removed by centrifugation from oil. The % 90 of stearin and waxes of oil is removed in cold neutralization. This application simplifies the winterization step in conventional chemical refining. The amount of perlite used in the winterization step decreases, it becomes easier filtration, the soap stock that contains waxes is sold as a more economical byproduct. In this research, the effects of hot and cold neutralization on oil quality especially waxes and oil loss during neutralization, the capacity usage and labor requirements in cold neutralization have been revealed.

Key words: cold neutralization, sunflower oil, oil refining

COMPARISON OF GAS CHROMATOGRAPHY AND NEAR-INFRARED REFLECTANCE SPECTROSCOPY METHODS FOR THE DETERMINATION OF FATTY ACID COMPOSITION OF SUNFLOWER SEED

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ABSTRACT

This study was performed in order to evaluate performance of Near-Infrared Reflectance Spectroscopy (NIRS) which is used to determine oil and fat content, oleic and linoleic acid percentages of sunflower seed by comparing with soxhelet extraction and gas chromatography methods (GC). Oil and fat contents of 34 different sunflower seeds were determined by soxhelet extraction method. Oleic and linoleic acid contents were then evaluated using GC equipped with capillary column and FID detector. Sample spectrums and approximate results were determined by a previously developed calibration method with determinate parameters (RSQ:0.8001, 0.9960, 0.9974 for raw oil content, oleic acid and linoleic acid, respectively) using Foos NIRS System XDS near-infrared Rapid Content Analyzer. Average oleic and linoleic contents of sunflower seeds using GC equipped with capillary column and FID detector were calculated as 53.24 and 33.13%. When the averages of oleic and linoleic acid contents were determined by NIRS, average oleic acid content were found to be 53.40% while 33. 48% was average linoleic acid content. The results of this study showed that NIRS-based method can be reliably used in the determination of oil and fat content, oleic and linoleic acid contents of sunflower seeds. The important contribution of this study is that NIRS-based method can be used as a quick and accurate method in the marketing of sunflower seed and as an environment-friendly method since no chemicals used in this method.

Keywords: Sunflower oil, Oil and fat content, Oleic acid content, Linoleic acid content, NIRS

INTRODUCTION

History dating back to 3000 B.C. and cultivating in a large area in Turkey, sunflower (Helianthus annuus L.) is considered to be one of the most important oil plants in both in Turkey and in the world with the rate of 22-55% oil content. With approximately six million decares planting area, sunflower can be grown in almost all regions in Turkey and contains high amount and good quality oil (TÜİK, 2016). It supplies half of our vegetable oil consumption (BYSD, 2016).

Sunflower oil containing approximately 15% saturated, 85% unsaturated fatty acid and consisting of 14 - 43% oleic and 44 -75% linoleic acid in its unstaurated fatty acids,standard type sunflower oil is one of the most important vegetable oil in terms of oil composition. It is

also among most important oils in human nutrition. In recent years, a range of variety and quality sunflower oil has been produced via the development of mid-oleic type (43.1-71.8%) and high oleic type (75-90.7%) sunflower varieties that has higher oleic acid content than standard sunflower type. There is a growing need for the oils that are less saturated, resistant to oxidation and durable to heat treatment by the change of our consumption habits. In last years, there is also another growing interest in sunflower oil due to its use in other fields apart from food industry. Since the development of high oleic sunflower hybrids, sunflower oil has become more important raw material for the oleochemical industry which includes cosmetics industry.

Food safety and food quality that have close relations with social development and human health are still considered as an important issue in all countries of the world. Day after day, consumers are searching for quality labels on food products and signs that are reliable, they expect high quality products from manufacturers. All of these factors emphasize the importance of reliable techniques for assessing the quality of food.

When the application needs are taken into consideration, the development of fast and effective methods like NIRS technology become apparent. In recent years, there is a growing interest in fast, reliable and enviromentally friendly technologies both in food production and food research. Consequently alternative technologies are being developed to conventional ones. One of the most important of these technologies widely used is the NIR spectroscopy (Cen and He 2007). Used in food analysis after appropriate calibration, fast, reliable and environmentally friendly, NIR Spectroscopy is a technology used for analysis and based on electromagnetic radiation absorbtion in 400-2500 nm wavelength range. (Davies ve Granth, 1987).

NIR spectroscopy , based on the resolution of the analytical and quality factors from food samples with correleation of electromagnetic absorption at aforementioned wavelength, allows to be used routinely in sensory, physical and chemical analysis of food and agricultural products. (Williams ve Norris 1987). For this purpose, studies were conducted to determine crude fat content of the oil plant and fatty acid composition by NIR spectroscopy. (Velasco ve ark. 2004; Koprna ve ark. 2006; Akkaya ve ark. 2015).

It is necessary to determine oil content and fatty acid composition by fast and reliable methods in sunflower seed which holds an important place both in national production and in importation. This study was performed in order to evaluate performance of NIR Spectroscopy which is used to determine oil and fat content, oleic and linoleic acid percentages of sunflower seed by comparing with soxhelet extraction and gas chromatography methods (GC).

MATERIALS AND METHODS

In this study, 34 pieces of different sunflower varieties in which reclamation and adaptation studies are done in East Mediterranean Agronomic Institute testing ground are used as material. Crude oil ratios were determined by Soxhlet extraction method, the ratio of oleic acid and linoleic acid was determined by FID detector gas chromatography capillary column method of sunflower seed oil samples.

FOSS NIRSystem XDS near-infrared Rapid Content Analyser apparatus is used to receive spectrums and determine estimated values of the spectrum of sunflower samples in which classical analysis were completed . Spectra of ground sunflower seed samples were taken to be every 2 nm in between 400-2500 nm wavelength. In determining the estimated

value, the information belongs to NIRS analysis calibration model which is previously developed by using WinISI III v1.61 software package, can be seen in Table 1.

Table 1 The statistics belong to calibration method developed to estimate dry matter, crude oil,oleic acid and linoleic acid rates

Properties	Average±SD	Min (%)	Max (%)	RSQ	SEP	Bias	Slope
Dry Matter Rate (%)	93.79±0.94	90.95	96.62	0.9026	0.286	0.000	1.000
Crude Oil Rate (%)	36.31±5.52	19.75	52.87	0.8001	2.394	-0.076	0.991
Oleic Acid Rate (%)	46.87±16.15	0	95.31	0.9960	0.946	0.043	0.998
Linoleic Acid Rate (%)	40.64±15.04	0	85.75	0.9974	0.793	-0.054	0.998

SD: Standart Deviation, RSQ (Coefficient of determination of Calibration), SEP (Standart Eror of Prediction)

RESULTS

The estimated values of the research in NIRS analysis device and the values determined by conventional analysis methods can be seen in Table 2. In this study, crude oil contents were determined between %32.80 and %48.40 by soxhelet oil extraction method, oleic acid ratio were determined between %34.41 and %80.29 by FID detector capillary column gas chromatography method while the ratio of linoleic acid was determined between %6.45 and %51.93 . Crude oil ratio estimated at NIRS is between 32.45% and 49.61%, oleic acid ratio is between 32.03% and 87.77%, linoleic acid ration is ranged from 3.66% to 51.29% . The average value of the crude oil determined by Soxhlet extraction method was %40.06 while the average values of crude oil estimated by NIRS was found to be %40.44. The average values of oleic acid determined by FID detector gas chromatography capillary column method was %53.24, the average value of linoleic acid was %33.13, while the average value estimated by NIRS was 53.40 for oleic acid and 33.48 for linoleic acid.

In conclusion, this study demonstrates that NIRS can be used reliably to determine crude oil, oleic acid and linoleic acid rates in sunflower seeds. In addition, it shows that NIRS analysis method can be fast and effective analysis method in both vegetable oil industry and sunflower seed trade and marketing and be greener compared to conventional chemical analysis methods due to fact that it has no use of any chemicals.

Table 2. Average values were determined by conventional analysis and average values were estimated by NIRS in sunflower seed samples

Sample No	Conventional Analysis Crude Oil Rate (%)	NIRS Crude Oil Rate (%)	Conventional Analysis Oleic Acid Rate (%)	NIRS Oleic Acid Rate (%)	Conventional Analysis Linoleic Acid Rate (%)	NIRS Linoleic Acid Rate (%)
1	44,93	45,80	60,64	64,30	25,88	22,84
2	40,96	38,86	43,12	40,40	44,24	45,06
3	41,43	41,19	44,56	42,83	40,14	44,09
4	43,68	42,18	46,61	49,13	40,79	39,78
5	33,01	32,45	75,03	70,02	12,24	14,56
6	32,80	33,62	80,09	75,89	7,11	10,20
7	40,73	41,08	34,41	32,03	51,93	51,29
8	41,00	42,63	78,53	78,66	7,72	7,00
9	46,96	47,23	45,01	43,17	42,35	42,49
10	39,11	39,13	55,3	53,97	31,65	30,70
11	44,47	43,76	80,29	87,77	6,45	3,66
12	34,22	34,93	46,47	43,25	36,31	40,78
13	34,52	33,71	49,78	49,84	36,11	34,73
14	47,53	48,23	45,06	45,92	41,01	40,39
15	40,11	41,16	47,04	46,41	40,43	42,48
16	42,67	41,10	58,14	61,41	23,74	25,15
17	43,15	40,57	49,85	54,37	36,91	34,52
18	42,20	43,97	48,24	44,76	37,81	39,34
19	37,16	39,25	52,55	58,27	33,14	31,38
20	48,40	49,61	43,67	44,43	44,56	44,31
21	38,32	38,25	43,36	48,00	42,79	42,43
22	41,65	42,80	51,79	51,62	34,78	33,94
23	43,67	44,72	46,2	45,63	42,51	43,39
24	23,57	33,48	44,32	44,86	40,2	39,41
25	40,83	41,60	48,04	45,94	39,6	42,77
26	47,26	47,61	50,88	54,99	35,69	34,86
27	39,05	36,80	52,27	49,88	32,86	36,08
28	33,81	34,99	43,19	41,40	43,72	42,68
29	43,25	44,14	44,08	40,15	40,46	45,19
30	39,81	38,86	56,28	59,38	28,39	28,52
31	39,17	39,64	50,58	54,86	36,18	33,62
32	33,86	33,64	61,86	59,02	26,42	27,53
33	43,79	44,64	55,06	59,40	32,96	28,98
34	35,07	33,27	77,87	73,57	9,36	14,29
Max Value	48,4	49,61	80,29	87,77	51,93	51,29
Min Value	32,8	32,45	34,41	32,03	6,45	3,66
Average	40,06	40,44	53,24	53,40	33,13	33,48

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AROMA DETERMINATION OF A REFINED SUNFLOWER SEED OIL BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY USING DIFFERENT EXTRACTION METHODS

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ABSTRACT

The sunflower (*Helianthus annuus* L.) seeds are eaten raw, roasted, cooked, dried, and ground, and used as a source of oil. Edible vegetable oils are important to our daily life by providing energies, nutritional compounds, and desirable flavors. Sunflower seeds are usually processed in large oil mills using solvent to extract oil and refining it. Three typologies of sunflower oil, characterized by diverse percentage of oleic acid are present on the market: a low, mid and high oleic sunflower oil. Refined sunflower oil, especially high-oleic, is very versatile and due to its neutral flavour and heat stability it can be consumed in many ways in the kitchen, such as frying and cooking. Edible oils play a significant role in the food industry due to both their functional and nutritional features and their impact on taste, aroma and health. Aroma is a main quality factor for edible vegetable oils as a characteristic parameter. Many extraction techniques have been carried out to extract the aroma compounds of oil. Therefore, in this study, aroma compounds of a refined sunflower oil obtained from a local market in Adana was extracted by different isolation methods including solid phase extraction (SPE), simultaneous distillation extraction (SDE) and purge and trap extraction (PTE). Afterwards, aroma compounds of the extracts were identified and quantified by gas chromatography (GC) coupled with a mass spectrometry (MS) and flame ionization detector (FID). Among the extraction methods, the PTE was quantitatively and qualitatively detected as the most suitable method for the extraction of aroma compounds in the studied sample.

Keywords: Refined sunflower oil, aroma profile, extraction techniques, GC-MS

THE EFFECT OF THE ESSENTIAL OIL FROM *CITRUS AURANTIUM* AS A SOURCE OF NATURAL ANTIOXIDANT IN SUNFLOWER OIL

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ABSTRACT

Edible vegetable oils undergo the oxidation, e.g. oxygen in the air during storage or heat process and etc. As a result of the oxidation, undesirable rancid taste, changes in colour, losses of odour and flavour, deterioration of essential fatty acids and vitamins occurs in oil. In the manufacturing, oxidation occurs spontaneously in oils because of the physical and technological methods. The synthetic antioxidants like butylated hydroxyanisole (BHA) , butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) have been popularly used as the antioxidants in oils; however these chemicals show some undesirable effects on health. The aim of the present study was to determine the effect of the essential oil from C*itrus aurantium (*bitter orange) as a natural source of antioxidant, which is an alternative to widely used and known synthetic antioxidants in the sunflower oil. Different concentrations (0, 200, 400, 600, 800 and 1000 ppm) of the essential oil and BHT (200 ppm) were added to sunflower oil emulsion in uncapped vials and then incubated in darkness for 7 days at 60^0C. Samples were examined at 24 h intervals. The oxidative stability of the samples was evaluated by peroxide value (PV) and free faty acid (FFA). ANOVA results showed that the peroxide value and acidity of the oils in treated with essential oil of C. *aurantium* at the following concentration of 200, 400, 600, 800, 1000 ppm and BHT were significantly lower than those of the control groups. Nevertheless, peroxide and acidity values of these samples increased with increasing time.

Key words: Sunflower Oil, Essential Oil, *Citrus aurantium,* Natural Antioxidant, BHT

INTRODUCTION

Vegetable oils, are very important components of our diet, which undergo oxidation during storage and heating process because of many factors especially oxygen in the air. In vegetable oils, oxidation results in many undesirable consequences such as rancid taste and odours, reduction in the shelf life, decrease the nutritional quality (Sikwese and Doudu, 2007). Therefore, manufacturers prefer to utilize the antioxidants in order to prevent the oxidation.

According to the Turkish Standards, there has been some limitations for the uses of synthetic antioxidants in the oil. It has been previously reported that syntetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) may cause many healthy risks, including cancer and carcinogenesis (Iqbal and Bhanger, 2007). Therefore, using natural antioxidants instead of synthetic ones has become popular in recent years., Today, essential oils are already

commercially available. Some of them are classed as generally recognized as safe (GRAS) food additives in the USA (Burt, 2004).

In recent years, uses of the plant extracts have received ongoing interest on the stabilization of the edible oils. For example, the pomegranate peels extract was found to be a potent antioxidant for the stabilization of sunflower oil (Iqbal et al., 2008); methanolic orange peel extract was reported more superior than that of BHT on the stability of crude peanut oil stored for twelve months at room temperature against oxidative rancidity (Arawande and Borokini, 2015). The essential oils of some medicinal and aromatic plants e.g. thyme, clove, orange peel, coriander, garlic and cumin have been tested for their antioxidant potential in different edible vegetable oils.

The aim of the study was to evaluate the antioxidative effects of the essential oil from bitter orange pell during the storage of sunflower oil. The hydrodistillated essential oil from the pell of *C. aurantium* at different concentrations ranging from 200 to 1000 ppm was tested in the sunflower oil. All treatments were stored at 60^0C during one week. The peroxide value and free fatty acid were analysed on each day. The results were compared with the synthetic antioxidant (200 ppm BHT) and that of the control groups.

MATERIALS AND METHODS

Preparing Essential Oil

The essential oil of the peel of *Citrus aurantium* was hydrodistillated for 3 h using Clevenger type apparatus. After distillation, essential oil was dried with anhydrous sodyum sulphate to remove the water from the distillate and then preserved in dark vials at +4°C for further analyses.

Addition of Additives to Sunflower Oil

The essential oil of the bitter orange peel at various concentrations ranging from 200 to 1000 ppm were separately added to sunflower oil in glass bottles and they were thoroughly shaken for proper mixing. Sunflower oil containing 200 ppm BHT and the one that had including no additive (also described as 0 ppm as the control groups) were also setup. Each glass bottle was appropriately labeled and stored in an open place at $60\ ^0$C.

Testing the Oxidative Stability

The stability of emulsions to oxidation was evaluated each 24 h over a 7-day period by analyzing the peroxide values (PVs) and free faty acid (FFA) levels.

PVs were measured on a daily basis. For this purpose, 2 g of oil was initially weigthed and then dissolved in chloroform (10 ml) and glacial acetic acid (15 ml). This was followed by adding 1 ml of saturated KI solution. The solution was throughly mixed for 1 min and then kept in the dark for 5 min. After addition of distilled water (75 ml), the mixture was titrated against sodium thiosulphate (0.01 N) using starch as an indicator. A blank titration was done parallel to treatment and PVs (meq of oxygen/kg) was calculated using the following formula:

$$\text{Peroxide value} = 1000\frac{SXN}{W}$$

In this formula, S is the volume of sodium thiosulphate solution (blank corrected) in ml; N is the normality of sodium thiosulphate solution (0.01 N) and W is the weight of the oil sample (g) (Anon., 1975).

FFA of each oil sample was monitored each day using the standard method for 7 days (Anon., 2003). For this purpose, a known weight of oil sample (3 g) was dissolved in 95% ethanol (75 ml). The mixture was titrated against KOH (0.01 N) using phenolphthalein as an indicator. A blank titration was done parallel to treatment and FFA (%) was calculated using the following formula:

$$\text{FFA} = \frac{SXNX28{,}2}{W}$$

In this formula, S is the volume of KOH in ml; N is the normality of KOH solution (0.01 N) and W is the weight of oil sample (g).

Statistical Analysis

One-way analysis of variance (one-way ANOVA) was carried out on the results. Data was processed using SPSS for Windows 18.0.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Figure I. depicts peroxide value (PVs) of sunflower oil stored with the essential oil from bitter orange peel and butylatedhydroxytoluene (BHT) for 7 days. It was observed that sunflower oil containing 200 ppm to 1000 ppm essential oil and 200 ppm BHT had lower peroxide values than those of the control groups during storage.

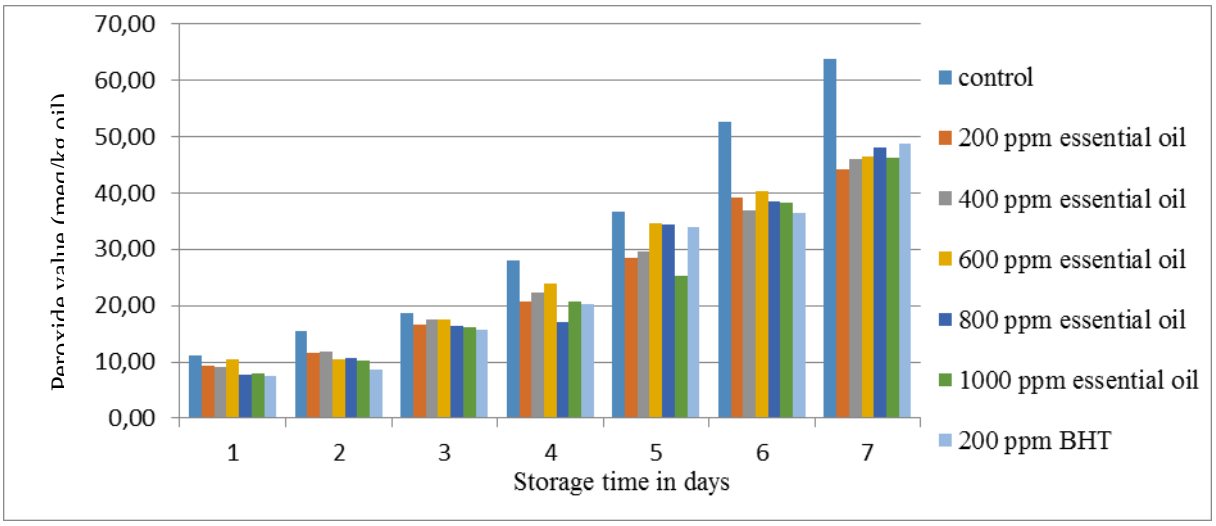

Fig. 1. Peroxide value (meq O_2/kg) of sunflower oil stored at 60^0C

This is in accordance with results of Kamkar et al. (2010) who reported that methanol and water extracts of Iranian pennyroyal in sunflower oil have better antioxidant activities than those of the control groups.

In a study of Shyamala et al. (2005), peroxide value of *M. pulegium* extracts was lowered than the control groups. The present values are in close agreement with findings of Shyamala et al. (2005) who found that extracts of four leafy vegetables which were added to refined sunflower oil conferred a protective effect on peroxide formation.

Figure II. depicts free fatty acid (FFA) of sunflower oil stored with bitter orange peel's essential oil and butylatedhydroxytoluene (BHT) under storage at 60°C for 7 days. It was observed that sunflower oil containing 200 ppm to 1000 ppm essential oil had lower FFA values than control groups during storage.

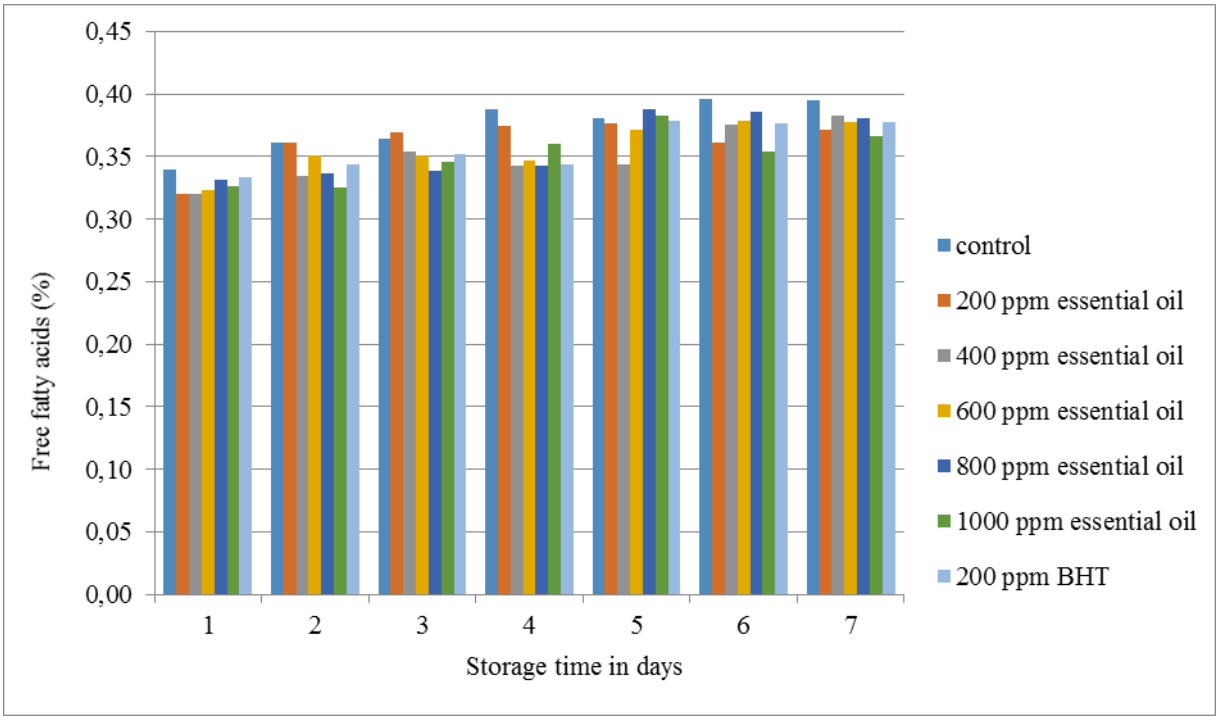

Fig. 2. Free fatty acid content (FFA) of sunflower oil stored at 60^{0}C

These results are in accordance with results of Arawande et al. (2014) who reported that the FFA of oil containing orange peel extract and 200 ppm of BHT was lower than the control groups.

Rehman (2006) also reported that after 6 months of storage, corn oil containing citrus peel extract showed lower FFA contents, and peroxide value levels than the control.

The present results show that all concentrations of the essential oil of *C. aurantium* showed more oxidative stability than that of the control groups. Furthermore, there were no significant differences among the oil groups which include essential oil ranging from 200 to 1000 ppm.

CONCLUSION

The results of the present study apparently indicated that essential oil distillated from bitter orange peels had significant antioxidant activity. It has been widely accepted that the stabilization of the sunflower oil is very difficult because of its high content of linoleic acid.

Two fold concentrations of the essential oil ranging from 200 to 1000 ppm were shown to be strong protective effects against lipid oxidation in the sunflower oil during the storage period. The findings of this study indicated that bitter orange peel exract could be suggested as a potential antioxidant for the stabilization of sunflower oil.

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CHARACTERIZATION OF SUNFLOWER OIL OLEOGELS PREPARED WITH BEESWAX AND SUNFLOWER WAX

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ABSTRACT

In this research we used the sunflower oil to create oleogels with beeswax and sunflower wax as the organogelators at 5% (w/w) addition level. The oleogels were prepared at 90 ºC isothermal conditions, and formed at room temperature overnight. Thermal behaviours of the oleogels were investigated using differential scanning calorimeter (DSC). The sunflower oleogel melting temperature was found to be between 60-65 ºC, while the beeswax oleogel were 45-50 ºC. The crystallization temperatures of the sunflower wax oleogel were ranged from 55 to 60 ºC and beeswax oleogel were from 35 to 40 ºC. The firmness values of oleogels determined by TA-XT Texture Analyzer. The firmness values of the beeswax and sunflower wax oleogels were found around 1.0- 2.0 N and 3.0 – 4.0 N, respectively. These parameters provide information about oleogels hardness and spreadability. The oil binding capacities of beeswax and sunflower wax gels were ≥ 99%. Solid fat contents of beeswax and sunflower wax oleogels determined at 35 ºC were ranged between 2.00 and 2.50%, and 3.00 and 3.50%, respectively. The X-ray diffraction peaks observed at 4.10 and 3.70 Å demonstrated that the oleogels had crystalline structure similar to β′ polymorphs of triglycerides. In conclusion *trans*-free spreads or margarines based on sunflower oil oleogels could be created as solid fat stock alternatives. Sunflower oil could be a valuable alternative to create oleogel stocks to produce margarine, spread, shortening and similar products.

Keywords: Sunflower oil, oleogels, hardness, melting point, X-ray diffraction

QUALITY CHARACTERISTICS OF THE OILS OBTAINED BY COLD PRESSING TECHNIQUE

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ABSTRACT

Cold pressing is used to extract oil from plant seed instead of conventional solvent extraction method since cold-pressing does not require the use of organic solvent or heat. The cold pressing procedure involves neither heat nor chemical treatments, and it is becoming an interesting substitute for conventional practices because of consumers' desire for natural and safe food products. Cold pressing also involves no refining process and may contain a higher level of lipophilic phytochemicals including natural antioxidants. Cold-pressed oils refer to oils that are extracted by cold-pressing plant seed with a screw press or hydraulic press. Cold-pressing is able to retain bioactive compounds such as essential fatty acids, phenolics, flavonoids and tocopherol in the oils. Hence, cold-pressed seed oils contain these bioactive compounds that exert health benefits. Sunflower (*Helianthus annuus*) and canola (*Brassica napus*) seed oils are examples of oils that are extracted by cold-pressing. Cold-pressed oils are considered as healthy oils that are important to human nutrition due to their favorable polyunsaturated fatty acid content, notably α-linolenic acid (C18:3; *n*-3) and linoleic acid (C18:2; *n*-2). Cold pressed oils are a good source of beneficial components, such as antioxidative phenolic compounds and other health-beneficial phytochemicals. Moreover, they are free of chemical contamination. The cold press which not exceed 50 °C preserves bioactive components, such as vitamins, provitamins, phytosterols, phospholipids and squalene). In addition, it has been proven that these components have a positive effect on human health.

Key words: cold press technology, quality characteristics, fatty acids.

EFFECTS OF TEMPERATURE AND VACUUM PARAMETERS APPLIED DURING DEODORIZATION STEP ON SUNFLOWER OIL QUALITY

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ABSTRACT

Deodorization is a crucial refining stage which aims to vaporize odoriferous compounds and free fatty acids from sunflower and other vegetable oils by applying high temperatures and low pressures. Deodorization, the common process used for the refining of edible fats and oils, has an important effect on the quality of refined sunflower oil. Although deodorization targets to remove only undesirable compounds, other components with comparable volatilities are also lost. Deodorization represents a critical step in the refining process as it involves high temperature (180–220 °C) that could induce degradation reactions under low pressure (1–10 mbar). The effect of deodorization temperature has been evaluated on the formation of *trans* fatty acids and long-chain PUFAs (LC-PUFAs) geometrical isomers that influence the final quality of vegetable oils. The main factor that controls the speed of the isomerization reaction is the deodorization temperature. Moreover, long time exposure of high temperature in deodorization enables carotene to be destroyed therefore the process needs to be controlled to minimize carotene decomposition. Distillation under vacuum is a principal process in deodorization. The purpose of this process is the removal of undesired volatile odoriferous components in sunflower and other vegetable oils, namely aldehydes, ketones, and free fatty acids. In conclusion, temperature and vacuum parameters should be under controlled during the deodorization process.

Keywords: deodorization, temperature, vaccum, oil quality

DIFFERENT EXTRACTION METHODS FOR SUNFLOWER AND OTHER EDIBLE OILS

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ABSTRACT

Nowadays, solvent extraction techniques are widely used in the production of sunflower and other vegetable oils. In recent years, increased sensitivity to the environment, efforts to improve the oil yield and quality, the desire to reduce the necessary of refining and to obtain more healthy edible oils are caused new extraction techniques development. These techniques can be considered as supercritical fluid extraction, ultrasound and ultrasound-enzyme assisted extraction, ultrasound-microwave assisted extraction, aqueous enzymatic extraction. Furthermore, these methods are also integrated with the solvent extraction process for sunflower and other edible oil production and applicability was investigated. In this review, the application of these methods in vegetable oil production were discussed.

Key words: ultrasound extraction, supercritical fluid extraction, aqueous enzymatic extraction, oil extraction.

FRYING PERFORMANCE OF HIGH OLEIC SUNFLOWER OILS

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ABSTRACT

Deep fat frying is one of the oldest and popular culinary techniques used in food cooking throughout the world. During the frying process, undesirable reactions like oxidation, hydrolysis, polymerization, isomerization etc. occur and these reactions cause quality deterioration in both oil and fried foods. The frying conditions as the temperature and time, ratio of food to oil, presence of oxygen and metals, and especially the composition of the frying oil used are the main factors that affect the deterioration of frying oil. Conventional sunflower oil is highly susceptible to lipid oxidation because it has approximately 70% linoleic acid content. High oleic (75-90%) sunflower oils have been shown to exhibit significantly higher oxidative stability than regular sunflower oil during frying. In addition, high oleic sunflower oil has the best characteristics (lower conjugated diene, total polar material and free faty acid content) and superior stability compared to the conventional sunflower oil and other commercial oils (soybean, corn, peanut oil etc.) at frying conditions. It was reported that high oleic sunflower oils could improve the health benefits as decreasing in the risk of coronary heart disease and plasma levels of LDL cholesterol susceptibility to oxidation. Hence, high oleic sunflower oils could be considered as a more suitable and cheaper alternative oil for catering and frying industries.

Keywords: Sunflower oil, high oleic, linoleic, frying, stability

COMPARISON OF PHYSICAL AND CHEMICAL PROPERTIES OF SUNFLOWER AND DIFFERENT VEGETABLE OILS BIODIESEL

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ABSTRACT

Biodiesel refers to a vegetable oil or animal fat based diesel fuel consisting of long chain alkyl (methyl, ethyl, or propyl) esters. It can fulfill energy security needs without sacrificing engine's operational performance. In this study, the relationship between the chemical structure and physical properties of biodiesel esters is analyzed and compared with sunflower oil and some vegetable oil diesel. The compared biodiesels were made using sunflower oil and different oils with sodium hydroxide as catalyst. The physico-chemical properties assessed includes, density, flash point, kinematic viscosity, sulfated ash, iodine value.

Key words: sunflower oil; density; flash point; kinematic viscosity

LC-DAD/ESI-MS/MS CHARACTERIZATION OF PHENOLIC COMPOUNDS OF SUNFLOWER OIL

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ABSTRACT

The study investigates the phenolic contents and antioxidant potential of sunflower oils from commercial markets in Turkey were extracted with methanol/water. Extracts were used for the phenolic and antioxidant studies. A simple and reproducible method for qualitative and quantitative analysis of phenolic compounds in sunflower oils, high performance liquid chromatography with diode array detector (HPLC-DAD), and HPLC-mass spectrometry (MS) in tandem mode was developed. Detection and quantification were performed at 280, 320 and 360 nm. For identification purposes, HPLC-MS/MS was equipped with electrospray ion source in the negative and positive-ion mode. Most of the compounds detected were mainly hydroxycinnamic acids. Chlorogenic acid was found as the major compound in the group of phenolic acids followed by vanillic acid, while rutin was determined as the most abundant compound in the overall phenolics of sunflower oil. Rutin has an average concentration of 2.70 mg/kg oil whereas chlorogenic acid has an amount of 1.66 mg/kg oil as the second most dominant phenolic compound. Antioxidant activities of sunflower oils were measured as a comparison of two methods; the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis- (3-ethyl-benzothiazoline-6-sulphonic acid) assays. Our results showed strong correlations between antioxidative capacity and total phenolic content of sunflower oils.

Keywords: Sunflower oil phenolics, phenolic characterization, LC-MS/MS analysis, antioxidant assays.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a crucial crop producing annually and native to North America (Amakura *et al.,* 2013). It is widely used in oil production and sunflower oil ranks fourth in world vegetable oil production, after palm oil, soybean oil and canola oil (Weisz *et*

al., 2009). Sunflower oil is also popularly used in Turkey and the seed production in 2013 was 1523000 tons (FAO-STAT,2013). Sunflowers date back to 26th century, are known and used till then (Pope *et al*., 2001).Sunflower oil (sunflower seed oil) is an abundant source of unsaturated fat, vitamin E, and some phenolic compounds (Fiori, 2009). Sunflower seeds are affluent in oil and with this oil having high ratio of polyunsaturated/saturated fatty acids and high linoleic acid content, sunflower oil is considered to be good for human consumption (Salgın *et al*., 2006).

Sunflower seeds have been shown to have antioxidant activities and are a very good source of vitamin E and several B-vitamins. Moreover, sunflower seeds contain a number of phenolic compounds largely responsible for the modifications occurring during the processing of sunflower seeds (Weisz *et al*., 2009). Phenolic compounds have been proposed to be the potent and important contributors in reducing oxidative stress due to their antioxidant activity, which are of great importance. Therefore, food industry is concentrating on foods containing various bioactive compounds for health promotion and disease prevention (Kelebek *et al*., 2015a).The major phenolic constituents of sunflower seeds are chlorogenic acid, smaller quantities of caffeic acid, cinnamic, coumaric, ferulic, sinapic and hydroxy-cinnamic and finally traces of vanillic, syringic and hydroxy-benzoic acids (Pedrosa *et al.,* 2000), but the phenols are present only in traces in sunflower seed oils due to the oil production process (Leung *et al.*, 1981).

In this research, the determination of phenolic content and their antioxidant activity was aimed. As regarding the lack of studies in these terms of sunflower oil, this paper will be helpful in understanding the characterization of sunflower oils.

MATERIAL AND METHODS

Chemicals

Methanol, acetonitrile, formic acid, and cyclohexane HPLC-grade solvents were purchased from Riedel-deHaen (Switzerland). All other reagents used were of analytical grade. Ultrapure water generated by the MilliQ system (Millipore, Bedford, MA) was used. Phenolic compounds (p-hydroxybenzoic, vanillic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, qurcetin-3-galactoside, kaempferol-3-glucoside and rutin) were obtained from Sigma-Aldrich (Steinheim, Germany).

Samples

Sunflower oil samples were collected from domestic markets in Adana, Turkey. 5 different commercial brands of oils were analysed.

Extraction of the Phenolic Fraction

According to Rotondi *et al*. (2004), 4 g of the oil sample was added to 2 mL of n-hexane and 4 mL of a methanol/water (70/30; v/v) solution in a 10 mL centrifuge tube. After vigorous mixing, they were centrifuged for 15 min at 5500 rpm. The hydro-alcoholic phase was collected, and the hexane phase was re-extracted twice with 2 mL of methanol/water (70/30; v/v) solution each time. Finally, the hydro-alcoholic fractions were combined, washed with 2 mL of n-hexane to remove the residual oil, then concentrated and evaporated in vacuum at 35 °C. The dry extracts were re-suspended in 0.5 mL of a methanol/water (50:50, v/v) solution and filtered through a 0.2 μm nylon filter (Whatman Inc., Clifton, NJ) before being analyzed by LC-ESI-DAD-MS/MS.

LC-DAD-ESI-MS/MS analysis of phenolic compounds

An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, California, USA) operated by Windows NT-based ChemStation software was utilized; the HPLC equipment was used along with a diode array detector (DAD). The system comprised a binary pump, degasser, and auto sampler. The column used was a Phenomenex reversed-phase C-18 column (4.6 mm × 250 mm, 5 μm) (Torrance, California, USA). The mobile phase consisted of two solvents: Solvent A, water/formic acid (99.5:0.5; v/v) and Solvent B, acetonitrile/solvent A (60:40; v/v). Phenolic compounds were eluted under the following conditions: 0.5 ml min^{-1} flow rate with temperature set at 25 °C; isocratic conditions from 0 to 5 min with 0% B; gradient conditions from 0% to 5% B in 20 min; from 5% to 15% B in 18 min; from 15% to 25% B in 14 min; from 25% to 50% B in 31 min; from 50% to 100% B in 3 min; followed by washing and reconditioning of the column. The ultra-violet-visible spectra (scanning from 200 nm to 600 nm) were recorded for all peaks. Triplicate analyses were performed for each sample. The identification and assignation of each compound was performed by comparing retention times and UV spectra to authentic standards; and confirmed by an Agilent 6430 LC-MS/MS spectrometer equipped with an electrospray ionization source. The electrospray ionization mass spectrometry detection was performed in negative ion mode with the following optimized parameters: capillary temperature 400°C, N_2 12 L/min; nebulizer pressure, 45 psi (Kelebek *et al*., 2015a). Data gaining was performed using the Multiple Reactions Monitoring (MRM) method that solely monitors specific mass transitions during preset retention times. The curves were obtained using the commercial standards of the concentrations normally present in sunflower oils (approximately 1-100 mg kg^{-1}), obtaining regression coefficients (r^2) above 0.995 in all cases.

Measurement of antioxidant activity

DPPH Assay: 0.1 mL of diluted sunflower oil extract was mixed with 3.9 mL of DPPH solution (2.36 mg/100 mL methanol) and vigorously vortexed. The solution was held in the dark at ambient conditions for 15 min. The absorbance was measured at 517 nm by a UV-Visible spectrophotometer (Shimadzu UV-1201, Kyoto-Japan). Trolox calibration curve was used to calculate the antioxidant activity of the oil extracts and to express the antioxidant capacity in mM Trolox equivalent per kg of sunflower oil. The mean and standard deviation were calculated for the three replicates (Kelebek *et al*., 2015a; Kesen *et al*., 2013).

ABTS Assay: The ABTS solution was created at a concentration of 7 mM and mixed with 2.5 mM of potassium persulphate, and stored after incubation at 23 °C in the dark for 12–16 h. The ready-made solution was diluted with 80 % methanol to measure an absorbance of 0.7±0.01 at 734 nm. Then, 3.9 mL of ABTS solution was added to 0.1 mL of the oil samples and mixed vigorously. Finally 10 min. were waited to ensure reaction and the absorbance was monitored at 734 nm [13, 14]. The calibration curve equations related to the Trolox standard were y=0.0004x + 0.0089 with R2= 0.9996 for ABTS and y=0.0004x + 0.0082 with R2= 0.9995 for DPPH within a concentration range from 5 to 150 μmol/L.

RESULTS AND DISCUSSION

Phenolic Compounds of Sunflower Oil

Table 1 lists the compounds identified according to different families, including the information provided by HPLC-DAD-ESI-MS/MS analysis: retention time, λ_{max} in the ultraviolet region, molecular ion, main fragment ions in MS/MS, and tentative identification. A total of 10 phenolic compounds were identified and quantified in oils, including p-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, quercetin-3-galactoside, kaempferol-3-glucoside and rutin. Rutin is the

major phenolic compound in sunflower oils followed by chlorogenic acid as they constitute the large proportion of the total phenolic content (Table 2). These two compounds were reported to present in the seed and kernel of the sunflowers in addition to caffeic and ferulic acid with chlorogenic acid being the most abundant compound that is one of the natural phenols includes one molecule of caffeic acid and one of quinic acid. Caffeic acid is reported to found more in the kernels of the sunflower than the chlorogenic acid Žilić *et al*., 2010. Phenolic acids have higher proportion in phenolic compounds of sunflower oils. Rutin is a flavonol formed by a quercetin which is a flavonol and a rutinoside, a dissaccharide (Kelebek *et al*., 2015b). This compoundhas the majority having a concentration between 2.23 and 2.99 mg/kg in flavonoids of sunflower oil Chlorogenic acid has a varying concentration between 1.40 and 1.80 mg/kg followed by vanillic acid (1.14-1.35 mg/kg) in phenolic acids. The other phenolic acids present in traces due to the refining process of the oil (Leung *et al*., 1981). Sunflower oil phenolic acids show similarity with phenolics of olive oil including vanilic, caffeic, ferulic and p-coumaric acid (Godoy-Cabarello *et al*., 2012; Kelebek *et al*., 2012) while olive oil having total phenol content approximately two or three times more than sunflower oil (Guzel *et al*., 2009). Also the amounts of phenolic compounds is known to vary according to the conditions of the region where in the crop grows, the extraction methods and the conditions of storage (Kelebek *et al*., 2012).

Antioxidant Activity of Sunflower Oil

Antioxidant capacity was measured by two methods namely, ABTS and DPPH assays. Table 3 presents the results of the antioxidant activities obtained by the sunflower oils. As it can be seen from the results, ABTS assay stated better the antioxidant activity of phenolic compounds than the DPPH assay as the method gave higher values.

DPPH is a free radical scavenging method, being simple, rapid and repeatable, preferably used in determining the antioxidant activity of compounds (Kelebek *et al*., 2015b). On the other hand, ABTS is used more in the food and agriculture industry which is clearly the better method for evaluating the antioxidant capacity of sunflower oils (Kelebek and Selli, 2011). Antioxidant capacities were found as 7.16 μM Trolox/kg oil using DPPH assay and 11.76 μM Trolox/kg oil by ABTS assay in average while the maximum values were 7.71 and 12.76 respectively. Rutin is known to have antioxidant, antiinflamatory activities and can be used in preventing cancer diseases (Kelebek *et al*., 2015b). In addition to the antioxidant behavior, chlorogenic acid shows antiviral, hypoglycaemic and hepatoprotective activities yet caffeic acid is reported to have higher antioxidant capacity (Dixon *et al*., 1995; Chen and Ho, 1997).

CONCLUSION

A total of ten phenolic compounds were isolated from sunflower oil samples and identified by HPLC-DAD-ESI-MS/MS analysis. Rutin is found as the most dominant phenolic compound with a concentration of 2.70 mg/kg oil followed by chlorogenic acid (1.66 mg/kg) and vanillic acid (1.35 mg/kg). The other phenolic acids present in sunflower oil are determined as p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid and sinapic acid while flavonoids include quercetin-3-galactoside and kaempferol-3-glucoside. Antioxidant capacity of these phenolic compounds was determined as a comparison of DPPH and ABTS methods. ABTS assay is found as more appropriate in determining antioxidant activity of sunflower oil. By using DPPH assay antioxidant capacity is determined as 7.16 μM Trolox/kg oil while ABTS had a result of 11.76 μM Trolox/kg oil. In conclusion, the phenolic content and antioxidant activity supply a beneficial contribution to the characterization of sunflower oils. Regarding this matter, further investigation is advised.

Table 1. HPLC-DAD-ESI-MS/MS identification of phenolic compounds

Peak	Compounds	MF	λ (nm)	Fragmentor (v)	Precursorion	Collision energy (v)	Quantitative transition (m/z)
	Phenolic acids (PA)						
1	p-hydroxybenzoic acid	$HOC_6H_4CO_2H$	256	90	137	15	137>93
2	Vanillic acid*	$C_8H_8O_4$	258, 293	90	167	15	167>123
3	Chlorogenic acid*	$C_{16}H_{18}O_9$	326	90	353	15	353>191
4	Caffeic acid*	$C_9H_8O_4$	325	90	179	15	179>135
5	*p*-coumaric acid*	$C_9H_8O_3$	236, 310	90	163	15	163>119
6	Ferulic acid*	$C_{10}H_{10}O_4$	323, 293	90	193	15	193>134
7	Sinapic acid*	$C_{11}H_{12}O_5$	324	90	223	15	223 >149
	Flavonoids (FLA)						
8	Quercetin-3-galactoside*	$C_{21}H_{20}O_{12}$	353	90	463	15	463>301
9	Kaempferol-3-glucoside*	$C_{21}H_{20}O_{11}$	348	90	447	15	447>285
10	Rutin*	$C_{27}H_{30}O_{16}$	360	90	609	15	609>301

Table 2. Phenolic content of sunflower oil extracts (mg/kg oil)

Phenolic Compounds	p-hydroxybenzoic acid	Vanillic acid	Chlorogenic acid	Caffeic acid	p-coumaric acid	Ferulic acid	Sinapic acid	Qurcetin-3-galactoside	Kaempferol-3-glucoside	Rutin	*Total*
Sample 1	0.61±0.01	1.46±0.00	1.80±0.03	1.04±0.01	0.52±0.01	0.38±0.01	0.33±0.00	0.09±0.00	0.13±0.00	2.75±0.04	***9.10±0.10***
Sample 2	0.54±0.01	1.40±0.01	1.72±0.02	0.98±0.01	0.57±0.00	0.44±0.01	0.21±0.01	0.11±0.00	0.15±0.00	2.99±0.01	***9.10±0.13***
Sample 3	0.58±0.01	1.43±0.02	1.76±0.03	1.01±0.02	0.55±0.01	0.41±0.01	0.27±0.01	0.10±0.01	0.14±0.00	2.87±0.03	***9.10±0.10***
Sample 4	0.53±0.02	1.31±0.01	1.62±0.02	0.93±0.01	0.50±0.03	0.37±0.01	0.25±0.02	0.09±0.01	0.13±0.00	2.64±0.02	***8.38±0.10***
Sample 5	0.47±0.01	1.14±0.03	1.40±0.02	0.80±0.01	0.42±0.01	0.31±0.00	0.23±0.01	0.08±0.00	0.11±0.00	2.23±0.01	***7.18±0.08***
Min	***0.47***	***1.14***	***1.40***	***0.80***	***0.42***	***0.31***	***0.21***	***0.08***	***0.11***	***2.23***	***7.18***
Max	***0.61***	***1.46***	***1.80***	***1.04***	***0.57***	***0.44***	***0.33***	***0.11***	***0.15***	***2.99***	***9.10***
Mean	***0.55***	***1.35***	***1.66***	***0.95***	***0.51***	***0.38***	***0.26***	***0.09***	***0.13***	***2.70***	***8.57***

Table 3. Antioxidant capacities of sunflower oil extracts (μM Trolox/kg oil)

	DPPH	**ABTS**
Sample 1	7.05±0.54	11.58±0.88
Sample 2	7.45±0.26	12.23±0.43
Sample 3	7.08±0.96	11.61±1.57
Sample 4	7.71±0.41	12.66±0.68
Sample 5	6.52±0.60	10.70±0.99
Min	6.52±0.26	10.70±0.43
Max	7.71±0.96	12.66±1.57
Mean	7.16±0.56	11.76±0.91

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COMPARISON OF ENZYMATIC PROCESS FOR BIODIESEL PRODUCTION FROM SUNFLOWER OIL

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ABSTRACT

The research on the production of biodiesel has increased significantly in recent years because of the need for an alternative fuel which endows with biodegradability, low toxicity and renewability. In order to design an economically and environmentally sustainable biodiesel production process, a proper understanding of the factors affecting the process and their relative importance is necessary. A comprehensive review of the literature on the subject of biodiesel production was carried out. Traditionally biodiesel has been produced using either acid or base catalysts. The multi-step purification of end products, wastewater treatment and energy demand of the conventional process has led to search for alternative option for production of biodiesel. The use the enzyme lipase as a biocatalyst for the transesterification reaction step in biodiesel production has been extensively investigated. The enzymatic process is known to be a clean and environment friendly technique for biodiesel production. The present review analyzes enzymatic process of some vegetable oils reported in literature and also suggests a suitable method for commercialization of the enzymatic process.

Keywords: biodiesel; enzyamtic process; sunflower oil, vegetable oils

ASSESSMENT OF SUNFLOWER OIL ADULTERATION

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ABSTRACT

The aim of this study is to estimate the sunflower oil adulteration with cheaper oils as raw cottonseed and canola oils. Sunflower oils, raw canola oil and raw cottonseed oil samples were supplied from market to investigate the possibility of adulteration. Main fatty acid composition of samples was detected by using GC-MS. L^*, a^* and b^* color values of the samples were also determined to detect the correlation with fatty acid composition. Increase of linolenic acid and palmitic acid percentages of sunflower oils samples was a good indicator for estimation of canola oil and palm oil addition, respectively. Some of the sunflower oil samples were suspected to be adulterated. L^*, a^* and b^* color values were also discussed on prediction of the possibility of adulteration. b^* values were detected to be higher in suspected oils. Detailed statistical modeling studies may be recommended for estimation of cheaper raw oil addition in sunflower oil.

Key words: Adulteration, Fatty acid, Sunflower oil, Color

INTRODUCTION

Sunflower is botanically classified as *Helianthus annuus* and is an annual plant. It is thought to have been domesticated around 1000 B.C. by Native Americans. People in many regions began to process vegetable oils, from many oil sources for cooking purposes, before thousands of years ago. In 1860, Russia farmers cultivated sunflower. At that time, they became the world's largest producer of sunflower seeds. (Anonymous, 2010).

Sunflower oil is rich in linoleic acid and it is one of the most economically important vegetable oil source, especially in Turkey. Also, the widely usage of cake/meal of sunflower, obtained after oil extraction, as livestock increases the economic value of sunflower (İncekara, 1972, Dayal et al., 2011).

The tendency of adulteration on olive oil is higher in comparison to other oils, so there are many researches on detection of the adulteration in olive oil (Gegiou and Georgouli, 1983; Mannina et al., 1999; Blanch et al., 1998, 1999, 2000; Salivaras et al., 1992; Dionisi et al., 1995; Flor et al., 1993). Sunflower oil is cheaper than many other oils and sometimes used for adulteration of olive oil (Savaş, 1969) but, in recent years sunflower oil is also subjected to be adulterated with some other cheaper oils.

The fatty acid composition of sunflower oil may vary by the effect of many reasons. Republic of Turkey Ministry of Food, Agriculture and Livestock published a regulation on the 12ht April 2012, called as 'Bitki adı ile anılan yağlar tebliği'. The Ministry announced the ranges of fatty acid composition of many vegetable oils (Anonymous, 2012). It is attractive that the lower and the higher limits of the ranges are at their maximum and in accordance with the literature.

Raw canola oil and raw cottonseed oil are cheaper than sunflower oil was subjected to this study for our suspect of their use in adulteration of sunflower oil. The ranges of fatty acid composition of sunflower, canola and cottonseed oil's, mentioned in regulation, are shown in Table 1.

Table 1. The ranges of fatty acid composition of sunflower, canola and cottonseed oil in Turkish Food Codex on vegetable oils (%)*

Fatty acids		Sunflower oil	Canola oil	Cottonseed oil
Caproic	(C6:0)	nd[a]	nd	nd
Caprylic	(C8:0)	nd	nd	nd
Capric	(C10:0)	nd	nd	nd
Lauric	(C12:0)	nd - 0.1	nd	nd – 0.2
Myristic	(C14:0)	nd - 1.0	nd - 0.2	0.6 - 1.0
Palmitic	(C16:0)	4.0 - 7.6	2.5 - 7.0	21.4 – 26.4
Palmitoleic	(C16:1)	nd - 0.3	nd - 0.6	nd – 1.2
Margaric	(C17:0)	nd - 0.2	nd - 0.3	nd - 0.1
Heptadecenoic	(C17:1)	nd - 0.1	nd - 0.3	nd - 0.1
Stearic	(C18:0)	2.1 - 6.5	0.8 - 3.0	2.1 – 3.3
Oleic	C18:1	14.0 - 71.8	51.0 - 70.0	14.7 - 21.7
Linoleic	C18:2	18.7 - 74.0	15.0 - 30.0	46.7 - 58.2
Linolenic	C18:3	nd - 0.5	5.0 - 14.0	nd - 0.4
Arachidic	C20:0	0.1 - 0.5	0.2 - 1.2	0.2 - 0.5
Eicosenoic	C20:1	nd - 0.3	0.1- 4.3	nd - 0.1
Behenic	C22:0	0.3 - 1.5	nd - 0.6	nd - 0.6
Docosahexaenoic	C22:1	nd - 0.3	nd - 2.0	nd - 0.3
Lignoceric	C24:0	nd - 0.5	nd - 0.3	nd - 0.1
Nervonic	C24:1	nd	nd – 0.4	nd

[a]: not detected (≤ % 0,05); *Anonymous, 2012.

The aim of this study is to estimate the sunflower oil adulteration with cheaper oils as raw cottonseed and canola oils by the aid of fatty acid composition.

MATERIALS AND METHODS

Thirtysix sunflower oil, one conola oil and one cottonseed oil samples were obtained from market from many regions of Turkey. Names of companies were hidden.

Color measurement:

Color measurements of the oil samples were carried out using a Minalto CR400 colorimeter. The instrument was standardized each time by a white (L=93.01, a=1.11, b=1.30) tile. The color values were expressed as *L** (whiteness or brightness/darkness), *a** (redness/greenness) and *b** (yellowness/blueness) (Hunter, 1948). 20 ml oil samples were

poured in a petric plate on a white tile for measuring the color values (Morello et al., 2004; Sikorska et al., 2007).

Determination of fatty acid composition:

Fatty acid composition was carried out by Agilent 6890 series GC system (Agilent Technologies, USA) fitted with a capillary column packed with 100% cyanopropyl methyl polysiloxane (Supelco SP-2380 model, 60 m × 250 μm × 0.2 μm i.d.; Bellefonte, PA, USA) and equipped with a flame ionization detector. Before injection, oil samples were converted to fatty acid methyl esters (FAMEs). 0.1 g of oil sample was weighed in a sample tube and dissolved in 10 mL hexane. Then 1 mL of 2 N potassium hydroxide in methanol was added and shaken for one minute before the centrifugation procedure. After centrifugation, the clear supernatant was transferred to a GC auto-sampler vials for injection. One μL FAMEs were injected into the GC-FID system using an auto-sampler with a split ratio of 100:1. The oven's initial temperature was set to 50°C for 2 mins and then increased at a rate of 4°C/min up to 240°C, where it was held for 10 min. Both the injector and the detector temperatures were set to 250°C. The flow rate of carrier gas (hydrogen) and make-up gas (nitrogen) were set to 1 mL/min^{-1} (AOCS, 1984). The data were recorded by using the Agilent ChemStation data processor. FAMEs peaks were identified by comparison with retention times of known standards (Sigma Chemical Co.) and quantification was determined as the percent area of each peak relative to the sum of all peak areas. All analyses were conducted in duplicate and results are provided as average values.

Statistical analysis:

Data were subjected to analysis of variance with mean separation by Duncan's multiple range tests. Differences were considered statistically significant at the $P < 0,05$ level. Statistical analysis was performed using SPSS 10.0 for Windows. The statistical results were evaluated according to Düzgüneş et al., 1987.

RESULTS

The detected *L**, *a** and *b** value ranges for 36 sunflower oil samples were 69,177-70,670, (-1.903) - (-4.233) and 7.597-16.060, respectively. *L** value of the samples were changed in a narrow range but the range for *a** and *b** were wide that reflects the sensitivity on them. *a** value of 30th sample were higher in comparison with other sunflower oil samples. And, the value of *a** was similar to values obtained for cottonseed and canola oils. *b** value was the lowest for 12th sunflower sample and was the highest for the 30th sunflower oil sample (Table 2).

10th and 26th sunflower oil samples were found to be higher in myristic acid content than the other sunflower oil samples as 1,529 % and 4,055 %, respectively. Myristic acid content of the samples doesn't give any confirmative idea on suspicion of adulteration of sunflower oils by the use of canola and cottonseed oil. Other fatty acid profile of these samples was belonging to fatty acid profile of sunflower oil. Especially the detection of high myristic acid content may cause a formation of doubt of adulteration with palm, coconut and babassu oil, but lauric acid was not detected in these samples which may be a parameter for removal of doubt (Table 2).

Table 2. Main fatty acid composition (%) and *L*, a*, b** values of oil samples

Samples	Myrstic acid	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	Behenic acid	L^*	a^*	b^*
1	0.045 e*	4.924 ö	31.523 ghıijk	62.173 ab	0 c	0.321 k	0.741 efghı	70.090 efgh	-2.720 l	10.613 l
2	0.059 e	6.782 klm	29.675 jklmno	61.371 abc	0 c	0.814 efghıij	1.037 cd	70.460 abcde	-2.757 l	10.790 jk
3	0 g	5.701 mnoö	32.482 fghıi	59.487 bcde	0 c	0.640 hıijk	0.954 cde	70.563 abc	-2.350 f	8.417 u
4	0 g	9.200 ı	28.039 oöp	59.398 bcdef	0 c	1.459 abc	1.535 ab	69.187 m	-2.417 g	8.947 sş
5	0 g	8.375 ıij	34.538 ef	56.430 fghı	0 c	0.334 k	0.380 jk	70.260 defg	-2.870 n	10.873 j
6	0 g	8.304 ıijk	30.959 ıijklm	57.496 efgh	0 c	1.255 bcde	1.628 a	69.693 jkl	-2.507 h	9.840 n
7	0.223 d	8.683 ıi	34.470 ef	55.405 hıi	0 c	0.417 jk	0.646 ghı	70.003 ghıi	-2.677 k	9.530 ö
8	0.044 e	6.368 lmno	29.976 ijklmno	62.506 ab	0 c	0.514 ijk	0.744 efghı	69.910 hıij	-2.940 o	11.193 i
9	0.022 efg	5.656 mnoö	34.957 ef	58.452 cdefg	0 c	0.405 jk	0.748 efghı	70.650 ab	-2.587 ij	9.137 pr
10	1.529 b	7.657 ijkl	40.863 d	48.427 mnoö	0 c	0.348 k	0.660 ghı	70.050 fghı	-2.720 l	9.763 no
11	0.056 e	5.351 oö	44.656 c	49.105 mno	0 c	0.568 ıijk	0.640 ghı	70.670 a	-2.150 c	7.653 v
12	0 g	8.487 ıij	33.855 fg	56.419 ghı	0 c	0.561 ıijk	0.644 ghı	70.527 abcd	-1.857 a	6.783 y
13	0 g	8.635 ıij	34.467 ef	55.406 hıi	0 c	0.524 ıijk	0.649 ghı	70.533 abcd	-2.530 hı	9.447 ö
14	0.038 ef	7.125 jkl	30.536 ijklmn	61.267 abcd	0 c	0.528 ıijk	0.704 fghı	70.403 cdef	-2.297 de	8.830 t
15	0 g	5.330 oö	29.133 klmno	64.480 a	0 c	0.648 ghıijk	0.657 ghı	69.827 ıijkl	-3.230 s	11.997 h
16	0 g	16.219 de	24.608 rs	57.652 efgh	0 c	0.941 defghı	0.730 fghı	70.430 abcdef	-1.903 a	7.597 v
17	0.040 e	13.417 fg	26.297 öpr	59.149 bcdefg	0 c	0.638 hıijk	0.610 hı	70.660 ab	-2.610 j	9.703 o
18	0.053 e	7.416 jklm	37.286 e	54.279 ıij	0 c	0.464 jk	0.632 ghı	69.887 hıijk	-2.407 fg	9.213 p
19	0 g	8.232 ıijk	31.736 ghıij	58.568 cdefg	0 c	0.548 ıijk	0.698 fghı	69.597 l	-3.010 ö	11.090 i
20	0 g	12.344 gh	30.751 ıijklmn	55.303 hıi	0 c	1.084 bcdefg	0.727 fghı	70.420 bcdef	-2.563 ıi	9.780 no
21	0 g	14.707 ef	28.657 mnoö	54.583 ıij	0 c	1.112 bcdef	0.732 fghı	70.163 efg	-2.720 l	10.037 m
22	0 g	16.465 d	33.662 fgh	47.653 noöp	0 c	1.265 bcd	0.740 efghı	69.773 ijkl	-3.643 ü	14.553 d
23	0 g	17.237 d	30.753 ıijklmn	50.470 klm	0 c	1.027 cdefgh	0.752 efghı	70.440 abcdef	-2.730 l	10.093 m
24	0 g	14.232 ef	31.222 hıijkl	52.481 jk	0 c	1.426 abc	0.682 fghı	70.513 abcd	-3.133 r	12.210 g
25	0 g	21.377 bc	23.522 s	52.640 ijk	0 c	1.345 bcd	0.810 defgh	69.660 kl	-2.013 b	8.257 ü
26	4.055 a	11.530 h	33.265 fghı	49.721 lmn	0 c	0.733 fghıijk	0.669 fghı	70.297 cdef	-2.843 m	10.573 l
27	0 g	22.614 b	28.394 noö	46.557 oöp	0 c	1.210 bcde	0.874 def	70.100 efgh	-2.940 o	10.737 k
28	0.054 e	5.678 mnoö	46.585 c	46.406 öp	0 c	0.549 ıijk	0.664 ghı	70.567 abc	-2.753 l	9.740 no

29	0 g	14.781 ef	33.259 fghı	50.478 klm	0 c	0.899 defghıi	0.709 fghı	70.550 abc	-2.347 ef	9.053 rs
30	0 g	6.622 klmn	58.456 b	26.634 r	5.251 b	2.058 a	1.169 bc	69.917 hıij	-4.233 y	16.060 c
31	0 g	14.427 ef	25.669 prs	58.113 defgh	0 c	1.110 bcdef	0.659 ghı	70.523 abcd	-2.413 g	9.767 no
32	0 g	22.327 b	28.820 lmno	46.459 oöp	0 c	1.123 bcdef	0.744 efghı	69.177 m	-3.403 t	12.967 e
33	0 g	21.618 bc	30.509 ijklmno	45.538 p	0 c	1.104 bcdef	0.816 defg	70.630 abc	-2.263 d	8.850 şt
34	0 g	20.440 c	23.464 s	49.369 mn	5.295 b	1.115 bcdef	0.339 l	70.573 abc	-3.333 ş	11.773 ı
35	0 g	11.518 h	28.450 mnoö	53.400 ij	5.400 b	0.734 efghıijk	0.357 k	70.233 defg	-3.470 u	12.530 f
36	0 g	9.199 ı	37.226 e	52.344jkl	0 c	0.578 ıijk	0.564 ıi	70.217 defg	-3.077 p	11.827 ı
Cottonseed oil	0.502 c	28.268 a	16.533s	53.392ij	0 c	0.818 efghıij	0 m	66.903 n	-4.120 v	32.057 b
Canola oil	0 g	5.475 noö	68.251a	17.414s	7.557 a	1.547 ab	0 m	64.933 o	-5.893 z	53.973 a

*Means with different superscript letters differ significantly.

The samples, 1, 2, 3, 8, 9, 10, 11, 14, 15, 18, 28 and 30 were found to be in the range in palmitic acid as mentioned in the regulation (4,0 - 7,6 %) announced by the Ministry. The palmitic acid content was ranged between 8,232 % - 9,200 % for the samples 4, 5, 6, 7, 12, 13, 19 and 36. The samples, 17, 20, 21, 24, 26, 29, 31 and 35's palmitic acid content were detected to be from 11.530 % to 14.781 %. It was surprising to detect the palmitic acid content of the samples 16, 22, 23, 25, 27, 32, 33 and 34 in between 16,219 % and 22,327 %. This classification aroused the suspicion of adulteration of sunflower oil with cottonseed oil for the last group, due to higher amount of palmitic acid.

Oleic acid content of sample 30 was 58,456 % which was found to be higher than other sunflower oil samples. Oleic acid content of samples 10, 11 and 28 were from 40,863 % to 46,585 %. The lower range of oleic acid content was from 23.464 % to 26.297 % for the samples 16, 17, 25, 32 and 34. The range for oleic acid content in sunflower oil, canola oil and cottonseed oil was announced as 14,0 - 71,8 %, 51.0-70.0 % and 14,7 - 21,7 %, respectively. Estimation of adulteration by the aid of data on oleic acid content of sunflower oils looks too hard to evaluate the suspicion of addition of canola and cottonseed oil.

The lowest linoleic acid content of sample 30 was 26,634 %. The linoleic acid content of sunflower oil, canola oil and cottonseed oil in the regulation announced by the Ministry was ranged as 18,7 - 74,0 %, 15,0 - 30,0 % and 46,7 - 58,2 %, respectively. Linoleic acid content of sample 22, 27, 28, 32, 33 and 34 was from 45,538 to 49,369 %. The other sunflower oil samples were detected to have a linoleic acid range in between 48,427 and 64,480 %. In general, the linoleic acid content of sunflower oil and cottonseed oil is similar and it is not possible use the linoleic acid data as estimation parameter on adulteration of sunflower oil by cottonseed oil. But the addition of canola oil in sunflower oil may cause a little decrease in linoleic acid content of sunflower oil.

Linolenic acid may be a good estimation parameter for addition of canola oil in sunflower oil due to apparent increase in percentage. In the announce of the Ministry's regulation, the range for linolenic acid was from 0 to 0,5. The detection of linolenic acid in sample 30, 34 and 35 was from 5,251 to 5,400 % that increases the suspect of canola oil addition in sunflower oil. If linolenic acid content is taken in to account, the possibility of estimation of cotton seed oil addition in sunflower oil is very poor due to low ranges of linolenic acid content in cottonseed oil (0 - 0,4 %). Detection of linolenic acid in sunflower oil arouses the suspicion of adulteration of sunflower oil with canola oil due to a visible

increasement. Linolenic acid was not detected in the sunflower oil samples except for the samples 30, 34 and 35.

Arachidic acid content of sample 30 was found as 2, 058 % and was higher than the other sunflower oil samples. It was the sample that was highly suspected to be adulterated with canola oil by the data on linolenic acid. The arachidic acid data was the second hint to strength this suspicion for the sample 30. The arachidic acid content of samples 4, 6, 20, 21, 22, 23, 24, 25, 27, 31, 32, 33 and 34 was from 1,084 to 1,459 %. These data on arachidic acid are higher than the announcement of the Ministry (0,1 - 0,5 %) for sunflower oil. According to these results, it may be offered to the Ministry to increase the limits of arachidic acid content up to 1,5 % in sunflower oil.

Behenic acid content of all tested samples was in the range that Ministry announced. Behenic acid is not a good parameter for estimation of adulteration of sunflower oil with the addition of canola and cottonseed oil.

DISCUSSION

Raw canola and cottonseed oils are cheaper than sunflower oil. By this study the suspense of adding these cheaper oils in sunflower oil was inspected by the evaluation of the possibility of the usage of fatty acid composition as a verification parameter.

Detection of linolenic acid in sunflower oil may strength the suspense of adding canola oil in suflower oil. Palmitic acid content increases by the addition of cottonseed into sunflower oil. Sample 30 is a special example that may be announced to be the most suspected sunflower oil to be adulterated by the addition of canola oil, individually. Linolenic acid was detected in sample 30 and also the amount of oleic acid was relatively higher enough to strength the possibility of suspicion. The palmitic acid and linolenic acid content of sample 34 and 35 were higher in comparison to other sunflower oil samples those shift the tendency of suspense on addition of both canola and cottonseed oils. Especially *b** value was found to be the highest for the sample 30. b* was also high in samples 34 and 35. Those oils were thought to be most suspected ones among the other samples which could be adulterated.

Detailed statistical modeling studies may be recommended for estimation of cheaper raw oil addition in sunflower oil. Detection of linolenic acid in sunflower oil may be a good indicator for addition of any other oil, especially the addition of canola oil. Palmitic acid may be a parameter for estimation of cottonseed addition but it is not a strong indicator individually. b* value was found to be high in the sample which was the most suspicious to be adulterated with the addition of raw canola oil. *b** value of other suspected samples were also high in comparison to the other sunflower oil samples. Additionally, revision of the arachidic acid range of sunflower oil in the related regulation may be referred to Ministry to increase the upper limit up to 1,5 %.

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EFFECT OF DIFFERENT STORAGE CONDITIONS ON QUALITY PROPERTIES OF RAW AND ROASTED SUNFLOWER KERNELS

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ABSTRACT

Quality of raw sunflower kernels changes due to the biochemical changes throughout the storage period. Thus, quality of sunflower kernels (SK) roasted after different storage periods may have different shelf lives. Relative humidity and temperature are the main factors affecting the quality of raw SK, whereas packaging material (O2 and water vapour barrier) properties and the gas composition in the package are the main factors affecting the quality of roasted sunflower kernels. The purpose of the present study was to explore the influences of storage conditions (room conditions-LOCAL and 10°C, Relative Humidity<%65 - MAM) on the quality of raw SK and to extend our knowledge concerning the changes in oxidative stability of roasted sunflower kernel processed at various storage periods (just after harvest, 8 and 12 months after harvest). Roasted products were packed in packaging material with high oxygen barrier (<0.008 ml/m²/day at 23°C) properties and kept at 10, 20 ve 30°C storage conditions under normal atmospheric conditions and nitrogen gas (>95%). Peroxide value, free fatty acids, contents of hexanal and vitamin E were determined at 2 months intervals during the storage for 12 months. Oxidative quality of the raw SK was similar when stored at cool (10°C, RH<65%) and local conditions (avg. 51 %RH, 19°C). SK roasted at 8th and 12th month storage periods lost quality more rapidly than the kernels roasted just after the harvest. Packaging under nitrogen gas rather than cold storage had the strongest influence in the prevention of oxidative changes of the roasted products.

Key Words : Sunflower kernel, oxidation, rancidity, peroxide value, free fatty acid, hexanal, vitamin E

QUALITY CHARACTERISTICS OF ROASTED SUNFLOWER SEEDS DURING STORAGE

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ABSTRACT

Sunflower seed being a part in both oil and dried nut industry is a highly nutritious oil seed. The oil and unsaturated fatty acids content plays an important role in determining the shelf life of seeds depending on lipid oxidation while increasing the nutritional value of the seeds. The oil seeds, like sunflower seed, which have high unsaturated fatty acid content, are exposed to oxidation during the long time storage that cause off-flavour, taste and rancidity. This may result in reduced overall sensory score when consumed. The packaging material properties (oxygen and water vapour permeability) have important effects on the shelf life of roasted dried nut products. The main objective of this study is to investigate the quality changes of sunflower seed in different packaging conditions and to optimize storage conditions for longer shelf life. In this study, the sunflower seeds obtained from different planting areas (Ankara, Kayseri, Bursa-İnegöl) were first roasted and then packaged under atmospheric and nitrogen gas conditions, and stored at 20°C for estimation of the shelf life. Peroxide value, free fatty acids, hexanal content, Vitamin E content and sensory quality properties were monitored during the shelf life study. As a result of this study; bio-chemical and sensory qualities of the stored products decreased within 2 months of storage period. It was observed that the product which is obtained from Bursa-İnegöl planting area packaged under nitrogen has the best chemical and sensory quality properties.

Key Words : Sunflower seed, oxidation, peroxide value, hexanal, Vitamin E, sensory

ACCEPTABILITY OF CHAPATI MADE WITH SUPPLEMENTATION OF SUNFLOWER (*HELIANTHUS ANNUS* L.) SEED MEAL

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ABSTRACT

The nutritional value of lab processed sunflower seed meal prepared from different sunflower seed cultivares i.e HSFM-848 and Morden as well as commercially processed cake (CPC) of sunflower seeds.*Chapati* was standardized in the lab by addition of sunflower seed meal and protein isolates (obtained from CPC) at 10,20 and 30% level. Nutritional evaluation revealed that lab processed seed meal of HSFM-848, Morden and CPC contained crude protein 42.51,51.44 and32.66%, fat 1.48, 0.86 and 0.55% crude fibre 4.16,2.48 and 14.56%, calcium 170.00,224.00 and 192.33mg/100g and iron 4.28, 25.12 and 22.13 mg/100g, respectively. Lab processed meals had significantly lower amount of polyphenols and higher amount of saponins as compared to the value of CPC. *in vitro* protein digestibility of lab processed seed meal as well as CPC was found to be improved after processing. *Chapatis* were found to be organoleptically acceptable. All the developed *chapatis* were rated in the range of like moderately to like very much category on Nine-Point Hedonic scale. Incorporation of sunflower seed meal and protein isolates at 10% level with wheat flour was the desirable level without altering the organoleptic traits and can be used for preparation of other traditional products like halwa suhali,cake & biscuits. These sunflower seed meal supplemented products if added in children diet can help in over coming protein energy malnutrition among infants & children in india.

Key Words : supplements, nutritional value, sunflower seed meal, acceptability, chapatti

SOME ANTINUTRIENTS AND IN VITRO PROTEIN DIGESTIBILITY OF HOME PROCESSED SUNFLOWER SEED MEAL

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ABSTRACT

Lab processed seed meals obtained from HSFM-848 was prepared by decorticating the seeds manually followed by grinding and extracting the oil with Hexane. Commercially Processed Meal contained average polyphenol content of 1675.00, 2001.66 and 1945.00 mg per 100g, saponin content of 1935.35, 1420.75 and 1112.25 mg per 100g respectively. Polyphenol content of CPC was significantly higher than those of Lab processed seed meals, whereas saponin content of CPC was significantly lower than those of lab processed seed meals Lab processed seed meals prepared from HS-1 and Morden cultivars and commercially processed cake contained on the average crude protein content (41.75, 50.68 and 31.75%), fat (1.45, 0.95 and 0.45%), crude fibre (3.75, 2.12 and 14.85%), respectively. But the polyphenol content of commercially processed cake (1936.00 mg/100g) was found to be significantly higher than those of both the lab processed seed meals. Saponin content of lab processed seed meal prepared from HSFM-848 variety (1922.68 mg/100 g) was significantly higher than that of Morden variety whereas the saponin content of commercially processed cake (1112.65 mg/100g) was found to be significantly lower than that of both the lab processed seed meals.It may be concluded from the study that the seed meal obtained from sunflower seeds after laboratory processing is nutritionally superior, in the preparation of various traditional food products. These food products if added in the diet will improve the nutritional quality of home diet .Processing has a significant effect on lowering antinutrients present in sunflower seeds which results in increase of *in vitro* digestibility of proteins and availability of minerals from sunflower seed meal.

Key Words : In vitro protein digestibility, home processed, sunflower seed, saponins, polyphenol

CONTENT AND OIL PRODUCTIVITY IN SUNFLOWER GENOTYPES PRODUCED IN CAMPO NOVO DO PARECIS – MT, BRAZIL

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ABSTRACT

This study aimed to evaluate genotypes of sunflower seeded second harvest in the year 2014 in Campus Campo Novo do Parecis, in the experimental field of the Instituto Federal de Educação Ciência e Tecnologia de Mato Grosso. The experimental design was a randomized block design with treatments 16 (16 genotypes) and four replications. The experimental plots consisted of four rows 6.5 m long with row spacing of 0.45 m, containing area of 11.7 m², totaling an area of 748 m². The population of 45000 plants per hectare is used. Data were subjected to analysis of variance and the Scott - Knott test at 5 % probability. The genotypes that stood out in relation to achenes productivity were the MG 360, AGUARÁ 06, MG 305, AGUARÁ 04, CF 101, SYN 045, GNZ NEON, HELIO 251 and SYN 3950HO. For the achenes oil content and productivity, the MG 360 genotype was the highest value and stands in relation to other genotypes.

Keywords: spectroscopy, *Helianthus annuus* L., lipids, oilseeds, achenes productivity.

INTRODUCTION

Among oilseeds grown in the world, the sunflower stands out among the main, both in production and in planted area. Sunflower (*Helianthus annuus* L.) is an annual cycle plant and its rapid growth characteristics, resistance to drought, cold and heat, more than most species of economic cultivation in Brazil and can be used for various purposes (Leite et al., 2005) as high quality oil extraction for human consumption or as raw material for biodiesel production, among others.

In general, sunflower seed it has about 45 to 65% oil in its composition (Grunvald et al., 2014A). Sunflower oil essentially consists of triglycerides (98 to 99%). It has a high content of unsaturated fatty acids (about 83%) and Vitamin E (alpha-tocopherol), but a reduced content of linolenic acid (≤ 0.2%). Sunflower oil is essentially rich in essential fatty acid (EFA) linoleic acid, about 60% that helps in reducing serum cholesterol and LDL. Thus contributing to the prevention of arteriosclerosis and cardiovascular problems (Turatti et al., 2002).

Changes in oleic are the result not only of the genotype, but also of climatic differences during their cultivation. Thus, among the various technologies developed for sunflower production, the appropriate choice of the genotype that has high yield and / or oil is important to ensure the success of the culture as a component of the production system (Porto et al., 2007).

In the region of Campo Novo do Parecis, sunflower is grown second summer harvest from February/March, due to the occurrence of rainfall conditions and temperatures suitable for its cultivation (Castro and Farias, 2005). However, despite being the main growing region in the country, little information is available on the agronomic characteristics of genotypes as content and productivity of oil, to facilitate the cultivation practices, reducing risk and increasing profitability.

MATERIAL AND METHODS

The work was carried out at the experimental fields and facilities of the Instituto Federal de Educação Ciência e Tecnologia de Mato Grosso - Campo Novo do Parecis in second-crop system in succession to soybeans in the agricultural year 2013/2014. The soil, according to the American System of Soil Classification (USDA, 1960) is the Typic Tropudox. The initial characterization of fertility, for the first layer of 0-0.20 m, presented the following values: pH ($CaCl_2$) = 5,7; MO = 26 g dm^{-3}; P (resina) = 5,9 mg dm^{-3}; K, Ca, Mg e H+Al = 1,5; 32; 11 e 40 $mmol_c$ dm^{-3}, respectively; with V = 54,8%.

Average temperatures occurred during the experimental period were: 30.3; 23.2 and 18.9 °C for maximum temperature, medium and minimum, respectively, and 570 mm rainfall, meeting the water demands required by sunflower between 500 and 700 mm distributed along its growing cycle (Castro and Farias, 2005).

The experimental design was a randomized complete block design with 16 treatments (genotypes) and four replications, as follows: ADV 5504, AGUARÁ 04, AGUARÁ 06, BRS 323, BRS G42, CF 101, GNZ NEON, HELIO 250, HELIO 251, HLA 2012, M734, MG 305, MG 360, PARAISO 20, SYN 045 and SYN 3950HO. The experimental plots consisted of 4 rows with 6.5 m long, with row spacing of 0.45 m, containing area of 11.7 m² (1.8 x 6.5 m). Only the two 5 meters central rows of each genotype were considered for data collection. The plotted area comprises 4.5 m².

The plot of the rows, was done on March 7, 2014, and the previous application of fertilizers was carried out with the aid of a sowing machine and was distributed at a depth of 0.10 m, 45 kg ha^{-1} Potassium Chloride + 267 kg ha^{-1} NPK 10-30-20, totalizing: 26.7 kg ha^{-1} N; 80 kg ha^{-1} P2O5; 80 kg ha^{-1} K_2O, according to the results of soil analysis and recommendation (EMBRAPA, 2004). Further, beside the row fertilization at 0.04 m deep, three seeds were placed in each hole, each 0.495 m, by manual planter.

The desiccation and the application of boron was performed on March 07, using trawl trailed sprayer with an application volume of 150 L ha^{-1} using glyphosate (648 g a.i. L^{-1}) at a dosage of 2 L ha^{-1} + Prometryn dosage 2 L ha^{-1} + mineral oil (0.5 L ha^{-1}) + boric acid dosage of 3 kg ha^{-1} (600 g ha^{-1} Boron).

Thinning was done 10 days after emergence (DAE) with a scissor, leaving only one plant per hole, reaching a population of 45,000 plants ha^{-1}.

The following coverage fertilizations were made: 1) 32 DAE with a dosage of 50 kg ha^{-1} N (urea); 2) foliar application of boron, with knapsack sprayer at 35 DAE using a dosage of 3 kg ha^{-1} (600 g ha^{-1} Boron), and 43 DAE with a dosage of 11 kg ha^{-1} (1.1 kg ha^{-1} of Boron). The source of Boron used was boric acid 150 L ha^{-1} according to the requirement of sunflower of 2 kg ha-1 B, Control of weed, pests and diseases have been carried out according to the recommendations of EMBRAPA (2004).

To avoid birds attacks, the plotted sections of the central rows were protected (stage R6) by using polypropylene based bags (30 x 30 cm) and fixed with clips.

The following agronomic characteristics were evaluated: productivity achenes (**PR**; kg ha-1), determined based on two central lines 5 meters, which is corrected for moisture condition of 11% (wet basis) obtained by reading the humidity value of the achenes; oil content (**OC**; %), predicted by near infrared spectroscopy (NIR) according to the methodology described by Grunvald et al. (2014b); and oil yield (**OY**, kg ha-1), calculated by multiplying the achenes oil content (%) and productivity achenes (kg ha-1) / 100.

The harvest of the capitulums was performed manually in the two of 5 meter central rows in R_9 with pruning shears aid. Later the capitulum inflorescence were the natural dryied, cleaned and weighed. The results were submitted to analysis of variance followed by the average test Scott-Knott, both 5% probability, with the statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

All variables showed significant differences ($p < 0.05$) in the analysis of variance (Table 1). The data from the achenes productivity variables, oil content and oil yield are shown in Table 2. For the achenes productivity, genotypes were stood SYN 3950HO (2205.5 kg ha^{-1}) and HELIO 251 (2204.1 kg ha^{-1}), but not statistically different genotypes GNZ NEON, SYN 045, CF 101, AGUARÁ 04, MG 305, AGUARÁ 06 and MG 360, which had average productivity ranging from 1836.8 e 2132.5 kg ha^{-1}. However, it appears that even the lowest yields were found in genotype HLA 2012 e BRS G42, with average 40% lower than those observed in the most productive genotypes.

Table 1. ABSTRACT of the analysis of variance for the sunflower productivitiy parameters (Campo Novo do Parecis, MT, 2014).

Parameters[1]	F[2]	CV (%)[3]	GA[4]
PR (kg ha^{-1})	6.4*	12.4	1846.9
OC (%)	27744.6*	0.1	43.2
OY (kg ha^{-1})	6.8*	12.4	796.5

[1] PR = achenes productivity, OC = oil content, OY = oil yield; 2 * significant at 5%; 3 CV = Coefficient of variation; 4 GA = General average.

Values higher than this study were found by Backes et al. (2008) for HELIO 250 genotypes (1849.0 kg ha^{-1}), M734 (2052.0 kg ha^{-1}), AGUARÁ 04 (2252.0 kg ha^{-1}) and below to HELIO 251 (1882.0 kg ha^{-1}) in second-crop cultivation in northern Santa Catarina. Additionally, Vogt et al. (2010), in sunflower crop sown in November in northern Santa Catarina, reported higher yields for genotypes AGUARÁ 04 (1916.0 kg ha^{-1}) e M734 (1962.0 kg ha^{-1}) and means inferior to HELIO 250 (1450.0 kg ha^{-1}). Already Capone et al. (2012) evaluated the performance of cultivars in southern Tocantins state reported productivities 2834.1 e 2997.6 kg ha^{-1} para os genótipos HELIO 250 e HELIO 251, respectively. Poletine et al. (2013) reported an assay developed in the northwestern region of the state of Paraná, for genotypes BRS G42, SYN 3950HO, M734 and MG 305, with productivities 715.5 kg ha^{-1}, 1215.0 kg ha^{-1}, 1225.0 kg ha^{-1} e 1592.0 kg ha^{-1}, respectively. These variations in productivity reveal the importance of evaluation of genotypes in different producing regions to verify the feasibility of its use.

Analyzing the oil content of genotypes, the MG 360 genotype had the highest oil content, 47.8% (Table 2), differing from the other investigated genotypes.

Table 2. Mean values for productivity achenes (PR), oil content (OC) and oil yield (OY) from different sunflower genotypes.

Genotypes	PR (kg ha^{-1})	OC (%)	OY (kg ha^{-1})
ADV 5504	1446.9 c	47.1 b	681.5 b
AGUARÁ 04	2084.1 a	45.9 d	956.6 a
AGUARÁ 06	1859.5 a	41.6 n	773.7 b
BRS 323	1782.0 b	42.1 l	750.2 b
BRS G42	1425.9 c	42.0 m	598.9 b
CF 101	2104.4 a	45.1 f	949.1 a
GNZ NEON	2132.5 a	37.8 p	806.1 a
HELIO 250	1694.7 b	43.5 h	737.2 b
HELIO 251	2204.1 a	39.1 o	861.8 a
HLA 2012	1313.0 c	46.7 c	613.2 b
M734	1673.7 b	37.6 q	629.3 b
MG 305	1993.8 a	43.3 i	863.3 a
MG 360	1836.8 a	47.8 a	878.0 a
PARAISO 20	1685.3 b	43.2 j	728.5 b
SYN 045	2108.5 a	43.6 g	919.3 a
SYN 3950HO	2205.5 a	45.2 e	996.9 a

Different letters differ by Scott-Knott test at 5% probability.

However, the ADV 5504 genotypes (47.1%) and HLA 2012 (46.7%) also showed considerable oil content. In contrast, the M734 genotype was presented the lower oil content, with the representative average 37.6%. Some industries have been remunerating the sunflower producers from the oil content contained in achenes and no longer by simple mass achenes, since not always the genotype with the highest productivity of achenes per area results in greater productivity of oil in the same area , and the oil product of greater interest at the end of the manufacturing process and currently the main commercial sunflower crop product.

Watching the oil yield data, the averages of the genotypes SYN 3950HO, AGUARA 04 CF 101, SYN 045, MG 360, MG 305, HELIO 251 and GNZ NEON were the ones that showed the highest values (Table 2), getting between 806.1 (GNZ NEON) and 996.9 kg ha^{-1} (SYN 3950HO), but all belonging to the same statistical group. Thomas et al. (2012), testing different planting dates mentioned lower oil yield for AGUARA 04 genotypes, with 928.0 kg ha^{-1}, and HELIO 250, with 717.0 kg ha^{-1}. For the M734 genotype, the value was 864.0 kg ha^{-1}.

CONCLUSIONS

For achene productivity variable stood out the AGUARA 04 and 06 genotypes, CF 101, GNZ NEON, HELIO 251, MG 305 and 360 and 045 and SYN 3950HO, whose values were ranging between 1836.8 and 2205.5 kg ha^{-1}. However, for the oil content of the MG 360 was the one with the highest percentage, especially also in the group of genotypes with the highest oil productivity values, confirming its high potential for use in production systems Brazilian savannah.

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DETERMINATION OF FATTY ACID COMPOSITION FOR FRYING SUNFLOWER OIL USING GAS CHROMATOGRAPHY

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ABSTRACT

Frying of sunflower oil has been carried out for 7 running days at 175°C±2 in this study. The aim of this study is to determine fatty acid composition of sunflower oil under real domestic frying conditions. In the frying processes, potato has chosen for food and the processes have continued during seven days. The composition, trans fatty acid (TFA) amount and average molecular weight of sunflower oil have been determined by gas chromatography (GC) technique. This work focuses on finding changes in free fatty acid after repeated batch potato frying. Unsaturated fatty acid (UFA) contents of sunflower oil have been decreased and saturated fatty acids (SFA) have also been increased during frying process. When the frying times run on, the analysis of oil samples have showed that trans fatty acids such as elaidic and linoelaidic acids occur quite slowly. At the end of the repeated frying series, the elaidic acid (C18:1 trans) has been determined in oils for sunflower 1.5%. And also linoelaidic acid (C18:2 trans) has been detected 0.3%. At the end of the seventh day of frying, the relative contribution of oleic acid has been decreased.

Key words: Sunflower, frying, fatty acid composition

INTRODUCTION

Today, frying is one of the most popular methods for the preparation of food stuff, because the method is fast and relatively cheap and results in yellow brown products with a typical taste and smell, preferred by the consumer. The oil plays a critical role as a heat transfer and impregnation medium, and it is the crucial component of the frying process. For the quality of products being fried the quality of the frying medium is very important, because during frying the food takes up the oil becoming a significant part of the product. (Taha et al., 2014). Many factors affect the deterioration of a frying oil, such as the presence of unsaturated fatty acids, the oil temperature, oxygen absorption, the presence of metals, and the type of food (Arroyo et al. 1992). During frying, oil or fat is subjected to high temperatures in the presence of air and water from the food, thus producing a wide range of compounds resulting from thermal, oxidative, and hydrolytic reactions (Chatzilazarou et al. 2006, Dobarganes et al. 2013). As a result of the deterioration, the oil sustains some physical changes: the colour darkens, the viscosity increases, and smoke appears (Paul and Mittal, 1997).

The fatty acid composition of the frying oil is an important factor affecting fried food flavor and its stability; therefore, it should be low level of polyunsaturated fatty acid such as linoleic or linolenic acids and high level of oleic acid with moderate amounts of saturated fatty acid (Kiatsrichart et al., 2003, Mehta and Swinburn, 2001). Partial hydrogenation decreases polyunsaturated fatty acid but increases saturated fatty acid and trans-fatty acid to

produce more stable frying oil. However, trans-fatty acids (TFA) adversely effects on cardiaovascular health (Rehab and Anany 2012). One approach to increasing the stability of unsaturated oil is partial hydrogenation (Li et al., 2008; Bysted et al., 2009), but hydrogenation also results in the formation of SFA and trans fatty acids. Trans isomers of fatty acids have been reported to increase the ratio of low-density-lipoprotein (LDL) to high-density-lipoprotein cholesterol (HDL) in the plasma and increase the risk of coronary heart disease (CHD), and play a part in atherosclerosis development (Willett et al., 1993; Dalainas and Loannou, 2008). Low levels of trans fatty acids and saturated fatty acids that are basis of nutritional and diet physiological aspects also play important roles in selecting a frying oil. Since the fatty acid composition alone is not enough to explain the stability of oils, a variety of minor components, such as tocopherols, polyphenols, phospholipids, caretonoids and certain sterols are also beneficial to oil stability during frying (İnanç and Maskan 2012).

Oil and fats are one of the important components of human diet and ingredients of food industry. Oils and fats are preferred as carriers of fat soluble vitamins (A, D, E and K) and source of essential fatty acids and energy (Öğütcü et al., 2015). Vegetable oils are recognized as important compounds of our life. Sunflower is between the five biggest oilseeds in world production (Anwar et al., 2008). Sunflower oil contains a wide range of unsaturated fatty acids and is rich in essential fatty acids. Sunflower oil is considered nutritious due to high content of polyunsaturated fatty acids (PUFA), mainly linoleic acid (18:2). However, due to high PUFA, it is more susceptible to oxidative degradation leading to rancidity, off-flavors, and discoloration (Gordon 1991). And also sunflower oil is characterized by high content of tocopherols (up to 935 ppm) higher than those of other oils such as soybean and peanut. It is considered an oil of high stability due to its high content in natural antioxidants (Bramley et al., 2000; Shahidi, 2005). The nutritional aspects of edible oils associated with the presence of minor and major components play an important role in preventing diseases and improving health. It is important to formulate vegetable oil blends with special composition in order to enhance their stability and nutritional value (Frankel et al., 1994; Shiela et al., 2004).

The objective of the present study was to obtain the fatty acids combination of refined sunflower oil under normal frying conditions. Frying processes were done with potato repeating seven days.

MATERIALS AND METHODS

Frying Process

At the beginning of frying, the fryers have been stuffed with 2 L of fresh oil samples, and then oils have been heated to 175 ± 2 °C. The frying temperature has been controlled using a probe joined to the thermometer. An electrical domestic deep-fat fryer has been used for frying experiments. Prior to frying, potato slices have been dried on both sides on filter paper to remove any excess water. The frying process started 30 minutes after the temperature reached at 175 ± 2 °C. The frying time has been 6 minutes for potato slices. One frying has been done per day for seven consecutive days. All physical and chemical analyses of oils have been performed immediately after the frying. During frying process, fresh oil has not been added to frying pans.

Determination of Fatty Acids Composition

Gas chromatography has been used for the qualitative and quantitative determinations of the fatty acids reported in relative area percentages. Fatty acids have been methylated prior to analysis by gas chromatography. Analysis have been performed on Agilent 9C 6890N gas chromatograph (CA, USA) equipped with a DB-23 capillary column (60 m, 0.32 mm, 0.25μm

film thickness) and a flame ionization detector. The oven temperature has been arranged from 160°C to 185°C at a rate of 7 minutes, later programmed from 195°C to 220°C for 3 minutes, finally kept 20 minutes at the last temperature. The injector and detector temperatures have been 230°C and 255°C, respectively. Nitrogen has been used as carrier gas at a flow rate of 1.0 ml/min. FAME has been identified by comparing their retention time with known commercial standard mixtures.

RESULTS AND DISCUSSION

The fatty acid compositions of sunflower oils are shown in Table 1. Composition of fatty acid in sunflower oil contained palmitic acid (7.1 %), stearic acid (4.3 %), oleic acid (19.0 %), linoleic acid (67.5 %) and linolenic acid (0.8 %). These results belong to before starting fryings. Linoleic acid (C18:2) is determinated the most abundant unsaturated fatty acid in the sunflower oil. Linolenic acid (18:3) is highly sensitive to oxidation because it contains three double bonds, while oleic acid (18:1) is less reactive as it contains only one double bond. At the end of the frying processes, composition of fatty acid in sunflower oil contained palmitic acid (11.4 %), stearic acid (4.9 %), oleic acid (9.1 %), linoleic acid (47.9 %) and linolenic acid (0.0 %). It is observed that there is a decrease in polyunsaturated fatty acids and resulting increase in the saturated acids content. When the frying times run on, the analysis of oil samples have showed that trans fatty acids such as elaidic and linoelaidic acids occur quite slowly. The elaidic acid ($C18:1_{trans}$) has been determined in oils for sunflower %1.5. And also linoelaidic acid ($C18:2_{trans}$) has been detected 0.3%. At the end of the seventh day of frying, the relative contribution of oleic acid has been decreased for sunflower oils.

Poor frying stability in sunflower oil comes primarily from the high level of linoleic acid. Therefore, sunflower oil must also be hydrogenated to reduce its linoleic acid content to 35% or lower for industrial frying. On the other hand, fatty acid compositions do not fully explain frying stability of oils. For understanding of the frying stability of oil, there are so many parameters. Stability of oil indicates that the oil must be low in free fatty acids, peroxide value, conjugated dienes, anisidine value, monoacylglycerols, diacylglycerols, and trace impurities, such as iron, phosphorus, calcium, and magnesium. All of these quality parameters have specific significance in influencing the performance of the frying oil.

Table 1 Changes in fatty acid composition (%) during frying processes.

Fatty Acids	Fresh oil	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
$C_{14:0}$	0.1982	0.2472	0.4000	0.5772	0.7579	0.9120	1.0700	1.2035
$C_{15:0}$	-	-	-	0.1859	0.3580	0.4727	0.5674	0.6498
$C_{15:1\ cis}$	0.2683	0.2037	0.1229	0.0680	0.0391	0.0253	0.0157	0.0102
$C_{16:0}$	7.0560	7.4007	8.0065	8.4157	9.2316	10.0710	10.8954	11.3895
$C_{16:1\ cis}$	0.1731	0.1710	0.1698	0.1687	0.1654	0.1619	0.1559	0.1463
$C_{16:1\ trans}$	-	-	-	-	-	-	-	-
$C_{17:0}$	-	-	-	-	-	-	-	-
$C_{17:1\ cis}$	-	-	-	-	-	-	-	-
$C_{18:0}$	4.3061	4.4458	4.5834	4.7435	4.8403	4.9146	4.9414	4.9502
$C_{18:1\ cis}$	18.9617	18.1325	17.2031	16.3104	15.1967	13.5689	11.2314	9.1256
$C_{18:1.\ trans}$	-	-	0.139	0.4793	0.7193	0.9486	1.2546	1.4876
$C_{18:2\ cis}$	67.5091	63.1032	59.9364	56.0213	53.4558	51.0132	49.0135	47.9356
$C_{18:2\ trans}$	-	0.0601	0.1147	0.1625	0.2053	0.2421	0.2748	0.3059
$C_{18:3\ cis}$	0.7778	0.5364	0.4915	0.3221	0.2287	0.2032	0.0913	-
$C_{18:3\ trans}$	-	-	-	-	-	-	-	-
$C_{20:0}$	0.2939	0.3192	0.3605	0.3989	0.4408	0.4854	0.5073	0.5231
$C_{20:1\ cis}$	0.1552	0.1187	0.0983	0.0812	0.0706	0.0567	0.0364	0.0286
$C_{20:1\ trans}$	-	-	-	-	-	-	-	-
$C_{20:2}$	-	0.0102	0.0243	0.0411	0.0618	0.0825	0.1026	0.1168
$C_{20:3}$	-	-	0.0306	0.0052	-	-	-	-
$C_{20:5}$	0.062	0.0245	0.0056	-	-	-	-	-
$C_{22:0}$	0.6325	0.6726	0.7094	0.7532	0.7831	0.8029	0.8203	0.8316
$C_{22:1}$	0.0153	0.0102	0.0044	-	-	-	-	-
$C_{23:0}$	0.0447	0.0635	0.0976	0.1368	0.1732	0.2123	0.2419	0.2604
$C_{24:0}$	-	0.1201	0.2032	0.2713	0.3404	0.3941	0.4402	0.4657
$C_{24:1}$	-	0.0223	0.0445	0.0614	0.0727	0.0802	0.0889	0.0901

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DETECTION OF REFINED MAIZE AND CANOLA OIL IN COLD-PRESSED SUNFLOWER OIL BY USING RAMAN SPECTROSCOPY

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ABSTRACT

The use of Raman spectroscopy for determination of food adulterations and fraudulent activities has received increased attention due to its simplicity and short response time in last decade. In the meaning of oil analysis, Raman spectroscopic methods positively differ from other analytic techniques such as chromatographic methods which include complex and time consuming sample preparation steps and involve toxic chemicals. Likewise most of the food products seed oils are attractive targets for malpractices. In this paper, the use of Raman spectroscopy for determination of canola and maize oil addition in cold-pressed sunflower oil is presented. The Raman spectra of the oil mixtures containing different amounts of abovementioned oils were collected in the range of 200-2000 cm-1 at a resolution of 5 cm-1. The strongest band at 1645 cm-1 belongs to C=C stretch was same for all three oils but its intensity increased according to the increase in canola oil ratio in the oil mixture. It may due to the highest monounsaturated fatty acid value of canola oil. Sunflower oil and maize oil have closer fatty acid composition compared with canola oil which results to very similar Raman spectra for them. However, some bioactive substances exist in cold-pressed oil affect the Raman signals and the findings showed that refined maize oil addition could be detected using Raman spectroscopy. As a conclusion, in this study, the detection of canola oil in cold-pressed sunflower oil seems possible using Raman spectroscopy at a level of 10 %.

Keywords : maize oil, canola oil, sunflower oil, Raman spectroscopy

DETERMINATION OF REFINED SUNFLOWER OIL IN COLD-PRESSED SUNFLOWER OIL USING RAMAN SPECTROSCOPY

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ABSTRACT

Seed oils are generally subjected to refining process to remove substances which would further catalyse oxidation or other undesirable reactions in commercialized product. On the other hand, cold-pressed oils obtained by mechanical processes without any chemical or heat treatment have been gaining more attendance according to the desire for minimally processed foods. However, cold-pressed oils are attractive targets for fraudulent activities due to their high price compared to refined oils. In this study, existence of refined sunflower oil in cold-pressed sunflower oil was investigated using Raman spectroscopy. Oil mixtures composed of 0-100, 5-95, 10-90, 20-80, 40-60, 60-40, 80-20 and 100-0 % (cold-pressed oil-refined oil) were prepared and analysed with Raman spectrophotometer. The spectra were collected between 200 and 2000 cm-1. The most intense band for both cold-pressed and refined sunflower oils was situated around 1646-1652 cm-1 and 1428-1432 followed this strong band. While the bands in the region of 1400 to 1500 cm-1 have been assigned to =CH2 scissoring, the band situated around 1650 belongs to C=C stretch. There was no missing or new-born band according to the mixture ratios but it was clearly seen that the intensity of the bands were significantly different for every sample. Raman spectroscopy was shown by this study as it is useful for differentiation of refined and cold-pressed sunflower oils. However, there is a need for extended studies to verify present method using more oil samples.

Keywords : Cold-pressed oil, sunflower oil, Raman spectroscopy

MONITORING THE CHANGES IN COLD-PRESSED SUNFLOWER OIL DURING HEATING BY RAMAN SPECTROSCOPY

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ABSTRACT

In the present study, the effect of continuous heating process on Raman spectrum of cold-pressed sunflower oil was monitored. Raman spectra were obtained using DXR Raman Microscopy system with a 532 nm laser source and CCD at 0 °C. Spectra were collected in the range of 200-2000 cm-1 at a resolution of 5 cm-1. Short-term heating process was applied for 30 m on direct heating system and the highest oil temperature was 173 °C measured by oil thermometer. Oil samples were collected in every 5 m through the heating. Continuous heating and especially frying process may result in peroxides degradation and polymerization reactions which are strongly related to the unsaturated fatty acid and other bioactive compounds exist in the oil. Our results showed that the main Raman bands were observed in 860, 960, 1067, 1144, 1255, 1290, 1428, 1509, 1651 and 1737 cm-1. The spectra of the samples collected at different times seemed to be similar. However, there were intensity differences in the same band intensity. Additionally there was a missing band at 1144 cm-1 (-C – O stretching) after 10 m heat treatment. The strongest band positioned at 1651 cm-1 belongs to C=C stretch which tends to loose intensity through the 30 m heating process may be explained due to the high unsaturated fatty acid exist in cold-pressed sunflower oil. As a result, our findings showed that Raman spectroscopy could be used to follow the heat-induced changes in cold-pressed sunflower oil.

Keywords : Sunflower oil, cold-pressed, Raman spectroscopy, heating

APPLICATION OF ARTIFICIAL NEURAL NETWORK ON PREDICTION OF MOISTURE CONTENT OF THE DEEP-FAT FRYING OF BEEF MEATBALLS IN SUNFLOWER OIL

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ABSTRACT:

In this study, it's aimed to predict moisture content of meatball samples fried in sunflower oil. A dataset of temperature, duration of sunflower oil and pH, *L**, *a**, *b**, Chrome, Hue, radius, height of meatballs were processed to develop a three layered artificial neural network. 85% of the objects were used as the training set and 15% as the test set in the application of artificial neural network model. The final developed model presented higher performance for the artificial neural-network than statistical regression model. Artificial neural network is shown to be a powerful and suitable tool for the prediction of moisture content of meatballs fried in sunflower oil.

Keywords: Sunflower oil, deep-fat frying, meatball, artificial neural network, moisture content.

DEEP FRYING QUALITY OF HIGH-OLEIC SUNFLOWER OIL

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ABSTRACT

Sunflower has high content and high quality oil is an important oil plant in all over the World. Sunflower oil has some differences for storage, consumption and industrial quality depending on fatty acid composition. In Turkey, linoleic type sunflower is usually cultivated and also used for industrial purposes. High-linoleic (omega 6) acid content increases to the nutritional quality for sunflower oil. However, this adversely affects to the industrial quality of high-linoleic sunflower because of high oxidative stability. Besides, high-oleic sunflower oil allows higher industrial quality and wide range of uses. Therefore, recent studies deal with breeding the high-oleic sunflower varieties and give to the industry. Frying is a major industrial uses for plant oils. It has critical importance from higher temperature than direct consumption. While polyunsaturated fatty acids (PUFA) are desirable in salads, PUFA is not wanted in frying oils. Therefore, all of the frying oils are originated vegetable have reduced the amount of PUFA through hydrogenation and/or interesterification after refining and hence frying oils have enhanced oxidative stability. Especially high oleic sunflower oil obtained by plant breeding programs, all vegetable oils have reduced content of PUFA is suitable source for frying. This is so important for our country has a very crucial position in sunflower production. Frying is the most preferred method for food cooking and preparing for last 50 years. Deep frying process has 20-200 mm oil height, 5-10 minutes processing time and fried oil is reusable. Firstly, oil is preheated to 150-180 °C for frying process. When the food contact to the oil, surface temperature reaches to the oil temperature rapidly. However, the inner part of food remains between 80-100 °C. Degradation products with hundreds of dissimilar structures occurred with different reactions via varied mechanisms and under varied temperatures. However, all of the degradation products are polar character and deep frying process is often used in fast food restaurants. These oils are subject to chemical and physical changes after 10-12 hours frying. Fried food consumption frequently and continuously increases the risk of cancer and cardiovascular and gastrointestinal diseases. The properties of the oil used in frying process are the biggest factor in the emergence of these risks. If the frying oil contains high amounts of PUFA, the resulting risks are that much bigger. Therefore, the use of high oleic oils for frying are recently encouraged. Modified sunflower seeds have a reduced linoleic acid and increased oleic acid content. Thus high oleic sunflower oil has both higher oxidative stability and positive effects on health. Therefore the aim of our work, to determine the thermal stability of high oleic sunflower oil (omega 9) and to compare with the linoleic sunflower oil (omega 6) and refined olive oil which has also high oleic acid content.

Keywords: High oleic, linoleic, refined olive oil, thermal stability.

THE DIFFERENCES BETWEEN LINOLEIC AND HIGH-OLEIC SUNFLOWER OIL

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ABSTRACT

Linoleic type sunflower oil is mostly preferred oil in Turkey for different purposes, such as salads, meals, frying etc. However, in recent years, oleic acid type sunflower oil is more suitable and healthy for both frying and biodiesel has begun to spread, particularly in US, France and Spain. High oleic sunflower production should be expanded and encouraged due to many advantages. In the last few years, Trakya Birlik which is the biggest oil growers cooperative encourages to high oleic sunflower production in Turkey. Linoleic sunflower varieties are generally grown and processed industrially in Turkey. Linoleic acid reduces to the saturation and facilitates to the digestion and passes the blood. The greater amount of linoleic acid in the oil increases the oil quality. However, high linoleic acid content in sunflower oil affects to the industrial value. Linoleic sunflower oil usually use in salads, meals, margarines and shortenings. High oleic sunflower oil is used in generally spray oil in crackers, dried fruits, bakery products, frying, deep oil frying, roast process, salads and sauces, food supplements specialized for elders and child and as a mixture oil in margarine and mayonnaise. Except food industry, high oleic sunflower oil uses for cosmetic-paint industry and biodiesel production. The farming of oleic type sunflower is increasing and getting more important because the usage and consumer preference of oleic type sunflower oil are also increasing. While the US prefers to farm mid-oleic types contain maximum 80% oleic acid, Europe prefers to high oleic types contain more than 80%. Recently, hybrid seeds are used for sunflower farming and breeding programs have begun to high oleic seeds. However, it is still widely used linoleic sunflower for industrial purposes. Fatty acid composition not only affects to the industrial quality but also nutritional value is also affected at the same time. Fatty acid composition affects to the taste and chemical quality of oil. The phenolics in sunflower oil have effective role on taste aroma, oxidation level and rate.

Keywords: High oleic sunflower, linoleic acid, mid-oleic sunflower.

APPLICATION OF SUPERCRITICAL CARBON DIOXIDE FOR SUNFLOWER OIL EXTRACTION

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ABSTRACT

Sunflower is an important source for edible oil and thus commercially widely cultivated in order to produce vegetable oil in the world. Industrial extraction of sunflower seeds is commonly carried out through mechanical pressing followed by hexane extraction. However, this procedure might causes the production of undesirable residues, and also the oil can undergo oxidative transformations during the removal of the solvent. Therefore, oil quality is influenced negatively. In this study, a comprehensive review is presented on the researches and developments related to supercritical CO2 extraction of oil from sunflower seeds. Supercritical carbon dioxide (SC-CO2) extraction method is a promising potential alternative method for vegetable oil extraction to replace traditional techniques like mechanical pressing, organic solvent extraction. CO2 is the most commonly used solvent because of being non-toxic, non-flammable, non-explosive, cost-efficient, readily available, and easy to remove from the extracted materials. In SC-CO2 studies pressure and temperature during the extraction and recovery of the oil are important parameters that are considered. Moreover, the yield of oil is affected by the size and physical structure of the sunflower seeds.

Keywords: Supercritical Fuid, Extraction, Carbon dioxide, Sunflower oil

EFFECT OF ENZYMATIC INTERESTERIFICATION ON OXIDATIVE STABILITY OF SUNFLOWER OIL

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ABSTRACT

A preliminary study was undertaken to investigate the effect of enzymatic interesterification on the oxidative stability of sunflower oil. Immobilized lipase from *Rhizomucor mihei* was used to catalyze the interesterification of sunflower oil at 25±3°C. The immobilization procedure involves electrostatic complex formation between lipase and a highly branched polycationic polymer. Interesterification procedures were carried out in a continuous stirred batch type reactor. The comparison of oxidative stability based on simply peroxide value was carried out between virgin and enzymatic interesterified sunflower oils. The differences in peroxide values of virgin and interesterified oil samples were evaluated at the first day and 21th day of storage under accelerated conditions. The presented results of this study showed that the enzymatic interesterification decreased the peroxide value of sunflower oil. At the end of the 21th day of storage at 80°C, peroxide value of interesterified sunflower oil showed lower increase tendency than virgin oil.

Keywords: Interesterification, lipase, oxidative stability, sunflower oil

EFFECT OF THE DEEP-FAT FRYING PROCESS ON AROMA COMPOUNDS OF SUNFLOWER SEED OIL

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ABSTRACT

Sunflower seed oil is the fourth largest edible oil in the world and the consumption of it has been increasing due to the aromatic, nutritive and economic reasons. This vegetable oil is mainly used for cooking especially as frying oil. Deep-fat frying is a significant process of food preparation and gives a unique color and desirable flavor to food which is accepted by consumers. Through the process, a variability of chemical reactions result in the formation of extensive aromatic compounds. Likewise any other food product, oil quality is closely related to the aroma detected by consumers, and this attribute will have a great influence on its acceptance or rejection. Therefore, the present study explores the effects of the deep-fat frying process on aroma composition of the sunflower seed oil. The aroma compounds of 1, 5 and 10 times used oils to fry potatoes and non-fried oils were analyzed. Volatile components of the oils were extracted by using of the purge and trap technique with dichloromethane and analyzed by gas chromatography mass spectrometry (GC-MS). Results showed that total aldehydes content increased with the frying treatment due to the Strecker degradation. In this reaction, dicarbonyls or hydroxycarbonyl intermediates deaminate and decarboxylate amino acids to produce the corresponding Strecker aldehydes. Among the aldehyde compounds, (E,E)-2,4-decadienal, (E)-2-heptenal and hexanal were the major aroma compounds in all sunflower seed oils.

Key words: Sunflower, Deep-fat frying, Sunflower seed oil, aroma, GC-MS.

INTRODUCTION

Frying is the cooking of food in oil or another fat. Foods can be fried in a variety of fats and vegetable oils. These oils play a significant role in the food industry due to both their functional and nutritional features and their impact on taste, aroma and health. Refined sunflower oil, especially high-oleic, is very versatile and due to its neutral flavor and heat stability it can be consumed in many ways in the kitchen, such as frying and cooking. Sunflower (Helianthus annuus L.) is globally one of the most important oil crops and in

Turkey sunflower oil is commonly used for frying. Especially, deep frying is now the basis of a very large and expanding worldwide industry. During deep-fat frying, fats and oils are continuously or repeatedly heated at high temperatures (up to 190 °C) for prolonged periods of time in the presence of air. Under these conditions both thermal and oxidative reactions of the oils occur, leading to the formation of volatile and nonvolatile decomposition products (Chang et al., 1978). Prolonged deep-fat frying results in poor acceptability and nutritive value owing to the thermal and oxidative reactions in the frying oil. Together with the generation of long-lived bubbles and an increase in viscocity, frying oil begins to generate a noticeable odor attributable to the various volatile decomposition products of the thermally oxidized oil (Fujisaki et al., 2002, Tekelioğlu et al., 2008). Aroma is a main quality factor for edible vegetable oils as a characteristic parameter. During heat treatment by frying beside the aroma compounds formed which are very appreciated by consumers also other compounds which are not desirable get accumulated in the products; those compounds are formed by partial or total alteration of thermolabile nutrients present in food and in the frying oil (Ghidurus et al., 2011). The frying oil decomposition products as well as products formed from reactions between food components (proteins, cabohydrates) and oil constituents may adversely affect the flavor, color, nutritive value, and safety of the fried food (Takeoka et al., 1995; 1996).

Therefore, the aim of the present study was to evaluate the aromatic extracts obtained by purge and trap technique and then investigate the influence of frying process on aroma compounds. In present study, the aroma compounds of 1, 5 and 10 times used oils to fry potatoes and non-fried oils were analyzed.

MATERIALS AND METHODS

Materials

The water used in the study was purified by a Millipore-Q system (Millipore Corp., Saint-Quentin, France). Dichloromethane, 2-octanol, and sodium sulphate were obtained from Merck (Darmstadt, Germany). Dichloromethane was freshly distilled prior to use. Sunflower oil samples were obtained from a local producer in Adana city, Turkey.

Methods

Treatment of Frying Process

Before frying process, potatoes were hand peeled and then cut into strips (1 × 1 × 6 cm) with a stainless steel slicer. A fraction of 200 g of potato strips were used at each frying process. The potatoes were deep fried in sunflower oil using an electrical fryer (Felix FL 269, Turkey) at the 190 ºC for 10 minutes. Frying process was repeated 10 times and oil samples were obtained after 1, 5 and 10 times frying.

Extraction of the Aroma Compounds

The volatile compounds of sunflower oils were extracted by purge and trap system which consists in a source of nitrogen, controlled by a flow-meter. For the extraction, 10 g oil sample transferred into 20 mL vial then the sample was pre-incubated at the extraction temperature for 10 min. After purging process, the compounds retained in the cartridge were eluted with dichloromethane. After dehydration by anhydrous sodium sulphate, the pooled

organic extract was reduced to 5 mL in a Kuderna Danish concentrator fitted with a Snyder column at 40ºC (Supelco, St Quentin, France) and then to 0.5 mL under a gentle stream of nitrogen. Extracts were then stored at -20°C in a glass vial equipped with a Teflon-lined cap before analysis. Each sample was extracted in triplicate.

Analysis of Aroma Compounds

The GC system consisted of an Agilent 6890 chromatograph equipped with a flame ionization detector (FID) (Wilmington, DE) and an Agilent 5973N -mass selective detector (MSD). Aroma compounds were separated on a DB-Wax (30 m, 0.25 mm, 0.5 mm thickness; J&W Scientific, Folsom, CA) column. Retention indices of the compounds were calculated by using the retention data of a linear alkane series. After identification, the concentrations of aroma compounds were calculated by GC–FID according to the internal standard (2-octanol). The condition details of GC-MS and GC-FID were described in our previous study (Amanpour et al., 2015).

The GC system consisted of an Agilent 6890 chromatograph equipped with a flame ionization detector (FID) (Wilmington, DE) and an Agilent 5973-Network-mass selective detector (MSD) (DE, USA). Aroma compounds were separated on a DB-Wax (30 m length x 0.25 mm i.d. x 0.5μm thickness, J&W Scientific Folsom, CA , USA) column. A total of 3 μL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. Injector and FID detectors were set at 270°C and 280°C, respectively. The flow rate of carrier gas (helium) was 1.5 mL/min. The oven temperature of the DB-Wax column was first increased from 50° to 200°C at a rate of 5°C min-1 and then to 260°C at 8°C min-1 with a final holds at 260°C for 5 min. The same oven temperature programs were used for the MSD. The mass detector was operated in the electron impact mode at 70 eV. The GC-MS interface and ionization source temperature was set at 250°C and 180°C, respectively. Identification and quantification were performed in full scan mode with a mass/charge range of 30-300 amu at 2.0 scan s-1 scan rate. The compounds were identified by comparing their retention index and Wiley-6 and NIST-98 mass spectral libraries. Standard compounds were injected and analyzed under the same conditions. Retention indices of the compounds were calculated by using an n-alkane series. After identification the concentrations of aroma compounds were calculated according to internal standard (2-octanol) (Kesen et al, 2014).

RESULTS AND DISCUSSIONS

The volatile compounds identified in sunflower oils subjected to different number of frying process were presented in Table 1. Mean values (μg kg^{-1}) of the GC analyses of triplicate extractions were reported. As can be seen in Table 1, the total content of volatile compounds in the non-fried sunflower oil was the lowest and it increased gradually depending on the number of frying. The main reason of this could be taking place of oxidation, polymerization or thermal decomposition reactions in the frying oils used repeatedly at high temperatures. Besides, there are certain proofs that secondary oxidation products such as aldehydes and ketones, polar compounds, and acrolein which are formed in oxidized and degraded oils. Volatile compounds formed in frying oil include aldehydes, ketones, hydrocarbons, alcohols, acids, esters, and aromatic compounds (Chang et al., 1978). The amounts of volatile compounds of non-fried and 1, 5, 10 times fried oils were 2289, 3748, 5074 and 6992 μg kg^{-1}, respectively.

Results showed that total aldehydes content increased with the frying treatment due to the Strecker degradation. In this reaction, dicarbonyls or hydroxycarbonyl intermediates

deaminate and decarboxylate amino acids to produce the corresponding Strecker aldehydes. Among decomposition products during frying process, aldehydes are the most important because they are the most abundant (Frankel, 1985) and their thresholds are lower than those of other secondary products that characterize the flavor of fried foods and oils. As for identified aldehyde compounds in this study, (*E*,*E*)-2,4-decadienal, (*E*)-2-heptenal and hexanal were the major aroma compounds in all sunflower seed oils and their contents were increased due to number of frying process. The amounts of (*E*,*E*)-2,4-decadienal, (*E*)-2-heptenal and hexanal were found as 1448.0, 905.6 and 535.6 μg kg^{-1}, respectively. Volatile aldehydes are generated mainly from frying oil via β-scission of alkoxy radicals formed by the homolytic cleavage of FA hydroperoxides (Frankel, 1985).

Alcohols which were the other aroma compounds whose amount increased with the frying process. While their concentration in non-fried oil was 463.6 μg kg^{-1}, it reached 1417.5 μg kg^{-1} after the tenth frying treatment. The total concentration of pentanol, 2-nonanol, 2-methyl-2-butenol, 1-octen-3-ol and 1H-indole-3-ethanol increased with frying process.

Looking at the acid, lactone, terpene and phenol compounds, it has been observed that the amounts of such compounds decreased with the frying process.

When compared to literature, the different pattern of the aroma compounds of oils that subjected to frying process was determined. In previous studies, Doleschall et al. (2003) showed the aroma compounds of refined sunflower oil before frying and after the 3rd cycle. On the results of the refined oil small amount of hexanal, (*E*)-2-heptenal and nonanal have been observed, while the fried oil contains more types of aldehyde in larger amount. Chang et al. (1978) showed that the volatile products formed from corn oil and hydrogenated cottonseed oil during deep-fat frying. Other researchers have also studied the volatile constituents resulting from the thermal treatment of vegetable oils (Snyder et al., 1985; Macku and Shibamoto, 1991; Wu and Chen, 1992; Chung et al., 1993). These results suggest that frying process can effectively decrease or increase the amount of volatile compounds.

Table 1. Volatile Compounds of Sunflower Oil Under the Influence of Frying Process

		Number of frying process		
Aroma Compounds	**Non-fried**	**1**	**5**	**10**
Aldehydes				
Pentanal	-	142.0	215.4	344.9
Hexanal	227.4	287.1	304.2	535.6
(E)-2-Hexenal	-	45.8	71.7	124.0
(E)-2-Pentenal	-	-	-	36.5
(E)-2-Heptenal	101.1	766.1	796.8	905.6
Nonanal	-	148.9	175.2	197.1
(E)-2-Octenal	-	259.9	189.5	392.1
(E,E)-2,4-Heptadienal	-	-	128.6	33.3
(E)-2-Decenal	-	-	132.1	137.7
(E,E)-2,4-Nonadienal	-	-	21.5	24.8
(E,Z)-2,4-Decadienal	25.9	171.8	425.5	487.8
(E,E)-2,4-Decadienal	27.8	332.0	1181.1	1448.0
2-Heptedecenal	101.4	-	-	-
Total	**483.7**	**2153.6**	**3641.7**	**4667.3**
Alcohols				
3-penten-2-ol	129.2	94.3	82.5	72.7
Pentanol	65.0	51.8	123.1	226.3

4-Heptanol		44.4	26.4	25.4	16.7
2-Nonanol		-	-	-	92.6
5-Methyl-2-hexanol		121.1	79.5	71.7	-
2-Methyl-2-butenol		15.4	13.5	17.1	26.9
3-Octanol		30.2	-	-	-
1-Octen-3-ol		-	99.8	116.7	215.6
2-Phenylethanol		58.3	30.4	10.3	7.2
1H-Indole-3-ethanol		-	-	-	759.5
	Total	**463.6**	**395.7**	**446.8**	**1417.5**
Acids					
Pentanoic acid		24.8	43.2	-	-
Hexanoic acid		189.4	138.5	123.3	53.0
Heptanoic acid		45.4	73.2	-	-
Octanoic acid		111.6	92.5	38.2	21.5
Nonanoic acid		73.8	54.5	-	-
Decanoic acid		88.4	43.7	32.9	-
	Total	**533.3**	**445.5**	**194.4**	**74.5**
Ketones					
6-Methyl-2-heptanone		41.0	-	-	-
4-Nonanone		-	19.2	21.1	41.8
4-Hydroxy-4-methyl-2-pentanone		316.9	399.1	348.5	346.3
3-Nonen-2-one		-	48.3	78.7	87.2
2,7-Octanedione		-	37.2	49.4	62.7
	Total	**357.9**	**503.7**	**497.6**	**538.1**
Lactones					
5-Pentyl-2(3H)-furanone		7.9	-	-	-
5-Pentyl-2(5H)-furanone		40.5	26.8	-	-
	Total	**48.4**	**26.8**	**0.0**	**0.0**
Terpenes					
dL-Limonene		140.8	116.0	62.3	23.7
Linalool		-	-	16.2	14.2
	Total	**140.8**	**116.0**	**78.6**	**37.9**
Phenols					
Phenol		20.1	-	-	-
2,3-Dimethyl phenol		93.9	-	-	-
	Total	**114.0**	**0.0**	**0.0**	**0.0**
Esters					
n-Butyl acetate		146.8	107.3	94.8	65.7
Methyl palmitate		-	-	35.8	48.5
Methyl oleate		-	-	83.8	142.5
	Total	**146.8**	**107.3**	**214.4**	**256.7**
GENERAL TOTAL		**2288.6**	**3748.5**	**5073.5**	**6991.9**

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BIOPELLET PRODUCTION FROM WASTE MATERIALS OF THE SUNFLOWER IS A MAJOR INDUSTRIAL PLANT

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ABSTRACT

Sunflower is the most important industrial plant with oil content and consumption percent in Turkey. The highest production of sunflower with 44% is made in Thrace and a large content of waste materials (core-shell, sunflower bat etc.) are obtained after harvest and processing. These materials have alternative assessment opportunities. Untreated agricultural waste is generally used for heating directly. However, this method is not economical, efficient and suitable for environmental point. Harmful gases such as CO2 release during the combustion process occur. These waste materials leave to the field and return to the land again because of the difficulties and the lack of economic benefits with usage of heating material. However, it is possible that the waste materials can be converted into heating material, biopellet, is not harmful and has higher energy value. Biopellet is important heating material for farmer and sunflower oil industry. Farmers have a large amount of waste after sunflower harvest. Besides, high content of core-shell and solid material also get to stay in oil factories and cooperatives. Sunflower oil industry only annually produces 800 000 tons of solid waste in Turkey as a byproduct. Failure in evaluation of sunflower waste materials is too big to ignore is a serious economical loss. There are various studies about converting the sunflower waste materials after harvest and/or oil extraction. All of them say that biopellet production is valuable method for both environmental and economical. At the same time, the waste materials used as a heating material directly but inefficient combustion and excess content of volatiles were determined. All for these reason, biopellet is environmental friendly waste is a great need to improve fuel production. Although the ban, a significant amount of agricultural waste are burned in the field or using as fuel in homes in our country for each year. However, biopellet is a modern technic for heating offers integrated solutions for sustainable development in developed and industrial countries. Besides, it also serves the purpose of preventing climate change, erosion and efficiency, ecosystem health and loss of biodiversity. So, biopellet production is an ecological solution.

Key Words : Biopellet, core-shell, sunflower bat, sunflower waste.

FACTORS AFFECTING THE NUTRIENT COMPOSITION OF SUNFLOWER MEAL

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ABSTRACT

Sunflower (*Helianthus annus L.*) is a high oil-yielding seed crop cultivated worldwide that adapts very well to a wide range of climates. Sunflower seed meal is a by-product of the oil extraction of sunflowers and it is produced in large quantities. Sunflower meal (SFM) is mainly used as feed source that offer cheap, eco-friendly substrates for the animal nutrition. The meal is initially used as a protein complement in ruminant diets, and also monogastric animal rations in appropriate amounts. The chemical compositions of SFM have been extensively evaluated and it has been found that the chemical composition of SFM is varied greatly. The mean moisture and dry matter contents of SFM were reported as 9.0 % and 91.0 %, respectively. SFM is composed basically on lignocellulosic fiber and proteins. The content of crude protein in SFM ranges from 23.0 to 42.0 % and the crude fiber level varies between 13.0 % and 35.0 % depending on the extent of dehulling. The concentration of ether exracts in SFM varies from 0.50 to 13.0 % depending on the extraction process. The large variation of ether exract level was mainly related to the different extraction process. The differences in production methods, such as heating temperature, pressure and time during the process might lead to the changes in ether exract values. The different production techniques also caused the variation of the other chemical components of SFM. The content of phenolic compounds such as chlorogenic acid and caffeic acid in SFM ranges from 3 to 4 %. The average ash composition of sunflower meal was reported to be 6. 0 %. In conclusion, the processing techniques is one of the major factor affects the nutritional composition of SBM. Processing techniques are initially effective in the levels of ether exracts, the crude fiber levels and other nutrients therefrom. The variations of nutrient composition in SFM might result from dehulling process too. SFM composition can vary somewhat according to extrinsic factors such as genetic, seed varieties, climate and soil conditions. In addition, the chemical concentration of SFM is also affected in each plant and collecting typical samples in person and the analysis method used.

Key Words : Crude protein, crude fiber, nutrient composition, processing techniques, sunflower meal

EFFECT OF HIGH OLEIC SUNFLOWER OIL INCLUDING OLEOGEL ON THE TEXTURAL AND SENSORY PROPERTIES OF CAKE

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ABSTRACT

The existence of the relation between health and diet has motived people to consume food products with lower adverse health effects. As known consumption of excessive saturated fatty acid increases the risk of cardiovascular disease. Therefore, decreasing saturated fatty acid content of the food materials without damaging the quality of the food products is important issue in the food industry. When considering the importance of fats in the quality of the products, liquid oils are structured to transform them to solid fats. Oleogelation is one of the way which has been recently used for this aim. In the present study, probable usage of oleogels prepared from high oleic sunflower oil (HOSO) in the formulation of cake was investigated. For this aim three different oleogel formulations were studied: (i). 50 % cottonseed oil (CSO) + 25 % shortening + 25 % HOSO, (ii). 50 % HOSO + 50 % CSO and (iii) it is the same with second formulation however, this oil blend was oleogelled with dehydrated wax. Textural and sensorial properties of oleogel including cakes and control sample were investigated. Hardness, chewiness and gumminess values of the cakes prepared by oleogels were found to be higher than those of control sample. According to sensory analyses, the sample prepared from third formulation had the highest overall acceptability value. Wax type used in the formulation as well as oil types significantly affected textural and sensory properties of cakes. The findings of the present study highlighted that oleogels rich in unsaturated fatty acid content could be used in the cake formulation instead of shortening rich in saturated fatty acids.

Key Words : Oleogel, cake, high oleic sunflower oil, texture, sensory

XYLOSE PRODUCTION FROM PRETREATED SUNFLOWER STALKS

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ABSTRACT

Sunflower is an important oilseed plant. After harvesting of seed, stalks are left behind in the fields and they are usually left to rot or burned in the field with associated environmental risks. However, this biomass can serve as an abundant and renewable source for the soluble sugar, especially xylose. Xylose or wood sugar, is a member of aldopentoses. It is a sugar that is found in many edible seeds of the plant and located in the structure of plants, such as wood or straw. It can be obtained from hard and the soft woody plants or can be produced from agricultural waste that has a lignocellulosic nature. The most important use of xylose is xylitol production. Production of xylose from lignocellulosic materials are genarally carried out by dilute acid. The dilute acid hydrolysis is affordable, easy, fast and effective method for the production of xylose, but the hydrolysis requires corrosive chemicals, neutralization process and produce some undesirable compounds. The use of xylanase to hydrolyze xylan may be another alternative to acid hydrolysis of xylan for the release of xylose. The aim of this study was to evaluate *Trichoderma reesei* xylanase for obtaining xylose from autohydrolysis liquors of sunflower stalk. The effects of substrate concentrations and enzyme activity were investigated for the production of xylose. In order to obtain high xylose yield and selectivity, the optimization study was conducted by response surface methodology. Under the optimum condition, xylose yield and selectivity were found to be 86.4% and 9.2 g/g, respectively.

UTILIZATION OF SUNFLOWER STALKS

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ABSTRACT

Sunflower is an important and major oilseed crop in both Turkey and worldwide. After harvesting of seed, stalks are left behind in the fields and they are usually left to rot or burned in the field with associated environmental risks. In Turkey, around 2,500,000 tons of waste per year in the form of sunflower stalks are produced, which is a serious problem for farmers growing sunflower. So researchers focused on the assessment of using sunflower stalks and other agricultural waste for food and non-food purposes. These biomass can serve as an abundant and renewable source for the production of various chemical. The main component of sunflower stalk is cellulose that can be used in a wide range such as food, textile, cigarette industry such as film and sheet production. Cellulose derivatives has been used in rubber, paint, oil rigs, coatings, ink food, pharmaceutical industry. Hemicellulose and its fragmentation products have very wide range of applications in various fields. They have been used in the medical, cosmetic and food industry as absorbant, gelling agent, stabilizer, emulsifier, thickening. It can be converted to the film that can be used used for food packaging or fragmented to the oligomers that serve as prebiotics in functional food industry. Due to its amorphous structure, hemicellulose can be hydrolyzed by dilute acid to soluble sugar that can be used for the production of xylitol, lactic acid or ethanol. The aim of this study is to give detail information about the utulization of sunflower stalk.

NATURALLY BLEACHED VEGETABLE OIL, SHAPED BY ONE ALL-ROUND SOLUTION: TONSIL®

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ABSTRACT

Powerful against undesired odor, flavor and impurities from crude sunflower oil and other crude oils and fats, Clariant's TONSIL® bleaching earths have now been in use for more than 100 years. To meet today's growing global demand and ensure certified solutions for the scope of applications, we constantly carry out research into new products as well as into the rapid and flexible optimization of TONSIL® qualities in Europe, America and Asia. In many countries, TONSIL® has already become synonymous with activated bleaching earths, which we view as both a challenge and an obligation for the future.

Key Words : Bleaching Earth, Crude Oil, Sunflower Oil

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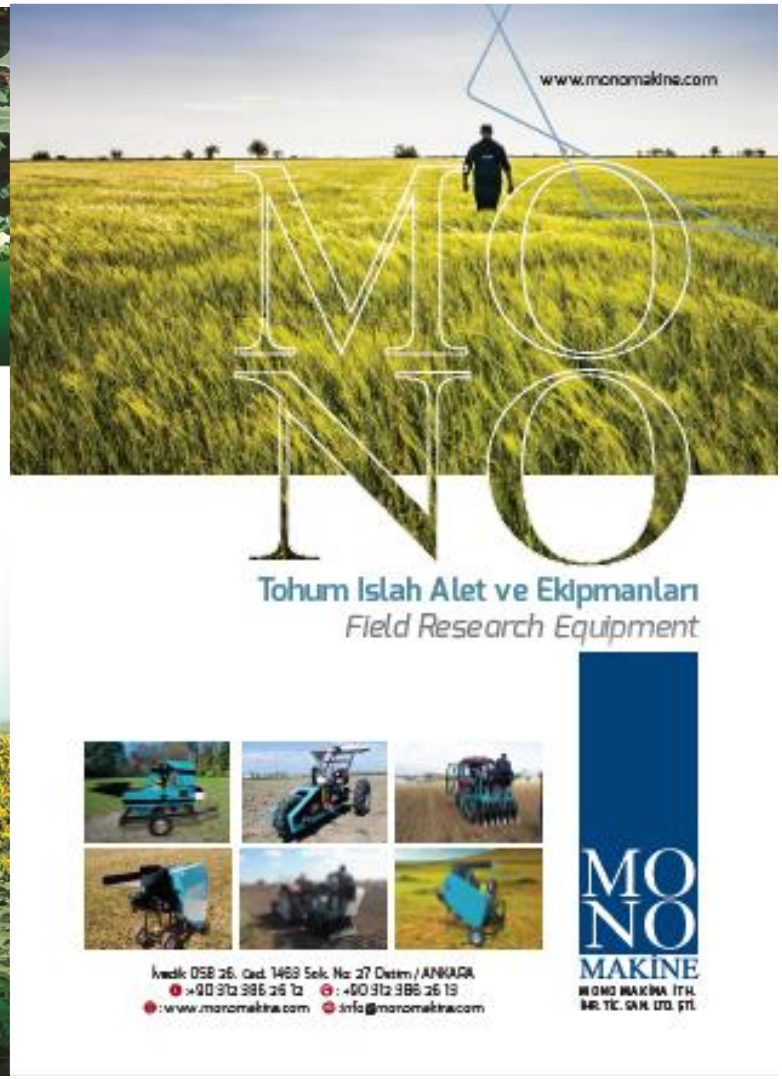

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