



# Innovations in Genetics and Breeding of Capsicum and Eggplant

Véronique Lefebvre, Marie-Christine Daunay

## ► To cite this version:

Véronique Lefebvre, Marie-Christine Daunay. Innovations in Genetics and Breeding of Capsicum and Eggplant: Proceedings of the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant September 11-13, 2019 | Avignon - France. Institut National de la Recherche Agronomique (INRA), 263 p., 2019, 9782738014290. hal-03552303

**HAL Id: hal-03552303**

**<https://hal.inrae.fr/hal-03552303>**

Submitted on 21 Jul 2023

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# Innovations in Genetics and Breeding of Capsicum and Eggplant

Proceedings of the 17<sup>th</sup> EUCARPIA Meeting on Genetics  
and Breeding of Capsicum and Eggplant,

September 11-13, 2019 | Avignon - France

Editors: Véronique Lefebvre & Marie-Christine Daunay

**Editors**

Véronique Lefebvre & Marie-Christine Daunay

**Title**

Innovations in Genetics and Breeding of Capsicum and Eggplant

**Sub-title**

Proceedings of the 17<sup>th</sup> EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant  
September 11-13, 2019 | Avignon - France

**Publisher**

Institut National de la Recherche Agronomique (INRA)  
Centre de recherche Provence-Alpes-Côte d'Azur  
228 route de l'aérodrome  
CS 40 509 - Domaine Saint Paul, Site Agroparc,  
84914 Avignon Cedex 9 - France

**Visual identity**

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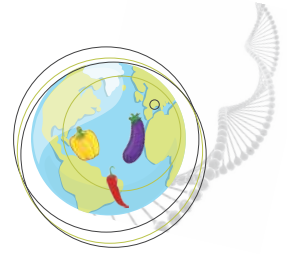
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**Layout design and editing**

Salima Kherchache

**Printed by**

SUD LABO, 35 avenue Pierre Sépard, 84000 Avignon - France



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# Foreword

*This book "Innovations on Genetics and Breeding of Capsicum and Eggplant" gathers oral and poster contributions presented at the eponymous 17<sup>th</sup> Eucarpia Meeting, held at the prestigious Popes' Palace in Avignon, between the 11<sup>th</sup> and 13<sup>th</sup> September 2019. Since its first edition in 1971, this triennial international meeting, held under the auspices of the Eucarpia association, is a fruitful forum for exchanges about the latest advances on Capsicum and eggplant genetics and breeding between c. 200 research and development experts from academic as well as private sectors. This 17<sup>th</sup> meeting is organized by INRA, as were its 3<sup>rd</sup> (1977) and 10<sup>th</sup> (1998) editions.*

*The five scientific sessions, Genetic Resources and Domestication, Resistance to Biotic & Abiotic Stresses, Nutritional Value & Fruit Quality, Biotechnology & Genomics, and Pre-breeding & Breeding, illustrate the commitment of Capsicum and eggplant scientists to sustainable breeding and crop production.*

*The scientific committee, together with the local organization committee, have introduced some new features into the 2019 meeting:*

- keynote speakers introduce each session with a talk providing an overview of each field of research;*
- the book includes abstracts of a standardized structure, instead of full papers, in order to provide complete and easy to handle information, and not to prevent subsequent publications in peer-reviewed journals;*
- authors of oral communications have been invited to write full original articles in the peer-reviewed and open-access journal 'Crop Breeding, Genetics and Genomics' (CBGG), which will publish a special issue on 'Genetics and Breeding of Pepper and Eggplant',*
- to promote participation of students in order to rejuvenate the Capsicum and Eggplant academic network, students have been offered reduced registration fees and awards will be attributed to the best oral and poster presentations of PhD students, thanks to public and private sponsorship;*
- free registration has been offered to a few PhD students and senior scientists from low-income countries, thanks to French institutional sponsorship.*

## Innovations in Genetics and Breeding of Capsicum and Eggplant



*We thank all oral and poster authors for their contributions and the special efforts made to comply with the specified abstract format together with the revisions suggested by the scientific committee aiming at ensuring uniform scientific quality. Members of the international scientific committee are warmly thanked for their noteworthy involvement in the scientific organization of the meeting. We are grateful to the local organization committee that has accomplished a tremendous and versatile 2 years' work. Our acknowledgements are also addressed to INRA, GEVES, CTIFL, Terralia, to several other national or regional French institutions, as well as to the vegetable seed companies, all of whose support and sponsorship was indispensable for the organization of the meeting.*

*We hope all attendees will enjoy the 17<sup>th</sup> Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant in Provence as much as the previous editions, and that this book will serve over the long term in testifying to its high scientific interest. Successive generations of researchers and breeders over almost 50 years have all paved the way to increased genetic knowledge and varietal improvement of Capsicum and eggplant.*

*We spare a special thought for Alain Palloix, an active member of our scientific community until 2016, on the occasion of this 17<sup>th</sup> Eucarpia meeting held in Avignon, and look forward to the next meeting in three years.*

*Avignon, France, 18<sup>th</sup> July 2019*

*Véronique Lefebvre & Marie-Christine Daunay  
Conveners of the 17<sup>th</sup> Eucarpia Meeting  
in Genetics and Breeding of Capsicum and Eggplant*



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In this book, abstracts are ordered by session. Within each session, abstracts appear in the same order as the oral presentations, as planned in the congress schedule. Following the oral presentations, the poster abstracts are ordered alphabetically by first author name.

Furthermore we have coded the abstracts according to the following system:

- The first two letters indicate the session:
  - "GR" for Genetic Resources and Domestication,
  - "RS" for Resistance to Biotic and Abiotic Stresses,
  - "NQ" for Nutritional Value and Quality Traits,
  - "BG" for Biotechnology and Genomics,
  - "PB" for Pre-breeding and Breeding.
- The following letter indicates the type of communication:
  - "K" for Keynote,
  - "O" for Oral,
  - "P" for Poster.
- The code ends with a number matching the order of the abstract within the session.
- Example given:
  - GR-K/01 for the keynote as the first communication of the session Genetic Resources and Domestication.



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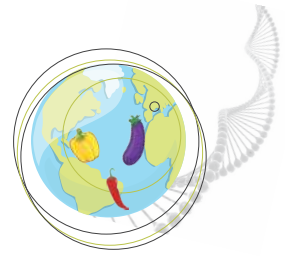
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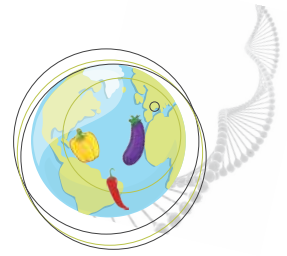
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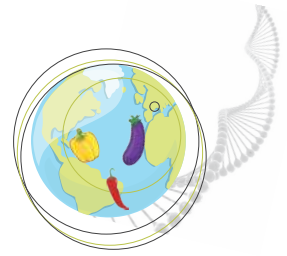
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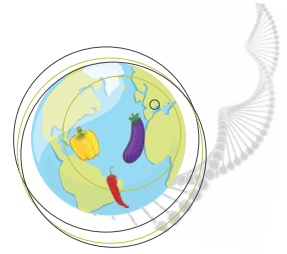
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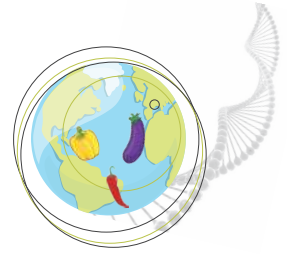
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# GENETIC RESOURCES AND DOMESTICATION

SESSION 1



# Eggplant (*Solanum melongena* L.) and its relatives: Overview of 50 years of research and breeding

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**BACKGROUND** The last fifty years have been the theater of outstanding breakthroughs for research in vegetables, including eggplant. Increasingly, breeding has become based on a more scientific approach. Erosion of cultivated diversity both national and international created a need to safeguard the constitution of ex situ collections throughout the world. The incomplete and confused taxonomy of eggplant relatives (wild and cultivated) has been treated in a series of major monographic works. These profound changes are surveyed here in the light of future challenges in eggplant breeding.

**MATERIALS & METHODS** The survey is based on the co-authors' decades of expertise on eggplant and its related species.

## RESULTS

### ***Evolution of production in Europe and the Mediterranean basin***

From the 1970s onwards, the traditional summer open field production of eggplant shifted progressively to round the year intensive practices. Technical changes accompanied the specialization of intensive production areas, in particular southern Spain, Sicily, and Turkey for eggplant (and pepper). Because of this shift in production method, fruits are now available on European markets year-round.

### ***Biotechnologies at the service of breeding and genetics***

The last fifty years are marked by the development of a wide and rapidly evolving range of bio-techniques. From the 1970s onwards, *in vitro* techniques such as embryo rescue, somatic embryogenesis, somatic hybridization, organogenesis, (di)haploid production and genetic engineering were successfully applied to eggplant. In practice, breeders use mostly (di)haploid production and embryo rescue. Out of the three countries that have engaged in the development of transgenic eggplants (*Bt* genes providing resistance against the fruit and shoot borer *Leucinodes orbonalis*) only Bangladesh achieved commercialization in 2013. Molecular genetics burst into eggplant research at the end of the 1990s. A first tentative linkage map of eggplant set up with RAPDs on an intraspecific F2 population (Eucarpia 1998) was followed by an interspecific map [1] that established for the first time the close synteny between eggplant, pepper, and tomato genomes, and located several major QTLs controlling plant and fruit traits. Thanks to marker diversification, further maps with better coverage and further QTLs controlling traits of interest were published. The first draft genome [2] was closely followed by the Italian sequencing initiative (presented at Eucarpia 2013, <http://www.eggplantgenome.org/> since October 2017). When compared to tomato, the gap in genomic and other -omics resources that eggplant suffered for many years is now rapidly decreasing.

### ***Evolution of breeding methods and cultivars***

Up to the 1970s, eggplant breeding was carried out mostly by local seed companies and based on mass or pedigree selection of landraces. Although the agronomic interest of F1 hybrids was known and used for commercial material in Japan since the 1930s [3], their development in Europe started only in the 1970s. The hybrid genetic structure, well-adapted to the production and distribution changes together with the growingly competitive seed market, rocketed the F1 hybrids from 0% in 1970 to 80% in 1992 for the French catalogue. In 2019, F1 hybrids represent 95% of the cultivars registered in the Netherlands. Innovative traits were progressively released in commercial cultivars, such as reduced vegetative development, ability to grow in limiting conditions, reduced plant and calyx prickliness, ability to set fruits under cold and low



light conditions, shiny dark and attractive fruit epidermis, firm fruits with improved shelf life and fruit shapes adapted to each production area. This evolution came with a narrowing diversity of the commercial cultivars and the disappearance of the diversified local open-pollinated (OP) varieties (heirlooms, landraces) although local growers still use them in countries like Italy where this type of material still represents 22% of the varieties registered in the national catalogue.

### **Eggplant germplasm**

In the 1970s very few *S. melongena* germplasm collections existed in public institutions. Private breeders worked mostly with local material for their national market. Thanks to the inputs of the International Board for Plant Genetic Resources created in 1971 (now Bioversity International) and of worldwide national initiatives, many public collections have been progressively assembled for saving local material endangered by the intensification of horticulture and/or for research purposes. *Solanum melongena* germplasm is relatively well represented worldwide in genebanks according to online databases, although a concern is the increasingly restricted access to seeds by both national and international policies. For *S. aethiopicum* and *S. macrocarpon*, the two indigenous African eggplants, efforts must be developed for prospecting their diversity and to complete *ex situ* collections.

### **Wild germplasm and relationships with eggplants**

Over 500 *Solanum* species, belonging to subgenus *Leptostemonum* and originating from western Africa to eastern Asia on one hand, and from Central to South America on the other hand, are considered related to eggplants. Thanks to ambitious international taxonomic projects from the 2000s onwards, the inventory of the whole genus *Solanum* was rearranged and completed, and phylogenetics became the backbone of its taxonomic treatment [4]. Based on large and representative species sampling, phylogenetic analyses have identified in particular the robust eggplant clade to which *S. melongena* and its closest relatives belong, as well as the poorly resolved *Anguivi* grade where the two cultivated African eggplants and part of the wild relatives are nested. Although further phylogenetic refinements are necessary to ascertain many nodes, a global phylogenetic picture of subgenus *Leptostemonum* is now available. The match between species phylogenetic relatedness and their crossability potential is however far from clear [5] and this apparent discrepancy questions once more the debated topic of the species concept [6]. The first botanical collection of eggplant and relatives was convened at the University of Birmingham (UK) and used intensively from the 1960s to the 1990s for taxonomic research. Thanks to the 1999-2004 European Union EGGNET project this collection was saved and split among European germplasm repositories [7]. Wild Asian species have been partly collected in the last decades via national and collaborative Asian projects, but apart from the World Vegetable Center (formerly AVRDC) little is known about these collections.

### **Germplasm management and use: strengthening interdisciplinary collaborations**

Bi-national projects (France, UK in the 1990s) and the EU ESIN project (1993-1994) set up for the first time “fruitful” collaborations among experts of complementary disciplines ranging from botany and taxonomy to germplasm collections and genetics. A few years later and within the framework of French and Dutch national agreements, vegetable breeding companies were connected to the management of eggplant and related species germplasm held by public institutions. These converging forces were further integrated at the European scale within the EGGNET project and from 2001 onwards within the European Cooperative Program on Plant Genetic Resources (<http://www.ecpgr.cgiar.org/working-groups/solanaceae/>). The challenges the eggplant community is facing nowadays invites further strengthening and widening of collaborations, for at least three main reasons:

First, the large number of species related to cultivated eggplants is both an outstanding reserve of genes for breeders and a burden for germplasm holders, the supervision of which requires close collaboration with taxonomists [4]. Living collections of eggplant wild relatives are very incomplete, both in terms of species and accessions per species, and their maintenance suffers from insufficient knowledge of each species’ biological peculiarities. Hence, there is a need to complete the collections with wild material and to upgrade

management both in terms of seed production and maintenance of accessions' original genetic integrity. Second, access to wide germplasm resources is necessary for optimizing the use of the powerful tools created by fast evolving genomics and bioinformatics. Quantification and structuration of genetic and phenotypic diversity, limited for decades to a handful of species and accessions is now accessible at whole collections and genome scales, as ambitioned by the EU G2PSol project (2016-2021). Hence, joint efforts among genebanks within and outside Europe are more imperative than ever to identify the strengths and weaknesses of the different collections, and to increase availability of accessions for research and breeding. Third, the exploration of phenotypic diversity for traits of interest within *S. melongena* and related species has been limited so far to a narrow range of accessions and traits, and is clearly a bottleneck on future research efforts. Increased knowledge of germplasm-wide diversity is indispensable, in particular for resistance or resilience to biotic and abiotic stresses that are expected to increase in our changing climate. Phenotyping methods must also gain in precision by intimate dissection of complex traits, for the purpose of identifying their key regulatory genes and QTL networks.

### **Eggplant domestication: first insights**

Phylogenetic relationships between *S. melongena*, its wild progenitor *S. insanum* and their closest African wild relative *S. incanum* were recently clarified [8]. *Solanum anguivi* and *S. dasyphyllum* were confirmed as the wild progenitors of *S. aethiopicum* and *S. macrocarpon*, respectively. Complex domestication events, as well as morphological changes associated with domestication are common to the three cultivated eggplants [9]. Similar seed, plant, and fruit traits were impacted in the same directions although it seems that the domestication process is more advanced for *S. melongena*. At the whole genome scale, the impact of domestication on *S. melongena* (and on pepper and tomato) has been shown to affect both genetic architecture and gene expression (Arnoux et al., in prep.). The comparison of the domestication signatures on *S. melongena*, *S. aethiopicum* and *S. macrocarpon* genomes should bring further insights into the similarities and differences between the three cultivated eggplants.

**CONCLUSIONS & PERSPECTIVES** In 50 years, eggplant breeding turned from an exclusively field activity of eggplant breeders focused on few traits and national markets, to a collective and highly technical process, targeting international vegetable seed trade with a diversity of targeted traits and breeding objectives. From hundreds of progenies screened mostly for quantitative and qualitative yield in the 1960s, eggplant breeders now work on thousands of plants, many varietal types grown year-round in several countries, and *in vitro* and molecular steps complete complex breeding schemes. However the transposition of swiftly changing molecular and data computing techniques to breeding schemes is conditioned by the limited research costs that companies can afford given the size of eggplant seed trade. Breeders can take advantage of the synteny between eggplant, pepper, and tomato genomes to efficiently and effectively improve their breeding programs. Access and characterization of germplasm diversity are now central issues for breeders as well as for academics. Breeders now realize the contribution of germplasm collections and the systematics knowledge that botanists have provided of the many peculiarities and potentialities of eggplant wild relatives. Future breeding challenges, linked to the evolution of horticulture towards sustainable practices including cultivar robustness and diversification, further imply the convergence of all these scientific disciplines.

### **REFERENCES**

- [1] Doganlar S et al., 2002, *Genetics* 161 (4):1697-1711
- [2] Hirakawa H et al., 2014, *DNA Res* 21 (6):649-660. doi:10.1093/dnares/dsu027
- [3] Kakizaki Y, 1931, *Genetics* 16: 1-25
- [4] Aubriot X, Daunay MC, 2019, chapter 10 *In The Eggplant Genome*, Chapman M. (Ed.), Springer: 91-134
- [5] Daunay MC, Aubriot X, 2019, these Proceedings
- [6] Knapp S, 2008, *Biol J Linnean Soc* 95 (1):17-25. doi:10.1111/j.1095-8312.2008.01090.x
- [7] Lester RN et al., 2001, *In Van den Berg RG, Barendse GWM, Van der Weerden G, Mariani C (eds) Solanaceae V, advances in taxonomy and utilization*. Nijmegen University press: 391-412
- [8] Knapp S et al. 2013, *PLoS One* 8 (2). doi:10.1371/journal.pone.0057039
- [9] Page AML et al., 2019, chapter 12 *In The Eggplant Genome*, Chapman M. (Ed.), Springer: 193-219

# Comparative demographic study: Reconstructing the evolutionary history of *Solanaceae* species to better understand the domestication process and outcome

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**BACKGROUND** Domestication is an ideal framework to study the interplay of evolutionary forces that induced phenotypic and molecular changes over short-time scales [1]. Molecular changes can now be tracked at the genome-wide scale, offering a unique opportunity to decipher the most probable demographic scenarios and characterize their parameters [2]. In this context and for the first time in plant, we performed a comparative genomic approach to infer the parallelism in the demographic history of three major crop species of the *Solanaceae* family including eggplant, pepper and tomato. In each species, we analyzed and compared the genetic summary statistics of two groups of crop and wild accessions, constituting prior knowledge for inferring demographic scenarios. The comparison between the three species demographic history is an unprecedented opportunity to further characterize each domestication event duration, and therefore confirm demographic history that were hypothesized through indirect means (human and cultivation history of the areas, ancient written records).

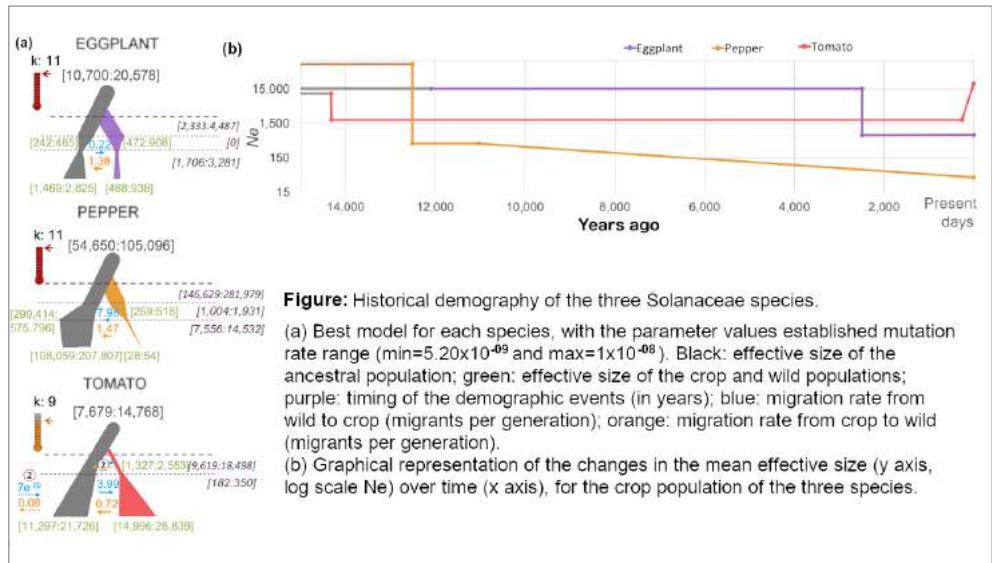
**MATERIALS & METHODS** We based our approach on the study of orientated site frequency spectrum in both crop and wild groups [3]. We implemented over 10 potential demographic models to test whether the divergence between the crop and wild groups occurred (i) in the absence/presence of gene flow, (ii) with gradual/instantaneous change in their effective population size, (iii) in one or two steps. For each species, the most probable scenario was selected according to its Akaike Information Criterion, and associated parameters and their precision were estimated. These parameters were translated to human timing by the use of a range of mutation rate to avoid any bias.

**RESULTS** The comparative study of the demographic inferences modeling the domestication of the three species revealed common features of the domestication processes in the *Solanaceae* family. In the three species the presence of a decrease in effective size corroborates the stage of domestication related to cultivation. That specific stage induced a reduction in effective size due to the extraction of few individuals from their wild environment to cultivate them in human managed fields. The demographic models inferred the duration of the domestication event such as the divergence time between the crop and their wild relatives, or when occurred the bottleneck. Consequently, using two mutation rates we could provide a range of estimation: the eggplant domestication occurred 4,039-7,768 years ago (ya), the pepper sampled allowed to estimate the stage of cultivation related to a bottleneck at 8,560-16,463 ya, and for the tomato the domestication seems to have occurred between 9,801 and 18,848 ya. Another parameter was the asymmetric gene flow between the wild and crop compartment. Our results highlight that both pepper and tomato have experienced a stronger gene flow from the wild to the crop compartment than crop to wild when it is the opposite for eggplant.

**DISCUSSION & CONCLUSION** Overall, our study provides insights into the convergence of the domestication processes in the *Solanaceae* family. Our results point out the influence of sympatry and allopatry between the crop and the wild compartments to explain the gene flows. These inferences bring as well new details about the timing of domestication and therefore insights into human history and how and when societies domesticated species. It confirms the importance of understanding how plant species respond to human manipulation. By knowing the past behavior of crops facing domestication events, we may potentially improve modern breeding efforts to sustain future crop breeding.

## REFERENCES

- [1] Meyer RS, Purugganan MD, 2013. Nat Rev Genet 14:840–52. doi: 10.1038/nrg3605
- [2] Gaut BS, Seymour DK et al., 2018. Nat Plants 4:512–520. doi: 10.1038/s41477-018-0210-1
- [3] Gutenkunst RN, Hernandez RD et al., 2009. PLoS Genet 5:e1000695. doi: 10.1371/journal.pgen.1000695



# High-throughput phenotyping to characterize *Capsicum* fruit shape diversity

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**BACKGROUND** Considering socio-economic importance of sweet and hot pepper; continuous selection and variety development resulted in poor understanding of capsicum diversity and subsequent overlapping of varietal identification, therefore it is essential to study existing diversity to preserve and better exploit the germplasm. New high-throughput (HTP) phenotyping tools are continuously developed and adapted for trait characterization and seen to be more sensitive and cost-effective in comparison to conventional phenotyping. In Solanaceous, HTP tool of tomato analyzer (TA) has been proven very useful to characterize fruit shape diversity in tomato [1], eggplant [2], and pepper [3]. In view of economic value of peppers, detecting pepper fruit shape diversity would be of immense value to plant breeders. In this study, we utilized TA to characterize fruit morphometric and colorimetric traits of Balkan pepper accessions so that essential information about unique pepper genetic resources could be better studied and utilized in future breeding.

**MATERIALS & METHODS** A total of 99 pepper accessions collected from different Balkan countries (Figure 1) were grown during 2017 at MVCRI and studied for fruit shape diversity. Based on adapted fruit usage, 8 fruits per accession were harvested before maturity or at maturity and were characterized using TA. A total of 47 different descriptors related to basic measurements (7), fruit shape index (3), blockiness (3), homogeneity (3), proximal (4), and distal fruit end (4), asymmetry (6), internal eccentricity (5), color values (9), and latitudinal section (3). Statistical analyses were conducted with SAS software and data visualization tools (R packages ggplot2, ggcorrplot, FactoMineR, Factoextra, and missMDA).

**RESULTS** Across different varietal groups, 46 out of 47 TA descriptor traits were highly significant ( $P < 0.001$ ) whereas within each varietal group, 38, 15, 43, 43 and 43 traits were highly significant for elongate, round, conical, bell, and pumpkin shape fruits, respectively. Most of the non-significant traits were related to asymmetry and internal eccentricity. Differences between conventional measurement and TA were tested using correlation between manually measured fruit length, width, and fruit wall thickness, which were equivalent to TA max height, width, and pericarp thickness and strong correlations ( $>0.80$ ) were reported for the first two traits. Considering genetic diversity of different fruit shape related traits we aimed to utilize multivariate analysis to identify potential trend and trait combination, which contributed and explained the variation. A total of 47 components were identified to contribute to total variation and the first two components explained ~54% of the variation (Figure 2) whereas 12 components contributed to around 90% of the variation.

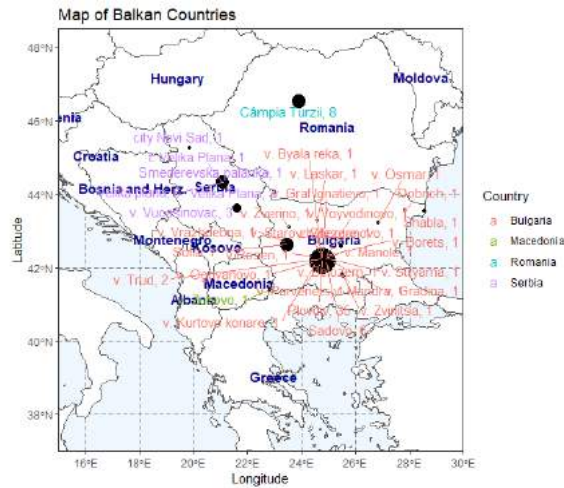
**DISCUSSION & CONCLUSION** Considerable grasping of phenotypic and genotypic diversity of region specific and geographical localized germplasm is essential to conserve and utilize genetic resources for germplasm enhancement and improvement. Previously defined TA descriptors from different solanaceous crops [1, 2, and 3] were proven useful to study fruit shape and reported diversity for morphometric and colorimetric traits and similar trends were also observed in our study. Diversity for fruit shape could be immensely crucial for further investigation of genome wide association (GWAS) to identify potential genomic regions and key genes related to fruit shape.

## REFERENCES

- [1] Brewer MT, Lang L et al., 2006, Plant Physiol, 141:15-25.
- [2] Hurtado M, Vilanova S et al., 2014, Genet Resour Crop Evol. 61(4): 787-795.
- [3] Tripodi P, Greco B. 2018, Plants, 7(4): 103.

## ACKNOWLEDGEMENTS

Velichka Todorova thanks the French “Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (program ACCES de CAMPUS France)” for covering the travel and accommodation expenses for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



**Figure 1.** Balkan Map of Pepper Accession Collection Sites.



**Figure 2.** Multivariate PCA Ellipse or Variable Plot.

# Inter- and intra-specific variations of the root system architecture among aubergines, capsicums and tomatoes

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**BACKGROUND** Genetic variations of shoot, flower and fruit development are relatively well described among *Solanaceae* species, because of their importance in qualitative and quantitative yield. However, despite its major roles in production, the development and the architecture of the root system were much less studied because of the difficult access to roots and because of the lack of relevant criteria to characterize the roots [1]. In this study, we compared a set of root traits related to root development and architecture for 10 genotypes in each group of species: aubergines, capsicums and tomatoes. The traits were chosen because they represent key indicators of root developmental processes (elongation, branching, production of adventitious roots), and they are used as input parameters in some simulation models of the root system architecture [2]. These traits are: extreme apical diameters (minimal and maximal), slope of the regression lines of elongation rate versus apical diameter; inter-branch distance and emergence rate of adventitious roots.

**MATERIALS & METHODS** Plants were grown in 1-meter long PVC tubes (10 cm in diameter), using a mixture of sepiolite and expanded clay pellets as substrate, in order to obtain vigorous growth and sufficient rooting volume. One month after sowing, plants were harvested, carefully separated from the substrate, and roots were sampled. We made high resolution (1200 dpi) images of the root samples on which we measured the root traits. We also measured the total leaf area and the total dry mass of the leaves, the shoot and the root system.

**RESULTS** Most traits showed significant genetic variations: extreme root apical diameters, inter-branch distances, and slopes of the regression lines of elongation rate versus apical diameter. On all genotypes, the initial root system issued from the elongation and branching of the taproot was complemented 2-3 weeks later by the emergence of a set of adventitious roots with large apical diameters emerged near the plant collar. On most root traits, inter-specific variations were higher than intra-specific variations, so that species could be separated. Correlations between traits were evidenced within the root systems on the one hand and between shoot and root on the other hand. They revealed both trade-offs in developmental processes and coordination between plant parts.

**DISCUSSION & CONCLUSION** Beyond these results, we demonstrated the importance of characterizing phenotypes through multiple criteria, considering the whole plant and including advanced stages of development. These new phenotyping methods are being used in current projects to analyze and characterize the genetic diversity of the root system architecture for various plant families.

## REFERENCES

- [1] Waisel Y, et al., 1991. Plant root: the hidden half (First edition). Books in Soils, Plants, and the Environment. Marcel Dekker, Inc.: 948.
- [2] Pagès L, et al., 2014. Ecological modeling 290, 76-84. doi 10.1016/j.ecolmodel.2013.11.014





# Genetic diversity between accessions of peppers (*Capsicum chinense* Jacq.) from the Brazilian Amazon region

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**BACKGROUND** *Capsicum* peppers, native to South and Central America [1], represent one of the most important spices in the world. The fruits of this genus are sources of natural antioxidants and have a characteristic pungency, which has promoted their use among different ethnicities. *C. chinense* peppers are native to Amazon Basin [2], and their cultivation and conservation have been carried out mainly by traditional communities. The studies of morpho-agronomic characterization of *C. chinense*, involving the identification of characteristics of interest, are essential in the conservation of the genetic variability of this species, and for assuring that this resource might be efficiently used. Thus, the aim of this study was to access the genetic variability of 55 *C. chinense* accessions, based on a joint analysis of 48 morpho-agronomic descriptors. This study aimed to select promising genotypes in terms of agronomic, chemical and nutritional aspects, especially those with fruits with a high content of vitamin C and low pungency.

**MATERIALS & METHODS** The experiment was conducted in a completely randomized design, with five replications and had one plant per plot. The plants were grown in pots under a protected environment, in the Experimental Garden of the Plant Science Department – UFV. We analyzed the qualitative descriptors by the frequency of distribution. The relationships between vitamin C and pungency, vitamin C and colour of fruits, and the colour of fruits and pungency were analyzed by Boxplot and histogram, with the aim of SigmaPlot. The genetic similarity between the accessions was estimated by the Gower general coefficient of similarity and the genetic diversity was evaluated by the UPGMA hierarchical method.

**RESULTS** The accessions expressed wide genetic variability especially for descriptors of fruits such as format, colour, size and, chemical traits. The content of vitamin C ranged from 734 to 1510 mg 100 g<sup>-1</sup>, and the capsaicin from 0 to 43, 69 mg L<sup>-1</sup>, that is 804818 – SHU. When we analyzed the relationship between the chemical traits of fruits, we observed that the vitamin C content for the fruits with red colouration ranged from 734 to 1371 mg 100 g<sup>-1</sup>. The vitamin C content for the fruits of high pungency (113105 to 804816 - SHU), ranged from 734 to 1510 mg 100 g<sup>-1</sup>. As to the relationship colour and pungency of fruits, we observed higher estimates of pungency for the red fruits. The genetic diversity of the accessions was confirmed upon the formation of seven groups. Group 7 allocated the BGH8321 accession, which has no capsaicin. This accession produced 190 fruits per plant and had additional chemical characteristics such as a vitamin C content of 1117 mg 100 g<sup>-1</sup>, soluble solutes content of 11 ° Brix, 5.0 pH and a titratable acidity 0, 15%. Group 5 allocated the BGH8320 accession, which has a peculiar characteristic of fruit colour: Group 3 allocated the BGH8328 accession, which had 1510 mg 100 g<sup>-1</sup> of vitamin C, a content 18 fold higher than that found in oranges (80 mg 100 g<sup>-1</sup>) [3].

**DISCUSSION & CONCLUSION** The variation found for the chemical aspects of fruits suggests that the accessions of *C. chinense* from the Brazilian Amazon Region have a great genetic variability. The clustering pattern of the accessions corroborates their high genetic diversity, suggesting the possibility of using these accessions in the pre-breeding for aspects such as the vitamin C content, capsaicin, besides the colour and format of fruits. This also suggests the possibility of implementing a participatory breeding for this species in the Brazilian Amazon Region, which will contribute to the conservation and sustainable use of the genetic resources of *C. chinense* in this region.

## REFERENCES

- [1] Carvalho et al., 2006, Embrapa. Docs. 94: 27
- [2] Reifschneider FJB, 2000, 1 Ed. Brasília: Embrapa Hortaliças, 113
- [3] TACO: Tabela Brasileira de Composição de Alimentos, 2011, NEPA, UNICAMP, 161

# Eggplants and relatives: Phylogenic relationships vs. crossability relationships

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**BACKGROUND** Cultivated eggplants and their closest relatives belong to the African and West Asian part of *Solanum* subgenus *Leptostemonum*, the phylogenetic treatment of which identified in particular the eggplant clade (*S. melongena*) and the less resolved Anguivi grade (*S. aethiopicum*, *S. macrocarpon*). Taxonomic revisions of tropical Asian [1], Australian and American *Leptostemonum* species are still ongoing. On the other hand the wealth of interspecific crosses between *Solanum* species carried out for decades provides another light on species relationships, based on reproductive affinity between species.

**MATERIALS & METHODS** Interspecific crosses best results are surveyed (no details here as regards to cross direction and results differences between publications) and discussed across two axes: (i) what is a successful interspecific cross, and (ii) to which extent do interspecific crosses results match species cross-partners phylogenetic relationships and continental origin.

## RESULTS

### 1. Interspecific crosses yielding partially fertile or fertile hybrids (Table 1)

- cultivated eggplants & wild progenitors: As expected *S. melongena*, *S. aethiopicum* and *S. macrocarpon* yield fertile hybrids (Pollen Stainability > 50%) when crossed with their respective wild progenitor i.e. *S. insanum*, *S. anguivi* and *S. dasyphyllum*. When crossed to each other, cultivated eggplants yield only partially fertile hybrids (10% < PS < 50%). Consistently, when each eggplant species is crossed with the wild progenitor of the two other eggplants, hybrids are partially fertile as well.

- cultivated eggplants & other wild *Solanum* species: Out of the crosses carried out between *S. melongena* and 61 wild cross-partners, only 25 yielded partially fertile or fertile hybrids, whereas out of the 16 crosses involving *S. aethiopicum*, only 2 yielded partially fertile hybrids (detailed information is not available for *S. macrocarpon*).

- wild species: Out of 116 crosses between 33 *Solanum* species, only 25 yielded partially fertile or fertile hybrids.

### 2. Progenies obtained from interspecific hybrids (Table 2)

This advanced step in the study of an interspecific cross is only occasionally reported in the literature. F2 or BC progenies were obtained whatever pollen fertility rates of the hybrids, including virtually sterile ones (PS < 10%).

**DISCUSSION & CONCLUSION** Results about interspecific crosses are very heterogeneously presented in the literature. Although the ultimate result that is significant for a breeder is the possibility to obtain progenies from an interspecific hybrid, this information is rarely available in literature. Hence there is a need to harmonize the inter crossability criteria for easing comparisons between publications. Although far from complete and definitive, available results shed a first light on the apparently loose relationship between the success of a cross (fertility of hybrids, progenies obtained from it) and the phylum to which cross partners species belong to. Species belonging to a same clade sometimes hybridize well with each other or not, and conversely species of more or less distantly related clades might hybridize or not [2]. Most crosses and successes concern Old World species. Unexpectedly a few crosses realized between *S. melongena* and American species were successful. Given (i) phylogenetic relationships between many species still need refinement [3] and (ii) cross-partners species used so far are insufficiently representative of each clade, the apparently loose relationship between phylogeny and crossability deserves further investigation.

## REFERENCES

- [1] Aubriot X et al., 2016, Bot. J. Linnean Soc., 181 (2):199-223. doi:10.1111/boj.12412  
 [2] Daunay MC et al., 2019, chapter 11 In The Eggplant Genome, Chapman M (Ed.), Springer: 135-191  
 [3] Aubriot X, Daunay M.C 2019, chapter 10 In The Eggplant Genome, Chapman M. (Ed.), Springer: 91-134

Table 1

cross-partner 1		cross-partner 2	
Phylum	species	Phylum	species
Anguivi grade	<i>S. aethiopicum</i>	Anguivi grade	<i>S. macrocarpon</i>
	<i>S. aethiopicum</i>		<i>S. anguivi</i>
	<i>S. aethiopicum</i>		<i>S. dasyphyllum</i>
	<i>S. aethiopicum</i>		<i>S. violaceum</i>
	<i>S. anguivi</i>		<i>S. capense</i>
	<i>S. anguivi</i>		<i>S. coccineum</i>
	<i>S. anguivi</i>		<i>S. platacanthum</i>
	<i>S. anguivi</i>		<i>S. rubetorum</i>
	<i>S. anguivi</i>		<i>S. violaceum</i>
	<i>S. capense</i>		<i>S. coccineum/S.tomentosum</i>
	<i>S. capense</i>		<i>S. giftbergense</i>
	<i>S. capense</i>		<i>S. supinum</i>
	<i>S. coccineum/S.tomentosum</i>		<i>S. giftbergense</i>
	<i>S. coccineum/S.tomentosum</i>		<i>S. rigescens</i>
	<i>S. coccineum/S.tomentosum</i>		<i>S. rubetorum</i>
	<i>S. coccineum/S.tomentosum</i>		<i>S. violaceum</i>
	<i>S. violaceum</i>		<i>S. giftbergense</i>
	<i>S. violaceum</i>		<i>S. rigescens</i>
	<i>S. violaceum</i>		<i>S. tomentosum</i>
	<i>S. macrocarpon</i>		<i>S. dasyphyllum</i>
Eggplant clade	<i>S. macrocarpon</i>	Eggplant clade	<i>S. anguivi</i>
	<i>S. aethiopicum</i>		<i>S. zanzibarensis</i>
	<i>S. aethiopicum</i>		Climbing clade
	<i>S. aethiopicum</i>		<i>S. melongena</i>
	<i>S. aethiopicum</i>		Eggplant clade
	<i>S. aethiopicum</i>		<i>S. melongena</i>
	<i>S. aethiopicum</i>		<i>S. incanum</i>
	<i>S. violaceum</i>		Giganteum clade
	<i>S. violaceum</i>		<i>S. pubescens</i>
	<i>S. violaceum</i>		<i>S. cinereum</i>
	<i>S. violaceum</i>		Sahul Pacific
	<i>S. violaceum</i>		unallocated
	<i>S. violaceum</i>		<i>S. virginianum</i>
	<i>S. melongena</i>		Acanthophora clade (America)
	<i>S. melongena</i>		<i>S. viarum</i>
	<i>S. melongena</i>		<i>S. aculeatissimum</i>
	<i>S. melongena</i>		<i>S. anguivi</i>
	<i>S. melongena</i>		<i>S. dasyphyllum</i>
	<i>S. melongena</i>		<i>S. violaceum</i>
	<i>S. melongena</i>		<i>S. burchellii</i>
	<i>S. melongena</i>		<i>S. catombelense</i>
	<i>S. melongena</i>		<i>S. coccineum</i>
	<i>S. melongena</i>		<i>S. cyaneopurpureum</i>
	<i>S. melongena</i>		<i>S. dinteri</i>
	<i>S. melongena</i>		<i>S. hastifolium</i>
	<i>S. melongena</i>		<i>S. lidii</i>
	<i>S. melongena</i>		<i>S. rigescens</i>
	<i>S. melongena</i>		<i>S. rigescensoides</i>
	<i>S. melongena</i>		<i>S. rubetorum</i>
	<i>S. melongena</i>		<i>S. supinum</i>
	<i>S. melongena</i>		<i>S. sessilistellatum</i>
	<i>S. melongena</i>		<i>S. tomentosum</i>
	<i>S. melongena</i>		Climbing clade
	<i>S. melongena</i>		<i>S. richardii</i>
	<i>S. melongena</i>		<i>S. insanum</i>
	<i>S. melongena</i>		<i>S. campylacanthum</i>
	<i>S. melongena</i>		<i>S. incanum</i>
	<i>S. melongena</i>		<i>S. lichtensteinii</i>
	<i>S. melongena</i>		<i>S. linnaeanum</i>
	<i>S. melongena</i>		<i>S. cerasiferum</i>
	<i>S. melongena</i>		<i>S. cerasiferum</i>
	<i>S. campylacanthum</i>		<i>S. campylacanthum</i>
	<i>S. incanum</i>		<i>S. insanum</i>
	<i>S. incanum</i>		<i>S. lichtensteinii</i>
	<i>S. incanum</i>		Giganteum clade
	<i>S. incanum</i>		<i>S. pubescens</i>
	<i>S. incanum</i>		unallocated
	<i>S. incanum</i>		<i>S. virginianum</i>
	<i>S. melongena</i>		unallocated (Australia)
	<i>S. melongena</i>		<i>S. melanosperrum</i>

Table 2

cross-partner 1		cross-partner 2	
Phylum	species	Phylum	species
Eggplant clade	<i>S. aethiopicum</i>	Eggplant Clade	<i>S. melongena</i>
	<i>S. trilobatum</i>	unallocated	<i>S. virginianum</i>
	<i>S. melongena</i>	Acanthophora (American)	<i>S. viarum</i>
	<i>S. melongena</i>	Anguivi grade	<i>S. tomentosum</i>
	<i>S. melongena</i>		<i>S. anguivi</i>
	<i>S. melongena</i>		<i>S. dasyphyllum</i>
	<i>S. melongena</i>		<i>S. macrocarpon</i>
	<i>S. melongena</i>	Elaeagnifolium clade (Amer.)	<i>S. violaceum</i>
	<i>S. melongena</i>		<i>S. elaeagnifolium</i>
	<i>S. melongena</i>	Eggplant clade	<i>S. incanum</i>
	<i>S. melongena</i>	Torva clade (American)	<i>S. insanum</i>
	<i>S. melongena</i>	<i>S. torum</i>	<i>S. torum</i>
	<i>S. melongena</i>	unallocated	<i>S. virginianum</i>

**Table 1:** Summary of the interspecific crosses yielding partially fertile or fertile F1 hybrids [2]

**Table 2:** Summary of the interspecific crosses yielding progenies issued from the F1 hybrid [2]

# Uncovering the diversity of eggplant wild relatives: Advances in taxonomy and phylogenetics of old-world spiny solanums

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**BACKGROUND** The spiny solanums clade (*Solanum* subgenus *Leptostemonum* Bitter) forms the most species rich major group within the giant genus *Solanum* L. (Solanaceae). It accounts for the cultivated eggplants (*S. melongena* L., *S. aethiopicum* L. and *S. macrocarpon* L.) as well as for more than 550 species; with a primary centre of diversity in the Neotropics it also diversified in the old world (c. 240 native species). Due to its large size and the great morphological variability of its component species, the spiny solanums group has always been regarded as taxonomically challenging. This hampered our understanding of the evolutionary context of *Solanum*. It also contributed to limit the work of plant breeders to a handful of crop wild relatives. This last decade has seen significant breakthroughs in the production and management of large amount of biological data. Here, we show how these technical advances contributed to radically increase our knowledge of the diversity of old world spiny solanums and shed new lights on the evolutionary history of eggplant closest relatives.

**MATERIALS & METHODS** Our taxonomical studies were designed to be global and account for most of the *Solanum* collections stored in herbaria worldwide. This work benefited from the building of a general database on Solanaceae collections, the Solanaceae Source Database; it was used to store all the data used to treat the nomenclature and taxonomy of old world spiny solanums. To untangle phylogenetic relationships and identify monophyletic groupings within spiny solanums, we sampled a large amount of historical collections. DNA extracts were submitted to Sanger and, for some, to NGS sequencing; phylogenies were inferred with model-based methods and, when possible, put into dated and geographic contexts.

**RESULTS** The investigations we led in more than 50 herbaria worldwide allowed us to identify c. 10,000 and 5,500 collections for African and Asian spiny solanums, respectively. These were used to produce accurate and thoroughly documented descriptions of the spiny solanums of Africa [1] and tropical Asia (Aubriot & Knapp, in prep.). Taking in account all the scientific names published for *Solanum* since Linnaeus, we provided full nomenclatural treatments of the 76 and 32 species that are native to Africa and Asia; we also generated distribution maps and IUCN Red List assessments for each of these species.

Although deep phylogenetic relationships within old world spiny solanums were particularly challenging to untangle, we recovered a number of clades that included African and/or Asian taxa (see for instance [2]). These groupings are homogeneous in terms of morphological traits or geographical provenance, or strongly heterogeneous; most of these were in contradiction with historical *Solanum* systematics. The first well-resolved and dated phylogeny for the species directly related to the eggplant was reconstructed using NGS data [3]; dispersion of the eggplant through Africa relate to large mammal grazers (elephants and impalas).

**DISCUSSION & CONCLUSION** Genus *Solanum* is acknowledged as a group of taxonomical complexity. Here, we show that by combining well-sampled morphological and molecular approaches, this complexity can actually be tackled. We hope that clarification of the taxonomy and systematics of spiny solanums will benefit the scientific community and in particular plant geneticists and breeders that are interested in eggplant wild relatives. Though, we are still facing a number of challenges such as the unstable taxonomy of the Australian spiny solanums and the unresolved deep phylogenetic relationships. Use of technologies that provide access to nuclear DNA data for a large number of accession should help in solving these issues.

## REFERENCES

- [1] Vorontsova MS and Knapp S, 2016, Syst. Bot. Monogr., 99:1–432
- [2] Aubriot X et al., 2016, Bot. J. Linn. Soc., 181:199–223
- [3] Aubriot X et al., 2018, Am. J. Bot., 105:1175–1187

# Genetic diversity and population structure analysis to construct a core collection from a large eggplant germplasm

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**BACKGROUND** The Institute of Vegetable and Floriculture Science, NARO plays a key role regarding the conservation of vegetable crops genetic resources in Japan. Currently, nearly 1,000 accessions of eggplant are conserved and propagated. Genetic resources containing wide genetic variation are widely considered indispensable materials with latent potential for future eggplant breeding. However, the status of genetic resources in eggplant is not completely organized. Therefore, the construction of a small subset that consists of a limited number of accessions but still retains the range of genetic variation of all the genetic resources as much as possible is useful for their efficient and pragmatic use. For this purpose, the construction of an eggplant core collection based on molecular genetic information is urgently required. Here we report the construction of a unique eggplant core collection that was accomplished using the genotype data of 831 SNPs and 50 SSRs obtained from 893 eggplant accessions collected from across the world.

**MATERIALS & METHODS** A dataset of 1,536 SNPs for 938 eggplant accessions was collected [1]. We used reliable markers to create a tentative core collection using Core Hunter II software. Adding microsatellite genotypic data [2], the core collection was then reconstructed. Clustering analysis of whole eggplant materials and the core collection was performed using STRUCTURE 2.3.4 software. GGT 2.0 program was used to formulate Jaccard similarity coefficient and an unrooted Unweighted Pair Group Method with Arithmetic mean (UPGMA) tree was constructed using MEGA program. Genetic diversity indices were defined using GenAlex 6.5 and PowerMarker 3.25.

**RESULTS** The data of 1536 SNPs of the 938 accessions were obtained. Firstly, 987 SNPs were selected as 'reliable markers'. Secondly, 893 accessions that exhibited stable genotypes for the 987 'reliable' SNP markers were selected as candidate accessions for constructing the core collection. Then 176 accessions were selected as independent members of a tentative core collection. Further, among 111 microsatellite markers, 50 microsatellites were selected as reliable markers based on the minor allele frequency and missing data ratio. Similarly, 831 SNP markers were selected. The genotype data of SNP and SSR were used to select 100 accessions for the final core collection, World Eggplant Core (WEC) collection. The WEC collection consisted of 3 African, 4 American, 80 Asian, 8 Europe, and 5 unknown accessions. STRUCTURE analyses on the WEC collection showed 4 cluster (S1-S4) corresponding to geographical groups as below, S1; the European, American, and African countries, S2; the East Asian countries, S3; the Southeast Asian countries, S4; Southeast Asian and South Asian countries. Basic data of the WEC collection are published in the VegMarks database (<https://vegmarks.nivot.affrc.go.jp/resource/>) and seeds of the WEC collection will be distributed by the NARO Genebank.

**DISCUSSION & CONCLUSION** Based on the genotypes of 831 SNP and 50 SSR of 893 eggplant germplasms, 100 accessions were selected as the eggplant core collection (WEC). Generally, the values of the genetic diversity indices were higher in the Asian countries and many accessions from these countries were selected as the WEC collection. Additionally, the collection maintains most of the genetic diversity of the whole collection and categorized into four groups. Obtained molecular information of the collection will help strategic planning of research and developing new breeding materials. Basic trait-based characterization of the collection is underway (Figure 1), which will contribute to the further utilization of the collection.

## REFERENCES

- [1] Hirakawa, H. et al., 2014, DNA Res., 21: 649–660
- [2] Nunome, T. et al., 2009, Theor. Appl. Genet., 119: 1143–1153



**Figure 1.** Pictures of mature and immature fruit of 100 accessions constituting the World Eggplant Core (WEC) collection.



# Capsicum seed viability monitoring for germplasm management

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**BACKGROUND** Kasetsart University by the Tropical Vegetable Research Center has held a large collection over 3,000 accessions of *Capsicum* germplasm. Seed viability is a key monitoring parameter for germplasm management. Low seed viability suggests the germplasm accession needs to be rejuvenated to maintain the collection diversity and seed stock for distribution. Regular monitoring for the large germplasm collection is costly and perhaps unnecessary. Initial seed quality including dry weight, moisture content and germination affect seed longevity [1]. Data of these seed qualities were collected from chili germplasm sampled from the core collection to identify factors most influencing the seed longevity and thus to provide guidance how to more efficiently monitor the chili seed viability.

**MATERIALS & METHODS** Total 181 chili accessions (214 samples from five species i.e. *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens*) were randomly selected from the core collection. The seed has been kept in a short-term storage (10°C, 45% RH) since 1995. Seed dry weight, moisture content and germination of all the samples were investigated in 2018 according to the ISTA rules (2016) [2]. Correlation between the seed qualities and germination were analysed.

**RESULTS** Chili accessions were divided into three groups according to the initial storage year; 23 samples were from 1995 (23 years old), 96 from 2001-5 (13-17 years) and 95 from 2009-13 (5-9 years). Average seed germination rate of the three age groups was slightly different ranging from 58.5-71.0% (Figure 1). Of the 181, 23 accessions had two seed lots from 1995 and 2011-12. Of the same chili genotypes, average seed germination of these 23 accessions decreased from 87.4 to 58.5% by the increased seed age. Seed moisture content was mostly below 10% (excepted for 3 accessions) with average of 7.0% (4.76-16.18%). Average 100-seed weight was 2.9 g (2.4-3.63). Correlation analyses revealed that seed germination tested in 2018 related to neither seed moisture content nor weight.

**DISCUSSION & CONCLUSION** Storage age did not seem to have major effect on the seed longevity of the chili germplasm. Of all the samples tested, only six had <10% germination, which originated from all the age groups. Although the correlation suggested seed moisture content did not affect the germination, it is interesting to follow up the germination tendency over years of the three accessions with high moisture content to prove if high moisture content shortens seed longevity. One accession was 17 years old having 64% germination, while the other two were 7 years old having 85% germination. Chili genotype and initial seed germination are also key factors that influence the seed longevity, unfortunately our genebank lacks such information. However, this study suggests that chili seed longevity can last at least 23 years in a short-term seed storage in a fair management.

## REFERENCES

- [1] Ellis RH, et al. 1992, Ann Bot 69: 53-58
- [2] ISTA, 2016, International Rules for Seed Testing.



# Screening of the *Pun1* gene in pepper genetic resources from the RDA (Rural Development Administration) genebank

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**BACKGROUND** Chilli or red pepper is commonly grown as a herbaceous annual dicotyledonous flowering plant in temperate areas. Chilli belongs to the genus *Capsicum*, twenty five species have been cultivated extensively, initially in the Americas and later spreading to Europe throughout the world. Pepper is used as a food flavouring, a colouring agent, a pharmaceutical ingredient, and in other innovative ways. Pepper (*Capsicum* spp.) is one of the main features of this crop because of its pungency, which is due to the presence of capsaicinoids. This compound is synthesized as a secondary metabolite and found only in the placental tissue of spicy fruit [1]. Stewart et al. [2] concluded that *Pun1* encodes the acyltransferase AT3 and they demonstrated its involvement in capsaicinoid metabolism. A DNA sequence possibly related to pungency with a high similarity to the *Pun1* locus was studied, and sequence analysis of this homologue revealed a 15 bp deletion in non-pungent pepper accessions. To analyze the pungency of a large number of pepper genetic resources in a short time, molecular markers for the *Pun1* gene were used in this study.

**MATERIALS & METHODS** Pepper accessions from different *Capsicum* species were used for the analysis. The 351 resources consisted of 12 resources for *C. annuum*, 36 resources for *C. baccatum*, and 303 resources for *C. chinense*. Fifteen varieties were used as control varieties. DNA extraction used the CTAB method. For SCAR (sequence characterised amplified regions) analysis two forward (5'-TCCTCATGCATCTCTTGCAG-3' and 5'-GCTCCACGGAAAAGACTCAT-3') and one reverse (5'-CAAATGGCACTTTCCCTTCTCTCATT-3') primer were used. The PCR program used was as follows: an initial denaturation at 95°C for 5 min; 35 cycles of amplification, each consisting of 95°C for 30 s, 60°C for 60 s, and 72°C for 2 min; and a final extension at 72°C for 10 min. The PCR product in SCAR analysis was directly separated on 1.0% agarose gels.

**RESULTS** The *Pun1* gene sequence was analyzed in the pepper germplasm using 3 individuals for each accession. Out of the 351 pepper genetic resources, the *pun* gene was not detected in 3 accessions. In the 12 *C. annuum* resources, 3 resources did not have the *Pun1* gene, and 9 resources had the *Pun1* gene. The 2 non-pungent resources of *C. annuum* were similar to sweet pepper. The 35 resources in *C. baccatum* had the *Pun1* gene and in one accession it was not detected. Of the 303 resources of *C. chinense* analyzed, 300 resources had *Pun1* genes, and one accession was heterogeneous. The others in *C. chinense* were not detected. The results of the marker analysis showed differences within pepper genetic resources. In general, *C. chinense* is known as the hottest amongst *Capsicum* spp., including Habanero. All *C. chinense* and *C. baccatum* studied had the *Pun1* gene.

**DISCUSSION & CONCLUSION** We analyzed the *Pun1* gene in 351 pepper genetic resources from the Korea RDA Genebank. As a result, it was possible to distinguish between pungent and non-pungent genetic resources. In general, HPLC is used to analyze the capsaicinoid content of pepper. HPLC requires much effort and cost in analysis. Using *Pun1* molecular markers, spicy pepper genetic resources could be distinguished at low cost. Both *C. chinense* and *C. baccatum* had a *Pun1* gene. *C. annuum*, which is widely cultivated, showed diversity in the *Pun1* gene.

## REFERENCES

- [1] Suzuki T et al., 1987, Plant Cell Physiol 21:83
- [2] Stewart C et al., 2005, Plant J 42:675-688



# The fascinating diversity of pepper and eggplant collections held at the INRA centre for vegetable germplasm

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**BACKGROUND** Both pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) are crops traditionally and currently grown in France, with 44 and 32 thousand tons produced respectively in 2016, and are economically important worldwide, with 34 and 51 billion tons produced respectively in the same year (source FAOSTAT). The INRA Unit for Genetics and Breeding of Fruit and Vegetables has carried out genetic research and breeding on both crops since 1962, and has progressively gathered numerous genetic resources, which are now managed by the INRA Centre for Vegetable Germplasm. No overview of the eggplant collection has been published so far while a publication on the pepper collection appeared in 2007 [1]. Our goal is to present a comprehensive overview of the current content of both collections, including their wild and cultivated relatives.

**MATERIALS & METHODS** The collection described is the one held by the INRA Centre for Vegetable Germplasm as of December 2018. It contained 3,574 pepper, eggplant, and related species, and we used data collected between 1962 and 2018. Passport data have been homogenized between years and between both collections. The primary descriptions were acquired on plants grown in the open field. As the descriptors have been modified over time, we have also homogenized them when necessary. We analysed the data covering almost 60 years in order to describe the taxonomic and geographic distribution of both collections together with the extent of their phenotypic diversity.

**RESULTS** The two main cultivated species of pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) represent the major part of each collection. Four other cultivated pepper species (*C. chinense*, *C. frutescens*, *C. baccatum*, *C. pubescens*) and 2 other cultivated eggplant species (*S. aethiopicum* and *S. macrocarpon*), together with wild related material, complete the collections (Table 1). The geographical origin of the accessions in collection is diverse, with accessions originating from sub-regions of almost the whole world as defined by the United Nations M49 standard. Most of the accessions originate from the main production areas and from areas of domestication or diversification. Phenotypic diversity of the collections is also high, with a well-balanced contribution of each of the main fruit types, that we defined as being a combination of shape (ratio length/diameter), colour at commercial stage and one other character (capsaicin content for pepper and calyx prickliness for eggplant).

**DISCUSSION & CONCLUSION** The pepper and eggplant collections from the INRA Centre for Vegetable Germplasm represent a wide diversity of species, origins and phenotypes depicting their domestication and diversification history. Their complementarity and overlap with other worldwide collections will be assessed through the H2020 G2P-SOL European project. The wide diversity of the INRA pepper collection has been used for in-depth research on disease resistance. Thanks to a former collaboration with R.N. Lester, the INRA eggplant collection has been enriched in wild relatives species, which are under-represented in most other genebanks [2], because of their difficult accessibility, botanical identification and *ex-situ* conservation.

## REFERENCES

- [1] Sage-Palloy A-M et al., 2007, Progress in research on Capsicum & Eggplant, 33-42  
 [2] Syfert MM, Castaneda-Alvarez NP, 2016, American Journal of Botany, 103 (4) 635-651. doi: 10.3732/ajb.1500539

Collection	Collection Sub-group	Number of species in collection	Number of accessions in collection
Eggplant	<i>S. melongena</i>	1	1103
	<i>Other cultivated Species</i>	2	462
	<i>Crop wild relatives</i>	126	556
Pepper	<i>C. annuum</i>	1	1148
	<i>Other cultivated Species</i>	4	337
	<i>Crop wild relatives</i>	6	21

**Table 1.** Number of species and accessions in the eggplant and pepper collections held at the INRA Centre for Vegetable Germplasm.

# New challenges and solutions in Hungarian spicy pepper breeding

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**BACKGROUND** Spice pepper production has more than 250 years history in Hungary. Due to the Hungarian soil and climate factors, the growers use only interior bred *Capsicum annuum* (L.) var. *Longum* cultivars for spice pepper production. The classic selection for high yield and standard 18% dry matter and high colour content led to sensitiveness for diseases. Considering the risk and expenses of the chemical treatments resistance breeding is a sustainable solution. According to the disadvantageous impacts of the climate change in the Carpathian Basin and the increasing demand for both quantity and quality of the crop, the cultivation technology requires continuous development and breeding high genetic potential virus resistant varieties and hybrids.

**MATERIALS & METHODS** The gene-pool of the breeding project is based on the landraces and cultivars from Szeged and Kalocsa Region. The resistance genes were obtained from wild *Capsicum* species. The breeding method consists of single cross, back-cross, endogamy and doubled-haploid technology in order to produce homogeneous breeding lines. The breeding lines were tested with direct infections of Leaf spot bacteria (*Xanthomonas campestris* pv. *vesicatoria* – Xcv) Cucumber mosaic virus (CMV) and Tobacco mosaic virus (TMV) races which are common in the Great Plains of Hungary. The resistant lines were tested for combining ability. The hybrid candidates were examined for main quality parameters like dry matter and colour content, pungency.

**RESULTS** The *Capsicum* gene pool in Hungary does not contain adequate resistance to the most frequent diseases (Xcv, CMV, TMV) causing economic losses in spicy pepper production every year. The commonly used varieties are predominantly sensitive to these diseases. Only a few exceptions (e.g. the 'Szegedi-178' and 'Kalocsai V-2' hot spice pepper varieties show tolerance against TMV) were found during the testing of approximately 150 breeding lines (including landraces, cultivars) [1]. The resistance genes can be primarily introduced in the breeding process by using wild *Capsicum* species [2]. Unfortunately, no resistance against CMV was found in the gene-pool of this project. The project was started with 20 selected parent lines which were inbred in greenhouse during 6 generations (two generations per year in a 3-year period). After the combining ability analysis 10 parent lines were selected in the interest of making hybrid candidates. As a partial result, 48 new hybrids passed the Xcv and TMV resistance test, but many of them failed to meet the demands of the growers (fruit size, shape, yield) and the processing factories (average weight, yield, pungency). Finally, 4 hybrid candidates were selected showing Xcv and TMV resistance and high yield with excellent quality parameters.

**DISCUSSION & CONCLUSION** The spicy pepper production industry needs new varieties and hybrids with complex resistance package. The gene pool of the Hungarian spicy pepper is very complex. Due to the association of wild characteristics to the resistance genes, it is difficult to obtain the required fruit size and yield common in disease sensitive varieties or hybrids. The results are encouraging, as new Xcv and TMV resistant lines were produced during the project. Nevertheless, further breeding is needed to find a resistance source against CMV.

## REFERENCES

- [1] Bráj R, 2017, Resistance testing of *Capsicum annuum* varieties and genotypes against ObPV and CMV, Thesis, Gödöllo, Hungary.
- [2] Somogyi N, 2010, Hybrid condiment paprika breeding and protected cultivation technologies, Thesis, Keszthely, Hungary



**Figure 1.** Necrotic local lesions on leaf of pepper genotype 0328 inoculated with ObPV-XM isolate.  
(Photo: Bráj R. 2017)



# Evaluation of agronomic and morphological traits of Balkan pepper (*Capsicum annuum* L.) accessions

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**BACKGROUND** Pepper is a traditional and most valuable vegetable crop all over the world and in different countries of the Balkan Peninsula as well. A great number of local populations with specific shape, color, taste, biological value and a great range of use of the fruits are grown across these countries in addition to major commercial cultivars [1], [2]. In recent years, the old native forms which are preserved in isolated areas are an object of considerable interest of contemporary breeding programs, because they could be valuable sources of novel traits. Collection, evaluation and exploitation of different pepper genetic resources are necessity and precondition for successful breeding. Given the enormous loss in global crop biodiversity witnessed in recent years, the preservation, conservation and utilization of the genetic resources is of utmost importance. Therefore a collection of Balkan pepper accessions was evaluated for different agronomic and morphological traits.

**MATERIALS & METHODS** One hundred diverse pepper accessions (cultivars, lines and local forms) collected from different Balkan countries (Bulgaria, Serbia, Macedonia and Romania) were evaluated during 2017-2018 in triplicated field trials at MVCRI, Plovdiv, Bulgaria. Nine traits - productivity, plant and stem height, number of the embranchments at first order, as well as fruit length, width, number of locules, wall thickness and weight of the fruits - were measured. A total of 51 accessions were harvested before maturity and 49 at maturity stage depending on their main usage. Statistical analysis of the obtained data was performed using SAS and R programs.

**RESULTS** Wide variation was observed for all plant and fruit related traits (Table 1). Coefficient of variation in the accessions less than 20% was reported for all traits, except plant productivity. More than 60% of studied accessions had plant productivity above 0.5 kg/plant with mean of 0.61 kg. Plant related traits as height, number of embranchments as well as fruit related traits as length, wall thickness, width and weight also displayed abundant diversity among evaluated genotypes. Accessions and Year effects were reported to be significant for the majority of the studied traits, whereas the interaction between Accession and Year was significantly different for all of the traits. Among all, Bulgarian local form CAPS-70 and breeding line CAPS-88 were the most productive genotypes. Prevailing part of the accessions (62%) was with conical fruit shape, followed by accessions with elongated (18%), pumpkin (10%), blocky (7%) and round (3%). Fruit weight varied from 1.28 g (CAPS-1) to 140.18 g (CAPS-98). Fifteen accessions had fruit weight above 100 g. Accessions with pumpkin shape CAPS-33, 97A, and 98 possessed the highest fruit wall thickness with above 5 mm. The majority of the accessions (83%) have sweet taste of the fruits, while the rest (17 %) are with variable level of pungency.

**DISCUSSION & CONCLUSION** This study provides useful knowledge for agronomic and morphological traits related to plants and fruits, within Balkan pepper (*Capsicum annuum* L.) germplasm collection. The obtained results show high variation in the studied traits among the accessions. They also enable to identify best accessions and to use them as parental components in the hybridization programs for breeding new cultivars with desirable characteristics [3]. The study is also essential in relation to the preservation and management of pepper genetic diversity on the area of Balkan region, which is considered as a center of diversity of this crop.

## REFERENCES

- [1] Krasteva et al., 2012, Proc. of the V Balkan Symposium on Vegetables and Potatoes, Acta Horticulturae, 960: 151-158. doi: 10.17660/ActaHortic.2012.960.21
- [2] Danojevic & Medic-Pap, 2018, Genetika, 50(1):121-129. doi.org/10.2298/GENSR1801121D
- [3] Oh et al., 2012, Korean J. Intl. Agri., 24(5): 547-556.

## ACKNOWLEDGEMENTS

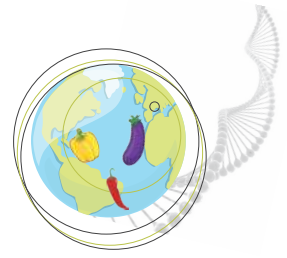
Velichka Todorova thanks the French "Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (program ACCES de CAMPUS France)" for covering the travel and accommodation expenses for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.

	PH (cm)	SH (cm)	EM	FL (cm)	FWI (cm)	FWT (cm)	Locules	FWE (g)	Productivity (kg/plant)
<b>Accessions</b>	77.20	24.00	2.65	9.79	4.14	3.46	2.67	61.70	0.61
<b>Range</b>	33.30- 125.00	14.90- 35.00	1.17- 4.00	1.18- 27.30	1.12- 7.99	0.73- 5.85	2.00- 3.92	1.29- 142.80	0.11- 1.26
<b>LSD<sub>0.05</sub></b>	11.60	5.66	0.62	1.60	0.67	0.69	0.56	15.90	0.27
<b>CV</b>	10.90	17.20	17.10	11.90	11.90	14.60	15.20	18.80	32.50
<b>ANOVA</b>									
<b>Rep</b>	7.35***	0.50	3.39*	1.27	0.47	1.35	0.58	1.20	8.19***
<b>Accession</b>	15.50***	5.20***	2.56***	64.20***	57.60***	31.10***	4.45***	56.20***	6.34***
<b>Year</b>	11.60***	270.0***	2.02	21.20***	5.33*	11.80***	2.61	322.8***	2.47
<b>A*Y interaction</b>	3.19***	1.94***	1.73***	2.88***	8.96***	4.53***	1.93***	6.73***	2.92***

**Table 1. Descriptive Statistics and Analysis of Variance (ANOVA) for plant and fruit traits of evaluated pepper accessions**

**NOTE:** Abbreviations are representation of following plant traits: PH-plant height, SH-stem height, EM embranchment, FL-fruit length, FWI-fruit width, FWT-fruit wall thickness and FWE-fruit weight. LSD: Least square difference; CV: coefficient of variation.





# RESISTANCE TO BIOTIC AND ABIOTIC STRESSES

SESSION 2



# Breeding for resistance to (a)biotic stresses in *Capsicum* and eggplant

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Crop plants are subjected to a variety of stresses during their lifecycle, including abiotic stress and biotic stress factors. Plants have developed a multitude of defence and adaption responses to these stress factors. In the past years, our understanding of plant-pathogen interactions has evolved rapidly. Pathogen genomics has allowed a genome-wide study on the structure, function and evolution of effectors in pathogen genomes. The so-called effectoromics offers a high-throughput functional approach to study effector-associated plant genes such as resistance (R) genes and susceptibility (S) genes. Together with the breakthrough and powerful techniques of genome editing, novel strategies are being developed for breeding crops with durable resistance to pathogens. In this talk, I will give an overview on the resistance and tolerance to biotic stresses in *Capsicum* and Eggplant. Taking this opportunity, I will present our most recent research on plant S genes and how to exploit these S genes in resistance breeding.

Further, I will briefly touch the topic of breeding for resilience to combined biotic and abiotic stresses. In the field, different stress factors mostly occur concurrently resulting in a new state of stress, the combined stress. There is evidence that plant resistance to pathogens can be attenuated or enhanced by abiotic stress factors. With stress tolerance research being mostly focused on plant responses to individual stresses, the understanding of a plant's ability to adapt to combined stresses is limited. In the last few years, we studied powdery mildew resistance under salt stress conditions in the model crop plant tomato with the aim to understand the requirements to achieve plant resilience to a wider array of combined abiotic and biotic stress combinations. We uncovered specific responses of tomato plants to combined salinity-pathogen stress, which varied with salinity intensity and plant resistance genes. Moreover, hormones, with their complex regulation and cross-talk, were shown to play a key role in the adaption of tomato plants to the combined stress. I will briefly present our recent research results on the complexity of plant responses to abiotic and biotic stress combinations. In addition, I will try to give an overview on resistance to combined stresses in *Capsicum* and Eggplant.

## ACKNOWLEDGEMENTS

Yuling Bai thanks the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant.

# Is wax composition playing a role in defense to chili anthracnose?

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**BACKGROUND** Anthracnose (*Colletotrichum* spp.) causes fruit rot in chili (*Capsicum* spp.). Systemic resistance to anthracnose, recognized as hypersensitive reaction [1], is rare and only found in few other species than *Capsicum annuum* [2], which is the most globally economically important species. However, fruits of different *C. annuum* genotypes exhibited various degrees of anthracnose infection after unwounded fruit inoculation, indicating that cuticle may play a role of defense in the fruit. Cuticle physical and biochemical properties have been investigated to prove their defensive role against anthracnose infection. The physical properties, i.e., cuticle thickness and wettability, showed insignificant relatedness to the anthracnose infection (unpublished). This study aimed at investigating the biochemical composition of chili fruit cuticular wax and its relatedness to defensive ability against anthracnose infection.

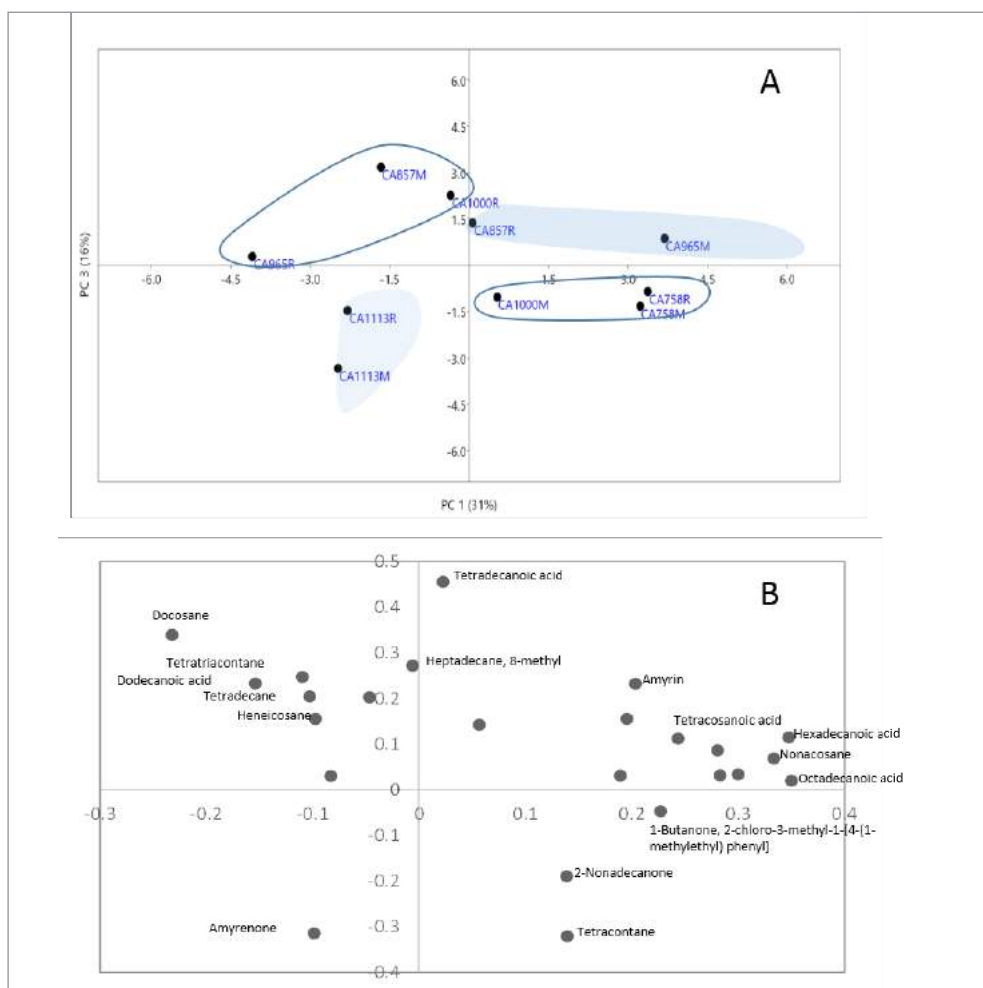
**MATERIALS & METHODS** Five chili genotypes with different degrees of infection (0-60%) after unwounded fruit inoculation were selected for the study. Fruit at two maturity stages including mature green (fruit having reached its full size with green color in appearance) and ripe (physiologically mature as fruit appearing red) were collected in three replicates. Fruit cuticular wax was extracted by chloroform, and the wax composition was identified and quantified using GC/MS technique. Unwounded inoculation with *Colletotrichum truncatum* was performed on the two fruit stages of each chili genotype.

**RESULTS** Chili fruit cuticular wax was composed of five compound groups including alkanes, alcohols, ketones, fatty acids and triterpenoids/sterols. Alkanes and triterpenoids/sterols appeared to be the major components in ripe fruits, accounting for 23% and 28% of total wax, respectively. However, different chili genotypes had different wax profiles. Also, the changes of the wax compounds during fruit development varied among the chili genotypes. At ripe fruit stage, 'CA758' and 'CA1000' displayed a triterpenoids/sterols increase while having slightly decreased alkanes when compared to the mature green stage. On the contrary, for 'CA965' and 'CA1113', triterpenoids/sterols and alkanes in the ripe fruit dramatically decreased from those in the mature green fruit. These two wax groups exhibited different trends during fruit development of 'CA857'. PCA analysis provided an overview of the interrelationships between wax composition and anthracnose susceptibility of different chili genotypes considering fruit at mature green and ripe stages separately (Figure 1). The first, second and third PC axes accounted for 31, 17 and 16%, of the total variance respectively (Figure 1A). The octadecanoic acid, hexadecanoic acid, nonacosane, heneicosane, tetracosanoic acid, docosane and 1-Butanone, 2-chloro-3-methyl-1-[4-(1-methylethyl)phenyl] had high loading score on PC1, while tetradecanoic acid, docosane, heptadecane 8-methyl, tetratriacontane, dodecanoic acid, amyirin, amyrenone, tetradecane, 2-nonadecanone, and tetracontane impacted PC3 separation indicating that they made the greatest contribution to this separation (Figure 1B).

**DISCUSSION & CONCLUSION** Based on the PCA result, the chili genotypes were divided into four groups according to their differential responses to *Colletotrichum truncatum*. Group I [CA857M, CA956R and CA1000R] and Group II [CA1000M, CA 758M and CA758R] were classified as resistant. Group III [CA1113M and CA113R] and Group IV [CA965M and CA857R] were susceptible. Further studies are needed to confirm the role of each wax composition on defense mechanism to anthracnose.

## REFERENCES

- [1] Mahasuk P et al., 2009, Plant Breeding, 128 (6):701-706. doi: 10.1111/j.1439-0523.2008.01615.x
- [2] Mongkolporn O et al., 2010, Plant Disease, 94 (3):306-310. doi: 10.1094/PDIS-94-3-0306



**Figure 1.** Principal component analysis of cuticular wax profiles of five chili genotypes (results from mature green and ripe stage fruits). (A) Four groups of varieties are defined on the basis of their wax composition together with their response to *Colletotrichum truncatum*. Filled/colored circles include varieties susceptible to *C. truncatum*. Blank circles include the resistant varieties. (B) Contribution of wax components to the definition of the two main PCA axes.



# Aphid populations showing differential levels of virulence on *Capsicum* accessions

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**BACKGROUND** The green peach aphid, *Myzus persicae*, is one of the most threatening pests in pepper cultivation and growers would benefit from resistant varieties. Previously, we identified two *Capsicum* accessions as susceptible and three as resistant to *M. persicae* using an aphid population originating from the Netherlands (NL) [1]. Later on we identified an aphid population originating from a different geographical region (Switzerland, SW) that was virulent on all tested *Capsicum* accessions [2]. The objective of this study is to describe in detail different aspects of the interaction between the two aphid populations and two selected *Capsicum baccatum* accessions, one that was susceptible (PB2013046) and one that was resistant (PB2013071) to population NL, including the biochemical processes involved.

**MATERIALS & METHODS** Aphid survival and reproduction were measured using clip cages containing five one-day-old nymphs placed on seven-week-old plants. The Electrical Penetration Graph (EPG) technique was used to monitor probing and feeding behaviour of the two aphid populations on the two *C. baccatum* accessions. We also studied the defense responses in plants of the two accessions after infestation with the two aphid populations. The accumulation of reactive oxygen species (ROS) on plant leaves was tested using 3,3'-diaminobenzidine (DAB) staining. Callose deposition was detected in leaves stained by aniline blue.

**RESULTS** Both *M. persicae* populations can survive and reproduce well on the susceptible accession PB2013046. The aphid population SW can also survive and reproduce well on the other accession PB2013071 that was resistant to aphid population NL. The EPG recordings showed similar feeding activities for both aphid populations on PB2013046. On accession PB2013071 the aphid population SW was able to devote significantly more time to phloem ingestion than population NL. We also found that plants of accession PB2013046 did not accumulate reactive oxygen species (ROS) and callose after infestation with either aphid population. However, plants of PB2013071 showed a strong defense response after infestation with the NL population, and only a weak one after infestation with the SW population. The defense response involves a stronger ROS production and more callose deposition after infestation with the NL population.

**DISCUSSION & CONCLUSION** Two populations of *M. persicae* (NL and SW) perform similarly with respect to survival and reproduction on an accession susceptible to the NL population, but significantly different on an accession resistant to that population. The performance difference between the two aphid populations is reflected in differences in feeding and probing activity as well as in levels of defense response (ROS accumulation, callose deposition). The results strongly suggest that the SW population has (partially) overcome the resistance that is effective against the NL population.

## REFERENCES

- [1] Sun M et al., 2018, BMC Plant Biology, 18: 138-151
- [2] Sun M et al., 2019, Insect Science, doi:10.1111/1744-7917.12648.

## ACKNOWLEDGEMENTS

Roeland Voorrips thanks the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



# Impact of host partial resistance on the gene expression of *Phytophthora capsici*: consequences for pepper breeding

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**BACKGROUND** Plant resistance to pathogens is either qualitative when the disease progression is stopped, or quantitative when the pathogen development is reduced. Qualitative resistance deployed in agrosystems applies a strong pressure on the targeted pathogen and frequently results in selection of virulent strains that break down the resistance. In contrast, quantitative resistance exerts a lower selective pressure limiting selection of a virulent strain, and thus promises a great sustainability potential. While the understanding on how the pathogen perturbs susceptible plants largely progressed, the impact of quantitative resistance on molecular mechanisms associated with pathogenicity is still poorly understood. Thus, we report here the *in planta* transcriptome analysis for two *Phytophthora capsici* isolates, one highly and one lowly aggressive on pepper. We describe how plant hosts with different resistance level modify the regulation of *P. capsici* genes at early stages of the interaction.

**MATERIALS & METHODS** Two *Capsicum annuum* genotypes, Yolo Wonder (YW) susceptible to *P. capsici* and CM334 partially resistant, were stem-inoculated [1], with two *P. capsici* isolates, Pc107 highly aggressive on pepper, and Pc273 lowly aggressive. An RNA-seq analysis was performed on live tissue cut under the necrosis at 24 hours post-inoculation (3 biological replicates per condition). We selected *P. capsici* genes with the highest numbers of reads to compare their expression between the 4 pairwise interactions. Molecular functions of *P. capsici* genes were determined by manually curated ontology searches (BLAST2GO Basic v4.1.9, PHI-base, InterProScan) and tested for enrichment using BLAST2GO.

**RESULTS** The highest percentage of *P. capsici* reads among all reads obtained from the tissue samples were observed in the interaction between the susceptible host and the high aggressive isolate, and the lowest in the resistant host whatever the isolate. 361 *P. capsici* genes out of 7,307 selected genes were differentially expressed between conditions. The highest expression variations were observed between isolates, with 136 DEGs (differentially expressed genes) in CM334 and 248 DEGs in YW. Only 48 genes of the isolate Pc107 were differentially expressed according to the infected host while no DEG of the isolate Pc273 were found between hosts. Out of the 48 DEGs of the aggressive isolate Pc107, 10 genes encoding putative transporters of nutrient and inorganic substances and 7 genes encoding hydrolase activity were more expressed in YW, suggesting their role respectively in facilitating the development of *P. capsici* by feeding nutrients from the susceptible host and in degrading the plant cell wall and supplying energy to help *P. capsici* development. The RNA-seq analysis also highlighted a single DEG encoding an RxLR cytoplasmic effector with a much higher expression in YW than in CM334 whatever the isolate.

**DISCUSSION & CONCLUSION** Here we compared *in planta* transcriptomes from two *P. capsici* isolates (one highly aggressive and another lowly aggressive) inoculated onto highly susceptible and partially resistant pepper germplasm [2]. Our findings shed light on the pathogen gene repertoire and regulation during these interactions; they provide novel insights into the effect of partially resistant plants to pathogen development and identify *P. capsici* genes responding to the plant resistance factors. Identified molecular functions will assist the discovery of genes responsible for the evolutionary adaptation of *P. capsici* isolates to plant hosts [3].

## REFERENCES

- [1] Lefebvre V, Palloix A, 1996. Theor Appl Genet, 93:503-511
- [2] Maillot G, 2018. PhD thesis, Avignon University.

## ACKNOWLEDGEMENTS

Authors acknowledge both the INRA Plant Breeding Division (project EFFECAPS) and Agropolis Fondation (project 'Protéines phytopathogènes I 300-002') for grants, and the French PACA sud region and the INRA Plant Breeding Division that offered a PhD fellowship to GM.

# The effect of plant and leaf age on thrips resistance in *Capsicum*

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**BACKGROUND** The Western flower thrips (*Frankliniella occidentalis*) is a worldwide pest insect that causes damage in pepper cultivation, so growers would benefit from host plant resistance. Earlier we identified strong resistance in *Capsicum* by screening plants at the age of 12 weeks [1]. Because thrips can attack a young crop as well, we wanted to determine how resistance develops over time. Also, because in a crop there are always leaves of different ages we were interested how resistance unfolds over the lifetime of a leaf and how this might affect the overall resistance of the crop. Finally, we wanted to know on which leaves the eggs are deposited: if females select leaves where resistance is not expressed the resistance would have little effect.

**MATERIALS & METHODS** The effects of plant age were studied in nine *Capsicum* accessions with different levels of resistance. These were sown at four-week intervals and the tests were performed when plants of 4, 8 and 12 weeks were available. The effects of leaf age were studied in one susceptible and one resistant accession. From 12-week-old plants we collected and tested leaves of 0-2-4-6-8 weeks old. Egg deposition was studied in 20 week-old flowering plants of resistant accession CGN16975 where entire branches were enclosed in a thrips-proof sleeve; after 3 days the 40 youngest and oldest leaves of each branch were detached and the larvae emerging in the next 10 days (a proxy for the number of eggs) counted.

**RESULTS** Our results show that the resistance (if present) starts to develop when the plants are between 4 and 8 weeks old. This transition was most marked on the resistant accession CGN16975, on which about 50% of the L1 larvae developed into the next larval stage on 4-week-old plants, whereas none of them developed beyond the L1 stage on 8- or 12-week-old plants. Furthermore, it was shown that youngest fully opened leaves of the resistant accession CGN16975 are significantly more resistant to thrips than older leaves; 89% of the L1 larvae did not develop into the next stage on the youngest leaves, whereas 57% did not develop beyond the L1 stage on the oldest leaves. In contrast, young leaves of the susceptible accession CGN17219 are more susceptible than older leaves; 9% versus 52% of the L1 larvae did not develop into the next stage on young and old leaves, respectively. The egg deposition experiment showed that on the resistant accession CGN16975, the average number of emerged larvae was significantly lower in old leaves compared to young leaves ( $P < 0.001$ ; 1.4 vs 0.1 larvae per cm<sup>2</sup> on young vs. old leaves, respectively).

**DISCUSSION & CONCLUSION** We showed that L1 larvae can develop to the L2 stage on 4-week-old plants of all accessions, although with significant differences between accessions and leaf age. In general, young leaves are better suited for thrips performance than older leaves, but resistance (if present) is mostly expressed in the younger leaves, resulting in a trade-off. Thrips females prefer the youngest leaves for oviposition, even on resistant plants where these youngest leaves have the highest level of resistance. This means that the resistance can be highly effective in reducing the thrips population, even though the older leaves in the crop would support larval development. This has also been shown in whole-plant population development tests reported previously [2].

## REFERENCES

- [1] Maharijaya A, Vosman B et al., 2011. *Euphytica* 177: 401-410
- [2] Van Haperen P, Voorrips RE et al., 2019. *Arthropod-Plant Interactions* 13: 11-18

### **ACKNOWLEDGEMENTS**

Roeland Voorrips thanks the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.

# Molecular mapping of the *Chili veinal mottle virus* (ChiVMV) resistance I (*CvrI*) gene in pepper

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**BACKGROUND** *Chili veinal mottle virus* (ChiVMV) is one of the important viruses causing severe losses of pepper yield in Asia and Africa. Nevertheless, only two ChiVMV resistance sources have been reported up to date. One is controlled by two complementary loci (*pvr1<sup>2</sup>* with *pvr6*) [1], and the other is the single dominant resistance gene, *CvrI*, on pepper chromosome 6 [2, 3]. Even though the *CvrI* locus was identified, highly repeated sequences in this region hampered the fine mapping of the *CvrI* locus. In this study, we fine-mapped the *CvrI* region and developed several molecular markers linked to the *CvrI* locus. Furthermore, we analyzed expression levels and sequence variations of *CvrI* candidate genes located in the *CvrI* region. The information obtained in this study will accelerate the breeding program for ChiVMV resistance in pepper.

**MATERIALS & METHODS** F2 populations derived from the cross between the ChiVMV resistant accession, *Capsicum annuum* 'CV3' and the susceptible accession, *C. annuum* 'Jeju' were used to fine map the *CvrI* locus. ChiVMV screening was performed with 2 g of ChiVMV inoculum per 10 mL potassium phosphate buffer and ChiVMV symptoms were analyzed at 21 days post inoculation (dpi). To delimit the *CvrI* region, high resolution melting (HRM) markers were developed using public pepper genome databases. Next, the candidate genes were predicted using the FGENESH program and annotated using the BLAST2GO program in the *CvrI* target region. *CvrI* candidate genes were subsequently analyzed by expression level and sequence variation between 'CV3' and 'Jeju'.

**RESULTS** No ChiVMV symptoms were observed in four resistant accessions, 'CV3', 'CV4', 'CV8', and 'CV9'. Low viral accumulation was also detected in these four resistant accessions compared to the susceptible control 'Jeju'. To fine-map the ChiVMV resistance locus in 'CV3', we challenged 750 'CV3' x 'Jeju' F2 populations with ChiVMV inoculum and confirmed dominant inheritance mode by a single gene called *CvrI* in 'CV3'. Using public genome databases, we developed closely-linked markers for *CvrI*, C10SNP1 and Z02SNP1. Then, six markers were developed between the region of C10SNP1 and Z02SNP1, which showed complete linkage (0 cM) with the *CvrI* locus. Based on genetic mapping data, we delimited the *CvrI* candidate region within 300 kb in the upper arm of pepper chromosome 6. Because the assembly quality of the *CvrI* candidate region is poor regarding nucleotide-binding domain leucine rich repeat (NB-LRR) sequences, we used high-quality pepper genome sequences to confirm *CvrI* candidate genes in the target region. As a result, 40 genes were predicted in this region including one NB-LRR gene. No difference of expression levels between 'CV3' and 'Jeju' was observed for this NB-LRR gene. However, there were some sequence variations between 'CV3' and 'Jeju' at the amino acid level, which could cause changes in ChiVMV resistance.

**DISCUSSION & CONCLUSION** In this study, we have assigned the *CvrI* target region to a 300 kb region on a new pepper reference genome with 0 cM markers. Gene prediction analysis predicted one NB-LRR gene as a *CvrI* candidate in this region. The NB-LRR gene sequence showed significant polymorphisms at the amino acid level between 'CV3' and 'Jeju'. However, as the current pepper genome was not completely assembled in this region (telomeric region of pepper chromosome 6), we are trying to sequence and detect the complete sequence variation in the *CvrI* target region between 'CV3' and 'Jeju' using targeted locus amplification (TLA) sequencing technology. This sequencing could reveal the complete sequence of the *CvrI* target region and haplotype differences between 'CV3' and 'Jeju'.

## REFERENCES

- [1] Hwang J et al., 2009, Mol Cells, 27(3):329-336. doi: 10.1007/s10059-009-0042-y
- [2] Lee HR et al., 2013, Euphytica, 193(2). doi: 10.1007/s10681-013-0897-z
- [3] Lee JH et al., 2017, Mol Breeding, 37:121. doi:10.1007/s11032-017-0717-6



# Genome-wide association mapping of loci involved in *Potato virus Y* resistance and tolerance in pepper germplasm

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**BACKGROUND** Plants have evolved two ways to decrease damage induced by parasite infections, resistance and tolerance. Resistance decreases the parasite load within plants whereas tolerance decreases the plant damage for a given parasite load. Resistance itself can be divided into qualitative resistance, usually conferred by a major-effect gene, and quantitative resistance conferred by quantitative trait loci (QTLs). In this study, we tested the following hypotheses:  
- In plants, do resistance and tolerance evolve as independent, complementary or alternative defence mechanisms?  
- Similarly, do qualitative and quantitative resistance evolve as independent, complementary or alternative mechanisms?

**MATERIALS & METHODS** We measured the resistance and tolerance levels of 276 accessions representative of the pepper (*Capsicum annuum*) germplasm against *Potato virus Y* (PVY; genus Potyvirus). Two resistance traits were measured in this core-collection: the number of infection foci in plant cotyledons inoculated with a GFP-tagged PVY and within-plant PVY accumulation. Tolerance was measured as the slope of the regression line of PVY-induced damage (plant fresh weight of infected versus mock-inoculated plants) against within-plant PVY accumulation. In addition, genotyping-by-sequencing (GBS) reads were aligned against the reference genome of *C. annuum* cv. CM334 v. 1.55 using the Burrows-Wheeler Aligner tool and the algorithm BWA-MEM. This provided 10,308 single nucleotide polymorphisms (SNPs) covering the whole genome of the accessions.

**RESULTS** Using these phenotype and genotype data, we performed genome-wide association studies (GWAS) to map PVY resistance and tolerance QTLs in the *C. annuum* genome. We identified four QTLs in the pepper genome that explained a significant proportion of resistance variation and one QTL associated with tolerance variation. These five loci were located on four chromosomes. One resistance locus located on chromosome 4 corresponded to the *elf4E* (eukaryotic initiation factor 4E)-encoding gene, which comprises many major-effect alleles in pepper. The confidence intervals of the other QTLs did not include obvious candidate genes for resistance or tolerance. Most of these QTLs coincided with QTLs that had been previously mapped with biparental progenies [1,2]. For each resistance QTL, the favourable allele was associated more frequently than expected at random with favourable alleles at the other resistance QTLs. In contrast, for the tolerance QTL, the favourable allele was less frequently associated with the favourable resistance allele on chromosome 4 than expected at random.

**DISCUSSION & CONCLUSION** These results show the efficiency of GBS and GWAS in *C. annuum* and indicate highly consistent results between GWAS and QTL mapping using biparental progenies. The fact that resistance alleles at different QTLs were more frequently combined than expected by chance may be explained by an increase in resistance efficiency and/or durability. In contrast, the repulsion observed between resistance and tolerance alleles suggests that, because these defense mechanisms are costly and redundant, plants have to invest either in resistance or tolerance, as suggested by theoretical studies [3].

## REFERENCES

- [1] Quenouille et al., 2014, *Heredity*, 112:579–587. doi: 10.1038/hdy.2013.138.
- [2] Tamisier et al., 2017, *Journal of General Virology*, 98:1923–1931. doi: 10.1099/jgv.0.000835
- [3] Pagán and García-Arenal, 2018, *International Journal of Molecular Sciences*, 19:810. doi: 10.3390/ijms19030810



# Combining resistance genes by crossing between an eggplant cultivar and its relatives

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**BACKGROUND** Cultivated eggplants produced in both greenhouse and open field are susceptible to pests and diseases [1]. *Solanum macrocarpon*, originated in African [2] shows multiple resistances to important pest for the cultivated eggplant. Resistance of *S. macrocarpon* to shoot and fruit borer; two spotted spider mite and *Cercospora* spot disease have been reported in several studies [1]. A study was carried out to disclose its resistance to *Fusarium oxysporum* f. sp. *melongenae* (FOM) which is destructive for eggplant. It has been proven that *S. macrocarpon* is resistant to FOM by classical test methods. A SCAR marker was found to be fairly close to the resistance gene from this resource which can be used for efficient molecular assisted selection. Nevertheless, this marker is not available in wild species of eggplant. In this study, an accession of *S. macrocarpon* with multiple resistance genes was crossed with a resistant inbred line of eggplant. In the F<sub>2</sub> generation marker-aided selection (MAS) and phenotypic characterization were performed for pyramiding the FOM resistance genes from eggplant and from *S. macrocarpon*.

**MATERIALS & METHODS** One African accession of *S. macrocarpon* and a FOM resistant inbred line of *S. melongena* were used. Reciprocal crosses were made among them in the greenhouse (Fig 1). F<sub>2</sub> plants were obtained from selfing the F<sub>1</sub> plants. *Fusarium* resistance tests were performed by classical and molecular methods. The SCAR marker (Me8/Em5) was used. The morphological characterization of the F<sub>2</sub> plants was performed by using morphological descriptors.

**RESULTS** The interspecific hybrid showed good fertility (Fig 1). However, undesirable characters were transmitted by the crossing. Although spines and hairs were absent in the parents, spines but not hairs were observed in the hybrids. Also, pollen sterility was observed in the F<sub>1</sub> plants and was the reason for low seed set in hybrid fruits in reciprocal crosses.

**DISCUSSION & CONCLUSION** It has been reported in a previous study that some F<sub>2</sub> progeny from *S. melongena* crossed with its relatives were very prickly although neither parents carried prickles [3]. A serial BC and selfing procedure will be applied to eliminate undesirable attributes. The SCAR marker (Me8/Em5) (426 bp in size) for eggplant is tightly linked to the FOM resistance gene and is a useful tool for disease resistance selection. Marker-assisted selection (MAS) of BC progenies having eggplant cultivar genetic backgrounds further supported the potential of the resistance gene in breeding. Thus, combined different resistance genes from both parents could be observed in their progenies, if a molecular marker associated to the *S. macrocarpon* resistance will be developed. This interspecific hybridization can confer multiple resistances. This information will be useful for the classification, management of genetic resources, selection and breeding of both crops. Consequently, the tests of advanced new eggplant BC progenies which will be continued in further studies. Moreover the FOM resistance genes from both parents will be followed in next progenies.

## REFERENCES

- [1] Rotino GL et al., 1997, Advances in Horticultural Science, 193-201.
- [2] Plazas M et al., 2014, Frontiers in plant science, 5, 318. doi: 10.3389/fpls.2014.00318
- [3] Lester RN, Daunay MC, 2003, Schriften zu Genetischen Ressourcen, 22: 137-152.



**Figure 1.** *Solanum macrocarpon* (a, b), its interspecific hybrids (c, d)

# Resistance of the pepper rootstock ‘Creonte’ to root rot caused by *Phytophthora nicotianae*

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**BACKGROUND** Thermal and water stresses are particularly problematic in North Africa since this region is becoming warmer and drier, which requires intense practice of irrigation. This trend towards global warming would lead to the appearance of adapted fungal diseases on pepper like *Phytophthora nicotianae*. Grafting is becoming a widespread eco-friendly technique in several horticultural crops to cope with different constraints. However, this technique is less applied in chili pepper due to lack of rootstock genotypes tolerant to biotic or abiotic stresses and improving commercial yields. Moreover, graft is not easy to take in chili plants. Spraying grafted plants with ascorbic acid enhanced the healing at graft union via callus formation, and reduced scion defoliation [1]. Grafting susceptible cultivars of chili pepper on the rootstock SCM334 was reported as a disease management approach where pepper rootstock caused by *P. nicotianae* may become an issue in pepper production [1]. Recently, a commercial rootstock named ‘Creonte’ has been considered as providing tolerance to thermal and water stresses [2, 3]. Besides to its tolerance to heat and water stresses already reported, the rootstock ‘Creonte’ was used in this work to study its reaction towards *P. nicotianae* infection.

**MATERIALS & METHODS** Seeds of the commercial pepper rootstock ‘Creonte’ (Monsanto BV, Holland) were received from DGSVCIA (Ministry of Agriculture, Tunisia) in order to test its degree of resistance to the oomycete *P. nicotianae*. The local cultivar ‘Baker’ was used as susceptible control. Pepper seeds were disinfected with 1% Sodium hypochlorite then let to germinate in Petri dish before transplanting without root injury to substrate previously pasteurized with steam. Seedlings were grown under 20-25°C and 16 hours light. Four isolates of *P. nicotianae* from our mycotheca were used for inoculation of seedlings at the stage of four true leaves. The control was distilled water. Rating the root necrosis intensity was done 29 days post inoculation when the majority of susceptible Baker plants showed generalized wilting. Scoring of root rot intensity was based on a scale ranging from 0 (healthy seedling) to 5 (whole seedling dead). Randomized block experimental design was used in this study. Each experimental unit contained at least 7 seedlings.

**RESULTS** The seedlings of Baker were highly attacked by different isolates producing intense root necrosis leading to their mortality, while the controls of the same variety were healthy (Table 1 and Figure 1). Regarding the rootstock ‘Creonte’, the average root necrosis score was low, varying between 0 and 0.5 indicating a good resistance of this cultivar to the pathogen. Moreover, in our essays ‘Creonte’ developed a root system too long showing increased biomass.

**DISCUSSION & CONCLUSION** Our results showed that ‘Creonte’ has good resistance to root rot caused by *P. nicotianae*, widely known in Tunisia as causative agent of root rot and wilting of chili pepper. Other pepper varieties like ‘SCM334’ [1] or ‘Brutus’ were also described as resistant to this pathogen. However, ‘Creonte’ appears superior to the others since three important traits are observed in the same genotype which are tolerance to thermal and heat stresses previously suggested and resistance to *P. nicotianae*. So, ‘Creonte’ appears a valuable plant material to be used as a rootstock of chili pepper in the context of global warming.

## REFERENCES

- [1] Saadoun M, Allagui MB, 2013, Phytopatol. Medit., 52(1):141-147
- [2] López-Martín J et al., 2013, Sci.Hort., 149:39-46
- [3] Lopez-Martín J et al., 2017, Sci.Hort., 214:9-17

### ACKNOWLEDGEMENTS

Bechir Allagui thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.

	Pnt 367-5	Pnt 367-3	Pnt 369-3	Pnt 378-5	Control
Creonte	0,3 (0-1,5)	0	0,5 (0-3)	0,1 (0-0,5)	0
Baker	5	4,5 (3-5)	5	5	0

**Table 1.** Root necrosis intensity in two pepper cultivars observed 29 days post inoculation with four isolates of *P. nicotianae* (extreme values between parentheses).



**Figure 1.** The two rows in the bottom: healthy seedlings of 'Creonte' that were inoculated with *P. nicotianae* compared to seedlings of Baker destroyed by the pathogen (row on top).

# The development of pepper lines resistant to yellow mite (*Polyphagotarsonemus latus*) and western flower thrips (*Frankliniella occidentalis*)

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**BACKGROUND** Pepper (*Capsicum annuum* L.) is one of the most grown vegetables in the world. Both diseases and pests cause crop losses. Some of these pests are yellow mite and thrips. There are many hosts of different families. *Tomato spotted wilt virus* (TSWV) is a serious plant pathogen affecting a lot of important crop plants. TSWV disease is transmitted and spread by thrips. For this reason, insect resistance has become important. Pesticides used by producers cause serious environmental pollution and health problems, and their cost is very high. The results obtained from the resistance studies against these pests in the world are not enough. In our country, no studies have been carried out in vegetables against these pests. The aim of this study was to determine the reaction/resistance of some genotypes against yellow mite and western flower thrips. In the study, crosses were carried out with the sources of resistance. This study will also provide the optimization of the test method against yellow mite and western flower thrips for the first time in our country.

**MATERIALS & METHODS** Susceptible Serademre 8, a widely grown commercial variety, was used as female parent. PI 152225 resistant to thrips and TSWV and SCM334 were used as male parent [1]. Appearance of parent's plants are given in Figure 1. SCM 334 was found as resistant to yellow mite in our previously work (unpublished data). All the genotypes were homozygous. The susceptible variety was crossed with resistant accessions to obtain F1 hybrids. Resistance or tolerance testing was carried out with insect contamination. Resistance to yellow mite was tested by standard procedure for scoring leaf curl index (LCI) [2]. This method was used for both thrips and mites resistance. [3]. Parents and F1 hybrids were evaluated by using 0-4 scale given in Table 1.

**RESULTS** The study was carried out for the determination of resistant lines to yellow mite and thrips. The study was done as a preliminary study for resistance breeding for pest. The test method has been optimized for the first time in our country. Newly developed pepper inbred lines will be evaluated for open fields and protected cultivation, in pepper breeding programs.

**DISCUSSION & CONCLUSION** Insects cause serious crop loss in plants and transmit diseases. On the other hand, the pesticides used adversely affect the health of people and the environment. Cost is very high due to product loss. Transmitted diseases can be prevented with insect resistance. In the past studies, the sources of resistance were very limited. It is important to search for new sources of resistance to pests and to develop new varieties. Different mechanisms related to insect resistance will be examined in the next studies on this population. Studies to determine new sources of resistance should be continuous.

## REFERENCES

- [1] Maris, et al. 2003. The American Phytopathological Society, vol.93 (1):96-101.
- [2] Niles, GA. 1980. In breeding plant resistance to insects. New York. 1980, 337-369.
- [3] Kurbett, A. et al. 2018. Journal of Entomology and Zoology Studies.2018. 6(3):696-701.



LCI Grade (0-4)	Category	Symptoms
0	Immune (I)	No symptom (No curling, completely healthy plant)
1	Resistant (R)	1-25 per cent leaves/plant show curling, less damage (low curling)
2	Moderately Resistant (MR)	26-50 per cent leaves/ plant show curling, moderately damaged (moderate curling)
3	Susceptible (S)	51-75 per cent leaves/plant show curling, heavily damaged, malformation of growing points and reduction in plant height (heavy curling)
4	Highly Susceptible (HS)	> 76 per cent leaves/ plant show curling, severe and complete destruction of growing points, and drastic reduction in plant height, defoliation and severe malformation (very high curling)

**Table 1.** Standard Procedure for Scoring Leaf Curl Index (LCI)



**Figure 1.** Appearance of parent's plants (Serademre 8, PI152225, SCM334)



# How to transform a genetic construct into an agronomic innovation meeting farmers' needs: A resistant pepper combining *Me1* and *Me3* used as a cover crop to trap root-knot nematodes

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**BACKGROUND** Root-knot nematodes (*Meloidogyne* spp., RKN) are causing increasing economic losses worldwide, particularly in vegetable production. Current agroecological control solutions are not effective enough to control this pest or are difficult to implement in farms. The use of a hybrid pepper in which the *Me1* and *Me3* RKN-resistant (R) genes are pyramided has proved highly effective as a crop used in rotation that strongly decreases the potential for soil infestation [1]. We developed an innovative cropping strategy in which the R-hybrid was used at high density as a dead-end trap cover crop, attracting RKN in the soil and preventing their development. It has several advantages: i/ the two genes are complementary and efficiently trap RKN; ii/ their activity is stable at high temperature and there is a low risk of overcoming their combined resistance; iii/ the use of this hybrid pepper as a cover crop between two cash crops should facilitate its introduction into highly constrained cropping systems (CS).

**MATERIALS & METHODS** Two 4-year trials were carried out under plastic tunnels. In the 1st and 3rd years, the R-pepper seeds were sown on plug trays in May-June, transplanted one month later (density = 12 plants/m<sup>2</sup>), and incorporated 2-3 months later (when 1.20m high) into the soil with a rotavator. Susceptible crops (lettuce/swiss chard in winter; melon in summer) were grown to determine the extent to which the soil had been disinfected. The effects of the pepper trap crop on plant damage and soil infestation were compared with a sorghum cover crop, sown at 50 kg/ha. Buried dry matter was evaluated to determine the R-pepper agronomic value as green manure. Soil colonization by pepper roots was also assessed.

**RESULTS** RKN soil infestation decreased by 80 to 99% after implementation of the R-pepper trap crop, similar to that after the sorghum crop, without the risk of RKN multiplication (which is the case when the sorghum is destroyed too late after one-month cultivation). The subsequent susceptible crops displayed low levels of RKN damage. The gall index (GI) measured on Swiss chard decreased from 2.5 to less than 1 after 4 years. GI were very low on lettuce throughout the trials (< 1). The highest GI (~6) were found on melon in summer; but yield was not affected. Ten weeks after planting 12 plants/m<sup>2</sup>, almost 80% of the soil in the first 30 cm was occupied by at least one pepper root, sufficient for successful RKN trapping. The amount of fresh organic matter incorporated into the soil was similar to that for sorghum green manure (~30 t/ha). 36 % of 28 local vegetable farmers, surveyed for their interest concerning the possible use of the pepper trap crop, found it partially, even completely acceptable. The most interested farmers were those having sufficient labour and available land in summer. Criticisms included higher nursery costs and planting duration compared to sorghum.

**DISCUSSION & CONCLUSION** Overall, this is the first design of a CS using an R-cultivar as a dead-end trap crop for RKN. The main issue now is its adaptation to farms. First, the generation of homozygous genotypes combining the 2 genes, which is currently underway [3], would lower the cost of the seed. Second, nursery practices and density for the pepper crop need to be adapted for cover crop production at a lower price. Finally, adaptation of the CS to farm constraints and searches for alternative strategies, combining plots and the CS at the farm scale, are required for a large agroecological transition.

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# Phenotyping *Capsicum* spp. for luminosity and temperature effects in the field and greenhouse

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**BACKGROUND** Temperature and light intensity are abiotic factors that play an important role in the growth, development and productivity of cultivated plants. High temperatures induce an increase in the photosynthetic rate, however, extreme temperatures can reverse this picture. Light intensity influences vegetative growth and productivity, but also dramatically affects fruit quality, especially on composition and coloring. The use of the foliar spectrometer allows the evaluation of abiotic stresses in plants through the analysis of leaves optical properties that reveal how these extreme climatic conditions influence foliar pigments, especially chlorophyll and carotenoids. This work reports the effect of temperature and luminosity as promoters of abiotic stress in three species of *Capsicum*.

**MATERIALS & METHODS** The experiment was carried out at the Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ-Brazil. Three species of *Capsicum*, *C. chinense* (UENF 2079), *C. baccatum* (UENF 1732) and *C. annuum* (UENF 1381) were cultivated in field and greenhouse. The experimental design was in randomized blocks with three replicates and four plants / plot. Temperature and humidity were measured with a digital thermometer (INSTRUTHERM, Model HT-600) and the brightness using a light sensor (Spectrum® Technologies). In order to quantify the influence of environmental conditions on the plants, a mini portable leaf spectrometer CI-710 (CID, Inc., Camas, Washington, USA) was used for measuring reflectance (R) in the visible and near infrared region at wavelengths between 400 and 1000 nm (spectral range of blue LED light and incandescent lamps).

The following variables were calculated:

- Photochemical Reflectance Index (PRI) =  $(R_{531} - R_{570}) / (R_{531} + R_{570})$  varies from -1 to 1. It informs about xanthophyll's pigments changes,
- Normalized Difference Vegetation Index (NDVI) =  $(R_{800} - R_{680}) / (R_{800} + R_{680})$ . This criteria, based on reflectance in Near Infra red (800 nm) and in red (680 nm) is directly related to the photosynthetic capacity and hence energy absorption of plant canopy. It ranges from -1 to +1,
- Carotenoid Reflectance Index (CRII) =  $(1 / R_{510}) - (1 / R_{550})$  varies from 0 to 15. It provides information about carotenoids and chlorophylls, both stress-related pigments,
- Plant Senescence Reflectance Index (PSRI) =  $(R_{680} - R_{500}) / R_{750}$ , ranges from -1 to 1, and indicates relative changes in chlorophyll and carotenoid contents,
- Water Band Index (WBI) =  $(R_{900} / R_{970})$ , varies from -1 to 1 and tracks changes in relative water content, leaf water potential and stomatal conductance.

These variables were analyzed using ANOVA and the means classified by the Tukey test ( $p < 0.01$ ).

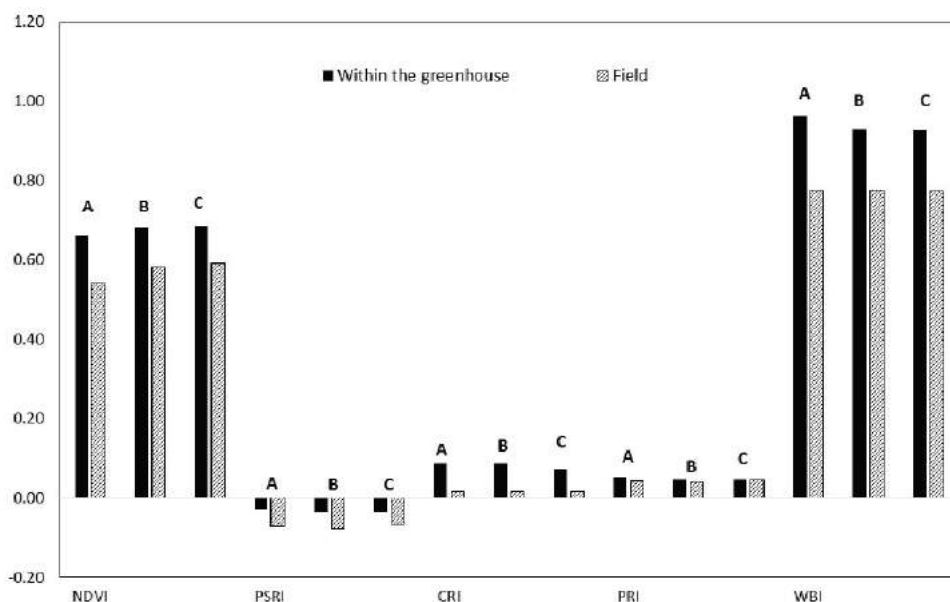
**RESULTS** Under greenhouse conditions, the brightness ( $926 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was 42% lower than in the field ( $2,213 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). However, higher temperature and humidity were observed inside the greenhouse ( $35.2^\circ \text{C} / 46\%$ ), with a variation of  $3^\circ \text{C}$  and 8.5% of humidity between both environments. Difference between species was not significant for the variables tested: this indicates that the species react in the same way when cultivated under the same climatic conditions. However, difference between environments was significant, indicating that plants react differently when subjected to different climatic conditions. Leaf parameters NDVI, PSRI, CRII, and WBI presented higher values in greenhouse. The value of 0.7 obtained for NDVI in greenhouse indicates greater density of green leaves in this environment than in open field.

The negative values of PSRI in greenhouse (-0.03) and in the field (-0.08) indicate that the leaves are not senescent whatever the environment. Low values of CRII mean lower concentration of carotenoids than of chlorophylls, corroborating in this way with low values obtained in the PSRI (higher values would indicate vegetation weakening, since carotenoids protect plants against excessive light harmful effects). The PRI values close to zero in both environments indicate a low chlorophyll/carotenoid ratio. WBI values close to 1 indicate that in both environments leaves water content is high although the lower field value (0.7) indicates that plants loose more water in the field than in greenhouse (Figure 1).

**DISCUSSION & CONCLUSION** Lower values of WBI and NDVI obtained in the field indicate that the plants experienced greater stress in this environment where high temperature together with low humidity lead to a higher transpiration rate than in greenhouse. NDVI field values indicate that the leaves became yellowish, due to light excess [3]. Low value of PSRI in the field indicates that plants are not in a senescence phase. Indeed PSRI detects agronomic problems related to premature loss of green leaf area and productivity capacity. We conclude that the plants grown in the field suffer greater stress than in greenhouse, given higher luminosity and temperature. The mini portable leaf spectrometer CI-710 is a useful tool in the functional investigation of *Capsicum* plants, and the foliar indexes studied were able to infer about the physiological state of the vegetation.

## REFERENCES

- [1] Jeeatid N, Techawongstien S et al., 2017, Horticult. Environment and Biotechnol., 58(2): 103-110, doi: 10.1007/s13580-017-0165-6
- [2] Gitelson A, Solovchenko A, 2018, J. of Photochem. and Photobiol. B: Biology, 178: 537-544, doi: 10.1016/j.jphotobiol.2017.11.023
- [3] Castro, S, Sanchez-Azofeifa A, 2018, Sensors., 18(10): 3302, doi:10.3390/s18103302



**Figure 1.** Reflectance indices in leaves of *C. annuum* (A), *C. Chinense* (B) and *C.baccatum* (C): NDVI - Normalized Difference Vegetation Index; PSRI - Senescence Reflectance Index; CRII - Carotenoid Reflectance Index; PRI - Photochemical Reflectance Index; and WBI - Water Band Index.

# Searching for the genes involved in the dramatic improvement of the percentage of fruit set in the F1 hybrid of *Capsicum chinense*

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**BACKGROUND** In *Capsicum* it is generally thought that inbreeding depression does not occur. We cultivated 'Sy-2' and 'No. 3686' which are *Capsicum chinense* inbred cultivars and 'Sy-2' × 'No. 3686' which is the F1 hybrid of these cultivars. In fact, there was no difference in their plant growth. On the other hand, in the high temperature period, 'Sy-2' and 'No. 3686' seldom set fruits, although F1 set a lot of fruits. It was hypothesized that F1 might have improved fruit set in the high temperature due to accumulation of multiple loci. It was thought to be important to improve the poor performance of fruit-set under the high temperature, thus, we investigated the mechanism. Previously, we have reported that the percentage of fruit set in F1 is higher at high temperatures than 'Sy-2' and 'No. 3686', and improvement of fruit-set involves both male and female fertility [1]. In this study, we searched for genes involved in fruit set using F2 lines obtained by self-fertilizing F1.

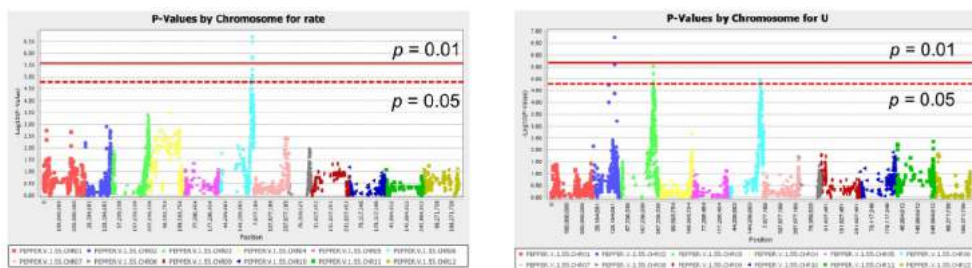
**MATERIALS & METHODS** 161 F2 lines obtained from self-fertilization of 'Sy-2' × 'No. 3686' (F1) were cultivated in a plastic house. From 1st to 31st August 2017, the number of flowers and of fruits of 156 F2 lines were counted and the percentages of fruit set were calculated. We also investigated pollen germination rates on 9th August. DNA was extracted from the young leaves of each F2 line and tested for ddRAD-seq analysis. DNA was sequenced in paired-end using Illumina HiSeq 4000. Mapped to the *C. annuum* reference genome Pepper v.1.55 [2], 3781 SNPs were extracted. We calculated GLM correlation between the percentages of fruit set or pollen germination rates and each SNP.

**RESULTS** In the F2 population, the percentage of fruit set, and the pollen germination rate were quantitatively segregated. ddRAD-seq analysis detected a gene region with high correlation with the percentages of fruit set under high temperature on chromosome 6. In addition, although there was no significant correlation, a gene region on chromosome 3 had relatively high correlation (Figure 1A). Also, gene regions significantly correlated with pollen germination rates at high temperature were detected on chromosome 3 and 6 (Figure 1B). The same or nearby SNPs correlated with the percentage of fruit set and the pollen germination rate. The genotypes of each F2 line were classified in each candidate gene region and it was found that the F2 lines having the No. 3686 allele for the chromosome 3 and the Sy-2 allele at the chromosome 6 had highest pollen germination rate under the high temperature period (Figure 2). It was suggested that the two gene regions have additive effects for the pollen germination rate in *Capsicum chinense*.

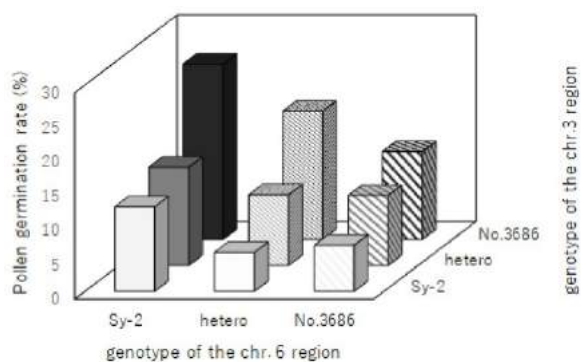
**DISCUSSION & CONCLUSION** We suggested that some gene could be involved in the improvement of fruit set, and that varieties which improve the fruit yield under high temperature could be made, even for inbred cultivars. Accumulation of genes involved in reproductive ability under high temperature was overlooked in *Capsicum*. Moreover, two same gene regions on chromosome 3 and 6 control the pollen germination rate and the percentage of fruit set under high temperature period, thus we concluded that we can search genes involved in fruit-set under high temperature using the pollen germination rate.

## REFERENCES

- [1] Yamazaki A, Hosokawa M, 2019, Sci. Hortic, 243: 421-427
- [2] Kim S et al., 2014, Nat. Gen. 46: 270-278



**Figure 1.** Manhattan plot of correlation of **(A)** the percentages of fruit set, or; **(B)** pollen germination rates and SNPs under the high temperature period.



**Figure 2.** Pollen germination rate under the high temperature period for each classification when the F<sub>2</sub> lines are classified according to the genotype of candidate gene regions of chromosomes 3 and 6.

# Novel sources of resistance to *Pepper leaf curl virus (Begomovirus)*

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**BACKGROUND** Pepper is an increasingly important vegetable and spice crop. Likely, the most devastating pepper-infecting viruses, especially in tropical and subtropical regions, are members of the whitefly transmitted *Begomovirus*, which cause *Pepper yellow leaf curl virus* (PepYLCV). Management of PepYLCV has been based primarily on insecticides against the whitefly vector. However, the use of insecticides has been found to be only partially effective, costly for producers, and represents a hazard to farmers and the environment. Generally, farmers apply the insecticides too late in response to seeing the early symptoms of disease by which time the whiteflies have already spread the virus to other plants [1]. An effective PepYLCV management strategy is the development of resistant cultivars. Genetic recombination, acquisition of extra DNA components, and synergistic interactions among different begomoviruses have resulted in the rapid emergence of new viruses that can infect new hosts, cause new disease symptoms, and overcome host resistance [2]. New sources of resistance to PepYLCV are required for effective breeding.

**MATERIALS & METHODS** For this project, 100 *Capsicum* accessions comprising breeding lines, open pollinated varieties, genebank accessions and wild species were screened for resistance to PepYLCV. The experimental design was a randomized complete block design with three replications and 10 plants in each replication and was conducted in field net-houses at two locations (Khon Kaen and Kamphaeng Saen, Thailand) using augmented inoculation by viruliferous whiteflies. Scoring was done at ~60, 90, 120, and 150 days after inoculation using a standardized 6-point scale (0 = no symptoms to 5 = very severe symptoms) and the average of the scores of 10 plants within each replication was used for analysis.

**RESULTS** While no accession was identified as being immune to the disease, several accessions were found to be highly resistant at both locations, with accession PP99 being the most resistant. The accessions PP1037-7644-1, PBC148, PBC149, PBC502, PBC518, and PBC601 were also identified as being highly resistant at both locations. The accessions including PI159236, VI012911, VI012528, and VI012642 were identified as being very susceptible, with high levels of symptoms occurring only 60 days after inoculation. Overall, the disease severity at the Khon Kaen location was greater compared to Kamphaeng Saen, highlighting the importance of multi-location testing for disease resistance. These resistant accessions can be used to study gene action and to move resistance genes into well adapted germplasm.

**DISCUSSION & CONCLUSION** In this project, we identified novel sources of resistance to PepYLCV, one of the most devastating pathogens. This work provides a basis for future research in the areas of plant breeding and plant pathology.

## REFERENCES

- [1] Kenyon L, Kumar S, et al., 2014. Virus diseases of peppers (*Capsicum* spp.) and their control. In: G. Loebeinstein and N. Katis (eds.). *Adv Virus Res* 90: 297-254.
- [2] Singh AK, Kushwaha N, et al., 2016. Synergistic interaction among begomoviruses leads to suppression of host defense-related gene expression and breakdown of resistance in chilli. *App Microbiol Biotech* 100:4035-4049.





# Effect of pesticide use on biochemical quality of the hybrid variety Vernal FI of *Solanum melongena* L. grown in the South-East region of Algeria

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**BACKGROUND** To protect crops, farmers use a variety of control methods. Among these, chemical control hold important role in conventional agriculture. But the intensive use of pesticides and their bad use can have serious consequences on the different components of the environment (soil, water, plant). Many pesticides currently in use tend to persist for a long time in fruits and vegetables as residues (Köhler and Triebskorn, 2013[1]). For all these reasons, this study aimed to verify the effects of three pesticides on the biochemical quality and in particular on compounds of primary metabolism and compounds of secondary metabolism of *Solanum melongena* L. Vernal FI hybrid variety grown in southeastern Algeria. To do this, these compounds were highlighted after a chemical screening of the cortex of *Solanum melongena* L. The dosage of the various components were then performed by standard spectrophotometric techniques from the methanolic and aqueous cortical extracts.

**MATERIALS & METHODS** The eggplants were obtained from the laboratory at a temperature of 24°C, and at a discontinuous light (12h / 12h). The pot containing a mixture of soil and compost (2/3: 1/3) have undergone treatments by spray of three fungicides (Azoxystrobin, Difeconazole, Cymoxanil). Primary and secondary metabolic products were evidenced by qualitative characterization reactions. These reactions are based on precipitation or coloring phenomena. These tests are carried out according to the technical techniques of Hargeman and al., 2000 [2] and Bekro et al., 2007 [3]. Dosage of the compounds in question such as carbohydrates, proteins, total polyphenols, total flavonoids, tannins, flavanols and flavonols were determined by spectrophotometric techniques from the methanolic and aqueous leaf extracts using specific reagents: gallic acid, rutin, catechin, and quercetin. They were then calculated from the calibration curves.

**RESULTS** The chemical screening revealed the presence of proteins, carbohydrates and six major families of secondary metabolites (alkaloids, flavonoids, tannins, saponosides, anthocyanins, terpenes and sterols) and the absence of other families such as cardenolides or leucoanthocyanins. The results expressed in milligram equivalents per gram of dry vegetable material reveal that the colorimetric assays of the various families of the secondary metabolites allowed us to record high levels rates between 228,582 and 336,756 mg / g dry matter for total polyphenols and from 0.958 to 17.056g mg / g dry matter for specific compounds such as flavonoids, tannins, flavanols and flavonols metabolites. These levels are, for the most part, greater than those of control plants not treated with pesticides. A similarity is recorded by comparison with previous work done on the whole fruit. In this same context, our results are completely different from those of other authors obtained from aqueous and methanolic extracts obtained of bark and fruits eggplant using other solvents of increasing polarity.

**DISCUSSION & CONCLUSION** The biochemical quality of the treated plants is not very affected by pesticides and in particular polyphenols; the rates are higher than those of the controls. In contrast, carbohydrate and total protein levels are slightly lower than those of controls. Maintained rates of secondary metabolites seem to affirm that the metabolic functions of the plant are not directly targeted by pesticides. Moreover, the plant seems to resist the effect of different pesticide molecules by the synthesis of these secondary metabolites having a determining role in the phenomena of defences against toxic products.

## REFERENCES

- [1] Köhler HR and Triebkorn R, 2013. Science 341, 759 (2013); DOI:10.1126/science.1237591
- [2] Hagerman AE et al., 2000. Joint FAO/IAEA Division of Nuclear Techniques in Food and agriculture, Vienna, 26p.
- [3] Bekro YA et al., 2007. Rev. Sci. Nat., 4, pp. 217-225

# Management of bacterial spot of pepper with genetic resistance and race dynamics of *Xanthomonas euvesicatoria*

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**BACKGROUND** Peppers (*Capsicum annuum*) are locally grown for capsaicin oleoresin extraction from super-hot chilies and for fresh market production of bell, jalapeno, and chili types. Bacterial spot, caused by *Xanthomonas euvesicatoria*, is the primary foliar disease of peppers in the region. The super-hot cultivars currently lack bacterial spot resistance, but hypersensitive response (HR) resistance from major genes is available in fresh market hybrids. The objectives were to evaluate types of HR resistance for control of bacterial spot and to assess impacts on pathogen race dynamics.

**MATERIALS & METHODS** Bell and jalapeno hybrids with the resistance gene *Bs2*, which confers resistance to races 1-3 of *X. euvesicatoria* and a bell hybrid with resistance genes *Bs1-2-3* which confer resistance to races 1-5 and 7-9 were grown in comparison to susceptible bell and jalapeno hybrids, and the super-hot chili cultivar Ocala in 2017 and 2018. Plots were inoculated in 2017 with a mixture of prevailing local races 1 (*AvrBs2-3-4*), 3 (*AvrBs2-4*), and 8 (*AvrBs2*). The genotypes received a full-season bactericide program (copper hydroxide + mancozeb) beginning in June, or were left untreated in a split-plot experimental design. Disease incidence (% symptomatic leaves) and defoliation were assessed in July and August, and yield was determined from June through August each year. In October 2018, diseased leaves were sampled from susceptible, *Bs2*, and *Bs1-2-3* hybrids and resulting isolates were race-typed on *Bs1* to *Bs4* differential cultivars [1].

**RESULTS** Bacterial spot pressure was moderate in 2017 reaching 40% disease incidence. In 2018, the disease was severe by the time the bactericide program was initiated and plots were not inoculated. The disease reached 80% disease incidence and nearly 70% defoliation on susceptible genotypes in 2018. The bactericide program was most effective early in the season when disease incidence was reduced up to 30%. By August, the effect of the spray program was significant ( $P=0.05$ ), but reductions in disease levels were generally less than 10%. Resistant genotypes were nearly disease free in 2017 and in July 2018. However in August 2018, bacterial spot incidence increased to over 50% in the *Bs2* hybrids, but remained near zero in the *Bs1-2-3* hybrid. Sub-plot yields were negatively correlated ( $P<0.01$ ) with bacterial spot incidence in July ( $r=-0.71$ ) and August ( $r=-0.40$ ), and with defoliation in July ( $r=-0.68$ ) and August ( $r=-0.57$ ). Despite the breakdown of *Bs2* resistance in 2018, yields of the resistant bell and jalapeno hybrids were 45% and 55% greater than respective susceptible hybrids. The bactericide program did not increase hybrid yields or plant fresh weight of the super-hot chili. Avirulence alleles and race of an isolate were dependent on the source plant genetics. *AvrBs2* was expressed in 86% of isolates from the susceptible hybrid which were predominately race 1 (*AvrBs2-3-4*) and race 7 (*AvrBs2-3*). *AvrBs2* was not expressed in any isolates from the *Bs2* hybrids, which were mostly race 4 (*AvrBs3-4*). Isolates from the *Bs1-2-3* hybrid were all race 6 (*AvrBs4*).

**DISCUSSION & CONCLUSION** HR resistance was effective in reducing yield loss to bacterial spot. However, a rapid breakdown of *Bs2* resistance was observed during the second year of deployment accompanied by the evolution of new races. The hybrid with pyramided resistance genes remained nearly disease free, but yielded race 6 isolates from lesions developing after the trial was terminated. The low disease on the *Bs1-2-3* hybrid may be from a residual effect of defeated genes [2]. While the HR resistances effectively protected yield, their deployment may not be sustainable without cultural practices such as crop rotation, sanitation, etc. that limit pathogen survival and disease carry-over to subsequent crops.

## REFERENCES

- [1] Stall RE et al., 2009, Annu Rev Phytopathol, 47:265-284
- [2] Kousic CS et al., 1999, Phytopathology, 89 (11) 1066-1072

# KASP assay for selection of *Tomato spotted wilt virus* (TSWV) resistance in pepper breeding

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**BACKGROUND** *Tomato spotted wilt virus* (TSWV) is one of the most important viruses that cause yield losses in pepper. Host plant resistance is the most efficient strategy to control TSWV. In pepper, the *Tsw* gene controls resistance to TSWV and has been widely used in pepper breeding [1]. In pepper, the screening of resistance has been facilitated by early selection tests using virus inoculation of plants in breeding process. However, the selection for TSWV resistance by bioassay has several drawbacks. For these reasons, molecular markers linked to the *Tsw* locus would be useful for breeders. Molecular markers are widely used for screening resistant genes in plant breeding. Kompetitive Allele-Specific PCR (KASP) is a novel molecular technique based on single nucleotide polymorphisms (SNPs) [2]. KASP markers have been developed for marker-assisted selection in crops [3].

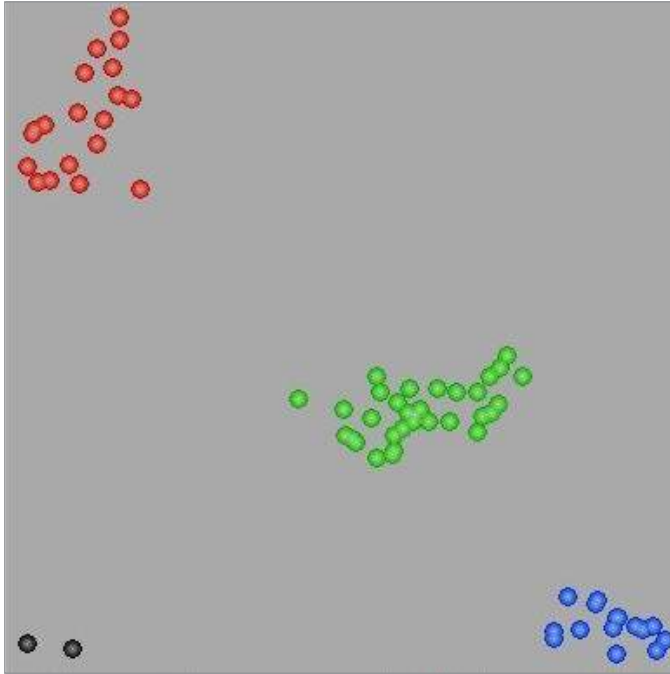
**MATERIALS & METHODS** Pepper plants were provided by the Yüksel Tohum and Multi Tohum companies. Pepper seedlings were inoculated with a TSWV isolate (Pathotype P0); after 3 weeks the plants were evaluated for resistance by symptoms and confirmed by DAS-ELISA. Genomic DNA was extracted from young leaves using a Wizard Magnetic Kit (Promega) according to the manufacturer's instructions. The *Tsw* KASP marker was used for screening in pepper breeding programs.

**RESULTS** The *Tsw* gene-specific KASP assay successfully distinguished between genotypes that were both homozygous-heterozygous resistant and susceptible to TSWV (Fig. 1). The KASP assay results were in accordance with the biological assay in all genotypes tested. Commercial pepper varieties carrying the *Tsw* gene were developed after further breeding.

**DISCUSSION & CONCLUSION** Molecular markers are widely used for screening in pepper breeding. Molecular markers tightly linked to the *Tsw* gene are highly desirable for fast and correct screening of resistance to TSWV in pepper. The *Tsw* KASP assay provided rapid and correct analysis for many pepper genotypes in a short time. This study showed a user-friendly KASP assay for screening of *Tsw* gene in pepper. The assay could be used for pyramiding of resistance genes in pepper.

## REFERENCES

- [1] Moury B et al., 2000, Genome 43: 137-142.
- [2] Semagn K et al., 2014, Mol Breeding 33:1-14
- [3] Devran et al., 2016, Horticulture, Environment, and Biotechnology 57:156-160.



**Figure 1.** Genotypic data from the Kasp Tsw assays. The X-axis indicates homozygous plants (blue) and the Y-axis indicates susceptible plants (red). Individuals clustered at the center (green) are heterozygotes. The black dots in the lower left indicate a water control.

# NSs gene sequence variability of resistance breaking TSWV pepper isolates from Hungary

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**BACKGROUND** In the past 25 years, the thrips transmitted *Tomato spotted wilt orthotospovirus* (TSWV) became the most important pathogen affecting forced pepper (*Capsicum annuum* L.) in Hungary. New cultivars carrying the *Tsw* resistance gene restrict the spread of the resistance-inducing P0 strains, although their fruits are often damaged, showing melanotic ringspots [1]. Besides the P0 pathotype, the resistant cultivars promoted the appearance of resistance-breaking (PI) strains. A previous study [2] demonstrated that Hungarian TSWV-PI strains have limited variation at the amino acid level in the viral NSs protein responsible for breaking the resistance and they concluded that the PI strains had most likely evolved from the local P0 strains. We have recently studied two PI strains, Cs and YR, collected in Hungary. Here we demonstrate that, based on the sequence data of their NSs gene, these isolates are more closely related to Italian and Asian isolates, than Hungarian ones.

**MATERIALS & METHODS** Diseased fruits of TSWV resistant peppers were collected at Csengőd (Cs) and Budakeszi (YR), Hungary. Viruses were sap transmitted to test plants, such as *Capsicum annuum* L. (genotype *Tsw/tsw*), *Nicotiana benthamiana*, *N. clevelandii* and *N. tabacum* cv. Xanthi-nc. Total RNA was extracted from the symptomatic tissues of the original pepper fruits (Fig.1) and also from *N. benthamiana*. For cDNA synthesis and PCR amplification of the NSs gene, we used the primers described earlier [2]. The sequence of the NSs proteins of PI isolates were compared with sequences available at the NCBI GenBank.

**RESULTS** Test plants inoculated with the extracts prepared from the symptomatic areas of diseased pepper fruits showed the local and systemic symptoms characteristic of mechanically transmitted viruses. Using TSWV NSs gene-specific primers, cDNA products of the expected size (1404 bp) were amplified, cloned and sequenced from the original pepper fruits and from *N. benthamiana* infected by isolates Cs and YR, respectively. Sequence similarity analysis demonstrated that both amplicons have 96-99 % sequence identity at the nucleotide and amino acid level with the NSs gene and protein of different TSWV strains. The NSs protein of isolate Cs showed the highest identity (99%) with an Italian TSWV isolate from pepper (ABD38688), while the same protein of isolate YR showed 99% identity with Chinese and Korean pepper TSWV isolates (ARX17066, AGM53739) (Fig. 2).

**DISCUSSION & CONCLUSION** *Nicotiana* plants inoculated with fruit extracts showed symptoms characteristic of TSWV and were devoid of other known pepper viruses, e.g. tobamoviruses or cucumber mosaic virus. It is worth noting that both Cs and YR isolates originated from TSWV resistant peppers produced at distant regions in Hungary. Accordingly, pathological tests proved that both isolates belong to the resistance-breaking strain. Molecular data revealed that they differed from each other and from PI strains isolated previously in Hungary [2]. The high similarity of Cs to the Italian PI05 and of YR to the Asian YN65 and TSWV7 suggests that they arose from abroad instead of having evolved locally.

## REFERENCES

- [1] Salamon P et al., 2016. Proc. XVIth Eucarpia Capsicum and Eggplant Working Group Meeting, 12-14 Sept. 2016, Kecskemét, Hungary, pp. 69-75.
- [2] Almási A et al., 2015. Virus Genes. 50: 71–78.

## ACKNOWLEDGEMENTS

This work was supported by the Hungarian National Research Fund/National Research, Development and Innovation Office grants OTKA- K-111935.



**Figure 1.** Symptoms on pepper fruits naturally infected with the isolate Cs.

	13	40	53	64	79	92	104	107	122	130	137	172	174	198
HUP1-AJA71431	R	P	K	N	I	D	A	T	D	T	K	I	T	S
HUP2-AJA71432	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HUP3-AJA71433	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NSs Cs	.	Q	.	D	.	.	T	A	A	I	T	.	M	.
P105-ABD38688	.	Q	.	D	.	.	T	A	A	I	T	.	M	.
NSs YR	K	.	R	.	T	N	T	.	A	.	I	V	M	C
YN65-ARX17066	K	.	.	.	N	T	.	A	.	T	V	M	C	.
TSWV7-AGM53739	K	.	.	.	N	T	.	A	.	T	V	M	C	.

	202	226	246	248	260	287	288	297	304	309	310	330	356
HUP1-AJA71431	S	E	K	S	V	Q	N	S	L	C	K	Y	N
HUP2-AJA71432	.	.	.	.	.	H	.	.	.	.	.	H	S
HUP3-AJA71433	.	.	.	.	.	H	.	.	.	.	.	H	.
NSs Cs	N	.	R	.	I	Y	S	T	.	F	.	H	.
P105-ABD38688	N	.	R	.	I	Y	.	T	.	F	.	H	.
NSs YR	N	D	.	.	I	H	.	.	I	.	Q	H	.
YN65-ARX17066	N	.	.	N	I	H	.	.	.	.	Q	H	.
TSWV7-AGM53739	N	.	.	.	I	H	.	.	I	.	Q	H	.

	369	381	387	389	390	436	437	450	459	462	463
HUP1-AJA71431	Y	R	E	I	C	P	V	R	S	Y	A
HUP2-AJA71432	.	.	.	.	.	.	.	.	.	.	.
HUP3-AJA71433	.	.	.	.	.	.	.	.	.	.	.
NSs Cs	H	K	.	A	Y	S	I	G	K	H	.
P105-ABD38688	H	K	.	A	Y	S	I	G	K	H	.
NSs YR	.	.	K	.	.	S	A	G	.	.	.
YN65-ARX17066	.	.	.	.	.	S	.	G	.	.	.
TSWV7-AGM53739	.	.	.	.	.	S	.	G	.	.	D

**Figure 2.** Differences in amino acid sequences of the NSs protein of Cs,YR and selected TSWV isolates deposited in GeneBank.



# Deciphering the genetic basis of tolerance to *Cucumber mosaic virus* in pepper

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**BACKGROUND** The use of resistant plant cultivars is an efficient, cost-effective and environmentally-friendly method of disease control, particularly against viral pathogens. However, resistance is subject to breakdown, through the often rapid adaptation of viral populations to newly deployed resistant genotypes. Plant defense against parasites can be divided into two distinct components: mechanisms that reduce parasite accumulation (resistance *sensu stricto*) and mechanisms that reduce the negative impact of infection on host fitness, health or yield without impacting parasite concentrations (tolerance). As it is expected to exert a weaker selection pressure on parasite populations, plant tolerance to pathogens appears as an interesting alternative to resistance *s.s.* for sustainable disease management. However, little is known about the genetic determinants controlling tolerance to parasites. We have chosen the interaction between pepper and *Cucumber mosaic virus* (CMV) as a model to study plant tolerance to viral pathogens.

**MATERIALS & METHODS** We performed a screen using a pepper doubled haploid (DH) mapping population [1] to map CMV tolerance and resistance QTLs. Both virus titer and plant health were simultaneously evaluated for each DH line. Virus accumulation was quantified using serological methods (semi-quantitative DAS-ELISA). The impact of infection on plant health was measured using different methods. These methods included calculating the AUDPC (Area Under the Disease Progress Curve) index, which combines time of symptom emergence and symptom intensity, measuring plant growth parameters, such as the reduction in fresh weight of infected plants compared to mock-inoculated plants, and measuring leaf chlorophyll content.

**RESULTS** Our screening efforts have shown that plant health and virus load are not correlated and allowed the identification of lines displaying contrasted levels of tolerance and resistance to CMV. Tolerance levels were measured as in [2] using the slope of the linear regression line between plant health and virus load. Based on the comparison of tolerance values calculated for 9 DH lines included in two independent screening rounds, broad sense heritability for this trait was estimated at  $h^2=0.74$ . Mapping efforts are underway to detect QTLs controlling tolerance or resistance *s.s.*

**DISCUSSION & CONCLUSION** Our screen has shown that tolerance is a highly heritable trait in the DH mapping population. Mapping of resistance and tolerance QTLs will allow us to compare the genetic architecture of these two defense mechanisms. Further efforts will aim at confirming tolerance and resistance levels of a small subset of DH lines with contrasted levels of resistance and tolerance to CMV, and testing their response to a set of isolates representative of CMV diversity. Experimental evolution assays using these DH lines should indicate whether tolerance is evolutionarily more stable than resistance *s.s.* and whether breeding tolerant crops may contribute to sustainable control of plant viruses.

## REFERENCES

- [1] Quenouille J *et al.*, 2014, *Heredity*, 112:579–587. doi: 10.1038/hdy.2013.138.
- [2] Råberg L *et al.*, 2009, *Phil. Trans. R. Soc. B*, 364: 37–49. doi: 10.1098/rstb.2008.0184.



# Screening of resistance to bacterial wilt (*Ralstonia solanacearum*) in 346 accessions of pepper germplasm

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**BACKGROUND** Bacterial wilt is a disease caused by *Ralstonia solanacearum*, and is widely distributed in the tropical, sub-tropical and warm temperate region of the world (Hayward, 1991). It is a major constraints of solanaceous crops; peppers, tomatoes, eggplants and potatoes. The pathogen invades plants through the roots, rapidly reaches the xylem vessels and then, spreads to aerial parts through the vascular system. It has been proved to be difficult to control due to its broad host range. Breeding resistant cultivars and grafting plants using resistant rootstock are the strategies to solve this problem (Wang et al, 1998). Though pepper varieties are resistant to wilt, the resistance often breaks in other growing areas due to different races or biovars of the pathogen or inadequacy of the resistance under environmental conditions favorable to the pathogen. This study aim to evaluate the resistance of 364 pepper germplasm to bacterial wilt caused by *R. solanacearum* and select resistant genetic resources to control this devastating disease.

**MATERIALS & METHODS** Resistance of pepper against *R. solanacearum* WR-I strain was assessed in 364 germplasm conserved in National Agrobiodiversity Center, RDA. Pepper cultivars 'Thankyou' and germplasm 'MC4' were used as resistant control. 'Manitta' was used as susceptible control. The roots of 10 seedlings per accession at four fully expanded leaves stage were inoculated with a 10ml bacterial suspension (108 CFU/ml) after blade wounding. Resistance traits were determined as disease index of plants in growth chamber setting at 28°C. Every 7 days for four weeks, each plant was evaluated visually for occurrence of bacterial wilt ranging from 0 (no symptom) to 4 (most wilted).

**RESULTS** In green house, where the temperature was not controlled, "Muhanjilju" and "Mutjinsanai" were evaluated as moderate resistant. But in growth chamber, where the temperature was constant and symptoms were the most severe, "Muhanjilju" and "Mutjinsanai" were evaluated as susceptible. Four weeks after inoculation, 39 accessions were showed as resistant as "Thankyou" to *R. solacearum* WR-I. With strong and moderated resistant germplasm, we performed iterative analysis in growth chamber and selected 4 resistant accessions. IT207293 (*Capsicum baccatum*), IT236750 (*C. galapagoense*), IT264141 (*C. chinense*), and IT305510 (*C. chinense*) showed resistance with lower 1.4 disease index than the resistant control. Forty eight accessions including IT183656 were assessed as moderate resistant with disease index between 1.4~2.0. The remaining 312 accessions were susceptible with a disease index over 2.0.

**DISCUSSION & CONCLUSION** We have evaluated the resistance of 364 pepper germplasm to bacterial wilt caused by *R. solanacearum* WR-I strain and select resistant genetic resources to control this devastating disease. IT207293, IT236750, IT264141, and IT305510 can be used as wilt resistant source in pepper breeding and further study is recommended to identify the genes of wilt resistance using molecular markers. These selected four genetic resources including IT207293 will be used as a resource for breeding or molecular markers linked to QTLs resistant to bacterial wilt in pepper plants.

## REFERENCES

- [1] Hayward AC, 1991, Annu. Rev. Phytopathol., 29:65-87
- [2] Wang et al, 1998, Springer-Verlag, Berlin

Acc. No.	Temporary number	Scientific name	Accession name	Origin	Disease index <sup>z</sup>
IT207293	709415	<i>Capsicum baccatum</i>	Aji verde	BOL	1.2±0.1
IT236750	K057566	<i>Capsicum galapagoense</i>	GRIF 1567	USA	1.4±0.2
IT264141	K157716	<i>Capsicum chinense</i>	C04745	BOL	0.9±0.2
IT305510	K161324	<i>Capsicum chinense</i>	Aji dulce	BOL	1.0±0.3

**Table 1.** Disease index and passport of selected resistant pepper germplasm.

Each value represents the mean ± standard error of two runs (growth chamber and green house) with 5 replications.



**Figure 1.** Morphological shapes of four accessions which were resistant (R) to the isolate of *Ralstonia solanacearum*, WR-I.

# A decade of studies in France to decipher the genetic/molecular basis of eggplant resistance to bacterial wilt

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**BACKGROUND** Bacterial wilt (BW) is caused by strains of the *Ralstonia solanacearum* species complex (RSSC) and widespread in the world. RSSC strains are phytopathogenic bacteria of primary importance in the world, due to their global distribution, broad-spectrum of host plants and the severity of attacks on food crops of major economic interest such as tomato, eggplant, and pepper. The varietal resistance is a major method for a sustainable control of BW. However, this resistance is unstable because of strong interaction with the huge genetic diversity of RSSC, which is structured into three species and four phylogenetic groups: phylotypes I, II, III, and IV. During the last decade, several sources of resistance to BW have been identified in eggplant accessions by CIRAD-INRA.

**MATERIALS & METHODS** A core-collection of eggplant lines was challenged with a core-collection of BW strains characterized by molecular genotyping techniques and representing the phylogenetic diversity of RSSC (Table 1). The GBS method was used for QTL mapping studies on RILs and DH populations from crosses between resistant and susceptible lines. Anchoring genetic maps on the physical maps of eggplant and tomato and RNAseq data from the parental lines identified candidate R-genes [1]. Functional genetics with RSSC mutants highlighted an avirulence gene in bacteria [2]. Breeder-friendly markers were developed in the region of the major gene for both fine mapping and marker-assisted selection (MAS).

**RESULTS** A total of six phenotypes ranging from highly resistant to highly susceptible were recorded when testing the interactions between eggplant lines and BW strains representing three out of the four phylotypes (Table 1). The MT035 haplotype of phylotype I, recently found prevalent in South Western Indian Ocean islands, was represented in Réunion by the aggressive strain RUN 3012 (I-31) that was observed throughout the vegetable production area up to 1200 m altitude [3]. The dissection of the resistance in accession E6 (AG91-25) revealed the presence of a major locus, *EBWR9*, controlling three strains of phylotype I, and two QTLs that revealed partially effective against strains of phylotypes I, IIA and III (Figure 1). Five candidates R-genes, polymorphic between parents, were found in the *EBWR9* genomic region, four of them being orthologs and in a syntenic position among eggplant, tomato, and potato. Studies with mutants of strains GM11000 and PSS4 demonstrated that RipAX2 type III effector of *R. pseudosolanacearum* was necessary to trigger resistance in AG91-25 eggplants, suggesting an R/Avr- type interaction [2]. We saturated the region of *EBWR9* with molecular markers for fine mapping and facilitating its transfer in commercial cultivars.

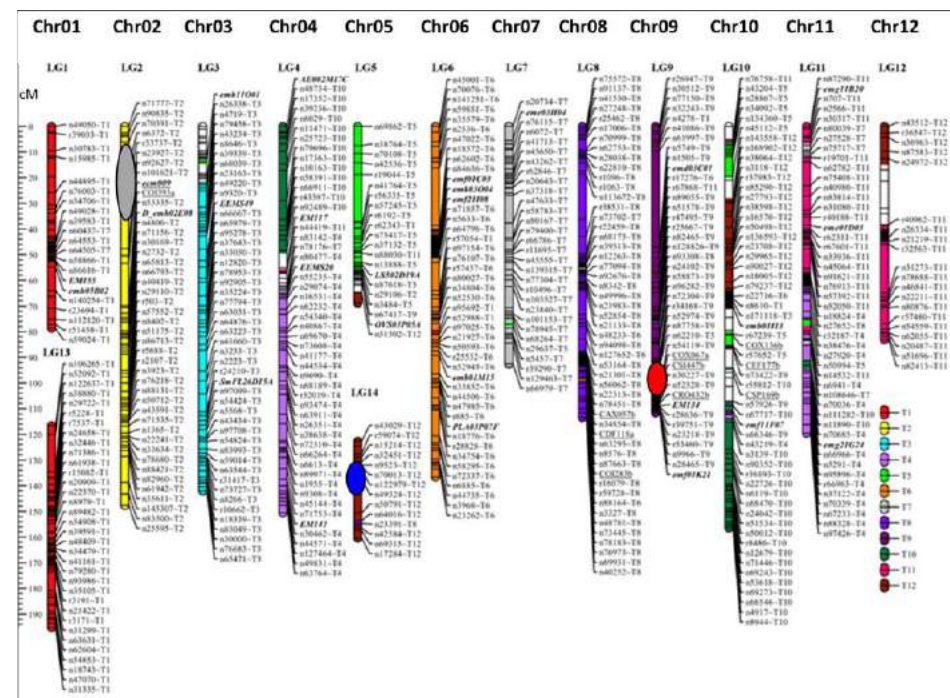
**DISCUSSION & CONCLUSION** The eggplant AG91-25 carrying *EBWR9* major gene recognizes the avirulence gene *RipAX2* to trigger resistance to BW. This R/Avr-type interaction, which is not conserved within a same phylotype, should be highly strain-specific. The identification of the *EBWR9* gene by functional genetics is necessary to understand this interaction, to search for orthologs in the genetic diversity of tomato and potato, and to assist breeding using molecular markers closely linked to the gene. Identification of complementary QTLs controlling resistance to other bacterial strains is ongoing in order to set up innovative breeding pyramiding strategies for the creation of wide-spectrum resistances controlling the disease in large production areas.

## REFERENCES

- [1] Salgon et al., 2017, *Frontiers In Plant Science*, 8. doi: 10.3389/fpls.2017.00828  
 [2] Morel et al., 2018, *Molecular Plant Pathology*, 19(11). doi:10.1111/mpp.12724  
 [3] Yahiaoui et al., 2017, *Frontiers In Plant Science*, 8. doi:10.3389/fpls.2017.02139

Strain Classification	CLIMATIC CHAMBERS ASSAYS										GREENHOUSE ASSAYS			
	CMR134	PSS366	PSS4	PSS358	GM1000	CFBP3959	CMR32	CMR15	CFBP2957	CMR39	CMR34	CFBP1873	RUN1012	RUN4509
	I-13	I-15	I-15	I-15	I-18	II-23	II-29	II-29	II-36	II-41	II-1	II-4NPB	I-31	II-1
HOT SEASON (La Reunion)														
COOL SEASON (La Reunion)														
E1	1	2	2	2	1	1	1	2	1	1	1	4	3.1	2
E2	1	4	4	2	1	1	1	1	1	1	3.2	1	2	3.2
E3	1	4	2	4	2	1	1	2	1	1	2	4	2	2
E4	1	1	2	1	1	1	1	4	1	2	2	2	2	1
E5	3.1	4	4	4	2	2	1	4	1	2	3.2	2	4	4
E6	2	1	5	2	3.1	4	1	5	2	1	5	4	2	3.1
E7	4	4	5	4	3.2	5	1	4	1	3.1	3.1	3.1	4	1
E8	4	4	5	5	5	5	3.1	5	4	4	5	4	5	4
E9	2	2	2	2	3.2	1	1	4	1	1	1	4	2	1
E10	5	4	5	5	4	5	4	5	2	4	5	5	4	4

**Table 1.** Eggplant accessions rank from highly resistant in dark green (1) to highly susceptible in red (5) when inoculated with representative RSSC strains.



**Figure 1.3** genetic factors of resistance in AG91-25 (E6) eggplant line  
 Grey, blue and red circles represent QTLs conferring resistance to phylotypes I, IIA and III strains (phenotypic variance = 13 to 38%); QTLs conferring resistance to phylotypes IIA and III strains (phenotypic variance = 17 to 45%) and EBWR9 major resistance gene conferring very high level of resistance to phylotype I strains.

# Response of pepper genotypes to resistance-breaking isolates of TSWV

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**BACKGROUND** Pepper is one of the most widely grown and economically important vegetables in the world. *Tomato spotted wilt virus* (TSWV) causes considerable losses in pepper, infecting over 1000 plant species. Control of TSWV is difficult because of its transmissibility by thrips species. Plant resistance is one of the most effective methods to control TSWV. The single dominant gene *Tsw* originating from *Capsicum chinense* confers resistance to the virus [1] [2] and has been introgressed into cultivated pepper varieties. However it has been reported that *Tsw* based resistance can be overcome by resistance-breaking (RB) isolates of TSWV [3] and RB isolates have been noted in several countries. In order to breed resistant varieties against RB isolates of the virus, sources of new resistance are needed.

**MATERIALS & METHODS** In total, 88 pepper genotypes including cultivated and semi-cultivated forms of different pod-types and 51 wild accessions of *Capsicum* species (*C. frutescens*, *C. chinense*, *C. baccatum*, *C. pubescens*, and *C. chacoense*) were tested in this study. The pepper genotypes were challenged with a local TSWV isolate (Pathotype PI). Pepper plants (*C. annuum*) possessing *Tsw* gene were used to multiply the virus. Inoculum, prepared from the infected pepper leaves in 0.01 M phosphate buffer (pH 7.0) containing 0.2 % sodium sulfite after adding carborandum, was rubbed on cotyledons of seedlings at the cotyledonary to the two true leaf stage. After three weeks of incubation in a growth chamber at 23 °C with a 16 h photoperiod, the plants were evaluated for resistance by symptoms and confirmed by DAS-ELISA.

**RESULTS** All of 139 pepper genotypes tested were found to be susceptible to TSWV pathotype PI isolate according to bioassays. Results were confirmed by DAS-ELISA. Biological assay results were compatible with DAS-ELISA in all genotypes tested.

**DISCUSSION & CONCLUSION** Virus resistance is the best way to protect against virus infection. In this study, all pepper genotypes tested were susceptible to TSWV pathotype PI isolate. Thus, additional germplasm, must be screened to determine new genetic sources of resistance.

## REFERENCES

- [1] Black et al., 1991, Plant Dis. 75:863. Boiteux, L. S. 1995
- [2] Boiteux et al., 1993, Euphytica 67:89-94
- [3] Boiteux et al., 1993, Plant Disease 77, 210





# A proteomic approach to elucidate the molecular interaction between the tracheomycotic fungi *Fusarium oxysporum* and *Verticillium dahliae* and eggplant

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**BACKGROUND** Accumulating evidence indicates that the application of proteomics for global investigation of plant-pathogen interactions can provide novel insights for molecular mechanisms involved in the defense processes [1]. The present study was carried out with the aim to elucidate, at the proteomic level, the defense mechanisms triggered by the resistance locus *Rfo-sal*. Proteomic analyses were performed on a *Rfo-sal* introgression line (IL) resistant to *Fusarium* (Fom) and on its susceptible recurrent line by using differential inoculations, Fom, *Verticillium* (Vd) and a mixture (Mixta) of both pathogens. The proteomic analysis of root extracts was performed at different times (0-8-24 hours) after fungal inoculations. The locus *Rfo-sal* is responsible for the resistance to *Fusarium* wilt, and, ILs introgressed with that locus showed an improved tolerance against *Verticillium* only in the case they were previously infected by *Fusarium* [2]. The present data provide important information on the molecular mechanisms of fungal defense in eggplant at protein level.

**MATERIALS & METHODS** '305E40' and 'TALI/1' genotypes, resistant and susceptible to Fom, respectively, were used. Samples of inoculated and Mock-inoculated roots were harvested at 0, 8 and 24 hours after the artificial inoculations. Total protein extracts were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D SDS-PAGE). Peptide mixtures, excised from the gel and digested, were analyzed by Ultra Performance Liquid Chromatography tandem mass spectrometry (UPLC/MS/MS) [3]. The identified proteins were annotated based on UniProt website information. To confirm the presence of some identified differentially expressed proteins (i.e. PR 10), western blotting analyses were performed.

**RESULTS** LC/MS/MS analysis allowed to identify 89, 127, 90 and 40, 48, 35 root proteins, after artificial inoculations with Fom, Vd and Mixta of '305E40' and 'TALI/1', respectively. The proteome analysis of '305E40' Fom-, Vd- and Mixta-infected roots at three different timings, compared to that of non-infected roots, unambiguously identified 46, 67 and 50 differentially expressed proteins, respectively; whereas 31, 28 and 30 differentially expressed proteins, respectively, were identified in infected roots of 'TALI/1' susceptible genotype (Table 1). In Fom inoculations, Defense related proteins contribute to 20% and 30%, ROS-scavengers/regulators proteins contribute to 21% and 15% and Stress related proteins contribute to 2% and 7% of total identified proteins in '305E40' and 'TALI/1', respectively. In Vd inoculations, Defense related proteins contribute to 20% and 23%, ROS-scavengers/regulators proteins contribute to 19% and 14% and Stress related proteins contribute to 2% and 4% of total identified proteins in '305E40' and 'TALI/1', respectively. In Mixta inoculations, Defense related proteins contribute to 19% and 11%, ROS-scavengers/regulators proteins contribute to 15% and 14% and Stress related proteins contribute to 3% and 3% of total identified proteins in '305E40' and 'TALI/1', respectively.

**DISCUSSION & CONCLUSION** The investigation confirmed the capacity of the locus *Rfo-sal* to protect eggplant under Fom inoculation and evidenced an improved tolerance of '305E40' genotype to Vd after simultaneous inoculation with Vd+Fom. The present study highlights the role of several important proteins like Defense related proteins (PR 10,  $\beta$ -1,3-glucanase, Xyloglucan endoglucanase inhibitor), ROS scavengers/regulators (Catalase, Glutathione S-transferase, Peroxidase) and their varied accumulation in susceptible and resistant plants. The functional characterization of these proteins could

help in directing crop improvement programs toward the obtainment of multi-resistant varieties to tracheomycotic fungal wilt.

## REFERENCES

- [1] Mehta A. et al., 2008, FEBS J., 275(15): 3731–46.
- [2] Barbierato V. et al., 2016, Plant Pathol., 65(8): 1297-1309.
- [3] Chatterjee M. et al., 2014, BMC Genomics, 15: 949.

Genotypes	Proteins	Fom inoculation			Vd inoculation			Mixta inoculation		
'305E40'	N° total proteins identified	89			127			90		
	N° differentially expressed proteins at T0, T0+8h, T0+24h	22	12	12	1	28	38	8	15	27
	More represented proteic functional categories	ROS-scavengers/Regulators; Defense related			Defense related; ROS-scavengers/Regulators; Synthesis, folding and degradation			Defense related; ROS-scavengers/Regulators; Synthesis, folding and degradation		
'Tall/1'	N° total proteins identified	40			48			35		
	N° differentially expressed proteins at T0, T0+8h, T0+24h	5	14	12	12	4	12	7	11	12
	More represented proteic functional categories	Defense related; Amino acid metabolism			Defense related; ROS-scavengers/Regulators; Synthesis, folding and degradation			ROS-scavengers/Regulators; Amino acid metabolism		

**Table 1.** Number of total and differentially expressed proteins identified in the three inoculations studied in '305E40' and 'Tall/1' genotypes.

# Diversity of Tobacco etch virus pathotypes faced with pepper resistance sources and durability potential of resistance genes

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**BACKGROUND** Potyviruses are an important agronomic constraint to pepper (*Capsicum* spp.) production, worldwide. Compared to other potyviruses, Tobacco etch virus (TEV) has rather limited natural host range and geographical distribution. The main crops affected by TEV are tobacco (*Nicotiana tabacum*) and pepper and TEV is mostly prevalent in the Americas, Turkey and sporadically in China. In pepper, several recessive resistance alleles at the *pvr1/pvr2* locus, which encodes the eukaryotic initiation factor 4E (eIF4E), are available to control TEV and the corresponding resistance-breaking factor in TEV is the VPg (viral protein genome-linked). Until now, the spectrum of action of these different alleles has only been evaluated with a limited set of TEV isolates.

**MATERIALS & METHODS** We used a worldwide collection of 16 TEV isolates to evaluate the spectrum of action and durability potential of five *pvr1/pvr2* resistance alleles in laboratory conditions and under mechanical inoculation.

**RESULTS** The 16 TEV isolates infected the susceptible controls 'Yolo Wonder' and 'Yolo Y' homozygous at the *pvr2*<sup>+</sup> and *pvr2*<sup>1</sup> alleles, respectively. Phenotypes of resistance (i.e. no detectable TEV accumulation at the systemic level) and of susceptibility (i.e. presence of symptoms and TEV detection at the systemic level) were observed in the four remaining pepper genotypes homozygous at the *pvr2*<sup>2</sup>, *pvr2*<sup>7</sup>, *pvr2*<sup>14</sup> or *pvr1* alleles, depending on the TEV isolate. Some genotypes had heterogeneous responses to inoculation with some TEV isolates, with both resistant and susceptible plants. In several of these cases, the TEV populations in susceptible plants showed amino acid changes in the VPg, indicative of resistance breakdown. The *pvr2*<sup>14</sup> allele had the broadest resistance spectrum (14 of 16 isolates), followed by *pvr1* (10/16), *pvr2*<sup>2</sup> (9/16) and *pvr2*<sup>7</sup> (4/16). In all, eight different pathotypes of a theoretical total of 16 were observed (Table 1). VPg mutations associated with resistance breakdown have been observed at amino acid positions 109 and 119 for the *pvr1* allele and at position 120 for the *pvr2*<sup>2</sup> allele.

**DISCUSSION & CONCLUSION** Although fewer *pvr2* alleles confer resistance to TEV than to the other potyvirus Potato virus Y (PVY) [1], there is a similarly high diversity of pathotypes in the two viruses. Such diversity is potentially driven by coevolution between pepper and potyviruses [2] but it is difficult to identify which potyvirus species has (or have) exerted a selection pressure on pepper. In addition, this study provides new informations about the resistance spectrum and potential of durability of different *pvr1/pvr2* alleles.

## REFERENCES

- [1] Moury B et al., 2014, Infection, Genetics and Evolution, 27:472–480. doi: 10.1016/j.meegid.2013.11.024.
- [2] Charron C et al., 2008, The Plant Journal, 54:56–68. doi: 10.1111/j.1365-3113X.2008.03407.x.

## ACKNOWLEDGEMENTS

This work was supported by the Plantum working group Phytopathology.

Bulent Arpacı thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.

Pathotype	<i>pvr2</i> <sup>2</sup>	<i>pvr2</i> <sup>7</sup>	<i>pvr2</i> <sup>14</sup>	<i>pvr1</i>
<b>1</b> ( <i>n</i> =2)	R	R	R	R
<b>2</b> ( <i>n</i> =3)	R	S	R	R
<b>3</b> ( <i>n</i> =3)	S	S	R	R
<b>4</b> ( <i>n</i> =2)	S	S	S	R
<b>5</b> ( <i>n</i> =2)	R	R	R	S
<b>6</b> ( <i>n</i> =2)	R	S	R	S
<b>7</b> ( <i>n</i> =1)	S	S	PR	S
<b>8</b> ( <i>n</i> =1)	S	S	S	S

**Table 1.** The eight TEV pathotypes revealed by this study using four pepper genotypes with *pvr2/pvr1* resistance alleles.  
R : resistant ; PR : partially resistant ; S : susceptible.  
*n* : number of isolates belonging to each pathotype.

# Marker assisted selection of *Tobamovirus* resistant pepper plants in a backcross PseudoF1 generation

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**BACKGROUND** Pepper is one of the most important vegetable crops in the Basque Country (Northern Spain). The main landraces cultivated are Gernika pepper (selected cultivar Derio) and Ibarra chilli peppers (selected cultivar Ibarroria), both of them susceptible to the group of Tobamovirus (TMV, ToMV, TMGMV, PMMoV and PaMMV). Tobamoviruses cause high yield losses in quantity and quality and are responsible for important reductions of crop profitability in this area. These viruses are difficult to control, since no curative treatments are available and the best strategy for their control is the use of resistant varieties. Thus, a marker assisted selection backcross plant breeding program was started in 2015 for the introgression of resistance genes *L3* and *L4* against Tobamovirus in both cultivars. The objective of this work was to evaluate molecular markers to select the resistant genotypes obtained in the PseudoF1 generation within the breeding program.

**MATERIALS & METHODS** A PseudoF1 generation was obtained from four different crosses: Ibarroria x L3 Hybrid, Ibarroria x L4 Hybrid, Derio x L3 Hybrid and Derio x L4 Hybrid. DNA extraction from plants leaves was carried out with the plant DNA commercial kit (InnuPREP plant DNA kit Analytik Jena). The molecular primers YB2A25 and YB2A19 [1] were used for the identification of plants with the *L3* resistance gene. The molecular marker L4SC340 [2] was used to identify plants with the *L4* resistance gene. The three molecular markers used were SCAR type.

**RESULTS** In the Derio x L3 Hybrid cross, 69 plants were genotyped with the YB2A25 primer, of which 31 (45%) showed PCR amplification (Table 1). Another 91 plants from the Ibarroria x L3 Hybrid cross were genotyped with the same primer, of which 45 (49%) showed PCR amplification. From the Derio x L3 Hybrid cross, 70 plants were analysed with the YB2A19 molecular marker, of which 33 (47%) showed PCR amplification. In the same way, 81 plants of the Ibarroria x L3 Hybrid cross were genotyped with this primer, and 39 individuals (48%) showed PCR amplification. Those plants with presence of the two primers associated to the *L3* gene were selected as resistant plants. In the two crosses with the L4 Hybrid, the L4SC340 molecular marker was used [2]. When plants from the Derio x L4 Hybrid cross were genotyped, 41 individuals out of a total of 89 (46%) showed PCR amplification. In the Ibarroria x L4 Hybrid cross, 91 individuals were genotyped, of which 39 (43%) presented the band corresponding to the molecular marker associated to the *L4* gene.

**DISCUSSION & CONCLUSION** With these molecular markers, resistant plants accounted for 45-49% of the plants for the *L3* gene (YB2A25, YB2A19) and 43-46% of the plants for the *L4* gene (L4SC340). These proportions were similar to the Mendelian segregation expected for a dominant monogenic character in a PseudoF1 generation, 50% resistant plants and 50% sensitive plants. The three molecular markers used in this breeding program were useful to select resistant individuals, which makes them very interesting to be used in future phases of the breeding program to introduce resistance in the Tobamovirus sensitive pepper cultivars Derio and Ibarroria.

## REFERENCES

- [1] Tomita R, Murai J, et al., 2008, Theoretical and applied genetics, 117(7):1107-1118, doi:10.1007/s00122-008-0848-6
- [2] Kim HJ, Han JH, et al., 2008, Molecules & Cells, 25(2):205-210

Crosses	YB2A25		YB2A19		L4SC340	
	Marker	No	Marker	No	Marker	No
		marker		marker		marker
<b>Derio x L3 Hybrid</b>	31	38	33	37	-	-
<b>Ibarroria x L3 Hybrid</b>	45	46	39	41	-	-
<b>Derio x L4 Hybrid</b>	-	-	-	-	41	48
<b>Ibarroria x L4 Hybrid</b>	-	-	-	-	39	52

**Table 1.** Number of plants of the generation PseudoF1 coming from different crosses that have shown or not the molecular markers YB2A25, YB2A19 and L4SC340.es.

# Susceptibility of pepper genotypes to root-knot nematode populations virulent on *Mi-1* tomatoes

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**BACKGROUND** Pepper is one of the most important vegetables in the world. Root-knot nematodes (RKNs) cause yield losses in pepper crops. Management tactics including soil fumigations, solarisation, biocontrol agents, nematicides and RKN resistant varieties are used to control RKNs. The use of resistant plants is one of the most effective and environmentally friendly methods against RKNs [1]. However, virulent RKN populations have increased in vegetable-growing areas. RKN populations have overcome nematode resistance genes such as the tomato *Mi-1* gene [2, 3]. The objective of this study was to determine the susceptibility of pepper cultivars to *Meloidogyne incognita* and *M. javanica* populations virulent on *Mi-1* tomatoes.

**MATERIALS & METHODS** Seven pepper genotypes (Safran F1, Mostar F1, Mert F1, B5 line, Carolina Wonder -homozygous for the *N* gene conferring resistance to RKNs-, B4 F1, and B6 line) were assessed for their resistance to four RKN populations virulent on *Mi-1* tomatoes (2 populations of *M. incognita* and 2 populations of *M. javanica*) with 5 plants for each treatment 'pepper genotype x RKN population'. Each pepper seedling was inoculated with 1000 second stage juveniles (J2). Plants were harvested 8 weeks after inoculation and the root systems were stained with 0.15 g/l Phloxine B solution for 10 minutes. Stained eggs, egg masses and galls were counted.

**RESULTS** The two *Mi-1* virulent *M. javanica* populations did not produce any egg mass and any gall on roots of the seven pepper genotypes. The two *Mi-1* virulent *M. incognita* populations multiplied very well on Safran F1, Mostar F1, Mert F1 and B5 line which are known susceptible to *Mi-1* avirulent populations. However, they did not produce any egg mass on Carolina Wonder line, B4 F1 and B6 line, known as resistant to *Mi-1* avirulent RKN populations.

**DISCUSSION & CONCLUSION** The *Mi-1* gene is commonly used for controlling RKNs in tomato. In this study, the seven assessed pepper genotypes showed different responses to *Mi-1* virulent *M. incognita* and *M. javanica* populations. The 3 pepper genotypes found as resistant to *Mi-1* avirulent RKN were resistant to the four tested *Mi-1* virulent populations. Some previous studies reported the weak reproductive ability of *Mi-1* virulent nematodes on pepper cultivars susceptible to RKNs. However, in this study, *Mi-1* virulent *M. incognita* populations produced numerous egg masses on the four susceptible genotypes. The different behavior of pepper genotypes can be explained by the different genetic backgrounds of plants and nematode populations.

## REFERENCES

- [1] Devran Z et al., 2010, Phytopathol. Mediterr; 49: 11-17 DOI: 10.14601/Phytopathol\_Mediterr-3116
- [2] Devran Z, Söğüt MA. 2010, Phytoparasitica 38:245-251 DOI: 10.1007/s12600-010-0103-y
- [3] Ornat C et al., 2001, Plant Dis. 85: 271-276 DOI: 10.1094/PDIS.2001.85.3.271





# Evaluation of Balkan pepper germplasm for resistance to tobamoviruses and tospoviruses

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**BACKGROUND** Pepper (*Capsicum annuum* L.) is a traditional vegetable grown in the Balkans. Tobamoviruses (*Tobacco mosaic virus*, TMV and *Pepper mild mottle virus*, PMMoV) are a serious threat for pepper production. They are grouped in four pathotypes (P0, P1, P1.2, P1.2.3), resistance to which is controlled by four allelic genes (*L1*, *L2*, *L3* and *L4*), respectively. Another virus of economic importance is *Tomato spotted wilt virus* (TSWV, genus *Tospovirus*). Resistance to TSWV is controlled by a single *Tsw* locus. Availability of the resistance sources to TMV, PMMoV and TSWV is of tremendous importance for breeders to introgress resistant alleles in elite germplasm background. To facilitate the process, molecular markers for *L3* and *L4* as well as for *Tsw* are currently available. The aim of the current study is to evaluate pepper accessions originating from the Balkans for resistance against TMV, PMMoV and TSWV by biological screening and molecular methods.

**MATERIALS & METHODS** Pepper germplasm, consisting of 94 accessions collected from Balkan regions, was the subject for investigation. Infectious tests with TMV (P0), PMMoV (P1.2) and a local isolate of TSWV were performed in growth chambers under controlled conditions. Resistance to PMMoV was assessed by a detached leaf method, whereas resistance to TMV and TSWV was evaluated by direct inoculation of the plants. Local and/or systemic symptoms were observed daily for a period of 21 days after inoculation. The resistant plants were subjected to molecular analysis using two markers for identification of *L3* (PMFRI1, [1]) and *L4* (087H3T7, [2]) genes.

**RESULTS** Most of the tested accessions appeared to be susceptible to both viruses. Resistance to TMV (P0) was discovered in seven accessions from Bulgaria, three from Romania and three from Serbia (Table 1). Resistance was expressed by necrotic local lesions and/or vein necrosis on inoculated leaves. Eleven accessions were homogeneous for TMV resistance, while segregation was observed only in two accessions from Bulgaria. One Romanian accession was resistant to both viruses - TMV and PMMoV - developing necrotic local lesions as primary symptoms. Complementary molecular analysis for *L3* and *L4* genes was performed only with the resistant accessions. Presence of the *L4* gene in the homozygous state was proved only in the Romanian local form.

**DISCUSSION & CONCLUSION** Application of a combined approach of virus inoculation techniques and use of molecular markers was found useful and effective for disease resistance screening. The obtained results suggest the presence of *L1* or *L2* in accessions resistant to TMV, while *L4* was present in the PMMoV resistant accession. This information will be valuable for future breeding programs.

## REFERENCES

- [1] Sugita T et al., 2004, *Breeding Science*, 54(2), 111–115. <http://doi.org/10.1270/jsbbs.54.111>
- [2] Yang HB et al., 2009, *Molecular Breeding*, 24, 433–446. <http://doi.org/10.1007/s11032-009-9304-7>

## ACKNOWLEDGEMENTS

Gancho Pasev thanks the French “Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (program ACCES de CAMPUS France)” for covering the travel and accommodation expenses for the 17th EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant.

**Table 1.** Resistance of 94 pepper accessions originating from Balkans after screening with three viruses.

Country	Accession #	TMV		PMMoV		TSWV	
		R	S	R	S	R	S
Bulgaria	70	7	63	0	70	0	70
FYROM	2	0	2	0	2	0	2
Romania	8	3	5	1	7	0	8
Serbia	14	3	11	0	14	0	14
Total:	94	13	81	1	93	0	94

**Legend:** R – resistant, S – susceptible.

# Identification and characterization of antimicrobial proteins from *Capsicum annuum* var. *annuum* and their action on the development of phytopathogenic microorganisms

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**BACKGROUND** Throughout evolutionary processes plants have developed innumerable strategies to overcome attacks by pathogens, including the production of antimicrobial peptides (AMPs). These small proteins, produced by all life forms, have a potent antimicrobial activity, in particular because of their interaction with cell membranes that they permeabilize. The production of AMPs by plants can be constitutive or induced after a pathogen attack [1]. Many resistances against phytopathogenic microorganisms exist in chilli peppers germplasm. Our work aimed at detecting and characterizing AMPs isolated from leaves and roots of UENFI 381, a *Capsicum annuum* L. accession resistant to bacterial spot caused by *Xanthomonas euvesicatoria* [2]. AMPs killing activity was evaluated on this pathogenic bacterium as well as on the fungus *Colletotrichum gloeosporioides*, and AMPs protease inhibitory effect was determined on trypsin.

**MATERIALS & METHODS** Self fertilized seeds of *C. annuum* accession UENFI 381 were cultured for 15 days in Petri dishes containing MS medium. Plantlets were then transferred in glass flasks containing 1/2-MS-medium and kept for 45 days. The leaves were inoculated with a culture of *X. euvesicatoria* (108 CFU/mL) or water (control). Leaf and root samples were collected 12, 24 and 48h after inoculation for protein extraction [3]. AMPs were characterized by electrophoresis. AMPs effect on *X. euvesicatoria* was assessed with an antibiogram. The bacterium was grown in Dygs medium in presence of 5µl of extracts (100 and 200µg/mL) or water (control) that were added to paper disks. Halos of inhibited bacterium growth were measured with a digital pachymeter 48h later. To assess the effect of AMPs on *C. gloeosporioides* growth, fungal cells and conidia (20 000 cells/mL) were incubated at 30°C in microplates in the presence of plant extracts (100 and 200 µg/mL) or water (control). Optical readings at 620 nm were performed after 48h. Membrane permeabilization was indirectly evaluated with fluorescent dye SYTOX green. Compounds signaling cells oxidative stress, namely reactive oxygen species (ROS), were measured with H<sub>2</sub>DCFDA. The ability of the extracts to inhibit trypsin (of insect) was determined in the presence of leaf and roots extracts.

**RESULTS** Electrophoresis revealed that protein extracts from leaves and roots of *C. annuum* UENFI 381 presented a majority of bands with a low molecular mass (6-8 kDa). At the concentration of 200µg/mL, all leaf extracts (issued from plants inoculated with *X. euvesicatoria* or with water) totally inhibited *C. gloeosporioides* growth. Only control root extracts, sampled 48h after water treatment, has a complete inhibitory effect on the fungus. At the concentration of 100µg/mL, leaf extracts permeabilize the membrane of *C. gloeosporioides*. They induce also an increase of endogenous ROS when compared to the control. Leaf extracts, sampled 48h after inoculation, induce a bacterium growth inhibition halo of 1.92mm, while the control extracts induce a much smaller halo of 0.47mm. Lastly, trypsin activity was almost totally inhibited by leaf extracts at the concentration of 30 µg/mL, whatever they originated from *X. euvesicatoria* or water inoculated plants.

**DISCUSSION & CONCLUSION** Our results demonstrate the antimicrobial activity of AMPs, which is of great importance for biotechnological applications. The adopted protein extraction methodology was efficient for isolating and concentrating proteins of low molecular weight, ranging from 6-8 kDa. We demonstrated that leaf and root extracts are potent growth inhibitors of *X. euvesicatoria* and *C. gloeosporioides*, and that leaf-extracts inhibit trypsin activity. AMPs based *Capsicum* spp. x microorganism interactions can be used for breeding chili resistant cultivars, and also for developing models for any pathosystem.

#### REFERENCES

- [1] Campos et al., 2018, Biochemical Journal, 475: 3359–3375. doi:10.1042/BCJ20180213
- [2] Pereira et al., 2018, Acta Physiologiae Plantarum, 22:40–107. doi: 10.1007/s1173-018-2685-9
- [3] Granier, 1988, Electrophoresis, 9 (11):712–718. doi.org/10.1002/elps.1150091106

# Development of a resistance test of pepper to *Meloidogyne incognita* and eggplant to *Verticillium dahliae* for DUS testing

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**BACKGROUND** *Meloidogyne incognita*, the causal agent of root-knot, is the most widespread nematode species causing important economic losses on pepper. Using resistant varieties allows reduction of the parasitic pressure in the soil. Breeding programs for the selection of resistant varieties have become a major issue. In *Capsicum annuum*, resistance to this pathogen is controlled by several independent dominant genes (the Me genes) which express different levels of resistance [1][2]. Verticillium wilt, caused by *Verticillium dahliae*, is a major plant pathogen affecting several solanaceous crops worldwide including eggplant. No commercial varieties are described as resistant yet. The resistant rootstocks which can be used are interspecific hybrids of tomato species [3]. The detailed objectives of this work were a first step to acquire a better knowledge of the levels of pepper resistance to *M. incognita* and of eggplant resistance to *V. dahliae*, then the definition of reference materials (isolates, controls and differentials), inoculation method and notation scale. The *Meloidogyne*/pepper protocol is now used to carry out official Distinction, Uniformity and Stability (DUS) tests for the registration of new varieties in the Catalogue of plant varieties. The *Verticillium*/eggplant protocol is used for evaluation of resistance of varieties.

**MATERIALS & METHODS** For both host/pathogen combinations, the study was performed on a panel of controls and commercial varieties with different levels of resistance. For pepper inoculated with *M. incognita*, types of containers, stage of inoculation, inoculation methods were compared. The notation scale was based on a visual evaluation of intensity of galls. For eggplant inoculated with *V. dahliae*, stage of inoculation, test conditions and inoculation methods were compared. The notation scale included foliar symptoms (yellowing and wilting) and vascular symptoms (brown vessel above cotyledons).

**RESULTS** For pepper tested with *M. incognita*, the sowing of plants on contaminated roots compared to deposition of contaminated roots between sowing line at 2 leaf stage gave better results in terms of repeatability. Using a tray compared to a pot allowed a better homogeneity in the mix of soil and contaminated roots and a homogeneous pressure of parasite on varieties. A notation scale was defined with 5 levels. The varieties of the panel were divided into three groups: resistant (Capital), intermediate (Yolo Wonder) and susceptible (Doux d'Espagne).

For eggplant assayed with *V. dahliae*, the main difficulty was the heterogeneity of plant stage for inoculation. Inoculation at an older stage (first true leaves) allowed limitation of this effect. The inoculation method with a higher aggressiveness (inoculation by soaking root in a spore suspension) was selected allowing identification of the different levels of resistance: resistant, intermediate, resistant and susceptible.

For both host/pathogen combination, representative controls were selected for each level.

**DISCUSSION & CONCLUSION** For pepper inoculated with *M. incognita*, this new protocol was accepted as a new characteristic by CTPS for registration of varieties in the Official Catalogue and will be proposed to CPVO and UPOV. The reference materials (strain and controls) have been included in MATREF (national network managed by GEVES in order to provide validated reference material) and is now available for breeders. For eggplant inoculated with *V. dahliae*, the development of a reliable resistance test will provide breeders with a new tool for screening resistant varieties.

## REFERENCES

- [1] Bleve-Zacheo T., Bongiovanni M. et al, 1998. Plant Science, 133: 79-90
- [2] Djian-Caporalino C., Palloix A., et al, 2013. Innovation Agronomique, 28: 187-199
- [3] Villeneuve, F., Latour, et al, 2014. Acta Horticulturae, 1044: 57-66

# Responses to water stress in four accessions of *Solanum melongena*

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**BACKGROUND** Most of the production of eggplant (*Solanum melongena*) takes place in tropical and subtropical areas where climate change, including increased drought stress, is expected to have a significant impact. Drought is one of the abiotic stresses that induces severe losses in eggplant production and also affects the quality of the berries [1]. However, little is known of the variation in the responses to drought among eggplant genotypes. For all these reasons, the main objective of this experiment was to study the biochemical response to water stress in eggplant aimed at unravelling what responses are activated in eggplant accessions that have a certain tolerance to drought. This information will be important for developing new resilient eggplant cultivars.

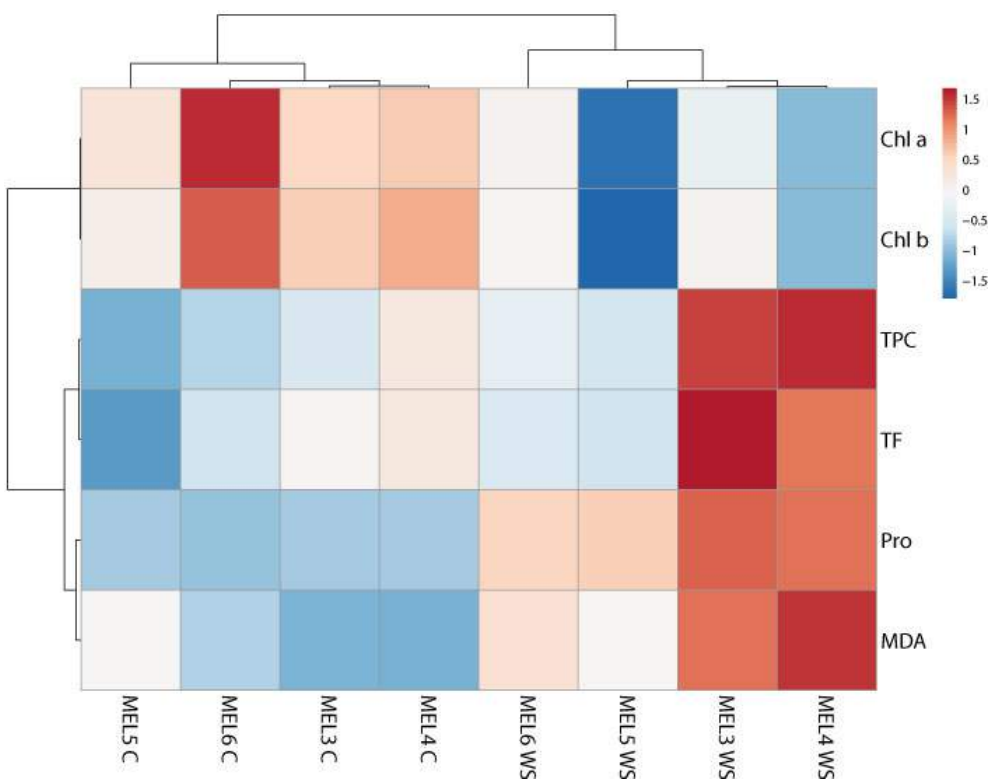
**MATERIALS & METHODS** In this work we evaluated four genetically diverse accessions of *S. melongena* (MEL3, MEL4, MEL5 and MEL6). Seeds were germinated in Petri dishes and transplanted to pots filled with growing substrate. When the plantlets reached the stage of four-to-five true leaves, a water stress treatment was initiated through the total interruption of irrigation; a control normally irrigated was used for comparison. Chlorophylls a and b, proline content, malondialdehyde (MDA), total antioxidant flavonoids (TF), and total phenolic compounds (TP) were determined following protocols described elsewhere [2] with slight modifications. The clustvis program (<https://biit.cs.ut.ee/clustvis/>) has been used as a tool to visualize the comparison of the measured traits.

**RESULTS** The experiment was finished after 11 days of initiation of the drought treatment. At that time plants from the drought treatment displayed severe drought symptoms and the moisture of the substrate of the water stress treatment had drastically reduced to less than 5%. In general, drought decreased the content in photosynthetic pigments, and increased proline, MDA, total phenolics and total flavonoids. However, differences were observed among accessions in response to drought for the biochemical traits measured (Figure 1). In this way, in MEL3 the reduction in photosynthetic pigments was less pronounced than in other accessions. Proline increased considerably by inducing drought stress in plants, but the increase was highest in accessions MEL3 and MEL4. The MDA, TPC and TF concentrations increased significantly in MEL3 and MEL4 but not in MEL5 and MEL6 (Figure 1).

**DISCUSSION & CONCLUSION** Our comparison of plants subjected to water stress versus control conditions shows a clear impact of water stress on the biochemical responses of eggplant to the drought. In particular, in the accession MEL3, where a lower reduction in chlorophylls was observed, proline MDA, TPC and TF in general increased more than in the other accessions. The information obtained suggests that differences in the biochemical response to water stress among eggplant varieties might be exploited to develop eggplant cultivars more tolerant to drought for being used in the adaptation of crops to climate change.

## REFERENCES

- [1] Díaz-Pérez JC, Eaton TE, 2015, HortScience 50 :1709-1714.
- [2] Kumar D et al., 2017, PLOS ONE 12:e0185017. doi: 10.1371/journal.pone.0185017



**Figure 1.** Responses to Water Stress in four Accessions of *Solanum melongena*.



# Genetic architecture of robustness in *Capsicum annuum* for resistance to *Phytophthora capsici* and *Potato virus Y* under a temperature stress

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**BACKGROUND** Current climate has an impact on the expression of plant diseases and therefore their management [1]. The plant-pathogen interaction resulting from an infection can be difficult to predict when one or several parameters of the environment are changed. For example, increased mean temperature was reported to inhibit the expression of resistance to pathogens. Usually, the higher the temperature, the higher the virus multiplication. However, the opposite can also be observed: Tobacco rattle virus multiplies faster at 18-22°C than at 26°C [1]. The pathogen multiplication rate is also linked to the host behaviour, which depends on its genetics. This leads to the definition of robustness, sometimes called canalization: the absence or low variation of a phenotypic trait faced to a specific environmental change [2]. Our goal is to identify Quantitative Trait Loci (QTL) and genes involved in the robustness of pepper resistance to *Phytophthora capsici* and Potato virus Y (PVY).

**MATERIALS & METHODS** A collection of 176 pepper (*Capsicum annuum*) accessions was phenotyped for resistance to *P. capsici* under two temperatures, 22/24°C or 28/30°C (the lowest being the night temperature) [3]. The same accessions were inoculated with PVY in two conditions (20°C or 28°C) and relative concentration of virus in apical leaves was measured with Enzyme Linked ImmunoSorbent Assay (ELISA). The collection was genotyped for 10,308 Single Nucleotide Polymorphisms (SNPs). Robustness was calculated as the inverse of the difference of resistance mean between both environmental conditions. Best Linear Unbiased Predictor (BLUP) values of robustness were used for Genome Wide Association Studies (GWAS).

**RESULTS** The temperature regime and the accession have an effect on the mean resistance level (Fig. 1). For both *P. capsici* and PVY, different responses were identified between the two conditions. Mortality at 30 dpi was more frequent at 20°C than at 28°C for PVY. Further analyses will underline the genetic part involved in robustness. QTL determining resistance for each pathogen at each temperature regime and robustness of resistance to these pathogens under thermic variation will be looked for with a GWAS approach. Then, a search into *C. annuum* reference genomes will enable us to identify robustness genes.

**DISCUSSION & CONCLUSION** Thanks to this project, we expect to identify different QTL of robustness for each pathogen. A larger temperature spectrum will allow the determination of reaction norms for each accession across multiple environments. A precise characterization of the response could help the breeding for changing environments. Robustness for different stress (flooding, drought, and other pathogens) and combination of stresses will also be assessed.

## REFERENCES

- [1] Jones and Barbeti, 2012, CAB Reviews 7:1-31. doi :10.1079/PAVSNNR20127022
- [2] Felix and Barkoulas, 2015, Nature Review in Genetics 16: 483-496. doi:10.1038/nrg3949
- [3] Lefebvre and Palloix, 1996, Theoretical and Applied Genetics 93:503-511. doi: 10.1007/BF00417941

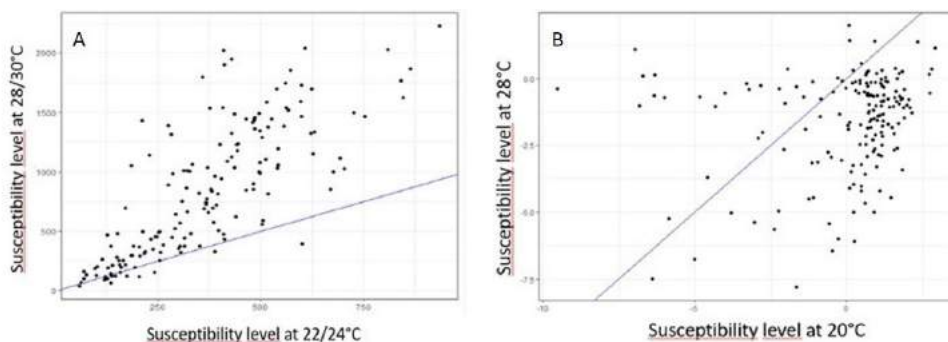


Figure 1: Plot of the susceptibility levels of the 176 accessions in both environments. The blue line corresponds to the first bisector *i.e.* the response is similar in both environments. A) For *P. capsici*, susceptibility level corresponds to the AUDPC index. B) For PVY, susceptibility level corresponds to the log transformation of virus concentration.

# Improving the durability and efficiency of plant resistance deployment using eco-evolutionary modelling

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**BACKGROUND** Genetically-controlled plant resistance can reduce the damage caused by pathogens. However, pathogens have the ability to evolve and overcome the resistance. This often occurs very quickly after resistance is deployed, resulting in significant crop losses and continuing needs to breed new resistant cultivars. To tackle this issue, several strategies have been proposed to constrain the evolutionary potential of pathogen populations and thus increase resistance durability. These strategies mainly rely on using different combinations of resistance genes (e.g. qualitative and/or quantitative resistance) in time, space, or both (e.g. via gene pyramiding, cultivar rotations, cultivar mixtures, field mosaics). However, experimental assessment of the efficiency (i.e. ability to reduce disease impact) and the durability (i.e. ability to limit pathogen evolution and delay resistance breakdown) of different deployment strategies presents a major challenge.

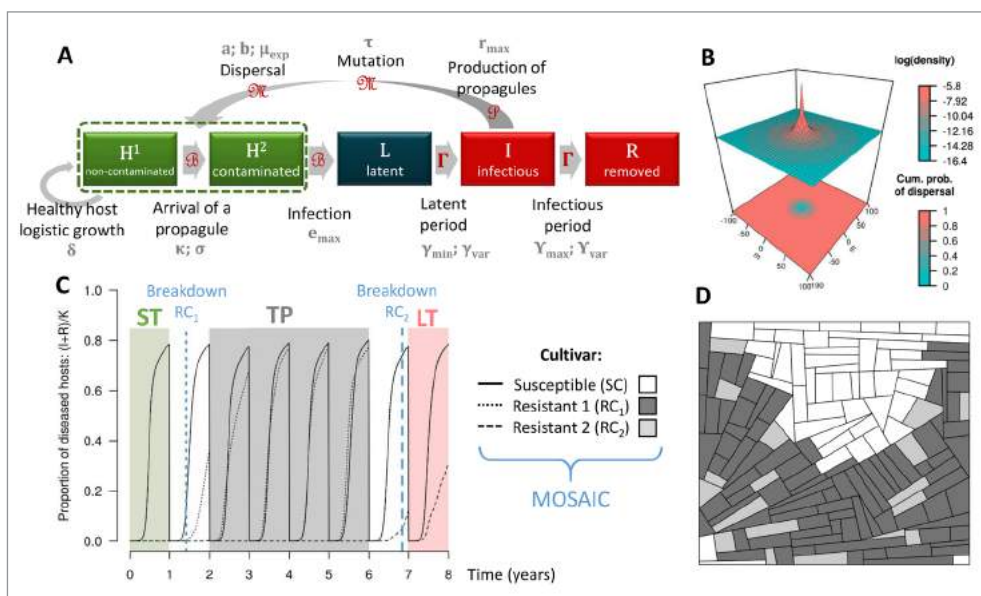
**MATERIALS & METHODS** Therefore, we developed a spatially-explicit stochastic model [1] to assess the epidemiological and evolutionary outcomes of the major deployment options described above, for both qualitative (major resistance genes) and quantitative resistance (e.g. QTLs affecting different pathogen life-history traits). In addition, we analyzed the impact of landscape organization (as defined by the proportion of fields cultivated with a resistant cultivar, and their spatial aggregation) and epidemiological or evolutionary parameters (e.g. mutation probability, cost of infectivity).

**RESULTS** Our main results on resistance to rusts indicate that evolutionary and epidemiological control are not necessarily correlated [2], and that no deployment strategies is universally optimal [3].

**DISCUSSION & CONCLUSION** The model was first parameterized for cereal resistance to rusts (caused by fungi of the genus *Puccinia*), and is destined to be applied to other pathosystems including viruses of vegetable crops, especially Potato virus Y on pepper (*Capsicum spp.*).

## REFERENCES

- [1] Rimbaud L, Papaïx J, et al. 2018. PLoS Computational Biology 14(4): e1006067. DOI: 10.1371/journal.pcbi.1006067
- [2] Papaïx J, Rimbaud L, et al. 2018. Evolutionary Applications 11(5): 705-717. DOI: 10.1111/eva.12570
- [3] Rimbaud L, Papaïx J, et al. 2018. Evolutionary Applications 11(10): 1791-1810. DOI: 10.1111/eva.12681



**Figure 1. Model overview.** (A) Model architecture. Healthy hosts can be contaminated by propagules and may become infected. Following a latent period, infectious hosts produce new propagules which may mutate and disperse across the landscape. At the end of the infectious period, infected hosts become epidemiologically inactive. Qualitative resistance prevents transition to the latent infected state (L). Green boxes indicate healthy hosts which contribute to crop yield and host growth, in contrast to latent hosts (dark blue box) and diseased hosts (i.e. symptomatic, red boxes). Model parameters associated with epidemiological processes are indicated in grey. Distributions used to simulate stochasticity in model transitions are indicated in red; B: binomial,  $\Gamma$ : gamma, P: Poisson, M: multinomial. Host growth is deterministic. (B) Two-dimensional representation of the power-law dispersal kernel calibrated for rust pathogens. Top panel indicates the logarithm of the probability to disperse from the origin to any point of the landscape; bottom panel indicates the cumulative probability of dispersing over a given distance. (C-D) Example of simulation with two major resistance genes deployed as a mosaic: (C) dynamic of diseased hosts; (D) landscape. Blue vertical lines indicate the durability of the two resistant cultivars. These lines delineate the three periods used to compute epidemiological outputs from AUDPC: short-term (ST, green area), transitory period (TP, grey) and long-term (LT, red).

# Resistance to melon aphids (*Aphis gossypii*) in *Capsicum annuum*

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**BACKGROUND** *Aphis gossypii* Glover (Hemiptera: Aphididae) or melon-cotton aphids, is one of the important insect pests in pepper in the tropics. Aphids can also transmit 22 viruses to *Solanaceae* crops, including non-persistent viruses such as CMV (*Cucumber mosaic virus*), Potyvirus (*ChiVMV*), and Polerovirus. Compared to other control practices, the use of host-plant resistance is one of the best management strategies against insect pests. Resistant varieties may also increase the suppression of the pest development in combination with biological control. Resistance sources have been identified in *Capsicum* collection. However, mostly are found in *C. baccatum* accessions including landraces and wild material. Since *C. annuum* is the major cultivated pepper species, the finding of resistance sources among *C. annuum* is still needed considering their compatibility to transfer the resistance into commercial varieties of pepper through conventional crossing and selection. Therefore this study is aimed at identification of more resistance sources in *C. annuum*.

**MATERIALS & METHODS** 40 genotypes of *Capsicum annuum* from Bogor Agricultural University and The World Vegetable Center collections were subjected to choice and non-choice tests. Melon aphids were collected from pepper cultivation at Unifarm of Bogor Agricultural University, Indonesia followed by the identification of the species to ensure that the aphid colonies were *A. gossypii* Glover. After the infestation with reared adult aphids (imago), observations were made on overall performance of the genotypes and the population development of aphids in each genotype. No insecticide was used during this experiment to avoid insecticide effects on the treatment.

**RESULTS** We detected less evidences of antixenosis based resistance; while antibiosis based resistance and induced resistance mechanisms were strongly identified in our study. In the no-choice test, all of the biological characters of aphid were affected by the genotype. There were significant differences ( $P < 0.05$ ) in response to the number of aphids infestation per leaf (number of aphid per leaf, total aphid per plant, and total winged aphid per plant) and population development among genotypes of pepper used in this study. For example, we found 3.0 adults in the most resistant genotype and 674.7 adults in the most susceptible genotypes. Interestingly, resistant genotypes with high productivity were also found in this study (Fig. 1).

**DISCUSSION & CONCLUSION** Wild relatives are already well known as good sources of resistance traits for plant genetic improvement including resistance to aphids in pepper. However, the use of wild relatives as source of resistance is constrained by biological constraints such as hybrid sterility and low crossability, retention of undesirable traits. Identification of more resistance sources in *C. annuum* in this study is important, as it will avoid cross ability constraint. It is also interesting that there is no correlation between resistance and yield. Therefore, the resistance sources in this study can be immediately used in breeding program toward aphid resistant varieties.

## ACKNOWLEDGEMENTS

Awang Maharijaya thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant.

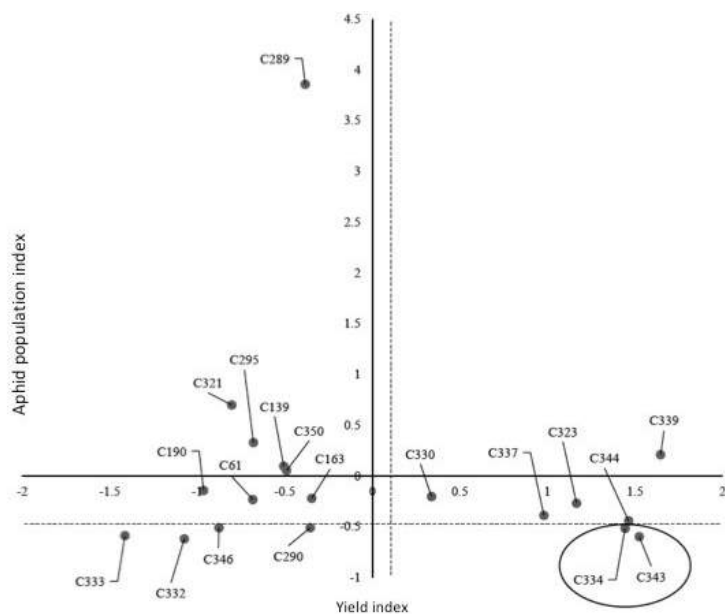


Figure 1. Biplot character of yield and Aphid population in pepper. Genotypes below the x-axis dotted line are the resistant genotypes

# Resistance to *Meloidogyne incognita* in pepper lines of the germplasm bank of IMIDA

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**BACKGROUND** *Meloidogyne incognita* is one of the main soil pathogens of the pepper crop in the greenhouses of Campo de Cartagena [1]. Disinfection with fumigants has been the most commonly used form of control to mitigate its effects. However, besides the restrictions concerning its use, these chemicals do not effectively control the nematode in greenhouses with high densities of the pathogen. The biosolarisation with organic amendments also presents some deficiencies for controlling the nematodes, while the use of resistant cultivars is increasingly considered as a viable strategy its control [2]. In pepper, the R-genes *Me1*, *Me3* and *N* confer resistance to the nematode, and the stability of this resistance is fundamental for its use in a continuous and stable way. The combination of R-genes and quantitative resistance in the same genotype is considered a good strategy to increase the durability of the R-genes [3]. The aim of this poster is to evaluate the behavior of pepper lines of the germplasm bank of Instituto Murciano de Investigación Agraria y Alimentaria (IMIDA) against *M. incognita* under controlled conditions for use in breeding programmes.

**MATERIALS & METHODS** Thirty-three genotypes were evaluated: 28 lines with no known resistance to the nematode and 5 lines with resistance to *M. incognita*, and the susceptible variety DLL was used as reference. The experimental design was of random blocks with five replications. The genotypes were inoculated with two populations of *M. incognita*, one virulent (MI-CH) and the other avirulent (MI-E) to the gene *Me3*. Plants were grown individually in 200 ml pots and were inoculated with 400 ( $\pm$  50) J2 juveniles. The test was carried out in a climatic chamber. The trial lasted 8 weeks, at the end of which the gall index, the percentage of affected plants and the number of egg masses were recorded.

**RESULTS** In the first trial, the lines P32, P33, MSS-I and PI3 showed low gall indices against the virulent and avirulent populations of the nematode and similar to those shown by Alcos (intermediate resistance) [3]. Two lines, P36 and P32, showed similar behaviour to HDA330 (carrying *Me1*). In the second test, the lines carrying the *N* gene were only resistant to the *Me3*-avirulent population. This same behaviour was shown by the hybrid P26 (Alcos  $\times$  SCM334), carrying *Me3*, and the lines S.G and K-522. Lines PI3, P32, P33 and P36 showed similar behaviour to the previous test. Line P36 showed a low level of gall index and number of egg masses with both populations, which would confirm the presence of a larger gene. The other three lines provided similar results to Alcos. The remaining genotypes showed similar results to the susceptible variety DLL.

**DISCUSSION & CONCLUSION** The results obtained demonstrated that some of the evaluated lines present a good behavior against *M. incognita*. It seems that some lines have an intermediate resistance similar to Alcos and there is a line that could be carrying the *Me1* gene. It would be interesting to corroborate these results in field condition and to further our study of the resistance of these lines, which could be used in pepper improvement programs to provide resistance to nematodes.

## REFERENCES

- [1] Ros C et al., 2014, Span. J. Agric. Res, 12(1): 225-232.
- [2] Djian-Caporalino C et al., 2011, Eur. J. Plant. Pathol, 31: 431-440.
- [3] Sánchez-Solana F et al., 2017, Pl. Pathol, doi: 10.1111/pbr.12517

Lines (R gene)	MI-E			MI-CH		
	GI <sup>1</sup>	EMs <sup>2</sup>	%plants <sup>3</sup>	GI <sup>1</sup>	EMs <sup>2</sup>	%plants <sup>3</sup>
Carolina Cayene (N)	0.2ab	0.2ab	20.0ab	1.2a	26.4b	100.0a
Carolina Wonder (N)	0.0a	0.0a	0.0a	5.1fg	235.8de	100.0a
Charleston Belle (N)	0.0a	0.0a	0.0a	5.0fg	224.8de	100.0a
K-522	1.1bcd	50.4b	40.0bc	4.6efg	191.8de	100.0a
K-801	3.6ef	213.0cd	100.0d	5.2fg	230.0de	100.0a
K.P.	3.9ef	288.6d	100.0d	4.0def	179.2de	100.0a
PI3	1.4cd	16.0b	100.0d	2.4bc	64.8bc	100.0a
P26 (Me 7 = Me3)	0.0a	0.0a	0.0a	3.6cde	69.0bc	100.0a
P32	1.2cd	12.8b	100.0d	2.0b	47.2b	100.0a
P33	1.2cd	8.4b	100.0d	NE <sup>4</sup>	NE <sup>4</sup>	100.0a
P36	0.6bc	6.6ab	60.0c	1.0a	10.2a	100.0a
PI-152225	2.8e	102.0c	100.0d	1.2a	38.6b	100.0a
PI-159536	3.9ef	278.8cd	100.0d	4.0def	136.6cd	100.0a
S.G.	0.2ab	0.4ab	20.0ab	3.2cd	62.8bc	100.0a
T.C.	5.1f	250.8cd	100.0d	4.5defg	257.0de	100.0a
Yahualica	1.6d	77.4b	40.0bc	3.5de	197.6de	100.0a
DLL (susceptible)	5.2f	283.0cd	100.0d	5.5g	299.4e	100.0a

**Table 1.** Gall index (GI), number of egg masses (EMs) and percentage of affected plants (% plants) of lines inoculated with Me3 (=Me7) avirulent (MI-E) or virulent (MI-CH) *Meloidogyne incognita* population.

The figures with the same letter in a column are not significantly different ( $P>0.05$ ) in ANOVA (1: LSD test,  $y = \log_{10}(x+1)$ ; 2: LSD test,  $y = \sqrt{x}$ ; 3: LSD test,  $y = \arcsin \sqrt{x}$ ; 4: not evaluated).



# Anatomical changes in *Capsicum annuum* leaves due to potentially toxic metals

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**BACKGROUND** The chili pepper (*Capsicum annuum*) accession UENF 1381, belonging to the UENF germplasm bank is used as a model plant because it has resistance to various diseases caused by *Xanthomonas euvesicatoria*, *Xanthomonas gardneri* and *Colletotrichum gloeosporioides*. In previous studies, differences in leaf mineral composition of macro- and micronutrients were detected in plants treated or not with potentially toxic metals. In order to understand the mechanisms of resistance to diseases and their interaction with these metals, we studied the anatomical structures of this accession treated with Hoagland nutrient solution with or without the potentially toxic metals Cd, Co, Cr, Li and Pb.

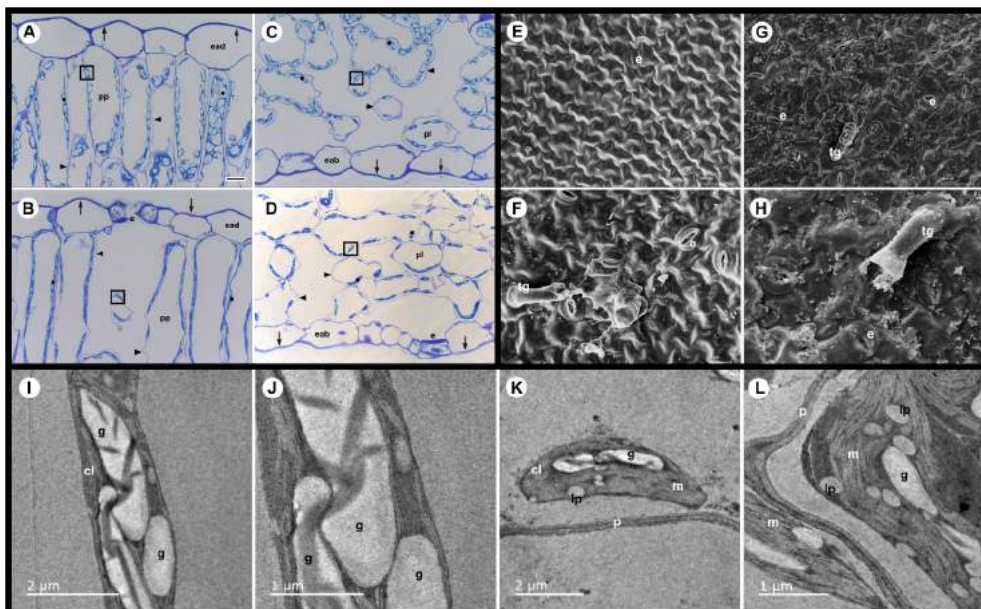
**MATERIALS & METHODS** Accession UENF 1381 was grown in pots containing sand and vermiculite mix in a greenhouse at Campos dos Goytacazes, RJ, Brazil. The experimental design was a randomized complete block with two treatments (a: UENF 1381 + Hoagland, and b: UENF 1381 + Hoagland with addition of Cd, Co, Cr VI, Li and Pb) and five replications [1]. Irrigation was performed daily. The leaves were collected in the second caulinar node from the apex with the aid of a 1 cm diameter circular cutter. Samples were fixed, washed, dehydrated and further processed for light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) [2].

**RESULTS** From LM analysis, it was observed that treatment without addition of metals caused an increase in cuticle thickness, cytoplasmic retraction (especially on adaxial side) and starch granule accumulation in both leaf sides (Fig. 1, A, B, C and D). When observed in SEM, leaves treated with nutrient solution with metals, showed a higher frequency of stomata and granular trichomes on the adaxial surface visibly with wider ornamentation (Fig. 1, E and G). Furthermore, after addition of metals, leaves showed thicker wax layer and wider ornamentation on the abaxial surface (Fig. 1, F and H). The amount of stomata and the arrangement of granular trichomes were similar in both treatments. TEM confirmed the significant differences in the chloroplast ultrastructure. Starch granules were observed, as well as cytoplasmic retraction, thylakoid membranes and lipid droplets. The addition of metals resulted in lower accumulation of starch in the chloroplasts (Fig. 1, I, J, K and L). It also allowed a better observation of thylakoid membranes and oil droplets on the treatment with addition of metals (Fig. 1, K and L).

**DISCUSSION & CONCLUSION** The treatment with addition of metals promoted anatomical and ultrastructural changes in UENF 1381 leaves. It reduced energy sources (smaller chloroplasts with less starch) and weakened the physical barriers, since it reduced the cuticle barrier. The addition of metals possibly favors mechanisms that decrease the resistance of the studied genotype. Due to the structural differences observed, they can significantly influence defense mechanisms. Studies relating defense with structure are needed to better elucidate how the nutrient solution influences the defense mechanisms.

## REFERENCES

- [1] Lahner B et al., 2003, Nature Biotechnology, 21(10): 1215-1221. doi: 10.1038/nbt865
- [2] de Boer P et al., 2015, Nature Methods, 12(6): 503. doi:10.1038/nmeth.3400



**Figure 1.** Leaf surface of *Capsicum annuum* UENF 1381. Treatments with Hoagland (A, C, E, F, I and J). Treatments with Hoagland and metals (B, D, G, H, K and L). Light micrographs of adaxial (A and B) and abaxial (C and D) leaf surfaces, bar: 10  $\mu$ m. Scanning electron micrographs of adaxial (E and G) and abaxial (F and H) surfaces, bars: EG = 50  $\mu$ m, FH = 20  $\mu$ m. Transmission electron micrographs are I, J, K and L. ● – indicative of primary starch; c – cytoplasmic membrane; ↓ – cuticle; cl – chloroplast; e – stomata; eab – abaxial epidermis; ead – adaxial epidermis; g – starch granule; lp – lipid droplets; m – thylakoid membranes; p – cell wall; pl – lacunar parenchyma; pp – palisade parenchyma; and tg – glandular trichoma. .

# Recombinant inbred lines of *Capsicum baccatum* var. *pendulum* resistant to Pepper yellow mosaic virus

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**BACKGROUND** Fruits of *Capsicum baccatum* var. *pendulum* have been largely widely used in food industry, especially in South America. The demand for new cultivars, that associate high yield, good culinary attributes and disease resistances, is challenging for plant breeders. Diseases caused by viruses are responsible for large crop losses and may even limit production of a particular species in regions whose conditions are conducive to plant-pathogen interactions. In sweet and chili pepper, *Pepper yellow mosaic virus* (PepYMV) is one of the most important diseases in Brazil. Preventive measures such as seedlings production in anti-aphids environment and elimination of host plants contribute to the disease control. However, the use of resistant cultivars is the most efficient alternative for virus disease control. Developing new commercial genotypes resistant to PepYMV is important to meet the needs of producers and consumers. In this work, we report the evaluation of F6 recombinant inbred lines of *C. baccatum* var. *pendulum* to PepYMV resistance.

**MATERIALS & METHODS** Forty-seven recombinant inbred lines - RILs (F6), from crosses between UENFI 616 (susceptible) x UENFI 732 (resistant), were evaluated for PepYMV resistance. The experiment was carried out in randomized blocks with eight replications and one plant per plot, totaling 416 plants. The plants were grown in cages with anti-aphid screen. *Nicotiana debneyi* plants were used to produce virus inoculum. Virus inoculated *N. debneyi* leaves were macerated and carborundum (600 mesh) were used to mechanically inoculated RILs. The Incubation Period-IP and the Area Under the Disease Progress Curve-AUDPC were calculated from the rating scale on alternate days for 60 days [2]. Genotypes 'Criollo de Morelos' and 'Ikeda' were used as resistant and susceptible controls, respectively.

**RESULTS** The incubation period-IP ranged from 14 to 59 days. AUDPC ranged from 17 to 72, showing different degrees of severity. The cultivar Ikeda, susceptibility pattern, had symptoms at 15 days. The 'Criollo de Morelos' resistance pattern had the highest IP (59 days) and the lowest AUDPC (17.5). Lines 345 and 414 stood out for presenting symptoms at 42 days post inoculation. Six lines were susceptible, 38 were moderately resistant, two resistant, and line 345 was considered highly resistant with AUDPC of 17.7 and 53 days of IP. Lines 414 and 506 had AUDPC of 20.2 and 20.7, with IP of 42 and 38, respectively. The evaluation of IP for resistance to PepYMV is of paramount importance, since the transmission of this virus is carried out by aphids during the «test bite» [3]. In this case, the use of pesticides to eliminate aphids is not efficient to control the disease. Thus, the classification for resistance to PepYMV should be judicious, considering only the resistant and highly resistant genotypes based on more than one resistance variable, always including the incubation period.

**DISCUSSION & CONCLUSION** The incubation period is fundamental in the evaluation of PepYMV resistance to identify promising genotypes. The F6 lines of *C. baccatum* var. *pendulum* 414, 506 and 345 were selected to final agronomic tests in order to recommend them as new commercial genotypes. In addition, these RILs could be used as sources of resistance to PepYMV.

## REFERENCES

- [1] Sricharoen P et al., 2017, Ultrasonics sonochemistry, 38, 629-639. doi: 10.1016/j.ultsonch.2016.08.018
- [2] Bento CS et al., 2013, Genet Mol Res, 12: 1074-1082. doi: 10.4238/2013.April.10.3
- [3] Gioria R et al., 2009, Scientia Agricola 66(2): 267-269. doi: 10.1590/S0103-90162009000200017



# Reduced phloem uptake of *Myzus persicae* on an aphid resistant pepper (*Capsicum baccatum*) accession

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**BACKGROUND** The green peach aphid (GPA), *Myzus persicae*, is one of the most threatening pests in pepper cultivation. It not only causes direct damage but it also transmits many viruses. Breeding aphid resistant pepper varieties is a promising and environmentally friendly method to control aphid populations in the field and in the greenhouse. Before this study no strong sources of resistance against the GPA had been identified. Therefore, the main aims of this study were to identify pepper materials with a good level of resistance to GPA and to elucidate possible resistance mechanisms.

**MATERIALS & METHODS** We initially studied 50 accessions of *Capsicum annum*, *C. chinense*, *C. frutescens* and *C. baccatum*. Subsequently 24 additional *C. baccatum* accessions were screened. The *Myzus persicae* strain originated from the Netherlands. Resistance tests were performed using clip cages containing GPA nymphs placed on eight-week-old plants. Survival of the original aphids and reproduction were scored. The feeding behaviour of GPA was studied using the Electrical Penetration Graph (EPG) technique, as previously described [1]. Callose deposition was studied by aniline blue fluorescent staining. The expression of callose related genes was analysed by quantitative real-time PCR.

**RESULTS** We screened 74 pepper accessions from different geographical areas for resistance to *M. persicae* [2]. After four rounds of evaluation, we identified one *Capsicum baccatum* accession (PB2013071) as highly resistant to *M. persicae*, while the *C. baccatum* accessions PB2013062 and PB2012022 showed intermediate resistance. The resistance of PB2013071 resulted in a severely reduced uptake of phloem compared to the susceptible *C. baccatum* accession PB2013046, as determined by Electrical Penetration Graph (EPG) studies. Feeding of *M. persicae* induced the expression of callose synthase genes and resulted in callose deposition in the sieve elements in resistant, but not in susceptible plants.

**DISCUSSION & CONCLUSION** Three aphid resistant pepper accessions were identified, which will be important for breeding aphid resistant pepper varieties in the future. The most resistant accession PB2013071 showed phloem-based resistance against aphid infestation.

## REFERENCES

- [1] Alvarez et al., 2013, Insect Sci. 20: 207–227.
- [2] Sun et al., 2018, BMC Plant Biol. 18: 138–151.

## ACKNOWLEDGEMENTS

Roeland Voorrips thanks the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



# Evaluation of GWAS performance to dissect *Phytophthora capsici* aggressiveness toward *Capsicum annuum*

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**BACKGROUND** Plant – pathogen gene interactions are extensively studied, with a major focus on major R genes – pathogenicity effector genes and their co-evolution. While providing molecular evidence for qualitative resistance breakdown, this candidate gene approach does not reveal much about quantitative resistance erosion. *Phytophthora capsici* is a major threat to *Capsicum annuum*. With only quantitative resistance, and no known interacting pathogenicity genes, detecting new candidate pathogenicity genes would be extremely useful to understand the interaction at the molecular level. Genome-wide association studies have a great potential for pathogens with sexual reproduction [1], and can overcome the frequent segregation distortion seen in Oomycetes biparental progenies. We therefore tested the feasibility of association studies on *P. capsici*, using a *Solanum lycopersicum* – *P. capsici* dataset.

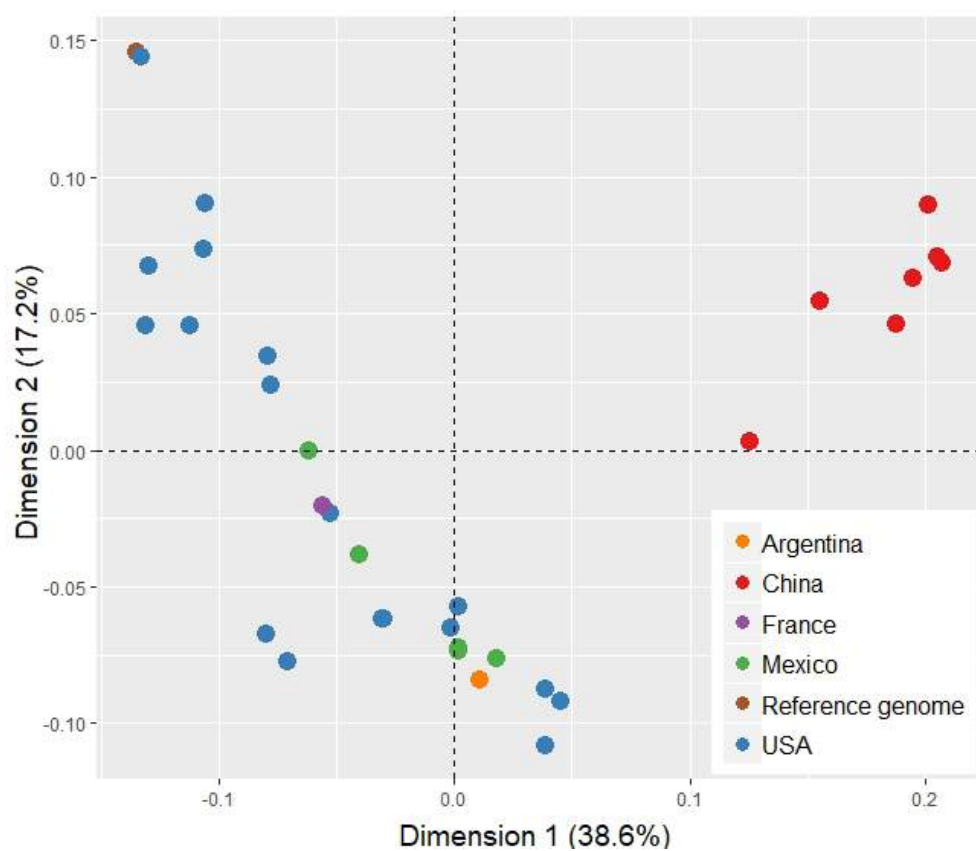
**MATERIALS & METHODS** A set of 34 *P. capsici* isolates were inoculated on tomato cv. MoneyMaker. After 24 and 72 hours post infection (hpi), lesions were sampled and sequenced (Illumina paired end RNA-Seq) with one replication. Single Nucleotide Polymorphisms (SNPs) were searched against the reference genomes [2][3] with GATK (following best practices protocol), and SNPs were conserved with a minimum allele frequency (MAF) of 0.2. Phenotypic values were approximated using the proportion of *P. capsici* read counts on the total mapped read counts for each library. Linkage Disequilibrium (LD), kinship matrix, genetic structure matrix, and genetic association were computed in Tassel. Bayesian clustering was performed on Structure 2.3.4 (50k burn-in, 50k bootstraps) and Evanno method for optimal cluster size estimation.

**RESULTS** *P. capsici* reads represented between 5.5% and 53.5% of libraries at 24hpi, and between 48.9% and 56.5% at 72 hpi. As phenotypic variation in aggressiveness was measured as the proportion of *P. capsici* per library, only 24hpi phenotypic data were retained for phenotype-genotype association. The 24hpi and 72hpi libraries were pooled, and 202 117 bi-allelic SNPs were found with a MAF > 0.2. Isolates were highly outcrossed with heterozygous SNPs representing between 47 % and 96 % of all SNPs. Average LD on the full population was estimated at 10 kbp. Multidimensional scaling plot shows a great differentiation of Chinese isolates over the rest of the world (Figure 1), as confirmed by Bayesian clustering (not shown). Trials to detect association on the 5 longest scaffolds using a mixed linear model with EMMA algorithm showed 2 SNPs on scaffold Sc\_01, 3 on Sc\_03, 12 on Sc\_04 and 7 on Sc\_05, with several of them positioned within known pathogenicity genes.

**DISCUSSION & CONCLUSION** These preliminary results reveal a small average LD that confirmed the frequent occurrence of sexual reproduction and large efficient population size, together with the lack of association between genetic structure and phenotype. Considering *P. capsici* small genome (65Mb), power of detection could be improved with whole genome DNA-Seq on a larger isolate collection. Genome-wide association studies for dissecting genetic determinants of aggressiveness in *P. capsici* has great potential, and will be performed on various *C. annuum* bearing quantitative resistance to identify novel candidate genetic factors in the interaction.

## REFERENCES

- [1] Plissonneau C et al., 2017, Frontiers in Plant Science, 8, 1–15.
- [2] Lamour K H et al., 2012, Molecular Plant-Microbe Interactions, 25(10), 1350–1360.
- [3] Sato S et al., 2012, Nature, 485(7400), 635–641.



**Figure 1.** Multidimensional scaling plot of 33 *P. capsici* isolates based on their geographical origin, using 202 117 biallelic SNPs.



# Development of male sterility-based powdery mildew-resistant high yielding F1 hybrid in chilli (*Capsicum annuum* L.)

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**BACKGROUND** The required goals of increasing productivity in the quickest possible time can be achieved only through heterosis breeding which is feasible in chili crop. Exploitation of natural out crossing could render commercial hybrid seed production technology economically viable through use of male sterile lines. Powdery mildew of chili incited by *Leveillula taurica* is one of the most serious diseases of chili. Chili production in India is responsible of heavy yield loss ranging from 14 to 30 per cent [1]. The use of systemic fungicides for control is not effective. Besides this, its indiscriminate use causes pathogen resistant strains development [2]. Perfect solution to keep disease away from the crop is to develop resistant variety/hybrid. Among the limited commercial hybrids available in chili none is resistant to powdery mildew. Heredity studies of *Capsicum annuum* showed that resistance to powdery mildew is dominant and polygenic [3]. Hence, it may be possible to develop high yielding hybrids resistant to powdery mildew. The present study was conducted for commercial exploitation of powdery mildew resistant hybrid in chili.

**MATERIALS & METHODS** Two geno-cytoplasmic male sterile lines GCMS lines of chili and 10 fertility restorer were used for development of hybrids and screening for resistance to powdery mildew. 20 hybrids were produced during 2010-11 using line x tester design. Station and multilocation trials were conducted using Completely Randomized Block Design with three replications during 2011-12 to 2013-14 and 2014-15 and 2015-16 respectively at University of Agricultural Sciences, Raichur, Karnataka, India. The promising hybrids were recommended for release by conducting farm trials during 2016-17. All of these sterile and restorer lines and 2 best hybrids were screened in natural epiphytotic conditions for powdery mildew resistance during 2018-19.

**RESULTS** The GCMS based hybrid UARCH42 registered significantly highest mean dry fruit yield (4900kg/ha) over non GCMS based hybrid Sitara (3936kg/ha) in station trials (Table 1). Multilocation trials were conducted over three locations and found that hybrid UARCH42 recorded significantly highest mean dry fruit yield of 4248kg/ha over standard non GCMS based check hybrid sitara (2272kg/ha). The high yielding hybrids were tested by conducting 6 large scale demonstrations and 17 farm trials in farmer's field and found that the hybrid UARCH42 registered highest average dry fruit yield of 3495kg/ha over check hybrid Sitara (3025kg/ha) which was 15.53 per cent superior. None of the male sterile lines, restorer lines and hybrids screened for powdery mildew disease was found to be Immune (I) in reaction in natural epiphytotic conditions (Table 1). However, one sterile line JNA1, one maintainer line JNB1 and one hybrid UARCH42 were found to be highly resistant and one restorer line BVC42 and one hybrid UARCH43 found to be resistant reaction to powdery mildew. Resistant and susceptible phenotypes are clearly distinct (Figure 1).

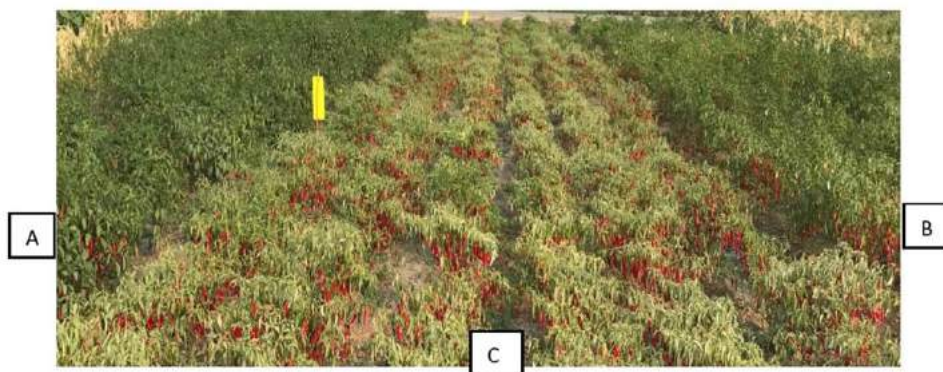
**DISCUSSION & CONCLUSION** The hybrid UARCH42 recorded significantly highest mean dry fruit yield in station trials and multilocation as well as farm trials indicating the stable performance of the hybrid over the year and the location. Hybrid UARCH42 and UARCH43 showed highly resistant and resistant reaction to powdery mildew respectively because of the high resistance of the male sterile line used to produce hybrid UARCH42 and the resistant male parent used to produce both hybrids. These results indicate that inheritance of resistance to powdery mildew is dominant. Both the resistant hybrids developed are useful to the farming community to boost the chili yield in commercial level.

## REFERENCES

- [1] Mathur RI et al., 1972, Indian J. Mycol. and Pl. Pathol., 2:182-183  
 [2] Bergamin FA et al. 1995, Manual de fitopatologia: princípios e conceitos. 3.ed. São Paulo: Ceres, 1:919p  
 [3] Murthy HMK, Deshpande AA, 1997, Veg. Sci., 24: p127131

**Table 1: Performance of High yielding hybrids and reaction to powdery mildew resistance in chili**

Hybrid/ Genotypes	Station trials				Multilocation Trials						Disease reaction		
	RA 11-12	RA 12-13	RA 13-14	Mean Yield Kg/ha	RA 14-15	RA 15-16	HMT 14-15	Bgudi 14- 15	HG15- 16	Mean Yield (kg/ha)	Hybrids/ Genotypes	Reaction	%leaf area infected
F1 UARCH-42	4008*	6033*	4661*	4900	3029*	6467*	4393*	1823*	3577*	4248	F1 UARCH42	Highly resistant	Upto 1
F1 UARCH43	3397*	5240	4026	4224	2875*	5896*	3292*	1474*	3530*	3716	F1 UARCH43	Resistant	1-10
F1 Indam5	--	--	--	--	908	3851	1273	975	2200	1841	F1 Indam5	Highly susceptible	>50
BVC1	2949	5137	--	4043	1757	2700	2051	--	2837	2905	BVC42	Resistant	1-10
B.Dabbi(c)	--	3468	2747	3107	1030	2750	441	615	-	1841	B.Dabbi	Highly susceptible	>50
F1 Sitara	2251	5420	4138	3936	1854	4329	708	1337	2743	2272	JNB1	Highly resistant	Upto 1
CV	9.18	4.98	5.11		14.16	13.2	7.24	6.9	4.72		JNA1	Highly resistant	Upto 1
CD 5%	222	309	204		451	1075	241	121	207				



**Figure I.** Reaction to powdery mildew resistance in chili. A- UARCH42, highly resistant, B –UARCH43 resistant; C- Indam 5 highly susceptible.

# ISF guidelines on the nomination of novel plant pest races

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**BACKGROUND** Disease resistance is a major goal in breeding new varieties and plays a key role in vegetable crop production and integrated pest management practices. It is also carefully described to differentiate new varieties from older ones on the market. The objective of the Working Group on Disease Resistance Terminology at International Seed Federation is to promote the consistent use of terminology in relation to disease resistance.

**MATERIALS & METHODS** The Working Group codes pathogens for which companies claim resistance in their varieties, promotes harmonized terminology across the industry to avoid any liability due to miscommunication, develops host differentials, and establishes procedures based on peer-reviewed scientific publication and industry practices to identify pathogen races/strains.

**RESULTS** To support the decision process associated with the nomination of novel races, the Working Group has formulated recommended guidelines. The recommendations constitute the minimum criteria that should be fulfilled in order to nominate a novel race for a specific plant pest. One of the most important selection criteria is the relative economic importance of the emerging resistance breaking event. The pest should have caused significant economic damage at least once, the geographical extent of this event should be of significance, and the event should be recurrent in time having been observed over multiple growing seasons and/or years. The guidelines include basic rules for nomination and numbering of novel races.

**DISCUSSION & CONCLUSION** The nomination criteria are:

The pathogenicity and/or resistance-breaking event observed on the pest-host interaction should be novel

The pathogenicity and/or resistance-breaking event should fulfil at least two of the three conditions below:

- The pest should have caused significant economic damage at least once.
- The geographical extent of this event should be of significance.
- The event should be recurrent in time having been observed over multiple growing seasons and/or years.

A stable isolate must be available and established as reference material.

Using the reference isolate, the characteristic of the event must be reproducible in a controlled disease test

Nomination of a new race cannot be done by a single stakeholder; but several independent stakeholders.

## REFERENCES

[https://www.worldseed.org/wp-content/uploads/2018/07/Guidelines-on-race-nomination\\_18.06.2018\\_FINAL\\_edited.pdf](https://www.worldseed.org/wp-content/uploads/2018/07/Guidelines-on-race-nomination_18.06.2018_FINAL_edited.pdf)



# Study of recessive bacterial leaf spot resistance genes in *Capsicum annuum* L.

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**BACKGROUND** Monogenic, dominant resistance to *Xanthomonas euvesicatoria* bacteria encoded by a few different genes (*Bs1*, *Bs2*, *Bs3*, *Bs4*, *Bs7*) is based on hypersensitive reaction (HR). Resistance gene acting by tissue retention (non-HR) was published at first in 1995 (*gds*) [1], then in 2002 (*bs5*, *bs6*) [2]. We examined the relation between *gds* and *bs5* genes. Both *gds* and *bs5* genes are recessive.

**MATERIALS & METHODS** Chile-type pepper lines containing *gds* gene in homozygous form (*gds/gds*), ECW50 pepper lines containing *bs5* gene in homozygous form (*bs5/bs5*), ECW60 pepper lines containing *bs6* gene (*bs6/bs6*) were planted in greenhouse. Susceptible *Szegedi-80* (+/+) chile-type variety was planted as control. Crossings were made in order to examine the relation between *gds* and *bs5* genes based on the segregation rates. Leafs of plants at ~8 leaf stage were infiltrated with 108 cfu/ml *Xanthomonas euvesicatoria* suspension. Bacteria were isolated from peppers in open field in Hungary. The identity of the isolate was validated by full genom sequencing [3]. The symptoms were evaluated 7 days after infiltration. ].].

**RESULTS** Reaction of homozygous plants with *gds* and *bs5* resistance gene for infiltration by *Xanthomonas euvesicatoria* were the same and couldn't be distinguished from each other. Plants containing *bs6* gene was susceptible like the control variety *Szegedi-80*.

The *gds*/+ as well as *bs5*/+ F1 plants showed uniformly susceptible symptoms 7 days after infiltration. In the *gds/gds* x +/+ F2 generation we found 261 susceptible and 94 resistant plants, which is in agreement with the expected 3:1 segregation rate. In the *bs5/bs5* x +/+ F2 generation we found 25 susceptible and 10 resistant plants, which also indicates 3:1 segregation rate.

The *gds/gds* x *bs5/bs5* F1 (n=40) and F2 (n=72) plants showed uniformly resistant symptoms.

The *bs5*/+ x *bs5/bs5* F1 crossing resulted 16 resistant and 13 susceptible plants, which was not different from the expected 1:1 segregation rate.

The *gds*/+ x *bs5/bs5* F1 crossing resulted 76 resistant and 89 susceptible plants, which also suggests 1:1 segregation rate.

The *gds*/+ x *bs5*/+ F1 crossing resulted 36 susceptible 15 resistant plants, in agreement with a 3:1 segregation rate.

**DISCUSSION & CONCLUSION** Resistance symptoms caused by *gds* and *bs5* genes are phenotypically the same. The test crossings confirmed that these two resistance genes are identical. The *bs6*, which was originally described as a resistance gene, gave susceptible plants in our study.

## REFERENCES

- [1] Szarka J and Csilléry G, 1995, Proceeding of 9th Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant: 184-187
- [2] Jones, JB, Minsavage GV. et al., 2002, Phytopathology: 92(3):273-7. doi: 10.1094/PHYTO.2002.92.3.273
- [3] Barak JD, Vancheva T. et al., 2016, Front. Plant Sci. 7:1805. doi: 10.3389/fpls.2016.01805



**Figure 1.** Symptoms induced by *Xanthomonas euvesicatoria* on *Capsicum annuum* L. A- homozygous for *gds* resistance gene; B- homozygous for *bs5* resistance gene; C -susceptible control.

# Tolerance of eggplant rootstocks to *Verticillium dahliae* and nematodes

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**BACKGROUND** *Verticillium dahliae* and nematodes are diseases affecting French eggplant production, causing significant yield losses and drastically modifying cropping systems from soil production to soilless culture. To limit and prevent these diseases, eggplants are grafted onto tomato rootstocks or *Solanum torvum* [1], but grafting can cause other problems due to insufficient resistance of the rootstock, low grafting compatibility, or other agronomic reasons. The two widely used cultivars of tomato rootstocks, Beaufort and Maxifort, are too vigorous, and can collapse from *Verticillium* wilt in cases of high disease pressure. *Solanum torvum* needs high temperature and thus cannot be used for very early production. For these reasons, new types of rootstock have been made available to producers, but without any indication of disease tolerance. This experiment evaluated under semi-controlled conditions with artificial inoculation, the resistance of the seven most used rootstocks in France.

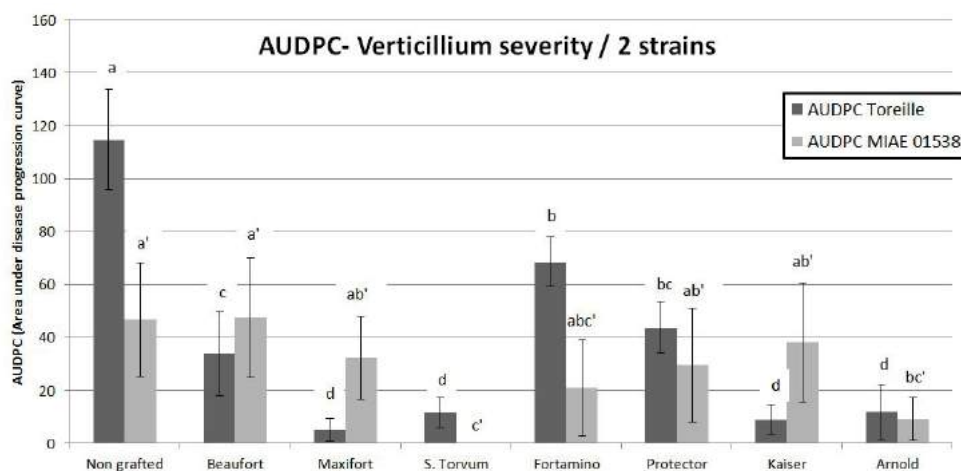
**MATERIALS & METHODS** Rootstocks evaluated were Beaufort, Maxifort, Fortamino, Protector, Kaiser, Arnold, and *Solanum torvum* STT3, grafted with Black Pear and compared with non-grafted plants. Plants were grown in 10 liter pots under greenhouse conditions. For *Verticillium* inoculation, pots were filled with 50% sand and 50% sieved compost. For nematodes inoculation, pots were filled with contaminated soil (1/3), sand (1/3) and sieved compost (1/3). Inoculation of *Verticillium* (French isolates Toreille and MIAE 01538) was done the day of planting, about 15 ml of 10<sup>6</sup> spores/ml of MS50 medium in each pot. Nematodes (*Meloidogyne arenaria*) were inoculated from natural infected soil localized on Aureille, France.

**RESULTS** Both inoculations with nematodes and *Verticillium* were effective, and first yellowing of plants appeared 17 days after planting. We measured twice a week incidence and severity of symptoms (yellowing and wilting plants). Concerning verticillium resistance of rootstocks, it appeared that behavior depends on strains of *verticillium*. Whereas 90% of non-grafted plants presented yellowing symptoms with Toreille, only 42% were noted with MIAE 01538. *Solanum torvum*, which is known to have resistance genes to *Verticillium*, is attacked by Toreille, but not by MIAE 01538. Among tomato rootstocks, the Toreille isolate affects the most Fortamino whereas, Arnold rootstock seems very resistant to both strains. At the end of the experiment, nematodes galls were counted on plants, and estimated with the Zeck Scale: Beaufort was the most susceptible, as compared to *S. torvum*, Protector, and Arnold where no galls were counted. However, it is important to note that the mean of Zeck notes were quite low, due to high temperatures in the greenhouse.

**DISCUSSION & CONCLUSION** Artificial inoculation permitted the characterize of tolerance of grafted plants to *Verticillium* and nematodes, with significant differences among tomato rootstocks. This experiment proved that tolerance is linked to the *Verticillium* strain. This has recently been mentioned by technicians located in the South West of France where severe wilting have been seen in the last years with *Solanum torvum* rootstock. The experiment characterized tolerance against two pathogens separately, whereas in standard crop production conditions, collapses can be due to a complex of pathogens including *Colletotrichum coccodes*, *Macrophomina phaseolina*, *Pythium* sp and *Rhizoctonia solani* [2]. Further investigations are warranted.

## REFERENCES

- [1] Inderbitzin P, Subbarao, KV, 2014. *Verticillium* Systematics and Evolution: How Confusion Impedes *Verticillium* Wilt Management and How to Resolve It. *Phytopathology* 104, 564–574.
- [2] Villeneuve F, Latour F et al, 2016, Greenhouse eggplant crop in France: success and failures of grafting for controlling *Verticillium* sp. XVI Eucarpia, Kecskemét, Hungary, *capsicum* and eggplant meeting, pp. 455-460.



Disease severity of seven rootstocks against two strains of *Verticillium dahliae*



# Fine mapping of the anthracnose resistance major QTL $AnR_{GO5}$ in *Capsicum chinense* PBC932

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**BACKGROUND** Anthracnose is a major disease affecting the yield and quality of pepper [1]. Anthracnose is caused by a variety of *Colletotrichum* species. Among them, *Colletotrichum acutatum* is widely spread in China, is harmful to fruits, and has strong pathogenicity and fungicide resistance, causing severe reduction in production. The symptoms of anthracnose appear as sunken necrotic tissue and the presence of acervuli. To date, 24 *Colletotrichum* species have been identified as pathogens causing chili anthracnose [2]. *Colletotrichum acutatum* has been reported as one significant pathogen in many countries such as China. *Capsicum chinense* 'PBC932' with immune resistance was identified by the World Vegetable Center-AVRDC. The goal of this work was to map the resistance gene(s) found in 'PBC932'.

**MATERIALS & METHODS** A F<sub>1</sub> generation was derived from single interspecific crosses of *C. annuum* '77013' × *C. chinense* 'PBC932'. Because the plants of BC1, BC2, BC3 generation were self-incompatible (difficult to harvest fruits), interspecific BC4F<sub>1</sub>, BC4F<sub>2</sub> and BC4F<sub>3</sub> progenies [( '77013' × 'PBC932' ) × '77013' ] were obtained to map QTL  $AnR_{GO5}$ . Using Kaspar and Indel markers linked to  $AnR_{GO5}$  at the green mature stage and Join Map 4.0 software, a genetic linkage map of BC4F<sub>1</sub> was constructed. Plants of BC4F<sub>1</sub>, BC4F<sub>2</sub> and BC4F<sub>3</sub> progenies were inoculated with *Colletotrichum acutatum*. Using MapQTL 6.0 with LOD 3.0 and a step size of 0.5, QTL  $AnR_{GO5}$  was identified. According to the phenotype of recombinant individual plants of BC4F<sub>2</sub> and BC4F<sub>3</sub> progenies, fine mapping allowed narrowing of the interval.

**RESULTS** Previous studies have shown that the resistance genes are on chromosome 5, and QTL  $AnR_{GO5}$  was located between InDel and HpmsE116 marker. Markers between InDel and HpmsE116 marker were developed, and a genetic linkage map with 42 markers, 24.4 cM in length was constructed. The map information was combined with the parameter of the true lesion diameter and a QTL related to anthracnose resistance was predicted using Inclusive Composite Interval Mapping (ICIM). The most closely linked QTL marker was P5L-P-67, with a contribution rate of 69.3% and a LOD of 24.37. With a 95% confidence interval for the QTL,  $AnR_{GO5}$  was identified between P5L-P-137 and UN16000\_1166-1. The recombinant individual plants of BC4F<sub>2</sub> and BC4F<sub>3</sub> and their progenies were screened with markers between P5L-P-137 and UN16000\_1166-1. According to the phenotype of fruits of recombinant individual plants, the major QTL  $AnR_{GO5}$  was finally located between P5in-2266-404 and P5in-2268-978, and the physical distance was 164 kb. According to the 'CM334' genome sequence (<http://passport.pepper.snu.ac.kr/?t=PGENOME>), 5 genes were predicted in the fine mapping positioning interval (Fig.1).

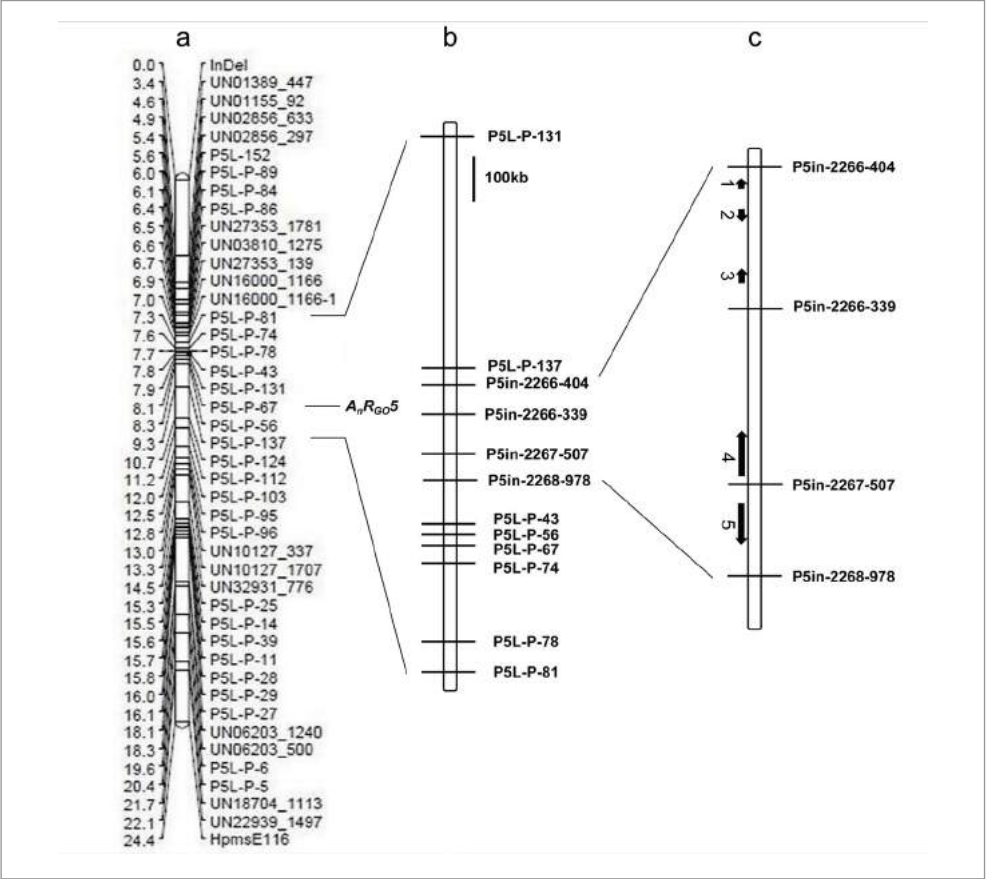
**DISCUSSION & CONCLUSION** One major QTL for anthracnose resistance,  $AnR_{GO5}$ , was located on chromosome 5, which fit the previous QTL studies that detected one major QTLs at the same location [3]. The markers developed in this study can be used for molecular marker-assisted selection (MAS). The genes in the fine mapping interval were CA05g17700, CA05g17710, CA05g17720, CA05g17730, and CA05g17740. Additional studies are needed to find the candidate gene.

## REFERENCES

- [1] Than PP, Jeewon R, et al., 2008, Plant Pathology, 57(3):562-572
- [2] Mongkolporn O, Taylor PWJ, 2018, Plant Pathology, 67(6):1255-1263
- [3] Sun C, Mao S L, et al., 2015, Scientia Horticulturae, 181: 81-88.

ACKNOWLEDGEMENTS

Lihao Wang thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



**Figure 1.** Genetic linkage map of chromosome 5 from the BC4F1 population.

a: The genetic linkage map constructed according to the BC4F1 population contained a total of 44 markers.

b: The  $AnR_{Go5}$  locus was confirmed and narrowed down to the P5in-2266-404 and P5in-2267-978 interval.

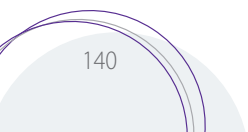
c: Predicted genes in the  $AnR_{Go5}$  region. Numbers indicate predicted genes and the arrows indicate the direction of transcription.





# NUTRITIONAL VALUE AND QUALITY TRAITS

SESSION 3



## Breeding for fruit quality traits in pepper

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Consumption of fruits and vegetables is regarded as an essential part of a healthy diet. *Solanaceous* crops such as tomato, pepper and eggplant are among the most widely consumed vegetables worldwide and are regarded as a valuable source of healthy ingredients. Although historically, breeding has focussed on producer traits, such as yield, disease resistance and shelf-life, consumer quality traits have gained increasing attention in the last two decades. Breeding for healthy ingredients has long been hampered by difficulties in detecting plant metabolites in an easy and reliable way. Advances in metabolomics technologies have made it possible to get a broad overview of the metabolic composition of *Solanaceous* fruits and vegetables. They have also given information on the metabolic variation existing within crop species and even between crop species. By combining genetic, next generation sequencing and X-omics approaches, metabolite QTLs (mQTLs) for the production of quality traits, such as flavour, nutritional value and colour can be found and further characterised to identify the underlying key genes and metabolic pathways. This approach was used to study the genetic regulation of several health and flavour-related compounds in pepper. In this lecture, several examples will be discussed in light of current and future perspectives.

Pepper fruits are regarded as an excellent source of metabolites with potential health-promoting properties, such as vitamin C, vitamin E, flavonoids and capsaicinoids. A metabolic screen of 32 pepper accessions revealed two accessions with outstanding levels of several health-related metabolites. Accession *C. annuum* Long Sweet was selected for its high levels of the flavonoid quercetin, while accession *C. annuum* AC2212 was above average in vitamin C and carotenoids, and showed exceptional levels of vitamin E (tocopherols). Based on these two parents, an F2 and an F6 RIL population were developed and phenotyped for variation in semi-polar (e.g. flavonoids) and non-polar (e.g. carotenoids, chlorophylls and tocopherols) metabolites.

QTL analysis of the F2 population revealed a strong QTL for quercetin on pepper chromosome P5, which was confirmed in NILs contrasting for the QTL region. A candidate gene in this region, homologous to the known tomato flavonoid pathway regulator *SlMYB12*, was differentially expressed in high- and low quercetin lines. RNA seq analysis of contrasting *MYB12*-like NILs provided a global view of the action of this transcription factor and supported its function as a regulator of the flavonoid pathway in pepper. Follow-up reverse genetics studies in pepper and tomato confirmed the role *CaMYB12*-like in the regulation of the flavonoid pathway.

A QTL for vitamin E co-localised with a QTL for immature green fruit colour and chlorophyll content. This QTL region harbored the gene encoding the golden 2-like transcription factor *CaGLK2*, which was shown to be a key regulator of chloroplast development, chlorophyll and vitamin E content, based on reverse genetic studies in tomato and genetic studies in pepper. To confirm the role of *CaGLK2* in chloroplast development in pepper and to test its possible role in regulating vitamin E content, virus-induced gene silencing (VIGS) was used to silence the expression of *CaGLK2* in the AC2212 background. VIGS-treated fruits showed clearly visible pale green sectors in immature fruits, which developed into light brown sectors in a dark brown background upon fruit maturation. Morphological and metabolic analysis of the VIGS plants is currently underway and will be discussed.

In addition to health-related compounds, pepper displays wide variation in fruit aromas. A set of 35 pepper accessions was screened for variation in taste and aroma, using sensory panels, as well as variation in sugars, acids and flavour-related volatiles. The *C. baccatum* accession PEN45 had a distinct fruity “papaya-like” aroma. To study the genetics underlying this aroma, a BIL population was developed based on the cross of this accession with a blocky pepper as recurrent parent. The distinct aroma was successfully introgressed into the blocky pepper background and was associated with a small introgression on chromosome P3. Metabolic analysis of BILs and NILs contrasting for this introgression region revealed four volatiles whose levels were linked to this *C. baccatum* introgression. Two volatiles (6-methyl-4-oxo-5 heptenal and a yet unknown volatile) showed strongly increased levels in the NILs containing the *C. baccatum* introgression, while the levels of (Z)-butanoic acid 3-hexenyl ester and 2-isobutyl-3-methoxypyrazine were decreased in the introgression-containing NILs. Detailed analysis of homozygous and heterozygous NILs suggested that perception of the “papaya-like” aroma is not only dependent on the presence of the volatiles that are increased in the NILs with the *C. baccatum* introgression, but may be partially masked by the presence of isobutyl-methoxypyrazine, which is responsible for the characteristic “green bell pepper” aroma. Further genetic and metabolic analysis of sub-NILs having the P3 introgression is needed to further elucidate the biochemical and molecular basis for the “papaya-like” aroma.

These examples, using fairly small accession panels, clearly show the potential of understanding and utilising pepper genetic resources for the improvement of fruit quality traits in pepper. In the frame of the G2P-SOL EU project, the diversity present in 10,301 pepper genebank accessions is currently under study. GBS analysis has provided insight in the genetic diversity of the accessions and enabled the construction of a GWAS core collection which is currently being phenotyped in different environments and evaluated for disease resistance, morphological and fruit quality traits [1].

[1] The G2P-SOL project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 677379.

#### **ACKNOWLEDGEMENTS**

Arnaud Bovy thanks the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.





# Intra-varietal diversity of aroma compounds in *Capsicum* peppers: from ancient landraces to modern FI

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**BACKGROUND** *Capsicum* peppers (*Capsicum* spp.) are profusely utilised worldwide in a range of recipes due to their colour and flavour. Capsaicinoids, as pungency components, and aroma volatiles are the main factors of flavour in peppers [3]. Capsaicinoids have been the most studied flavour components, while work on the volatile fraction has been more recent: bell pepper pyrazine was identified as the most specific volatile in peppers in the 70-80s. Studies in the last decade revealed a considerable interspecific and intraspecific diversity in terms of the volatile composition [1,2,3]. In contrast, the knowledge on intra-varietal diversity is still scarce and this fact could be of paramount importance for breeders aiming to improve flavour in modern cultivars or to recover landraces based on high-added value. Here we present the preliminary results of a study on the volatile fraction of peppers from the jalapeno varietal type as a case study.

**MATERIALS & METHODS** Eight jalapeno accessions including 4 ancient cultivars (Espinaltecos), 1 open-pollination cultivar (Jalapeno M), 2 modern jalapeno FI varieties (Delicias and Jalapa) and 1 Serrano, considered to be the ancestor of jalapenos, were studied. The volatile profile was studied at the unripe stage, the most economically important in jalapenos. Three samples (1 g) per accession were each prepared with fruits from five plants. Each sample was introduced into a 10 mL vial and sealed. Samples were extracted by head-space solid phase microextraction and analyzed by gas chromatography mass spectrometry, using an Agilent GC/MS with an autosampler and following the protocol reported by Rodríguez-Burruezo et al. [3].

**RESULTS** A total of 40 volatile compounds have been preliminarily identified among the accessions studied, including 20 terpenoids (6 monoterpenes), 10 esters, 2 alkanes and another 8 compounds, aldehydes, lipoxygenase-derived compounds like the bell pepper pyrazine, methyl salicylate, etc. Considerable qualitative and quantitative differences were found among the studied materials. Thus, the highest levels of total volatiles corresponded to ancient jalapenos, i.e. espinalteco landraces, with values ranging from  $645 \times 10^6$  to  $8180 \times 10^6$  GC peak area units (p.a.u.) of Esp10402 and Esp10397 (Table 1). In contrast, the lowest levels in total volatiles were found in modern Jalapeno FI (Delicias and Jalapa), values were comprised between  $40 \times 10^6$  and  $80 \times 10^6$  p.a.u., while Jalapeno M, a traditional open pollinated jalapeno, and the ancestor Serrano showed intermediate levels,  $250\text{--}585 \times 10^6$  (Table 1). This pattern was also found within the main groups of volatiles and also many individual compounds, like those particularly relevant for unripe peppers, i.e. several terpenoids (3-carene,  $\alpha$ -longipinene,  $\alpha$ -copaene,  $\beta$ -caryophyllene,  $\alpha$ - and  $\beta$ -himachalene), esters (4-methylpentyl-3-methylbutanoate, hexyl 2-methylbutanoate, 4-methylphenyl pentanoate), and the bell pepper pyrazine and methyl salicylate.

**DISCUSSION & CONCLUSION** Our findings indicate that modern breeding, mainly focused on improving new material for resistance to disease, high yields, morphological uniformity, good appearance and other marketing and postharvest objectives, may have been to the detriment of flavour. Such a fact, which is also obvious in other vegetables like tomato (in particular due to the decrease in sugars and acids), must be studied in depth in peppers. We have found that ancient landraces are very rich in the total volatile fraction and individual volatiles. This offers breeders the opportunity of selecting and breeding ancient cultivars for their enhanced flavour or, alternatively, using them for improving the flavour of modern cultivars.

## REFERENCES

- [1] Eggink PM, et al., 2014, Theor. Appl. Genet. 127:373-390
- [2] Kollmannsberger H, et al., 2011, J. Sci. Food Agric. 91:1598-611
- [3] Rodríguez-Burruezo A, et al., 2010, J. Agric. Food Chem. 58:4388-4400

VOLATILES	Esp10397	Esp10400	Esp10402	Esp10415	Jalap M	DeliciasF1	JalapaF1	Serrano
<b>TERPENOIDS</b>								
<b>Monoterpenes</b>								
Tricyclene	-	3.47	0.29	-	-	0.26	3.01	5.22
$\alpha$ -Pinene	-	tr	tr	tr	0.44	0.52	0.38	tr
Camphene	-	tr	tr	-	0.20	0.27	0.30	0.28
$\beta$ -Pinene	-	tr	-	-	-	-	tr	tr
(Z)- $\beta$ -Ocimene	-	0.31	0.87	-	1.50	0.77	0.65	-
Limonene	tr	5.08	1.10	0.52	2.50	1.50	3.06	0.70
<b>Total Monoterpenes</b>	<b>tr</b>	<b>8.86</b>	<b>2.26</b>	<b>0.52</b>	<b>4.64</b>	<b>3.32</b>	<b>7.40</b>	<b>6.20</b>
<b>Other terpenoids</b>								
3-Carene	72.82	140.81	9.80	2.25	3.40	20.92	6.33	26.40
$\alpha$ -Longipinene	203.30	33.84	8.45	8.57	4.20	0.13	1.27	5.16
(+)-Cyclosativene	tr	13.29	30.95	tr	0.17	0.66	0.29	tr
$\alpha$ -Ylangene	tr	-	-	-	18.02	tr	0.50	-
$\alpha$ -Copaene	171.30	12.39	-	42.69	8.31	4.13	5.42	24.68
$\alpha$ -Cedrene	tr	1.39	-	tr	0.92	0.42	0.22	-
$\beta$ -Caryophyllene	11.75	-	1.94	3.58	1.46	0.23	0.14	1.61
Himachala-2,4-diene	35.78	1.46	6.32	11.43	4.54	tr	tr	6.52
$\alpha$ -Bergamotene	2.82	1.47	1.39	4.50	3.88	2.50	1.52	1.65
$\alpha$ -Himachalene	-	66.99	37.14	47.58	22.64	0.29	1.83	34.00
(+)-Valencene	tr	-	-	1735.14	tr	-	tr	tr
$\delta$ -Himachalene	6005.91	50.79	41.84	78.06	27.48	0.14	1.23	40.00
$\delta$ -Cadinene	4.73	-	-	0.50	0.55	tr	0.39	tr
Longipinocarvone	-	-	5.68	-	-	tr	0.24	6.02
<b>Total Other Terpenoids</b>	<b>6508.41</b>	<b>322.43</b>	<b>143.51</b>	<b>1934.30</b>	<b>95.57</b>	<b>29.42</b>	<b>19.38</b>	<b>146.04</b>
<b>ESTERS</b>								
Methyl 3-methylbutanoate	tr	tr	-	-	0.40	tr	0.05	1.21
3-methylbutyl, butanoate	2.35	-	1.03	tr	0.51	-	0.40	-
Pentyl 3-methylbutanoate	10.05	1.36	1.01	tr	tr	tr	0.25	0.79
Hexyl 2-methylpropanoate	tr	20.03	11.78	6.43	4.06	tr	0.26	10.43
4-methylpentyl-3-methylbutanoate	256.86	257.04	63.80	14.27	5.06	0.42	3.52	19.82
Hexyl 2-methylbutanoate	14.36	13.96	5.87	1.82	0.52	tr	0.21	2.07
Hexyl 3-methylbutanoate	1.41	3.08	2.08	-	-	-	-	-
4-methylphenyl pentanoate	58.36	22.74	33.59	23.81	8.27	tr	1.63	26.39
Heptyl 3-methylbutanoate	-	-	-	tr	-	-	-	-
Hexyl decanoate	tr	2.51	-	1.58	1.07	-	-	tr
<b>Total Esters</b>	<b>360.85</b>	<b>320.72</b>	<b>119.16</b>	<b>50.34</b>	<b>19.89</b>	<b>0.42</b>	<b>6.32</b>	<b>62.12</b>
<b>ALKANES</b>								
10-Methylnonadecane	1151.25	443.78	271.74	722.01	58.96	2.56	21.57	336.49
Tetradecane	108.85	68.24	45.35	111.31	4.33	0.52	tr	28.69
<b>Total Alkanes</b>	<b>1260.10</b>	<b>512.02</b>	<b>317.09</b>	<b>833.32</b>	<b>63.29</b>	<b>3.08</b>	<b>21.57</b>	<b>365.18</b>
<b>MIXCELANEA</b>								
Hexanal	-	-	-	-	-	-	-	0.29
Anisole	23.29	7.10	38.08	19.97	38.83	tr	2.88	-
Pentanoic acid	-	tr	0.44	tr	tr	-	0.07	tr
$\alpha$ -Phellandrene	-	tr	-	-	tr	-	0.06	tr
1-Butanol, 3-methyl	20.67	6.89	5.88	1.89	0.73	1.05	-	4.10
2-methoxy-3-isobutyl pyrazine	6.94	2.96	5.33	2.26	1.51	0.50	1.15	0.86
Methyl Salicylate	-	tr	tr	tr	tr	tr	tr	tr
(Z)-9-Octadecen-1-ol	tr	tr	0.23	tr	tr	0.35	0.21	0.59
<b>Total Volatiles</b>	<b>8180.26</b>	<b>1195.71</b>	<b>644.82</b>	<b>2850.22</b>	<b>248.21</b>	<b>40.08</b>	<b>79.88</b>	<b>585.38</b>

**Table 1.** Volatile profile of jalapeño peppers at the unripe stage. Content is expressed in GC peak area units  $\times 10^6$ .

# From farm to cell: a look at carotenoids and phenolics in diverse *Capsicum* species and their nutraceutical properties

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**BACKGROUND** Chile peppers (*Capsicum* species) are known to ripen to a variety of colours and have a significant amount of health benefits. They produce compounds that are powerful antioxidants and also help prevent diseases like age-related blindness. These compounds include phenolics and carotenoids, which serve as plant pigments and protect plants from abiotic and biotic stresses. As one of the few plant compounds that enter the bloodstream, the yellow carotenoid, lutein, accumulates in ocular retina and brain tissues. Studies have found that lutein is able to delay the onset of and reduce the risk of cataracts and age-related macular degeneration. Chile peppers, *Capsicum* spp., are among one of the few fruits and vegetables that produce both lutein and phenolics [1]. Considering that there is a demand for lutein and phenolic dietary supplements, our goal is to find a chile pepper rich in lutein and phenolics which can be used for breeding purpose or in the dietary supplement industry. In addition, it can also be a source of lutein-rich and phenolic-rich natural yellow pigment.

**MATERIALS & METHODS** Thirty-seven yellow *Capsicum* accessions (*C. annuum*, *C. chinense*, and *C. baccatum*) from the Chile Pepper Institute were analyzed for nutraceutical composition. Peppers were harvested at ripeness. Carotenoids were extracted and lutein, beta-carotene and total carotenoids were identified and measured employing high performance liquid chromatography (HPLC). Lutein and beta-carotene standards were purchased to identify unknown peaks in the extracts. Total phenolics were extracted with acidified methanol and measured spectrophotometrically [2]. To measure bioaccessibility of lutein in humans, we employed an in vitro digestion method to measure lutein that is present for absorption in the human gut.

**RESULTS** After HPLC analysis, we observed differences in carotenoid and phenolic amounts among varieties. We found that Yellow Habanero had the highest total carotenoid and beta-carotene amounts with 767.20 µg/g dry weight (DW) and 70.62 µg/g DW, respectively. Several varieties had low amounts of beta-carotene (less than 0.5 µg/g DW). A yellow *C. annuum* (a Mulato x Permagreen hybrid) had 84.98 µg/g DW of lutein. Our observations showed that out of 35 peppers, 30 had less than 50% lutein of the total carotenoid amount. Three peppers, a yellow serrano, Wild Chinense, and Aji Limon, contained 67.2%, 66.3% and 66.1% lutein of their total carotenoid amounts, respectively. A *C. chinense*, Fatali, contained the highest amount of total phenolics with 4.64 mg/g gallic acid equivalents. The second highest was the *C. annuum* Mulato x Permagreen hybrid, which contained 4.54 mg/g of gallic acid equivalents. To assess nutraceutical potential, simulated human digestions were performed to measure the bioaccessibility of lutein from NuMex Lemon Spice (*C. annuum*) and Aji Limon (*C. baccatum*) powder in the human gut. Data indicates that 22.8% of the lutein in NuMex Lemon Spice is available for absorption and 18.71% of the lutein in Aji Limon is available for absorption in the human gut.

**DISCUSSION & CONCLUSION** *Capsicum* spp. phytochemicals have potential as nutraceuticals. However, the variability of amounts in fruits and vegetables depends on the variety. We have shown diverse amounts of carotenoids and phenolics in 37 chile peppers. The implication is that not all yellow fruits contain equal amounts of health promoting compounds. The cultivars presented have unique genetic and biosynthetic pathways that influence carotenoid and phenolic synthesis and accumulation, and therefore can be used as breeding material to increase lutein and phenolic content in *Capsicum* species. In addition, these unique phytochemical mixtures can also influence the bioaccessibility of these compounds in our gut.

## REFERENCES

- [1] Guzman I et al., 2010, Plant Science, 179:49-59
- [2] Grace M et al., 2013, Journal of Agricultural and Food Chemistry, 61 (28):6856–6864

# The *f-al* mutation responsible for anthocyanin-less Hungarian wax peppers results from a deletion in flavonoid 3',5'-hydroxylase (F3'5'H)

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**BACKGROUND** Certain varieties of peppers (*Capsicum annuum*) are harvested unripe, meaning that they have not yet changed from their immature colour to their mature colour. The accumulation of anthocyanin (*al*+) in these types of peppers is undesirable because anthocyanin appears in non-uniform streaks, reducing post-harvest quality. Hungarian wax peppers, for example, are harvested and consumed white making anthocyanin very visible (Figure 1). Thus, the market demands anthocyanin-less plants (*al*-). Anthocyanin can accumulate in anthers, stems, leaves and fruits but whether or not an *al*+ genotype produces the phenotype depends on the plant organ and whether there is stress, eg. high light or low temperature [2]. The anthocyanin biosynthesis pathway has been well-studied [2] and many pepper mutants have been identified [1], including the *afx* mutant (anthocyanin less cv Fehérözön plant), here referred to as *f-al*. The *f-al* mutant was crossed with elite Rijk Zwaan Hungarian wax pepper breeding material and many years of phenotypic selection maintained the mutation in the breeding pool. However, breeders desired a linked molecular marker to improve breeding efficiency. Thus, our goal was to identify the gene and causal mutation behind the *f-al* mutant.

**MATERIALS & METHODS** We used the existing knowledge on the anthocyanin biosynthesis pathway and took a candidate gene approach. We used a literature mining technique and then found orthologs in pepper, resulting in a list of 15 candidate genes. We extracted sequences of these candidates from NCBI and mined our in-house sequence database for polymorphisms between *al*- and *al*+ genotypes.

**RESULTS** Since the *f-al* mutation is linked to the *L3* gene of TMV resistance on chromosome 11 [3], we reduced our list to the only two candidates which are also on chromosome 11: Chalcone-flavonone isomerase (CHI) and Flavonoid 3',5'-hydroxylase (F3'5'H). There were no polymorphisms in CHI correlating with the phenotype. However, we found a deletion of one base pair in the coding region of F3'5'H of the *f-al* genotype which was not present in any of the *al*+ genotypes. This deletion caused a premature stop codon and therefore a non-functional protein that is 255 amino acids long instead of 513. We developed a marker for this deletion and verified the marker using an F2 population derived from an *al*+ parent and a parent known to carry the *f-al* mutation. We phenotyped the anthers (yellow or blue) of 100 F2 plants. The marker correlated 100% with the F2 phenotypes, supporting the hypothesis that this was the correct gene and likely to be the causal polymorphism.

**DISCUSSION & CONCLUSION** Using a candidate gene approach we identified the causal gene, F3'5'H, behind the anthocyanin-less mutant known as *afx* or *f-al*. We used sequence data to find the causal deletion in this gene and we developed an in-gene marker, allowing for efficient selection of the *al*- phenotype. Breeders can also use this marker to distinguish this particular mutation from other *al*- mutations at an early stage of plant development.

## REFERENCES

- [1] Csilléry G, 2016. Pepper Mutant Collection. Budakert Ltd.
- [2] Liu Y *et al.*, 2018, *Frontiers in Chemistry* 6 (MAR), art. no. 52, DOI=10.3389/fchem.2018.00052
- [3] Zatykó L *et al.*, 1998, *Proc. Eucarpia Meeting on Genetics and Breeding of Capsicum & Eggplant*. 10: 249



**Figure 1.** Example of harvested Hungarian wax pepper fruits showing anthocyanin accumulation.

# Determination of capsaicin content and pungency level in some pepper genotypes and varieties

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**BACKGROUND** Peppers are known as a good source of important vitamins (ascorbic acid-vitamin C, carotenoids-vitamin A, tocopherols-vitamin E), minerals [Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Phosphorus (P), Sulphur (S), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn) and Selenium (Se)], antioxidant, flavonoids and capsaicinoids [1,2]. Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin have been identified as naturally occurring capsaicinoids, however the most abundant ones are capsaicin and dihydrocapsaicin [1] and the pungency level of peppers has been determined by using a formula containing these two compounds. Genotype, fruit maturity and climate factors have an effect in the concentration of capsaicinoids [1]. In this study, the capsaicin content and pungency level of some pepper genotypes and varieties are described.

**MATERIALS & METHODS** Turkish materials (Alata 10, 15 and 111) were selected from the pepper collection of Alata Horticultural Research Institute-Turkey. The Rwandan variety Pili-Pili is consumed in Rwandan cuisine. The capsaicin content of the Alata collection and Pili-Pili were identified both at green and red ripening stages. In addition some accessions carrying various resistances were evaluated: Er-Fu-Tou (Cucumber Mosaic Virus), C29 (*Phytophthora capsici* and Potato Virus Y), PI 260429 (Tobacco Mosaic Virus) and PI 152225 (Tomato spotted wilt virus); for these accessions, sampling was done at red ripening stage. High-performance liquid chromatography (HPLC) was used for identifying of capsaicinoids and extraction was performed by 95% methanol.

**RESULTS** The highest capsaicin content and pungency level were detected in genotype PI 152225. For dihydrocapsaicin content, Er-Fu-Tou variety was the first of the list. It was followed by PI 260429 and PI 152225. Pungency involves the relationship between capsaicin and dihydrocapsaicin and therefore, it is important to convert to the Scoville Scale. The most pungent genotype PI 152225 was followed by Pili-Pili variety (red stage), Er-Fu-Tou, Rwandan Pili-Pili variety (green stage), Alata 111 (red stage) and PI 260429 (Table 1).

**DISCUSSION & CONCLUSION** Hot peppers are desirable and popular in Turkish cuisine. Therefore, knowing the capsaicin content and pungency level of pepper genotypes is important for future breeding projects, which will be performed in Turkey. In particular, obtaining reliable data from disease-resistant genotypes is important for breeders. The plant materials including both high capsaicinoid content and disease resistance will be priceless for planning of future projects.

## REFERENCES

- [1] González-Zamora A *et al.*, 2013, Molecules, 18: 13471-13486. doi: 10.3390/molecules181113471
- [2] Guil-Guerrero JL *et al.*, 2006, Eur. Food. Res. Technol, 224: 1-9. doi: 10.1007/s00217-006-0281-5

## ACKNOWLEDGEMENTS

Bulent Bechir Arpaci thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.

Genotype	Botanical name	Type	Ripening stage	Capsaicin (mg kg <sup>-1</sup> )	Dihydrocapsaicin (mg kg <sup>-1</sup> )	Scoville Scale SHU
Pili-Pili	<i>C. chinense</i>	-	Green	2949	Not detected	44241
Pili-Pili	<i>C. chinense</i>	-	Red	3590	430	59157
Alata 10	<i>C. annuum</i>	Capia	Green	Not detected	Not detected	0
Alata 10	<i>C. annuum</i>	Capia	Red	Not detected	Not detected	0
Alata 111	<i>C. annuum</i>	Turkish-Kahramanmaraş	Green	Not detected	Not detected	0
Alata 111	<i>C. annuum</i>	Turkish-Kahramanmaraş	Red	1805	53	27741
Alata 15	<i>C. annuum</i>	Turkish-Sivri	Green	Not detected	Not detected	0
Alata 15	<i>C. annuum</i>	Turkish-Sivri	Red	Not detected	Not detected	0
PI 152225	<i>C. chinense</i>	Wild	Red	90722	17700	105236
Er-Fu-Tou	<i>C. annuum</i>	Long type for dried	Red	28083	20668	45032
C29	<i>C. annuum</i>	Kahramanmaraş for dried	Red	6453	3805	9574
PI 260429	<i>C. annuum</i>	Wild	Red	7906	18758	23288

**Table 1.** Capsaicin-Dihydrocapsaicin contents and pungency level of pepper genotypes-varieties tested in this study



# Identification of a novel locus controlling pungency in *Capsicum annuum*

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**BACKGROUND** *Capsicum* is an early domesticated genus synthesizing unique pungent substances, the capsaicinoids. Because of the distinctive pungency, chili type peppers are in high demand in Asia and South America. Despite of its commercial importance, only three genes (acyltransferase (Pun1), putative aminotransferase (pAMT), ketoacyl-ACP reductase (KR)) have been identified as factors controlling biosynthesis of capsaicinoids in pepper [1]. To better understand capsaicinoid biosynthesis, it is necessary to identify more genetic factors. Ethyl methanesulfonate (EMS) treatment is one of the useful methods to develop new genetic diversity for both fundamental and breeding studies. In this study, we used a non-pungent mutant line, *Capsicum annuum* '221-2-1a', derived from EMS-treated Korean landrace 'Yuwol-cho' to identify a novel gene controlling pungency. We performed genetic mapping of non-pungency in '221-2-1a' using SNP markers and MutMap analysis, and identified the non-pungency locus on chromosome 6. To identify candidate genes, we are performing fine mapping of the non-pungency locus.

**MATERIALS & METHODS** A total of 89 F<sub>2</sub> plants obtained from a cross between a non-pungent mutant line *Capsicum annuum* '221-2-1a' and the pungent accession *C. annuum* 'Lam32' were used. Capsaicinoid content in the placenta tissues was evaluated by combining Gibb's reagent screening [2] and high-performance liquid chromatography (HPLC). To map the locus in '221-2-1a', Fluidigm EPI system was used. A genetic map was constructed with a LOD 3 and maximum genetic distance of 30 cM. MutMap analysis between '221-2-1a' and 'Yuwol-cho' was carried out and variant SNPs were called against CM334 (ver.1.55) chromosome as a reference. Furthermore, CM334 peptide sequences (ver.2.0) was annotated to find the candidate genes controlling non-pungency.

**RESULTS** In the F<sub>2</sub> population, the segregation of pungency and non-pungency fit a 3:1 ratio, supporting that pungency in EMS-induced mutant line is controlled by a single recessive gene. To map the non-pungency locus, a total of 143 SNP markers were genotyped using Fluidigm EPI system. Linkage analysis between SNP markers and pungency phenotype identified 11 SNP markers linked to pungency trait on pepper chromosome 6. Furthermore, we used MutMap analysis to identify candidate genes controlling pungency and reveal SNPs at 215 Mb region on chromosome 6. Then, we annotated pepper peptide sequences using protein database. A total of 21 annotated proteins were detected as capsaicin biosynthetic gene homologs, and 10 genes can be candidate genes in the target region.

**DISCUSSION & CONCLUSION** In conclusion, the position of a non-pungency locus in a mutant line was too large to identify the exact region because the SNP markers were biased to telomeric region. To further delimit the target region, it is necessary to saturate the genetic map with more markers on pepper chromosome 6. MutMap data could narrow down the target region and enable candidate identification. However, the candidate region from MutMap was not well aligned with the position mapped using Fluidigm EPI system. To overcome this discrepancy, MutMap analysis needs to be tested with other pepper genome.

## REFERENCES

- [1] Koeda S, Sato K et al., 2019, Theor. Appl. Genet., 132(1):65-80, doi: 10.1007/s00122-018-3195-2
- [2] Jeong HJ, Hwang D et al., 2012, Kor. J. Hort. Sci. Technol., 30(3):294-300, doi:10.7235/hort.2012.12011



# Total phenol content of some pepper genotypes originating from Turkey and Rwanda at green and red ripening stages

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**BACKGROUND** Pepper is one of the most consumed vegetables with high nutritional content and functional properties. Many parameters are used to determine the functional properties of foods, and content of phenols is one of them. Phenolics are important for human health, due to their effects on taste and fragrance, their role in colour formation and its variation, their antimicrobial and antioxidative effects and their enzyme inhibition [1]. Antiallergic, anti-inflammatory, antidiabetic, antimicrobial, anti pathogenic, antiviral properties of phenolic compounds and their protective effects in cardiovascular diseases, cancer, osteoporosis, diabetes mellitus and neurodegenerative diseases have been highlighted in many studies [1]. In this study, total phenol content of four pepper genotypes originated from Turkey and one from Rwanda were assessed at both in green and red ripening stages.

**MATERIALS & METHODS** *Capsicum annum* Turkish genotypes and breeding lines (Alata 10, 15 and 111) were selected from the pepper collection of Alata Horticultural Research Institute (Mersin, Turkey). The Rwandan Pili-Pili variety of *C. chinense*, widely consumed in Rwandan cuisine, was also chosen. Gallic acid was used as a standard phenolic compound, and total phenolic contents were determined with Folin–Ciocalteu reagent. The concentration of total phenolics was calculated as milligram of gallic acid equivalent by using an equation obtained from standard gallic acid graph [2].

**RESULTS** Our results showed that the total phenolic content of the pepper genotypes in study ranged from 184.07 to 217.61 mg/100 g at the green stage and from 241.13 to 412.74 mg/100 g at the red stage (Table 1). The highest phenolic content was obtained from the genotype 'Alata' 10 at both stages. In green stage, 'Alata' 10 was followed by 'Alata' 15, 'Pili-Pili' and 'Alata' 111, while at the red stage by 'Pili-Pili', 'Alata' 111 and 'Alata' 15. By comparing the *Capsicum chinense* ('Pili-Pili' variety) with *C. annum* ('Alata' 10, 15 and 111), we found out that, at the green stage, the mean phenolic content of the three *C. annum* genotypes was higher than the one of *C. chinense*, while at the red stage was the opposite. In a study carried out in India, phenolic content of nine Indian varieties were determined during ripening [3], and their contents varied between 64.0 and 188.0, 85.0 and 220.0, 110.0 and 266.0 mg/100 g at green, intermediate and red ripening stages, respectively.

**DISCUSSION & CONCLUSION** Our data demonstrate that peppers are richer in phenols at the red stage. Knowing the phenol content of different pepper varieties is important for both consumers and scientists. If consumers know the phenol content of different varieties, they will have the opportunity to choose the ones containing higher amounts. From a scientific point of view, it is important to know the phenol content in germplasm collections for future breeding programs.

## REFERENCES

- [1] Kolaç et al., 2017, İ.Ü. Sağlık Hizmetleri Meslek Yüksekokulu Dergisi, 5(1): 26-42.
- [2] Sadasiyam & Manickam, 1992, Wiely Estern Ltd., Madras.
- [3] Kumar et al., 2010, Journal of Phytology, 2(6): 87-90.

Genotype	Species	Type	mg/100 Green stage	mg/100 Red stage
Alata 10	<i>C. annuum</i>	Capia	199.10	356.46
Alata 10	<i>C. annuum</i>	Capia	236.13	469.02
Mean			217.61±26.18	412.742±79.59
Alata 15	<i>C. annuum</i>	Sivri	248.42	238.71
Alata 15	<i>C. annuum</i>	Sivri	179.52	243.56
Mean			213.97±48.72	241.14±3.43
Pili-Pili	<i>C. chinense</i>		229.80	368.60*
Pili-Pili	<i>C. chinense</i>		160.90	296.70*
Mean			195.35±48.72	332.65±50.84*
Alata 111	<i>C. annuum</i>	Kahramanmaraş	202.59	259.19
Alata 111	<i>C. annuum</i>	Kahramanmaraş	165.56	281.05
Mean			184.07±26.18	270.12±15.45

\*: Pili-Pili variety was in orange step

**Table 1.** Phenolic content of pepper genotypes used in this study.

# Phytochemical assessment of Ecuadorian peppers (*Capsicum* spp.) and correlation with fruit horticultural traits

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**BACKGROUND** The genus *Capsicum* has its origins in tropical South America. It harbours five domesticated species, *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*, which are cultivated around the world. After the discovery of the New World, pepper spread to several civilizations, which were able to adapt it to different environmental conditions, resulting in a vast phenotypic and compositional diversity. Thus, *Capsicum* species can be sorted into different classes based on fruit features such as shape, size, pungency, flavour, and colour. In addition to sensory characteristics, pepper fruits are a well-known source of bioactive compounds, such as vitamin C, phenols, or capsaicinoids. These compounds primarily protect cells against oxidative damage, promoting human health, and preventing common degenerative diseases [1]. The identification of a phytochemical profile for each *Capsicum* species and their correlation with well-defined fruit morphology traits allows for the selection of promising accessions for future breeding programs and commercialization.

**MATERIALS & METHODS** Forty-two *Capsicum* spp. accessions from Ecuador, provided by the USDA-ARS (USA), the CGN (the Netherlands), and the COMAV (Spain) were used: *C. annuum* (8), *C. chinense* (7), *C. frutescens* (8), *C. pubescens* (7), *C. baccatum* (12). Plants were grown in an experimental field at the Universidad Técnica de Machala (El Oro, Ecuador). Four descriptors from the IPGRI [2] were recorded in mature fruits. Additionally, fruits were oven-dried and crushed. Powder was employed to quantify total polyphenol content, capsaicinoids, and the ability to prevent cholesterol oxidation. Vitamin C, titratable acidity, pH, soluble solid content (SSC), and antioxidant capacity were evaluated in fresh fruits.

**RESULTS** Overall features were assessed within species. Fruit length, width, weight, and wall thickness were analysed. ANOVA showed significant ( $p < 0.05$ ) differences among the 5 domesticated *Capsicum* species for the four fruit traits under study (Table 1). *C. annuum* exhibited the highest values for length and fruit weight, whereas *C. frutescens* displayed the smallest fruits. The highest fruit width and wall thickness were recorded for *C. chinense*. ANOVA also revealed significant differences among *Capsicum* species for various phytochemical parameters. The greatest capability to inhibit cholesterol oxidation was found in *C. chinense* and *C. annuum* accessions, whereas *C. annuum* and *C. frutescens* showed the highest content in vitamin C. Fruits from *C. frutescens* exhibited high capsaicinoid contents and antioxidant activity. Pearson correlation analysis detected positive and negative associations (Figure 1). Interestingly, the majority of bioactive compounds negatively correlated to fruit traits. Capsaicinoids showed a strongly significant negative correlation with fruit width, fruit weight and pericarp thickness. Similarly, significant negative correlations were evidenced between titratable acidity and both fruit weight and wall thickness.

**DISCUSSION & CONCLUSION** Peppers exhibit an immense phenotypical diversity, which varies worldwide according to consumer preferences. Fruits range from sweet, large and thick, to pungent, small and thin. This vegetable also represents an extraordinary source of many human health-promoting components [3]. Few attempts have been performed to correlate phytochemical and horticultural traits. Our work revealed that longer and bigger pepper fruits are expected to contain lower amounts of bioactive compounds. According to this, the highest capsaicinoid concentration and antioxidant capacity were found in *C. frutescens* accessions, which exhibited the smallest fruits.

REFERENCES

[1] Antonio et al., 2018, RSC Adv. 8: 25767–25784. DOI: 10.1039/C8RA02067A.  
[2] IPGRI, 1995, International Plant Genetic Resources Institute, Rome, Italy, 49 p.  
[3] Baenas et al., 2019, Food Chem. 274: 872–885. DOI: 10.1016/j.foodchem.2018.09.047.

	Phenols (µg FAE/mg)	Vitamin C (mg/100g)	Remaining cholesterol (%)	Capsaicinoids (mg/g)
<i>C. annuum</i>	25,50 a	272,03 a	80,39 a	0,34 a
<i>C. baccatum</i>	21,72 a	198,21 ab	66,65 b	0,20 a
<i>C. chinense</i>	23,62 a	202,77 ab	80,66 a	0,20 a
<i>C. frutescens</i>	26,58 a	229,66 a	65,66 b	1,84 b
<i>C. pubescens</i>	23,36 a	142,43 b	58,39 b	0,13 a
	SSC (°Brix)	pH	Titrateable acidity (%)	Antioxidant capacity (mg/g)
<i>C. annuum</i>	9,92 ab	5,26 ab	0,18 a	381,65 a
<i>C. baccatum</i>	11,35 ab	5,26 ab	0,20 a	475,15 a
<i>C. chinense</i>	8,95 a	5,36 ab	0,14 a	404,92 a
<i>C. frutescens</i>	13,58 b	5,78 a	0,25 a	1919,01 b
<i>C. pubescens</i>	10,58 ab	4,96 b	0,21 a	389,74 a
	Fruit Length (cm)	Fruit width (cm)	Fruit weight (g)	Wall thickness (mm)
<i>C. annuum</i>	7,67 a	2,24 ab	16,96 a	3,25 ab
<i>C. baccatum</i>	6,68 ab	1,70 ab	10,07 ab	2,58 bc
<i>C. chinense</i>	5,74 ab	2,74 a	12,11 ab	3,85 a
<i>C. frutescens</i>	2,61 c	1,09 b	2,87 b	1,43 c
<i>C. pubescens</i>	4,23 bc	2,18 ab	8,32 ab	3,14 ab

Different letters in the same column indicates significant differences (p < 0.05)

Table I. Means of phytochemical parameters in *Capsicum* spp.

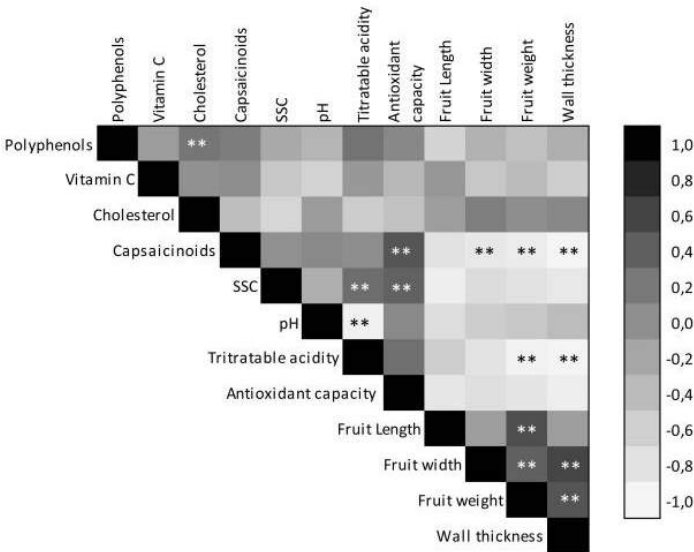


Figure I. Correlation matrix among 12 traits. \*\*: significant at p<0,01.

# Analysis of pepper (*Capsicum annuum*) diversification in primary and secondary centres based on horticultural fruit traits

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**BACKGROUND** *Capsicum annuum* is the most commonly *Capsicum* cultivated species worldwide. It was domesticated in Mexico from the wild bird pepper, and then it probably migrated to the Andean region, which constituted one of the primary and most significant centres of specialization. Likewise, *C. annuum* was the first *Capsicum* introduced to Europe in post-Columbian times. Its arrival likely occurred in the Iberian Peninsula, from where it dispersed to Mediterranean countries, Africa, India and China, which turned into valuable secondary diversification centres. Since then, peppers have been under strong selection for horticultural fruit traits, such as shapes and size, founded on regional preferences and agro-climatic conditions [1]. The description of the extraordinary phenotypic diversity reached at primary and secondary centres will allow, not only to understand the evolution of fruit morphology, but also a more efficient management of pepper genetic resources, which constitute the basis of any crop breeding procedure.

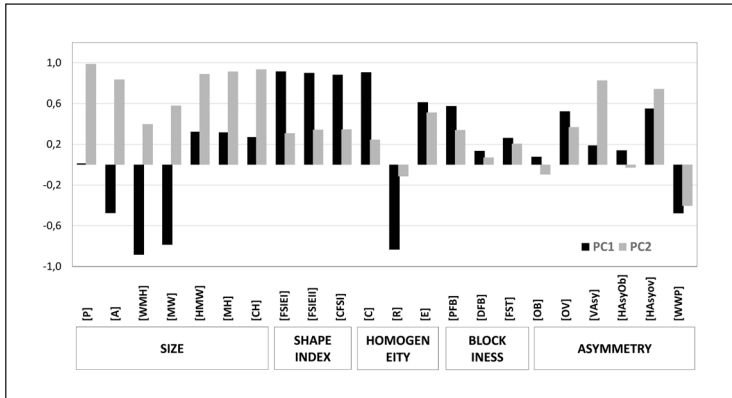
**MATERIALS & METHODS** Eighty-five peppers (*C. annuum*) provided by the CGR (Netherlands), the BGHZ (Spain), and the IPK (Germany) were selected. Accessions come from primary centres: México (9) and the Andean Region (11), and secondary centres comprising the Iberian Peninsula (31), and the African (16) and European (18) Mediterranean areas. Plants were grown in an experimental field at the Centro de Investigaciones Agrarias de Mabegondo (A Coruña, Spain). Twenty fully-ripe fruits per accession were subjected to automated phenotyping with Tomato Analyzer (TA) 3.0 software [2]. Twenty-two descriptors into the categories of fruit size, shape index, homogeneity, blockiness and asymmetry, were analysed.

**RESULTS** A principal component analysis was performed. Four principal components with eigenvalues > 1 were identified, cumulatively counting for 90.2 % of the total variance. The first and second components explained 48.9 % and 22.8 %, respectively. The first component was positively and strongly correlated with Fruit Shape Index and Homogeneity, except for Rectangular, and negatively correlated with Width Mid-Height and Maximum Width. The second component showed a strong and positive correlation with the Fruit Size category, with the exception of Width Mid-Height and Maximum Width (Figure 1). A two-dimensional graph was drawn to observe the divergences among the five geographical regions under study (Figure 2). Fruit Shape Index External I and II, which represent the ratio between fruit height and width, were the principal variables discriminating the genotypes on the first axis, while Perimeter, Maximum Height, and Curved Height, strongly contributed to the location of fruits on second axis. Pepper accessions displayed a wide dispersion. However, American peppers (from Mexico and the Andean region) mainly plotted in the IV quadrant, while Iberian and European Mediterranean accessions tend to place in the II and III quadrants. Curiously, Mediterranean peppers from Africa plotted all around the bi-dimensional space (Figure 2).

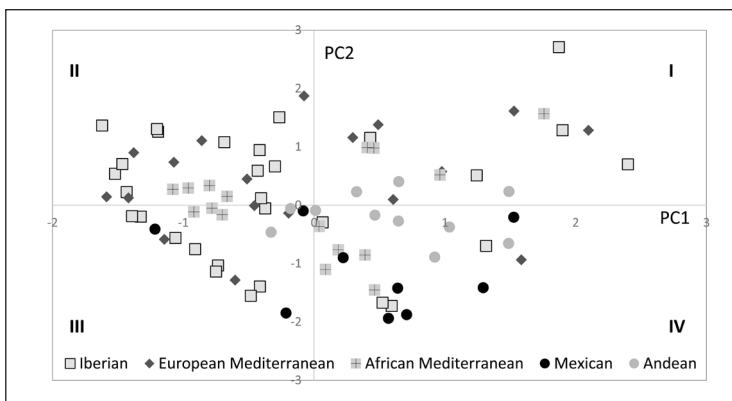
**DISCUSSION & CONCLUSION** After its domestication in Mexico, *C. annuum* spread around the world, becoming an essential component of human diet. Such migrations led to an endless array of phenotypic diversity, primarily for fruit traits. Our data suggest that fruit diversification on faraway geographical areas may occur in a different manner. Hence, peppers from primary centres mostly showed small and elongated fruits, whereas accessions from the secondary centres tend to be bigger and blocky. These results agree with the previously proposed evolution of *C. annuum* fruits, starting from the bird pepper ancestor [3] and bring to light the type of variability harboured in American and European areas.

## REFERENCES

- [1] Bosland PW, Votava E, 2000, CABI Publishing, Oxon. 204 pp.
- [2] Brewer MT, Lang L et al., 2006, Plant Physiol. 141: 15-25
- [3] Nicolai M, Cantet M et al., 2013, Genet Res Crop Evol. 60: 2375-2390



**Figure 1.** Correlation coefficients for TA fruit descriptors in the two first principal Components. [P] Perimeter; [A] Area; [WMH] Width Mid Height; [MW] Maximum Width; [HMW] Height Mid Width; [MH] Maximum Height; [CH] Curved Height; [FSIEI] Fruit Shape Index External I; [FSIEII] Fruit Shape Index External II; [CFSI] Curved Fruit Index; [C] Circular; [R] Rectangular; [E] Ellipsoid; [PFB] Proximal Fruit Blockiness; [DFB] Distal Fruit Blockiness; [FST] Fruit Shape Triangle; [OB] Obovoid; [OV] Ovoid; [VAsy], V. Asymmetry; [HAsyOb] H. Asymmetry.ob; [HAsyov] H. Asymmetry.ov; [WWP] Width Widest Pos.



**Figure 2.** Scatter plot of the first (PC1) and second (PC2) principal components based on 22 TA descriptors.



# Assessment of fruit quality and fruit morphology in androgenic pepper lines (*Capsicum annuum* L.)

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**BACKGROUND** Production of haploids and doubled haploids (DH) is an important plant-breeding tool allowing rapid recovery of unique homozygous genetic recombinants, and quick detection of recessive mutations. DH lines are immensely valuable to speed up the breeding process and have been identified as essential breeding material of crop improvement with its proven practical applications for the development of hybrid displaying maximum heterosis and/or improved traits [1]. Pepper is economically pivotal crop from Solanaceae family characterized by wide variation for fruit colour, shape and size. In recent years a great attention has been focused on studies associated to fruit quality traits with enhanced antioxidant and health-promoting properties [2]. Creation of bio-diversity using advanced cytological and tissue culture methods has been proven as a unique opportunity for pepper germplasm improvement with novel value added traits. Therefore, this study was designed to evaluate fruit quality characters including vitamin C, dry matter content and fruit morphology from diverse fourteen androgenic pepper lines.

**MATERIALS & METHODS** Different 14 DH lines in R4 generation were obtained as a result of anther culture of four parental genotypes – one hybrid '202' and three varieties – Slonovo uvo, Stryama and Zlaten medal 7. R4 plants and original parental genotypes were grown under field conditions with two replications, each composed of 10 plants. Data from different fruit and plant morphological traits were collected (Table 1). Total 47 different descriptors for fruit morphology and colour were characterized using Tomato Analyzer v. 3 software [3]. Freshly homogenized juice from a bulk sample of 10 fruits/accession was used for analyses of dry matter and Vitamin C content.

**RESULTS** The results showed significant phenotypic homogeneity within androgenic lines and considerable variation for main fruit characters and productivity among different androgenic lines of same background (Table 1). The lines originating from 'F1 202' were distinguished with higher values of the studied fruit morphology traits compared to parental genotype, while the highest productivity, vitamin C and dry matter content was observed only in line '211'. The statistical analysis of the lines deriving from variety Slonovo uvo showed that line '214' exceed the parental genotype for studied morphology traits. In terms of Vitamin C content the higher value was measured in both androgenic lines. Among androgenic lines, originating from variety 'Stryama' the highest fruit weight, length and width were recorded in lines '220' and '216'. The lines originated from variety Zlaten medal 7 were not distinguished with higher values of the fruit morphology traits, but lines '221' and '224' showed 2.5 fold increase in vitamin C content.

**DISCUSSION & CONCLUSION** Pepper belongs to recalcitrant species characterized by low frequency of embryo induction from anthers and low rate of subsequent conversion to normal plant-regenerants. The percentage of spontaneous doubling of the chromosomes depends on genotype and culture conditions. In the recent years application of anther culture as a method to speed-up the breeding process have significantly increased. Results from the study demonstrate that application of anther culture can assist to obtain genetic variation and transgressive traits. Widened genetic diversity of pepper genepool can be resourceful in development of novel breeding lines with improved quantitative and qualitative fruit quality traits.

## REFERENCES

- [1] Germana MA, 2011, Plant Cell Tiss. Orgn. Cult., 104:283-300. doi 10.1007/s11240-010-9852-z  
 [2] Stommel JR., Albrech E, 2012, Genetics, In V.M. Roso (ed.). Peppers: botany, production and uses, pp. 29-45.  
 [3] Brewer MT, Lang L et al. 2006, Plant Physiol., 141:15-25.

Line	Fruit					Productivity/ plant g	Fruit per plant No.	Vit. C mg%	Dry matter content %
	weight g	length cm	width cm	wall thickness mm	usable part g				
211	141.3 <sup>a</sup>	14.0 <sup>ns</sup>	6.2 <sup>ab</sup>	6.5 <sup>a</sup>	124.5 <sup>a</sup>	2047 <sup>a</sup>	15 <sup>ns</sup>	190.1	8.9
SD	5.26	0.28	0.08	0.42	4.38	900.85	7.07		
212	127.0 <sup>a</sup>	14.4 <sup>ns</sup>	6.4 <sup>a</sup>	6.5 <sup>a</sup>	108.3 <sup>a</sup>	1398 <sup>b</sup>	12 <sup>ns</sup>	167.8	7.8
SD	6.08	1.34	0.85	0.71	10.23	308.30	2.40		
213	125.8 <sup>a</sup>	15.1 <sup>ns</sup>	6.6 <sup>a</sup>	6.5 <sup>a</sup>	110.1 <sup>a</sup>	1449 <sup>b</sup>	12 <sup>ns</sup>	136.1	8.2
SD	7.71	0.35	0.57	0.71	7.71	236.70	1.52		
F <sub>1</sub> 202	90.8 <sup>b</sup>	13.8 <sup>ns</sup>	5.0 <sup>b</sup>	4.0 <sup>b</sup>	83.3 <sup>b</sup>	1150 <sup>b</sup>	12 <sup>ns</sup>		
SD	4.10	0.14	0.00	0.01	6.29	0.71	0.71		
214	201.5 <sup>a</sup>	15.0 <sup>a</sup>	7.5 <sup>a</sup>	9.1 <sup>a</sup>	181.5 <sup>a</sup>	2043 <sup>a</sup>	11 <sup>ns</sup>	192.2	9.7
SD	8.35	0.54	0.37	0.14	10.54	89.57	0.71		
215	146.0 <sup>b</sup>	13.0 <sup>b</sup>	6.5 <sup>b</sup>	5.1 <sup>b</sup>	130.5 <sup>b</sup>	1414 <sup>b</sup>	11 <sup>ns</sup>	237.7	10.1
SD	26.67	0.68	0.16	0.42	24.61	175.01	0.19		
S. uvo	105.6 <sup>b</sup>	13.6 <sup>ab</sup>	6.4 <sup>b</sup>	5.5 <sup>b</sup>	92.0 <sup>b</sup>	1193 <sup>b</sup>	11 <sup>ns</sup>	151.9	11.3
SD	0.57	0.07	0.14	0.71	0.42	37.71	0.47		
216	100.1 <sup>ab</sup>	11.9 <sup>ab</sup>	5.1 <sup>a</sup>	5.5 <sup>ns</sup>	85.2 <sup>a</sup>	1166 <sup>ns</sup>	12 <sup>ab</sup>	40.8	4.7
SD	14.20	0.49	0.17	0.99	12.53	5.30	2.14		
217	86.3 <sup>bc</sup>	10.4 <sup>b</sup>	5.0 <sup>a</sup>	4.9 <sup>ns</sup>	74.6 <sup>ab</sup>	1141 <sup>ns</sup>	13 <sup>ab</sup>	55.5	4.5
SD	1.57	0.45	0.20	0.42	1.97	100.76	2.03		
218	77.7 <sup>c</sup>	11.0 <sup>b</sup>	4.9 <sup>a</sup>	4.9 <sup>ns</sup>	64.2 <sup>b</sup>	1415 <sup>ns</sup>	18 <sup>a</sup>	70.2	4.6
SD	5.73	1.20	0.04	0.42	6.03	141.93	0.28		
219	86.1 <sup>bc</sup>	10.8 <sup>b</sup>	5.1 <sup>a</sup>	4.9 <sup>ns</sup>	71.3 <sup>ab</sup>	1250 <sup>ns</sup>	15 <sup>ab</sup>	35.9	4.3
SD	6.02	0.54	0.28	0.78	5.29	229.81	3.18		
220	106.0 <sup>a</sup>	13.0 <sup>a</sup>	5.3 <sup>a</sup>	5.1 <sup>ns</sup>	89.0 <sup>a</sup>	1190 <sup>ns</sup>	12 <sup>b</sup>	21.1	4.2
SD	0.27	0.80	0.23	0.71	1.99	216.73	1.80		
Stryama	76.4 <sup>c</sup>	11.5 <sup>ab</sup>	4.5 <sup>b</sup>	4.0 <sup>ns</sup>	65.7 <sup>b</sup>	1193 <sup>ns</sup>	17 <sup>a</sup>	39.2	5.7
SD	8.72	1.13	0.07	1.41	8.08	152.03	2.12		
221	70.7 <sup>ns</sup>	12.1 <sup>ns</sup>	4.4 <sup>ns</sup>	4.7 <sup>a</sup>	59.4 <sup>ns</sup>	1237 <sup>ab</sup>	17 <sup>ab</sup>	106.1	8.7
SD	9.63	1.16	0.45	0.14	7.68	4.71	2.04		
222	85.7 <sup>ns</sup>	13.7 <sup>ns</sup>	4.8 <sup>ns</sup>	4.7 <sup>a</sup>	72.4 <sup>ns</sup>	1105 <sup>b</sup>	14 <sup>b</sup>	52.2	6.7
SD	6.46	1.82	0.08	0.99	7.60	1.10	1.16		
223	80.4 <sup>ns</sup>	13.4 <sup>ns</sup>	4.8 <sup>ns</sup>	4.0 <sup>ab</sup>	68.4 <sup>ns</sup>	1296 <sup>ab</sup>	17 <sup>ab</sup>	84.9	4.6
SD	8.66	0.23	0.08	0.57	7.21	165.41	0.56		
224/	80.3 <sup>ns</sup>	12.1 <sup>ns</sup>	4.9 <sup>ns</sup>	4.0 <sup>ab</sup>	70.2 <sup>ns</sup>	1418 <sup>a</sup>	18 <sup>a</sup>	106.1	5.1
SD	10.25	0.21	0.35	0.00	6.60	139.06	3.06		
Z. medal	77.8 <sup>ns</sup>	14.3 <sup>ns</sup>	4.7 <sup>ns</sup>	3.0 <sup>b</sup>	67.1 <sup>ns</sup>	1303 <sup>ab</sup>	17 <sup>ab</sup>	24.2	7.2
SD	6.75	0.42	0.49	0.00	4.90	102.53	2.12		

a.b.c....p≤0.05 Duncan's Multiple Range Test ; ns – not significant

**Table 1.** Morphology evaluation of fruit and productivity in R4 androgenic lines and four initial genotypes (Mean value and standard deviation (SD)).

# Nutritional and organoleptic quality of two major *Solanaceae* species: a comparative study

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**BACKGROUND** Pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) are two of the most important *Solanaceae* species grown worldwide. They play an important role in human diet as source of vitamins, minerals and fibers. Some current breeding programs focus on the improvement of bioactive compounds present in these two crops. In this regard, peppers have remarkable antioxidant activity due mainly to their content in ascorbic acid, and carotenoids as vitamin A precursors [1]. Eggplants have been reported to show outstanding contents in phenolic compounds, with chlorogenic acid being the major phenolic constituent in its fruit flesh [2]. Herein, we measured some major nutritional compounds in landraces of pepper and eggplant. Our aim was to compare the nutritional and functional quality profile of these two solanaceous crops and provide relevant information to consider in a well-balanced diet and future breeding programs.

**MATERIALS & METHODS** A collection of pepper (10 accessions) and eggplant (10 accessions) landraces from the Spanish region of Valencia was used. For each accession, ten plants were grown during the spring-summer of 2017 under organic open field conditions. Commercially ripe fruits were harvested, processed and analysed for the following traits involved in nutritional and/or organoleptic characteristics: soluble solids content (SSC), glucose (GLU), fructose (FRU), citric (ACIT) and malic (AMAL) organics acids, vitamin C (VITC), protein (PROT), total carotenoids (TCAR) and total phenolics content (TPC). An ANOVA and a PCA were performed to evaluated differences among the accessions and species.

**RESULTS** Significant differences were found between the pepper and eggplant average values for every trait analysed (Table 1). The heatmap in Figure 1 obtained from PCA values groups the 20 accessions into two clusters that match the corresponding species and shows a distinct quality profile between eggplant and pepper accessions. Pepper had around 2-fold more soluble solids, glucose and fructose average content than eggplant. Unlike eggplant fruits, peppers presented more fructose than glucose content, which may have implications in the sweet taste balance. Eggplant stood out for having high malic acid content, which contributes to the sour taste of fruits. Eggplant also had the highest average content of crude protein, making it a better source of amino acids. Eggplant had by far the highest total phenolics content, with average contents 2.4-fold higher more than pepper. On the other side, pepper had a much higher content in vitamin C (50-fold over eggplant), besides the highest citric acid and total carotenoids content. Important differences were also observed among varieties within each crop, with some overlap between the two species for some traits such as SSC, GLU, FRU, AMAL, PROT and TPC (Table 1).

**DISCUSSION & CONCLUSION** Our results suggest that pepper and eggplant have very distinct profiles of composition for traits involved in organoleptic and nutritional quality. Due to their complementary nutritional quality profiles, a combined regular consumption of eggplant and pepper would supply the Dietary Reference Intake (DRI) of several of the analysed phytochemicals. Our work also reveals wide variation within each species and enhances the potential of landraces in plant breeding for developing improved varieties of pepper and eggplant with enhanced organoleptic and nutritional properties.

## REFERENCES

- [1] Ribes-Moya, A. M., Raigón, M. D. et al., 2018, PLOS ONE, 13(11), e0207888
- [2] Plazas, M., López-Gresa, M. P et al., 2013, J. Agric. Food Chem., 61(37), 8871-8879

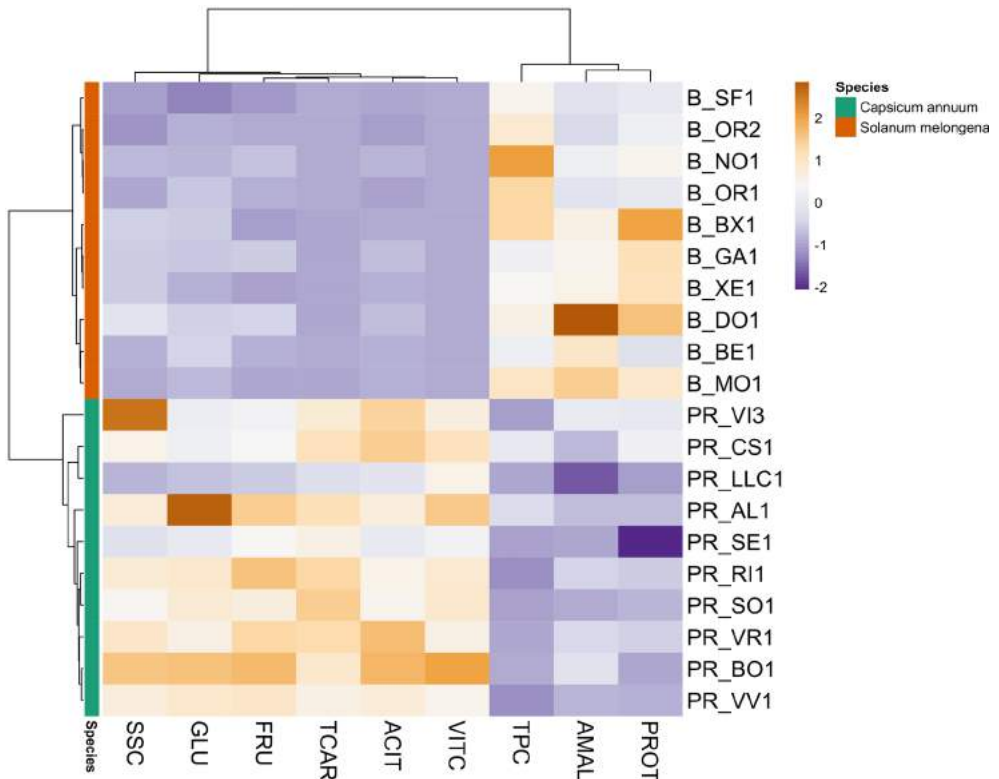
Traits	Pepper ( <i>C. annuum</i> )	Eggplant ( <i>S. melongena</i> )
SSC (*Brix)	11.0 <sup>b</sup> (5.6-17.2)	6.0 <sup>a</sup> (4.6-8.3)
GLU (mg/100g FW*)	3044.2 <sup>b</sup> (1569.6-5180.7)	1580.8 <sup>a</sup> (795.9-2145.9)
FRU (mg/100g FW)	3553.3 <sup>b</sup> (1532.4-5048.6)	1210.4 <sup>a</sup> (636.7-2034.5)
VITC (mg/100g FW)	236.2 <sup>b</sup> (148.2-378.9)	4.7 <sup>a</sup> (3.1-6.6)
ACIT (mg/100g FW)	535.7 <sup>b</sup> (259.5-793.8)	80.5 <sup>a</sup> (28.1-132.2)
AMAL (mg/100g FW)	191.0 <sup>a</sup> (62.6-305.3)	355.9 <sup>b</sup> (224.0-632.2)
PROT (% DM**)	8.6 <sup>a</sup> (6.1-10.5)	11.1 <sup>b</sup> (9.3-13.3)
TCAR (mg/100g DM)	60.4 <sup>b</sup> (21.1-82.1)	0.5 <sup>a</sup> (0-1.2)
TPC (mg GAE***/100g DM)	57.7 <sup>a</sup> (40.0-99.4)	138.5 <sup>b</sup> (94.7-215.2)

\*FW: fresh weight

\*\*DM: dry matter

\*\*\*GAE: gallic acid equivalents

**Table 1.** Average values and accession means range for 10 accessions of pepper and 10 accessions of eggplant for traits involved in organoleptic and nutritional quality. For each trait, average values separated by different letters indicate significant differences ( $P < 0.05$ ) between pepper and eggplant.



**Figure 1.** Heatmap representing the hierarchical clustering of the 20 accessions studied based on their nutritional profiles. Columns represent the nine traits measured and rows represent the accessions. Unit variance scaling was applied to columns. Both rows and columns are clustered using correlation distance and average linkage. The scale of the colour intensity is shown in the top corner and it represents a proportional value of the nutritional content.

# Effects of different growing methods on the production of a new paprika variety 'Hetényi Parázs'

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**BACKGROUND** In Hungary spice paprika is an important species. In 2018, paprika was produced in cca. 2000 ha. Because of the high cost of seeds, hybrid cultivars are not popular among farmers. Although hybrids are necessary in the case of intensive cropping methods [1], open-pollinated varieties with direct seeding are decisively used. The objective of this study was to investigate the performance of a new hybrid variety 'Hetényi Parázs' using different growing methods. Open field experiments were carried out at Univer Product Plc. in 2018.

**MATERIALS & METHODS** Planting methods were direct seeding in April (with two stocking densities: 17.3 (S1), and 26.0 seeds/m<sup>2</sup> (S2)) and seedling planting in May in twin rows, 0.4 m spacing inside the rows and 1.1 m between adjacent twin rows. The space between the plants was 0.2 m, with a plant density of 6.67 plants/m<sup>2</sup> (P). This method was combined with mulching (M) and mulching with nonwoven fleece (MF). Due to the high amount of precipitation in June, the growing conditions were not optimal. After harvesting in August, the samples were dried in a covered place for 40 days. In order to determine the best growing method, chemical components were measured right after harvesting and drying (Table 1).

**RESULTS** We measured the highest yield (32.7 t/ha) and capsaicinoid content (1,736 mg/kg) in S2 (Table 1). With a rate of deterioration of less than 7% and a dry matter content of over 78%. The highest pigment content was found in M (114ASTA). In the S1, we only had to face a loss of 3.6%.

**DISCUSSION & CONCLUSION** From our experiment it can be concluded that the different growing methods resulted significant difference in yield and chemical components. The direct seeding method gave better commodity for industrial use than the seedling planting method. Determination of optimal number of plants/m<sup>2</sup> should be a topic of further study for the most economical production of this cultivar.

## REFERENCES

[1] Bosland PW, Votava EJ, 2012, Peppers: vegetable and spice capsicums, 2nd ed. 104-117 ISBN 978-1-84593-825-3

Technology	Avg. yield (t/ha)	Avg. dry matter content (%)	Avg. capsaicinoid content for dry matter (mg/kg)	Avg. pigment content at harvest (ASTA)	Avg. fruit weight (g)	Avg. post-ripening deterioration (%)	Avg. post-ripened dry matter content (%)
S1 (direct seeding 17.3 seeds/ m <sup>2</sup> )	20.12	<b>23.43</b>	1632	103	13.0	<b>3.6</b>	<b>82.2</b>
S2 (direct seeding 26 seeds/ m <sup>2</sup> )	<b>32.73</b>	21.49	<b>1736</b>	111	13.6	6.7	78.1
P (Planting 6.67 plants/m <sup>2</sup> )	14.13	21.08	1157	66	<b>20.6</b>	19.0	43.0
M (Planting with mulching)	11.40	21.66	1296	<b>114</b>	14.3	10.1	66.0
MF (Planting with mulching and nonwoven fleece)	18.82	19.97	1568	76	13.3	5.7	46.0

**Table 1.** The summary of results.

# Evaluation of fruit mineral composition in a diverse Balkan pepper collection

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**BACKGROUND** Vegetables are known to be an excellent source of nutrients, such as minerals and vitamins. Mineral ions are of prime importance in determining fruit nutritional value, the major ones being potassium, calcium, and magnesium – very important to human health. Trace elements on the other side are essential components of enzyme systems involved in key physio-regulatory, organelle, and cellular functions. Biofortification of vegetables may be accomplished through breeding targeting higher mineral content or varieties that uptake minerals with higher efficiency. Surveying pepper germplasm for nutrient content is a useful strategy to identify accessions rich in biologically active substances, minerals, and trace elements [1, 2, 3]. The current study is a part of a large-scale phenotyping of *Capsicum annuum* L. accessions, collected from the Balkan region. The aim is to determine the content of some of the most important minerals and trace elements in the pepper fruits, which will give additional information about the biological value of the tested accessions.

**MATERIALS & METHODS** A total of 66 pepper accessions (all *Capsicum annuum* L.) were cultivated in a triplicated field trial at MVCRI, Bulgaria. Evaluated accessions belonged to five pre-defined groups: pungent green (10), sweet green (21), pumpkin (6), kapia (23), and for powder - sweet paprika (6). In the first two groups, fruits were harvested before maturity, as they are commonly used, while the remaining three groups were harvested at the fully ripe stage. Freeze dried samples were subjected to microwave digestion in closed PTFE (polytetrafluoroethylene) vessels, and subsequently analyzed by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) with dual-view configuration. Ten different macro and microelements were quantified and their concentrations expressed relative to dry matter content.

**RESULTS** Quantified macro and microelements were ranked in the following descending order: K > Mg > Ca > Na with an average composition of 1.58, 0.10, 0.07, and 0.004 %, respectively, whereas Fe > Zn > Mn > Cu > Ni > Cr were reported at 376.7, 232.4, 102.7, 55.2, 10.4, and 2.56 ppm, respectively (Table 1). Accessions from the 'pungent green' and 'sweet green' groups were distinguished with the highest amounts of most macro- and micronutrients, while accessions from the 'kapia' group displayed the lowest content of these elements. Principal component analysis (PCA) showed that factors contributing to total variation were mainly associated with dry matter, and the first and second principal components explained around 40.5% and 19.6% of variation, respectively (Fig. 1A and 1B). Clear distinction and varietal separation were seen based on the fruit harvesting stage, where 'pungent green' and 'sweet green' were seen to be located on the right side of the PCA plot, whereas varietal types harvested at the fully ripe stage, 'kapia', 'pumpkin' and 'for powder' were located on the left side of the PCA plot (Fig. 1A).

**DISCUSSION & CONCLUSION** The obtained results revealed a wide range of variation for mineral composition profiles between and within the five pre-defined groups. In each group, accessions with a high content of individual elements were distinguished. The observed variation within each group suggests that mineral composition is vastly influenced by genotype. This variability is anticipated to enable selection and breeding of novel varieties with enhanced mineral composition.

REFERENCES

[1] Fernandez-Ruiz V et al., 2011, Biol.Trace Elem. Res. 141:329-339.  
[2] Guil-Guerrero, JL et al., 2006, Eur Food Res Technol, 224:1-9.  
[3] Wahyuni Y et al., 2013, Metabolomics, 9(1):130–144.

ACKNOWLEDGEMENTS

Velichka Todorova thanks the French “Ministère de l’Enseignement Supérieur, de la Recherche et de l’Innovation (program ACCES de CAMPUS France)” for covering the travel and accommodation expenses for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.

Varietal type	Dry matter (%)	K (%)	Na (%)	Mg (%)	Ca (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Ni (ppm)	Cr (ppm)
Pungent green	11.25 <sup>b</sup>	2.00 <sup>a</sup>	0.0048 <sup>ns</sup>	0.12 <sup>a</sup>	0.12 <sup>a</sup>	347.56 <sup>b</sup>	113.77 <sup>ab</sup>	318.22 <sup>a</sup>	71.47 <sup>a</sup>	7.51 <sup>b</sup>	4.19 <sup>ab</sup>
Sweet green	8.25 <sup>c</sup>	1.66 <sup>ab</sup>	0.0038 <sup>ns</sup>	0.11 <sup>a</sup>	0.09 <sup>ab</sup>	418.30 <sup>a</sup>	117.57 <sup>a</sup>	256.52 <sup>b</sup>	71.80 <sup>a</sup>	12.38 <sup>a</sup>	5.68 <sup>a</sup>
Pumpkin	10.02 <sup>b</sup>	1.68 <sup>ab</sup>	0.0033 <sup>ns</sup>	0.10 <sup>ab</sup>	0.07 <sup>bc</sup>	365.77 <sup>ab</sup>	80.02 <sup>c</sup>	194.40 <sup>c</sup>	46.45 <sup>bc</sup>	7.47 <sup>b</sup>	0.22 <sup>b</sup>
Kapia	10.01 <sup>b</sup>	1.33 <sup>b</sup>	0.0036 <sup>ns</sup>	0.09 <sup>b</sup>	0.04 <sup>c</sup>	364.53 <sup>ab</sup>	91.37 <sup>c</sup>	188.16 <sup>c</sup>	35.32 <sup>c</sup>	10.50 <sup>ab</sup>	0.20 <sup>b</sup>
For powder	14.13 <sup>a</sup>	1.48 <sup>b</sup>	0.0033 <sup>ns</sup>	0.09 <sup>b</sup>	0.05 <sup>c</sup>	337.33 <sup>b</sup>	98.43 <sup>bc</sup>	212.78 <sup>bc</sup>	54.37 <sup>ab</sup>	10.70 <sup>ab</sup>	0.37 <sup>b</sup>
LSD 0.05	1.69	0.38	0.0040	0.02	0.04	69.64	19.09	51.34	17.55	3.89	4.12

NOTE: a, b, c. Values followed by the same letter are not significantly different according to LSD test at P=0.05

Table I. Means of macro and microelement content in pepper fruits from five varietal types within a collection of 66 Balkan pepper accessions (on fresh weight basis).

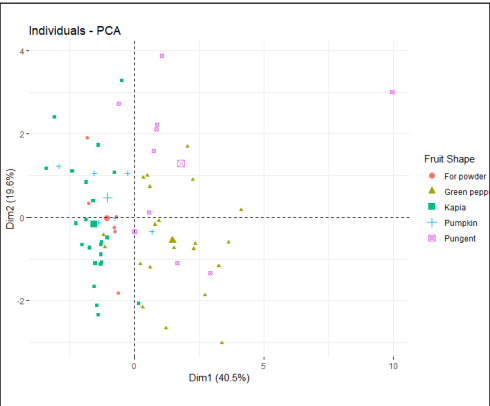


Figure 1.A PCA plot

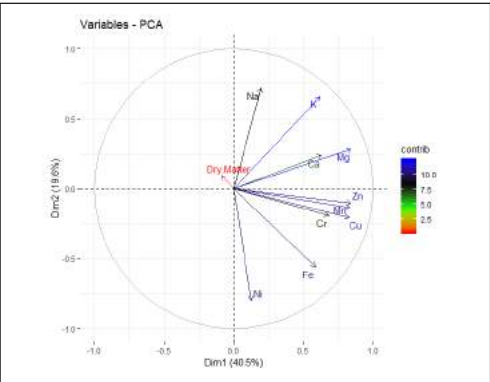


Figure 1.B Variable plot



# Evaluation of $\alpha$ -glucosidase inhibitor (AGI) activity for the selection of leaf-using pepper varieties with high AGI activity

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**BACKGROUND** Pepper is a valuable vegetable containing various functional ingredients such as vitamins, carotenoids and capsaicin, and is known to be effective in preventing diabetes. Once diabetes occurs, it needs to be managed throughout life, and various complications can arise. The  $\alpha$ -glucosidase inhibitor (AGI) functions to lower postprandial hyperglycemia by inhibiting  $\alpha$ -glucosidase, an enzyme that accelerates the absorption of carbohydrates in the small intestine by decomposing disaccharides into monosaccharides. It is known that AGI is suitable for diabetics in Korea who have high carbohydrate intake and high blood sugar. The AGI activity of pepper leaves has been reported to be high, and the National Institute of Horticultural and Herbal Science (NIIHHS) has developed 'Wongi No. 1' which has 4 times higher AGI activity than the commercial variety. The purpose of this study is to evaluate the AGI activity and horticultural characteristics of the plant material for the development of a new commercial pepper variety using leaves.

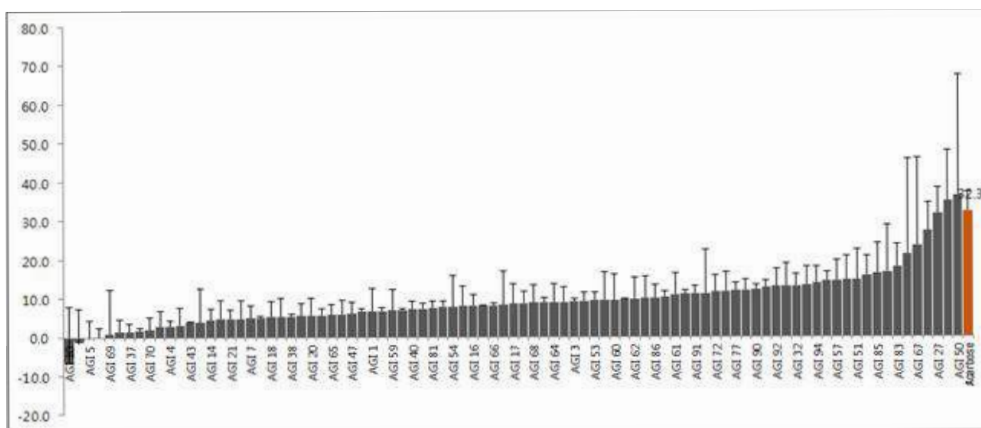
**MATERIALS & METHODS** The AGI activity analysis was based on the method of Chonbuk National University by adjusting the  $\alpha$ -glucosidase and substrate (pNPG) capacity, extraction method and dilution factor in the previously reported AGI activity assay. A total of 97 samples were collected from the inbred lines, introduced resources and commercial varieties using the analytical samples prepared by drying harvested leaves from the 3rd branch at 55°C.

**RESULTS** The fruit and leaf characteristics of tested materials were as follows: fruit weight was 4.9~219.5 g, fruit length was 6.0~28.0 cm, fruit width was 1.2~9.3 cm, leaf length was 8.0~22.3 cm, and leaf width was 3.3~11.7 cm. As a result of AGI activity analysis on the leaves of the 3rd branch for the 94 evaluation materials, AGI10 showed the lowest inhibition rate of 6.8% and AGI50 showed the highest inhibition rate of 36.4% (Figure 1). AGI 50 showed higher activity than ACARBOSE, a diabetic agent used as a control, and it will be used as a potential breeding material after progress through the generations. As a result of the analysis, we have selected 6 resources with high AGI activity.

**DISCUSSION & CONCLUSION** The selected resources are planned to be used as a material for breeding anti-diabetic pepper varieties.

## REFERENCES

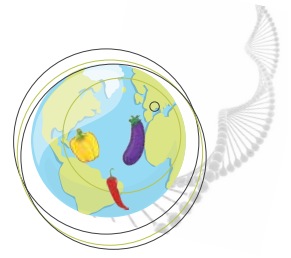
- [1] Kim MJ et al., 2015, J Korean Soc Food Sci Nutr., 44(7):993-999
- [2] Kim YM et al., 2005, Nutrition, 21:756-761
- [3] Tattersall R, 1993, Diabetic medicine, 10:668-672



**Figure 1.** Inhibition rate of AGI activity (y axis) on leaves of the 3<sup>rd</sup> branch for 94 pepper accessions (x axis).



Capsicum and Eggplant EUCARPIA Meeting 2019



# BIOTECHNOLOGY AND GENOMICS

SESSION 4



# Capsicum spp. and eggplant genome sequencing and resequencing provide new tools for the characterization of genetic resources

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**BACKGROUND** We report the current state of the art on genome sequencing and resequencing studies in *Capsicum annuum* L. and *Solanum melongena* as well as some of their cultivated and wild related species. The availability of high quality genome sequences in both species has opened the way to the characterization of genetic resources through genotyping-by-sequencing (GBS) approaches. Among them, we focus on the recently developed Single Primer Enrichment Technology (SPET) technique, which we have applied for targeted sequencing of a wide collection of *S. melongena* accessions and representatives of its primary, secondary and tertiary gene pools.

## RESULTS

### Pepper

Since the beginning of 2014, various consortia have released the genome sequences of domesticated and wild *Capsicum* species. The whole-genome sequences of *C. annuum* CM334 and *C. chinense* P1159236, which carry important disease resistance traits and have been widely used as parental lines of mapping populations, were the first to be released [1]. Next, the genome sequences of *C. annuum* Zunla-I and of the wild species Chiltepin (*C. annuum* var. *glabriusculum*) were published [2]. Both studies highlighted that the pepper genome size is ~3-3.5 Gb, has a high percentage (over 80%) of repetitive elements, and encodes about 35K genes. Later, the improved version of the reference genome of CM334 and *C. chinense* P1159236, together with the sequencing of the domesticated *C. baccatum* were also made available, and contributed to deciphering the evolutionary relationships among the three species as well as to estimating the lineage-divergence times occurring in *Capsicum* [3]. By adopting the linked-read sequencing technology, the genome sequence of an F1 hybrid obtained by crossing CM334 with a non-pungent *C. annuum* breeding line was also published [4]. The availability of pepper genome sequences has allowed the resequencing of *Capsicum* accessions at both targeted genomic regions and the whole genome level. By applying a bulk segregant analysis, it has been possible to identify markers tightly linked to the *Pvr4* locus, conferring dominant resistance to three pathotypes of Potyvirus (PVY, [5]). Furthermore, after the resequencing of resistant and susceptible cultivars, SNPs and putative alleles related to resistance against powdery mildew and bacterial wilt have also been detected [6,7]. To provide insights into the process of pepper domestication, the Zunla-I genome sequence was analysed together with resequencing (20-30X) information of 18 cultivated accessions representative of the major varieties of *C. annuum* and two semi-wild/wild peppers [2]. This resulted in the identification of 115 genomic regions affected by artificial selection in cultivated peppers. Lastly, genome resequencing (30X) of four genotypes, representative of the main varietal types grown in the Mediterranean region, has been performed. Distinctive variations in miRNAs and resistance gene analogues (RGAs) have been highlighted as well as mutations in the coding sequences and regulatory regions of genes affecting fruit size and shape (Barchi et al., SOLCUC meeting 2017). Within the G2P-SOL EU project ([www.g2p-sol.eu](http://www.g2p-sol.eu)), the analysis of genome-wide genotyping-by-sequencing (GBS) data on 9,659 pepper accessions retrieved from the major European (CGN, INRA, IPK, UPV) and Asian (AVRDC) gene banks, Universities and Research Centres has been recently performed and the results obtained reported by Tripodi et al. (see Proceedings of this Meeting).

## Eggplant

In 2014, Hirakawa and co-authors [8] produced the first unanchored draft of the *S. melongena* genome sequence using the Nakate-Shinkuro accession. The obtained sequence covered about 70% of its projected 1.2 Gb genome size and more than 42K genes were identified. More recently, the Italian Eggplant Genome Consortium (IEGC) [9] developed a high quality and anchored genome assembly of the eggplant line 67/3, the male parent of an F6 RIL (Recombinant Inbred Line) mapping population. A hybrid assembly, covering 1.22 Gb, was obtained by merging Illumina sequencing data and optical mapping. The female parent of the RIL mapping population (line '305E40') was also sequenced (coverage of 34X), and thanks to low coverage resequencing (1X) of the F6 RILs, the genome assembly was anchored to the 12 chromosomes. Recently, based on Illumina sequencing data, a draft genome assembly of 1.02 Gb in size was developed for the cultivated species *S. aethiopicum*, which contained as for eggplant, about 76% of repetitive sequences. Furthermore, compared to *S. melongena*, an expansion of gene families involved in drought or salinity tolerance as well as disease resistance including defence responses was identified. Recently, the resequencing of seven eggplant accessions and one accession of the wild relative *S. incanum*, which are the parents of a MAGIC population, has been performed (Gramazio et al., see Proceedings of this Meeting). The set of identified SNP polymorphisms has been annotated and currently is being used for further analyses in order to efficiently genotype the MAGIC population with the goal of dissecting key agronomic and morphological traits. More recently, 60 genotypes of the cultivated scarlet eggplant (*S. aethiopicum*) belonging to the varietal groups, "Gilo" and "Shum", as well as 5 accessions of its ancestor *S. anguivi*, were sequenced at a coverage of 30-60X, with the goal of investigating the evolution, population demography and domestication history of the species [10].

## Population structure of eggplant germplasm based on Single Primer Enrichment Technology (SPET) genotyping

Within the G2P-SOL EU project ([www.g2p-sol.eu](http://www.g2p-sol.eu)), which brings together the major European (CGN, INRA, IPK, UPV) and Asian (AVRDC) gene banks, Universities and Research Centres, 2,912 *S. melongena*, 305 *S. aethiopicum* and 122 *S. macrocarpon* accessions as well as a set of 266 accessions belonging to 29 wild species have been inventoried. The Single Primer Enrichment Technology (SPET) genotyping, recently developed by Nugen® was applied for their targeted genotyping.

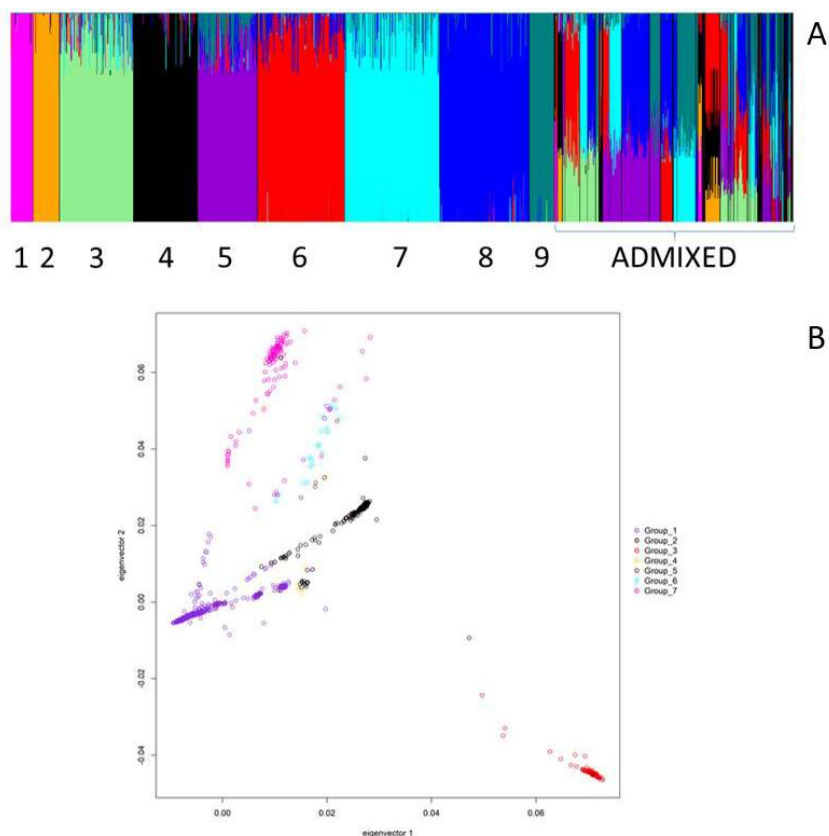
Starting from more than 12K polymorphic sites found in both coding regions and in the introns/UTRs, a set of 5K best performing SPET probes was used for diversity analyses, and a panel of about 25K high confident (min mean read depth of 30 and max missing data of 0.5%) SNPs evenly distributed throughout the genome were detected. The FastSTRUCTURE analysis (Fig. 1) identified 9 main clusters (K), with *S. melongena* accessions grouping into 6 sub-clusters. Three further clusters were found, including accessions belonging to *S. macrocarpon* and *S. aethiopicum* as well as species belonging to the subgenus *Solanum*, in good agreement with clustering obtained with a maximum likelihood phylogenetic tree. A total of 1,114 accessions were finally classified as admixed. The first two components of the PCA (Fig. 2) explained about 26% of the genetic variation. The first axis, explaining 15% of the genetic variation separated *S. macrocarpon* from the other species. The second component grouped separately *S. melongena* and *S. aethiopicum* as well as species from the *Leptostemonum* and *Solanum* subgenera. Several accessions had an unclear assignment, presumably due to misclassifications in the genebanks or gene flow among species. The gathered information was used for the development of a core collection of genotypes for future Genome Wide Association (GWA) studies. For this purpose, 15 *S. melongena* accessions and 5 wild species will be re-sequenced at 30X with the goal of identifying new SNPs useful for high resolution SPET genotyping.

## REFERENCES

- [1] Kim S et al., 2014, Nat Genet. 46, 270–8
- [2] Qin C et al., 2014, Proc Natl Acad Sci. 111, 5135–5140
- [3] Kim S et al., 2017, Genome Biol. 18, 210
- [4] Hulse-Kemp AM et al., 2018, Hortic Res. 5, 4
- [5] Devran Z et al., 2015, Mol Breed. 35, 101
- [6] Kang YJ et al., 2016, BMC Plant Biol. 16, 235
- [7] Ahn YK et al., 2018, Sci Reports 8, 5188
- [8] Hirakawa H et al., 2014, DNA Res. 21, 649–60
- [9] Barchi L et al., Sci Rep Submitted.
- [10] Song B et al., 2019, bioRxiv 532077

## ACKNOWLEDGEMENTS

Lorenzo Barchi thanks the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



**Figure 1.**

**A** The FastSTRUCTURE genetic architecture of the full germplasm panel at  $K = 9$ . **Group 1** includes subgenus *Solanum* species, **Group 2** mainly includes *S. macrocarpon* and closest relatives, **Group 3, 5, 6, 7, 8 and 9** includes *S. melongena* and closest relatives, **Group 4** includes *S. aethiopicum* and closest relatives. Admixed groups include among others other African, Asian and Australian wild relatives of eggplants as well as the Torva group-American species.

**B: PCA results:** **Group 1** includes *S. melongena* and closest relatives, **Group 2** includes *S. aethiopicum* and closest relatives, **Group 3** includes *S. macrocarpon* and closest relatives, **Group 4** includes other African, Asian and Australian wild relatives of eggplants, **Group 5** includes the *Solanum* section Torva group-American species, **Group 6** includes other American subgenus *Leptostemonum* species and **Group 7** includes subgenus *Solanum* species.



# Resequencing of seven eggplant (*Solanum melongena*) accessions and one wild relative (*S. incanum*) used for the development of a MAGIC population

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**BACKGROUND** Common eggplant (*Solanum melongena* L.) is the fifth economically most important vegetable crop in the world, but despite its importance in many tropical and subtropical areas has lagged behind other major crops in the development of genetic and genomic tools [1]. The first draft of an eggplant reference genome was released in 2014 and a new high-quality genome sequence, accession «67/3», has been recently assembled resulting in lower scaffold fragmentation and higher reference sequence coverage (1.06 Gb of 1.20 Gb estimated genome size) [2]. We present the first whole-genome resequencing study in the eggplant genepool including a comprehensive structural and functional characterization of seven *S. melongena* accessions and of one wild eggplant relative (*S. incanum* L.) of interest for eggplant breeding. These eight accessions are the founders of a MAGIC (Multi-parent Advanced Generation Intercrosses) population that is under development.

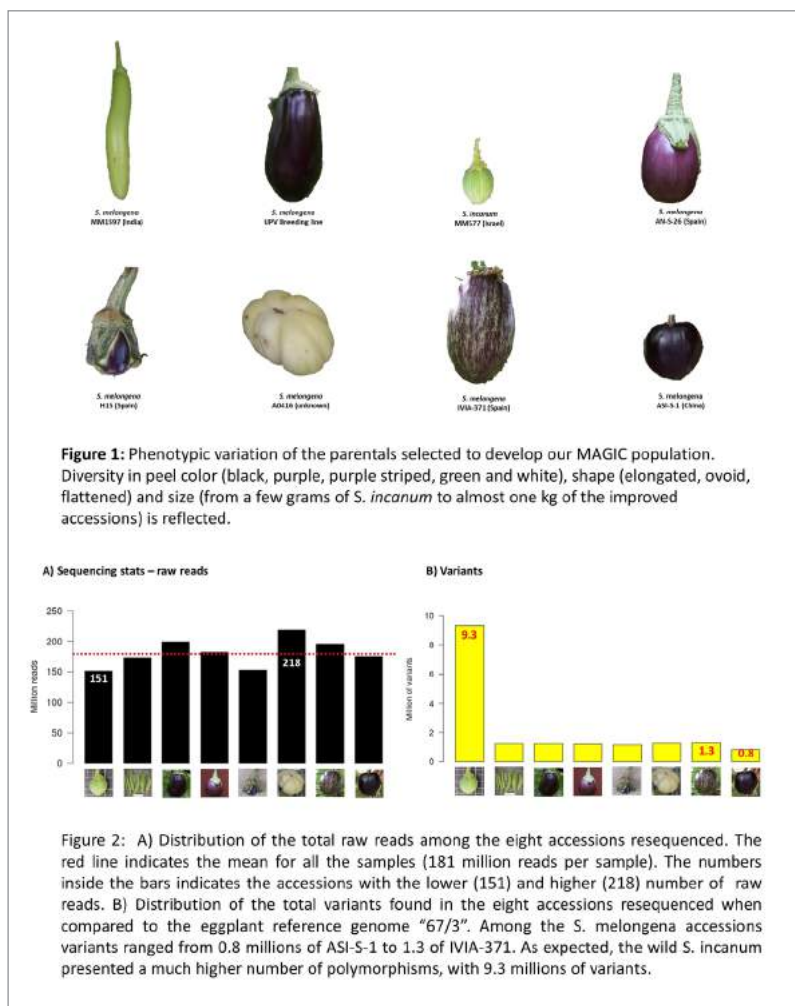
**MATERIALS & METHODS** Seven *S. melongena* accessions were chosen to maximize the phenotypic, genetic and geographic variation in the common eggplant. In addition, one *S. incanum* accession (MM577) was selected for its interest in eggplant breeding, displaying phenolic contents several times higher than those of common eggplant and being tolerant to some abiotic and abiotic stresses, mainly drought (Figure 1). Paired-end libraries were prepared with an insert size of approximately 300 bp and sequenced on two lines of an Illumina HiSeq 4000 sequencer as 2 × 150 bp. The raw sequences were processed with the Ea-utils package for barcode demultiplexing, adapter trimming, quality filtering and stats. The processed reads were then mapped to the «67/3» high-quality eggplant reference genome. Variant calling was performed using the Bayesian-based FreeBayes software and the variant effects were annotated based on their genomic position using the SnpEff software. Using the whole set of SNPs, a genetic matrix distance was calculated among the different accessions using the TASSEL software.

**RESULTS** The genome sequencing of the eight accessions generated over 1.4 billion paired-end raw reads, with a mean of 180.9 million reads per sample (Figure 2A). After the cleaning step, 97.6% of sequences were kept and mapped onto the eggplant reference genome «67/3». The mapping rate was quite similar across the accessions with an average of 85.4% and a mean coverage depth of 19.8x. A total of 10,916,466 high-quality variants polymorphisms were detected among the eight resequenced accessions (Figure 2B). Of those, 9,228,065 were SNPs (84.5%), 705,687 InDels (6.5%), 275,467 MNPs (multiple-nucleotide polymorphisms) (2.5%), and 707,247 complex variations (6.5%). As expected, the wild *S. incanum* presented a much higher number of polymorphisms, with 9,343,703 variants. According to the annotation and effect prediction, 98.72% of the variants were classified as «modifier». The second most abundant variants impact class was «moderate» (0.77%) and the «low» impact effects represented an average of 0.46%. Finally, the less abundant impact class corresponded to the «high» variation effects (0.07%). Genetic identities among the accessions were calculated on 9,109,331 SNPs after removing all the missing data. High values of genetic identities were obtained among common eggplant accessions, while lower values were obtained when *S. melongena* accessions were compared with the *S. incanum*.

**DISCUSSION & CONCLUSION** This first resequencing study in eggplant has gathered relevant genomic data and information that will be extremely useful for the genotyping and genetic analysis of a MAGIC population. This population will provide relevant information to face the present and future challenges in eggplant production and quality, as well for marker-assisted mapping of important eggplant breeding traits, and shed light on eggplant domestication history events. In this way, this first study paves the way and encourages other breeders to perform the resequencing of more eggplant accessions, especially crop wild relatives, where many valuable alleles for eggplant breeding have still to be discovered and exploited.

## REFERENCES

- [1] Gramazio et al., 2018, Not Bot Horti Agrobo, 46(1):1-13  
 [2] Barchi et al., 2016, In 20th EUCARPIA General Congress (pp. 122-122). Ed. Agroscope



# Unlocking genomic diversity of pepper (*Capsicum* spp.) collections held in genebanks: perspectives for breeding and germplasm management

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**BACKGROUND** The genetic diversity present in cultivars, landraces and wild relatives of modern crops, represents the source of agriculturally important genes to be discovered in our attempts of making crops more resilient and better adapted to the challenges of modern agriculture. In this context, genebanks are pivotal for the collection, preservation and distribution of germplasm; however, better knowledge of the available genetic variability would help improve the management of plant genetic resources. Here we report the analysis of genome-wide genotyping-by-sequencing (GBS) data on 9,659 pepper accessions retrieved from the major European (CGN, INRA, IPK, UPV) and Asian (AVRDC) genebanks, universities and research centres. The collection analyzed is so far the largest and the most diverse ever studied in pepper. The main objectives of this study are to: a) evaluate the genetic diversity using a large set of single-nucleotide polymorphism (SNP) data; b) detect the overlaps within collections and between genebanks; c) detect misclassification of accessions; d) develop core collections for association mapping studies.

**MATERIALS & METHODS** Germplasm studied comprised 7,253 *C. annuum* accessions, 1,795 accessions belonging to the other four domesticated species (*C. frutescens*, *C. chinense*, *C. baccatum*, *C. pubescens*) and 43 accessions belonging to the wild species (Table 1). About 3% of the collection was represented by unknown species. Passport information and 42 phenotypic descriptors for plant, flower and fruit traits have been provided by genebank curators in order to enhance the description of plant material. A two restriction enzyme GBS protocol was used [1]; *Pst*I-*Msp*I have been selected based on in silico digestion of the reference genome accession (CM334) [2].

**RESULTS** GeGBS data provided more than 10,000 SNPs evenly distributed on the genome and used to characterize genetic diversity, population structure and phylogenetic relationships. The analysis allowed identifying five main clusters (K) corresponding to the most represented *Capsicum* species in the whole collection (Figure 1). *Capsicum annuum* was subdivided into two sub clusters and include admixed samples. Three other clusters were observed for *C. baccatum*, *C. frutescens* and *C. chinense*, respectively. The remaining species, including those corresponding to the group of purple flowers clustered in an admixed group. The principle component analysis (PCA) plot for the first two principal components suggests a geographical differentiation of the Caribbean, Central Asia and European gene-pools, and a distinction of the three main clades according to the most updated classification [3]. Moreover, the observed ~1,000 admixed accessions, and the incomplete separations between *C. frutescens* and *C. chinense*, revealed possible taxonomic misclassifications in the genebanks or gene flow between species resulting in introgressions. The genetic and phenotypic variation existing in the collection studied will be exploited for genome-wide association studies (GWAS).

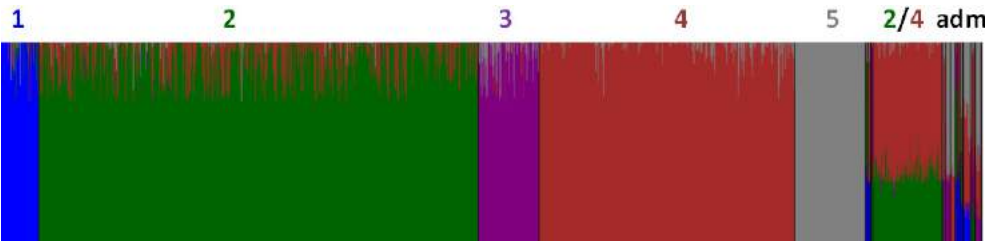
**DISCUSSION & CONCLUSION** The genetic information provided in this study is appropriate for a deep investigation of the diversity present across the different *Capsicum* collections maintained by genebanks. Appropriate strategies including "germplasm genomics" can optimize the management of resources, the exchange of material between genebanks and their use for breeding purposes. Beyond genetic diversity, data can be used for the development of core collections to be exploited in GWAS, facilitating the identification of genomic regions associated with valuable traits and enhancing the efforts made by genebanks in the extensive collection and characterization of *Capsicum* germplasm resources.

**REFERENCES**

[1] Elshire et al., 2011, Plos One 6: e19379. doi.org/10.1371/journal.pone.0019379  
[2] Kim et al., 2014, Nature Genetics 46:270–278  
[3] Carrizo-Garcia et al., 2016, Ann. Bot. 118:35–51. doi: 10.1093/aob/mcw079

**ACKNOWLEDGEMENTS**

Lorenzo Barchi and Arnaud Bovy thank the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.  
Gancho Pasev thanks the French "Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (program ACCES de CAMPUS France)" for covering the travel and accommodation expenses for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



**Figure 1.** Population structure of the *Capsicum* germplasm collection. Five subpopulation clusters inferred by STRUCTURE are represented by different colors : 1) *C. baccatum* ; 2) *C. annuum* ; 3) *C. chinense* and *C. frutescens* ; 4) *C. annuum* ; 5) *C. chinense*, *C. frutescens* and *C. annuum* ; adm = admixed

Species	N° of accessions sequenced	AVRDC	IPK	UPV	INRA	WUR	ARO	CREA	Unito	BATEM	MVCRI
<i>C. annuum</i>	7523	4166	908	935	639	264	215	209	94	48	38
<i>C. annuum</i> var. <i>glabriusculum</i>			4					3			
<i>C. frutescens</i>	728	474	160	10	39	15	21	8	1		
<i>C. chinense</i>	627	325	50	7	104	71	31	37	2		
<i>C. baccatum</i>	395	101	3	24	86		8		1		
<i>C. baccatum</i> var. <i>baccatum</i>		3	3			7		2			
<i>C. baccatum</i> var. <i>pendulum</i>		115	23			17		2			
<i>C. pubescens</i>		45	8	21	7	2	2	2	3		
<i>C. chacoense</i>	26	10	5			6	4	1			
<i>C. eximium</i>	8	2	4		2						
<i>C. praetermissum</i>	7		5*					1	1		
<i>C. cardenasii</i>	1		1								
<i>C. tovari</i>	1					1					
Undefined	298	286	7	3	1				1		
<b>TOTAL</b>	<b>9659</b>	<b>5490</b>	<b>1194</b>	<b>979</b>	<b>878</b>	<b>383</b>	<b>281</b>	<b>265</b>	<b>103</b>	<b>48</b>	<b>38</b>

\* Listed as *C. baccatum* subsp. *praetermissum*

**Table 1.** Summary of plant material provided by genebanks and used in this study

# Transcriptomic changes induced by drought stress and recovery in *Capsicum annuum*

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**BACKGROUND** Pepper is a major crop in the Mediterranean basin, where its cultivation in open field is vulnerable to drought. Water stress tolerance is under the control of several genes including transcription factors (TFs), key molecular switches orchestrating the regulation of plant development in response to stresses. We previously performed the re-sequencing at -39X of the breeding line of 'Cuneo' pepper CCu07 ([www.pepper-genomics.unito.it](http://www.pepper-genomics.unito.it)), and following reads alignment to the CM334 reference genome [1] we estimated about 0.11 % of heterozygosity. Here, we report on RNA-sequencing profiles obtained from CCu07 leaves after induced water stress as well as recovery (re-watering) at three plant development stages. Attention was focused on NAC, one of the largest families of plant TFs, which play key roles in regulating the transcriptional re-programming associated with plant stress responses.

**MATERIALS & METHODS** Water stress was induced by stopping irrigation on three plants of the breeding line 'CCu07' at three development stages: (i) production of five true-leaves; (ii) production of third flower; (iii) setting of first fruit. When stomatal conductance (gs) of water stressed plants was close to 0 mmol·m<sup>-2</sup>·s<sup>-1</sup>, they were re-watered until they reached the standard gs values of control plants (recovery phase). Leaf samples were collected (3 replicates per stage) after water stress and recovery, total RNA was extracted and directional cDNA libraries sequenced by Illumina (2x150bp paired-end). Reads were aligned against CM334 genome with Hisat2 [2] and counts of mapped reads per gene obtained with Stringtie [3]. Differentially expressed genes (DEGs) of stressed, recovered and control plants were identified with DESeq2 ([bioconductor.org/DESeq2](http://bioconductor.org/DESeq2)). Gene ontology (GO) enrichment was performed on the DEs with AgriGO ([bioinfo.cau.edu.cn/agriGO](http://bioinfo.cau.edu.cn/agriGO)). By searching the NAC domain Hidden Markov Model (HMM) profile (PF02365) against the pepper proteome using Hmmer ([hmmer.janelia.org](http://hmmer.janelia.org)) we manually detected positive matches.

**RESULTS** Hundreds of genes were differentially expressed after water stress and recovery at each development stage. In stressed plants a common set of 198 genes were always up-regulated, while just 5 always down-regulated (Fig. 1). Recovered plants shared a common set of 91 up-regulated and 188 down-regulated genes. GO enrichment analyses highlighted that most of the GO terms for abiotic stress response were spotted, confirming the reliability of our analyses: i.e. response to abiotic stimulus (GO:0009628), oxidation-reduction process (GO:0055114), nucleic acid binding TF activity (GO:0001071) and response to water (GO:0009415) for up-regulated genes after stress. Among the differentially expressed TF genes, those encoding MYB, bHLH, ERF and CO-LIKE were the most represented in both stressed and recovered plants (Fig 2). A set of 115 sequences were putatively assigned to the TF family of NACs, of which 24 were up-regulated after drought stress and 19 were down-regulated after recovery at all development stages. Interestingly, 5, 8 and 6 NACs at the vegetative, flowering and fruit setting stages, respectively, were always up-regulated after drought stress and down-regulated after recovery (Fig 3). Future studies will be focused on the their functional characterization.

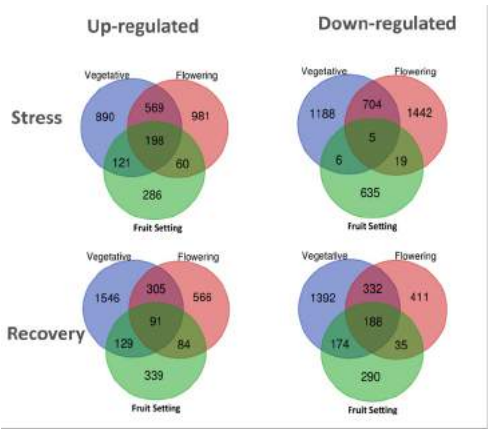
**DISCUSSION & CONCLUSION** Our results confirm that the effect of water stress and recovery in pepper is associated with multiple processes and mechanisms including stress-related genes and TFs. Drought stress and recovery elicited common up- or down-regulated genes as well as unique sets of responsive genes and TFs, within which our future studies will be focused on members of the NAC family. The identification of drought stress or recovery responsive genes in this study provides novel insights into the molecular basis for water stress tolerance in *Capsicum annuum*.

REFERENCES

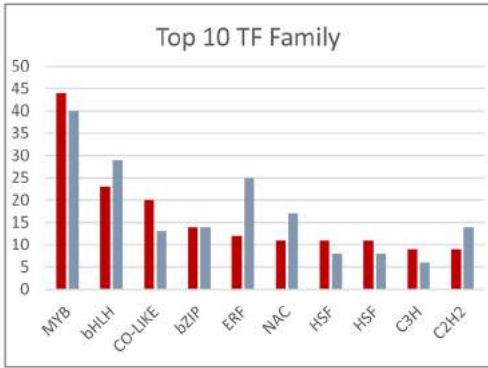
[1] Kim et al., 2017, Genome Biology 18: 210.  
[2] Kim et al., 2015, Nature Methods 12: 357-U121.  
[3] Pertea et al., 2015, Nature Biotechnology 33: 290-295.

ACKNOWLEDGMENTS

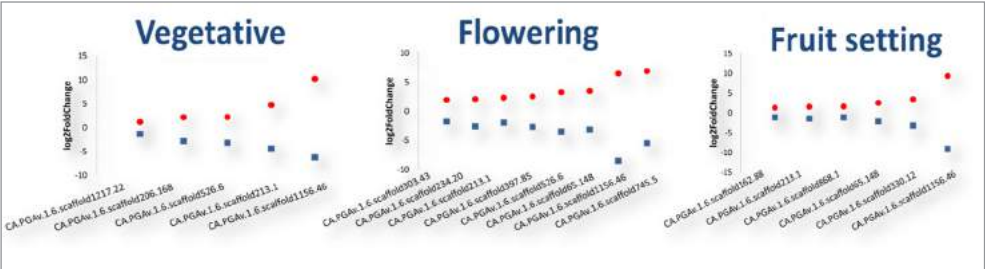
This research was supported by Cassa di Risparmio di Cuneo (CRC) Foundation ([www.fondazioneirc.it](http://www.fondazioneirc.it)). Lorenzo Barchi thanks the Agropolis Fondation and the SFR Tersys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



**Figure 1.** Venn diagram showing up (on the left) and down-regulated (on the right) genes specific or in common between the three stages (Vegetative, Flowering and Fruit setting) after stress or after recovery.



**Figure 2.** Vegetative Stage TFs Family. The TOP 10 differentially expressed transcription factor genes in response to drought stress. The X-axis reports the TF family members. The Y-axis reports the number of differentially expressed (DE) family members. Red columns represent DE TFs after stress while blue columns DETFs after recovery.



**Figure 3.** Genes with a NAC domain up-regulated after stress (red dots) and down-regulated after recovery (blue dots) at the vegetative (left), flowering (center) and fruit setting (right) stages.

# Optimization of VIGS system and function identification of the *SmMsrA* gene in eggplant

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**BACKGROUND** Virus-induced gene silencing (VIGS) is transcript suppression technique for characterizing the function of plant genes. The method includes cloning a short sequence of the targeted plant gene into a viral delivery vector. Then the vector infects a young plant, and natural defense mechanisms of plant directed at suppressing virus replication also result in specific degradation of mRNA from the endogenous plant gene which is targeted for silencing. This technology was widely used in plants [1]. No VIGS system was established in eggplant until the PDS (phytoene desaturase) gene was silenced by TRV (tobacco rattle virus) based VIGS technology [2], but some influencing factors in eggplant VIGS system also need to be further explored. In this study, we optimized the inserted gene fragment size, plant growth environment condition and infected seedling age of VIGS system in eggplant. The optimized VIGS system was used to assay gene function of Methionine Sulfoxide Reductase gene A (*SmMsrA*), which can reduce methionine-S-sulfoxide to methionine and restore the activity of the protein.

**MATERIALS & METHODS** Three *SmMsrA* coding sequence fragments with 597bp, 442bp and 205bp were cloned from eggplant, which were used to construct TRV2 mediated VIGS vectors. The pTRV2 empty vector and pTRV2-PDS were used as control. The VIGS expression vectors infected cotyledons of 10-day-old seedling and leaves of six-week-old seedling by *Agrobacterium-syringe*. The infected plants were put in spring greenhouse (9-22°C/night and 20-41°C/day), autumn greenhouse (8-20°C/night and 19-38°C/day) and temperature controlled greenhouse (25°C/day and 20°C/night). Phenotypes observation and qRT-PCR detection technology were used to evaluate the efficiency of VIGS system and the function of *SmMsrA*.

**RESULTS** The results showed that the silencing efficiency was higher in temperature controlled greenhouse (25°C/day and 20°C/night) than in spring greenhouse and autumn greenhouse. After inoculation of the cotyledons of eggplant, the plants had obvious silencing symptom, but there was no obvious silencing effect when the six-week-old true leaves were inoculated. The silencing frequencies of the gene fragments 597 bp, 442 bp and 205 bp were 56.67%, 62.5% and 68.75% respectively, indicating that about 200 bp might be the most suitable fragment size for TRV mediated VIGS system. The positive control of silencing reporter gene PDS showed photo-bleaching phenotype in eggplant leaves. Silencing *SmMsrA* gene were observed pale yellow (mosaic) leaves and bore smaller fruits. The expression of *SmMsrA* gene was decreased obviously in the leaves and fruits of the infected eggplant plants. These results suggested that the *SmMsrA* gene was a related gene in positive regulation of fruit development in eggplant.

**DISCUSSION & CONCLUSION** In this study, the TRV-mediated VIGS system was successfully constructed and optimized in eggplant. The expression level of *SmMsrA* gene in leaves and fruits was successfully silenced by this VIGS system. After the silencing of *SmMsrA*, the fruits became smaller, which proved that *SmMsrA* was an important gene that positively regulated the development of eggplant fruits. The establishment of VIGS system in eggplant laid a foundation for rapid identification of gene function in eggplant.

## REFERENCES

- [1] Burch-Smith TM, Anderson JC et al. *The Plant Journal*, 2004, 39(5): 734-746
- [2] Liu H, Fu D et al. *Journal of Integrative Plant Biology*, 2012, 54(6): 422-429





# Loss of function mutation in the putative *ketoacyl-ACP reductase* *CaKRI* induces non-pungency in *Capsicum*

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**BACKGROUND** *Capsicum* had already been domesticated by around 6000 B.P in the Americas, making it one of the earliest domesticated plant genera [1]. *Capsicum* was introduced into Europe at the end of the fifteenth century, and its use as a horticultural crop spread rapidly across the Old World. Pungent peppers are widely cultivated and consumed as spices and non-pungent peppers are also valuable vegetable crops because they are concentrated sources of vitamins. The pungency of chili pepper fruits is due to the presence of capsaicinoids, which are synthesized through the convergence of the phenylpropanoid and branched-chain fatty acid pathways. The extensive, global use of pungent and nonpungent peppers underlines the importance of understanding the genetic mechanism underlying capsaicinoid biosynthesis for breeding pepper cultivars. Although *Capsicum* is one of the earliest domesticated plant genera, the only reported genetic causes of its loss of pungency are mutations in *acyltransferase* (*Pun1*) and putative aminotransferase (*pAMT*) [2, 3].

**MATERIALS & METHODS** A single recessive gene responsible for the non-pungency of pepper No.3341 (*C. chinense*) was analyzed using 98 F2 individuals derived from a cross between Habanero and No.3341 by RAD-seq. Sequence and gene expression analyses were conducted for five candidate genes identified in the target region. Also, virus-induced gene silencing of a candidate gene, a putative *ketoacyl-ACP reductase* (*CaKRI*) was conducted and the capsaicinoid content of the fruits was confirmed using HPLC. Moreover, presence of 8-methyl-6-nonenic acid was confirmed using GC-MS.

**RESULTS** For linkage analysis of non-pungency in No.3341, 244 polymorphic SNPs from RAD-seq were used, among which a total of 242 were successfully mapped onto 13 linkage groups (LGs). The results suggested that the candidate gene was closely linked with RAD107981 on LG 10. The sequences of RAD107981 and nearby RAD tags were localized at chromosome 10. To reduce the size of the candidate region, an addition sixteen markers were newly developed based on the RAD tags and the comparison of the whole genome sequences between Habanero and No.3341. The candidate gene was delimited to an interval with a physical distance of 220 kb. Five candidate genes were identified in the target region. The No.3341 allele of *CaKRI*, a candidate gene, had an insertion of a 4.5-kb transposable element (TE) sequence in the first intron, resulting in the production of a truncated transcript missing the region coding the catalytic domain. Virus-induced gene silencing of *CaKRI* in pungent peppers resulted in the decreased accumulation of capsaicinoids, a phenotype consistent with No.3341. Moreover, GC-MS analysis of 8-methyl-6-nonenic acid, which is predicted to be synthesized during the elongation cycle of branched-chain fatty acid biosynthesis, revealed its deficiency in No.3341.

**DISCUSSION & CONCLUSION** In the present study, we identified *CaKRI* by a map-based cloning approach, in an attempt to identify the mutation responsible for non-pungency in *Capsicum* without the bias of previous hypotheses. Because none of the previous studies identified *CaKRI* as a candidate capsaicinoid biosynthesis gene, analysis of naturally occurring genetic variation in capsaicinoid biosynthesis is still a crucial approach to deepen our understanding of pepper pungency.

## REFERENCES

- [1] Perry L et al., 2007, Science, 315(5814):986–988
- [2] Stewart C Jr et al., 2005, Plant J, 42(5):675–688
- [3] Lang Y et al., 2009, Plant J, 59(6):953–961

# Polymorphism of anthocyanin I orthologs in pepper and eggplant

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**BACKGROUND** Anthocyanins are high value plant antioxidants, which not only give specific coloration to fruits and seeds, but also determine biotic and abiotic stress resistance. Regulation of their accumulation in plants is an important object of study for many researchers. Taking into account that the structural genes of the anthocyanin biosynthetic pathway function under control of a regulatory complex called MYB-bHLH-WD40 (MBW) [1], the allelic polymorphism study of these regulatory genes is important. It is known that the tomato gene Anthocyanin I (AntI) encodes a Myb transcription factor (TF) regulating the flavonoids' accumulation in fruits. After testing the CAPS marker AntI-NcoI for the detection of the allele AntI C [2] forming the Aft (Anthocyanin fruit tomato) phenotype with a characteristic coloration of the vegetative mass and dark blue coloring of fruits, the aim of our research was to study the allelic polymorphism of Anthocyanin I orthologs in the vegetable Solanaceae crops of *C. annuum* and *S. melongena*.

**MATERIALS & METHODS** The search for orthologs to the allele AntI (EF433416) in the GenBank database revealed the following closest by the nucleotide composition sequences of MybI13-like TF: in *C. annuum* - mRNA XM\_016689227, mRNA NM\_001324618 and in *S. melongena* - mRNA KT259043. Based on the Solgenomics database, the primers completely overlapping the exons of the above genes were selected. Using the primers in PCR on genomic DNA, amplicons were obtained and then sequenced in pepper and eggplant collection samples with a contrasting anthocyanin fruit coloration. Markers for the identified polymorphisms were developed and their correlation with the anthocyanin accumulation in fruits was studied.

**RESULTS** Comparative analysis of *C. annuum* sequences under study allowed to identify the following polymorphisms: 4 SNPs and a single base deletion in the 3rd exon of MybI13-like factor (XM\_016689227) (in Belosnezhka, LI 60-10) and 2 SNPs in the 4th exon of MybI13-like factor (NM\_001324618). Comparison of the obtained sequences of the mybI gene in *S. melongena* revealed varied polymorphism: a deletion of 6 bp at the end of exon 1, leading to the loss of 2 amino acids in the protein (Zelenenky variety), or a deletion of 26 bp at the end of intron 1 - the beginning of exon 2 and 11 SNPs (Snow and Pelican varieties). Based on the identified polymorphism, SCAR and CAPS markers were developed and tested. As a result of DNA typing of 55 *C. annuum* samples and 34 forms of *S. melongena* using the developed markers, a close correlation was found between the minimum accumulation or complete absence of anthocyanin synthesis in fruits with a single nucleotide deletion (loss of T in the +1217 position) in pepper samples detected using CAPS marker MybI13-AccI, as well as deletions of 6 and 26 bp in eggplant samples detected using the SCAR marker MybMel and CAPS marker Mybmel-PstI.

**DISCUSSION & CONCLUSION** The disturbance of anthocyanin synthesis in pepper forms with 1 Indel in the 3rd exon of the MybI13-like factor (XM\_016689227) is determined by a shift in the reading frame and the synthesis of a truncated regulatory protein. In eggplant, a deletion of 6 bp (mybI exon 1) leads to the loss of ala and arg in the protein. A deletion of 26 bp (mybI intron 1-exon 2) is likely to cause disorder during mRNA maturation. The developed markers allow identifying alleles of myb-like TF under study resulting in anthocyanin synthesis disturbance in fruits. *C. annuum* and *S. melongena* samples with different alleles have been selected for further study and new variety development for agriculture.

## REFERENCES

- [1] Liu Y. et. al., 2018, Front. Chem., 6:52. doi: 10.3389/fchem.2018.00052  
 [2] Sapir M. et al., 2008, J. Hered., 99 (3): 292-303. doi: 10.1093/jhered/esm128

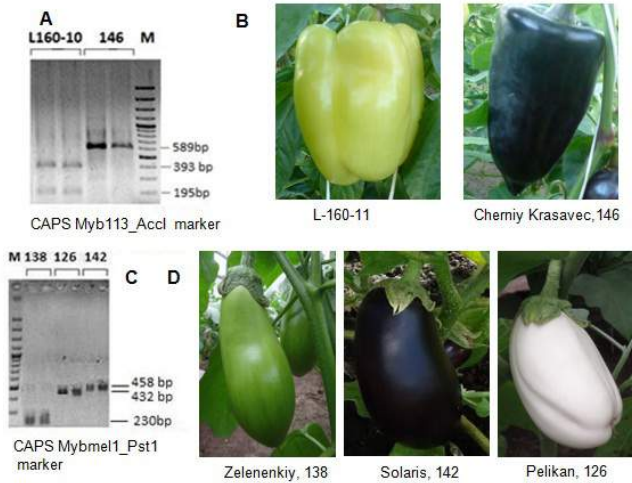


Figure 1. DNA identification of *myb* allele polymorphisms using CAPS markers for the collection samples of pepper and eggplant (A,C) and their phenotypic manifestation (B,D)

# Optimization of a DNA extraction protocol for *Capsicum* spp. without liquid nitrogen

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**BACKGROUND** The basic step for studies based on the application of molecular markers, mainly the ones requiring the polymerase chain reaction (PCR), is the extraction of genomic DNA. The latter must be in adequate quantity and quality to avoid enzymatic inhibition during PCR or to cause interferences in migration patterns during electrophoresis. Different methods of DNA extraction from leaf tissue have been used for different species. The standard protocol used in *Capsicum* spp. is the CTAB [1], however, it is possible to adopt modifications and adaptations in this protocol to obtain clear and reproducible patterns of DNA bands using simple, fast and inexpensive techniques [2]. One modification avoids the use of liquid Nitrogen, as required by the original process. The aim of this work was to check if this modification of the conventional CTAB method does not alter the quantity and quality of extracted DNA, thus simplifying the protocol.

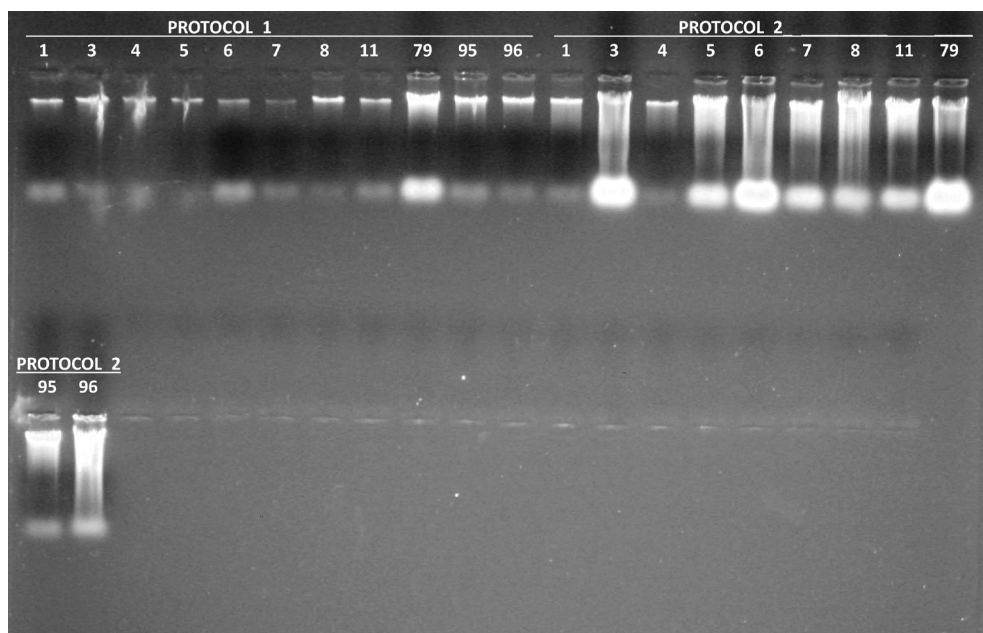
**MATERIALS & METHODS** The DNA of 11 accessions of pepper (*Capsicum* spp.) was extracted using two protocols. In the first one, the leaf fresh tissue was triturated with liquid nitrogen and incubated at 65°C for 30-40 minutes in tubes with extraction buffer (2% CTAB; 1.4 M NaCl; 20 mM EDTA pH 8.0; 100 mM Tris-HCl pH 8.0, 2% PVP, 0.2% mercaptoethanol). In the second DNA extraction protocol, each sample was obtained by macerating leaf tissues in the extraction buffer solution, without liquid nitrogen. The agarose gel was used for assessing the quality of DNA after staining with ethidium bromide solution. The DNA concentration was estimated by spectrophotometry at 260 nm. The total DNA quantification was expressed in ng [3].

**RESULTS** As regard DNA integrity, only the protocol using liquid nitrogen maceration demonstrated satisfactory efficiency in all the tested samples (Figure 1). In the samples macerated directly in the extraction buffer, it was observed that, in addition to the vertical drag of the DNA samples on the gel, no clear bands were detected, indicating that the DNA was very degraded. However, samples 1 and 4 were of good quality, demonstrating the potential of the protocol, with some modifications such as the cooling of the extraction buffer. Taking into account that the purpose of an extraction is to obtain a good quality DNA sample for the PCR, our results demonstrate that both protocols guarantee a satisfactory concentration following quantification of DNA at the spectrophotometer, although the DNA concentration obtained by applying the protocol based on liquid nitrogen was significantly higher.

**DISCUSSION & CONCLUSION** The use of liquid nitrogen in the DNA extraction of *Capsicum* spp., following the Doyle and Doyle protocol made it possible to obtain an higher concentration and integrity of DNA samples. However, new modification in the protocol can avoid DNA degradation so as to reduce the costs of extraction reagents.

## REFERENCES

- [1] Doyle & Doyle, 1990, Focus, 12: 13-15.
- [2] Danner et al., 2011, Ciência Florestal 21: 363-367.
- [3] Barbosa, 1998, In: Marcadores moleculares em plantas, Milach SCK. (ed.), Porto Alegre, Brazil, pp. 99-106.



**Figure 1.** Electrophoretic analysis of the *Capsicum* spp. varieties DNA using DNA extraction protocol 1 by leaf maceration with liquid and protocol 2, directly in extraction buffer solution.

Accessions of pepper	Protocol 1		Protocol 2	
	260	DNA (ng/uL)	260	DNA (ng/uL)
1	0.0600	280.00	0.0134	65.00
3	0.0580	225.00	0.0470	260.00
4	0.0800	420.00	0.0450	225.00
5	0.0770	325.00	0.0340	190.00
6	0.0730	325.00	0.0520	270.00
7	0.0570	245.00	0.0310	210.00
8	0.0780	335.00	0.0310	190.00
11	0.0630	290.00	0.0440	250.00
79	0.0670	285.00	0.0570	240.00
95	0.0350	200.00	0.0670	300.00
96	0.0760	325.00	0.0400	205.00

**Table 1.** Quantification of DNA samples, extracted from foliar tissue of *Capsicum* spp. varieties, using liquid nitrogen (protocol 1) and without nitrogen (protocol 2), in absorbance readings at 260 nm.

# High-frequency *in vitro* androgenesis for production of doubled haploids (DHs) in Chilli (*Capsicum annuum* L.) cv. Byadgi Dabbi

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**BACKGROUND** The chilli cv. Byadgi dabbi is a very famous and a widely grown commercial dry chilli variety in Karnataka, India. It is characterized by deep red, highly wrinkled fruits with characteristic flavor, aroma, low pungency, high color level, and oleoresin that makes it the most preferred variety for export. It has been accorded with Geographical Indication. In a recent survey conducted by scientists (Salimath et al., 2008), farmers, traders, and consumers alike stated that yield and more specifically quality feature of Byadgi chilli is deteriorating year after year because of introgression of undesirable genes from other chilli varieties/hybrids. Hence, for restoring the past glory of this variety, there is an urgent need for genetic purification and improving the yield potential of this variety without sacrificing the good quality, which is a challenging task for the breeders. Biotechnological tools such as tissue culture techniques and specifically anther culture may be applied successfully for plant breeding and genetic improvement to generate complete homozygous lines in a shorter time in comparison with the classic breeding methods. Our main objective was to standardize the protocol for the higher rate of in-vitro production of DHs in Byadgi dabbi through anther culture.

**MATERIALS & METHODS** In the present study, the anthers isolated from flower buds of cv. Byadgi dabbi grown in a plant growth chamber were cultured on solid induction medium. Three basal induction media viz., Sibi, Dumas and Modified Dumas media were tested in eight replications. Method/steps shown here in the flow chart were followed in all the media and all the replications. Collection of floral buds → Detection of the pollen development stage → Surface sterilisation → Anther dissection → Anther placement on the medium → Embryo development → Haploid plantlets → Confirmation of ploidy → Chromosome doubling → Doubled haploids (DHs) → Hardening. The data were subjected to Analysis of Variance (ANOVA).

**RESULTS** The embryo induction was observed in all three media. The type and color of the embryos induced were solitary and white in the three media. The growth rate was medium in all media. The highest frequency of embryo induction was observed in modified Dumas media (6.25 %) followed by Dumas media (3.38 %) and Sibi Media (2.23 %). It was observed that modified Dumas Media was superior over the original Dumas Media (45.92 %) and Sibi Media (64.32 %) (Table.1). The most promising Modified Dumas Media was supplemented with 20.13mg/l MnSO<sub>4</sub>.H<sub>2</sub>O, 3.22mg/l ZnSO<sub>4</sub>.7H<sub>2</sub>O, 1.55mg/l H<sub>3</sub>BO<sub>3</sub>, 0.33mg/l KI, 0.13mg/l Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O, 0.01mg/l CuSO<sub>4</sub>.5H<sub>2</sub>O, 0.01mg/l CoCl<sub>2</sub>.6H<sub>2</sub>O and 0.20 mg/l 2,4-D.

**DISCUSSION & CONCLUSION** Anther culture of chilli is an attractive system for the production of haploid and doubled haploid plants. Modified Dumas de Vaulx Media was found to be most promising in inducing high-frequency *in vitro* androgenesis for production of Doubled Haploids (DHs) in chilli (*Capsicum annuum* L.) cv. Byadgi Dabbi.

## REFERENCES

- [1] Dumas de Vaulx et al., 1981, Agronomie, 1:859-864
- [2] Salimath PM et al., 2008, SpiceIndia, 21 (7):6-8
- [3] Sibi M et al., 1979, Annales de l'Amelioration des Plantes, 29:583-606

<b>Media</b>	<b>Embryo Type and color</b>	<b>Embryo Induction (%)</b>	<b>Root Regeneration (%)</b>	<b>Shoot Regeneration (%)</b>	<b>Per cent Increase</b>
Sebi et.al., 1979	Solitary and White	2.23	88.88	88.88	64.32
Dumas de Vaulx et al., 1981	Solitary and White	3.38	84.61	84.61	45.92
Modified Dumas Media	Solitary and White	6.25	87.50	87.50	--

**Table 1.** Comparison of Embryo induction frequency in the three media tested.



# A high-density genotyping strategy based on gene capture in pepper: perspectives for genome wide association study and genetic mapping

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**BACKGROUND** The genetic diversity within cultivated peppers (*Capsicum annuum*) has been reduced through its domestication and since its first introduction from the West Indies into Europe during the first travel of Christopher Columbus in XVth. For a long time, it was consequently difficult to get dense genotyping in *C. annuum*. Moreover, pepper is characterized by a large genome size (~3.5 Gb) resulting from its expansion by accumulation of transposable elements (81.5% of the genome), preventing the discovery of SNPs evenly distributed throughout the genome [1]. To overcome this difficulty, we chose a targeted sequence gene capture strategy [2]. We present here the design of baits combining different approaches. First, in a genome wide approach, baits were designed in polymorphic regions identified in RNAseq and genotyping by sequencing (GBS) datasets from pepper genome. Second, in a candidate gene approach, genes of interest from published datasets of other species were in the focus of our investigation in pepper.

**MATERIALS & METHODS** In order to identify SNPs, reads from two sequencing datasets (21 genotypes from RNAseq, 282 from GBS) were mapped to the 35,884 genes from the reference pepper genome CM334 v1.6. In addition, a set of 10K SNPs from the G2P-SOL project on 871 INRA accessions was included. SNPs calling was performed using the pipeline of Holtz et al. [2]. Since the bait hybridization is efficient when >92-95% similarity occur, we designed baits on polymorphic sites of exons as they are well conserved between genotypes. Plant genes involved in oomycete, virus and nematode resistance as well as in abiotic stresses were selected from literature and their homologs identified in pepper using BLAST.

**RESULTS** After filtering a total of 463,525 unique SNPs, 26,777 genes (74.6% of the genes annotated on the genome) were found to contain at least one SNP. The majority of SNPs, and consequently the majority of the genes containing SNPs, were found using the RNAseq dataset compared to GBS datasets (Figure 1). The genes containing at least one SNP were evenly distributed throughout the genome. We identified 700 candidate genes from literature led to 1,646 homologous in CM334 (Table 1). A total of 1,352 candidate genes contained SNPs (82% of the candidate genes).

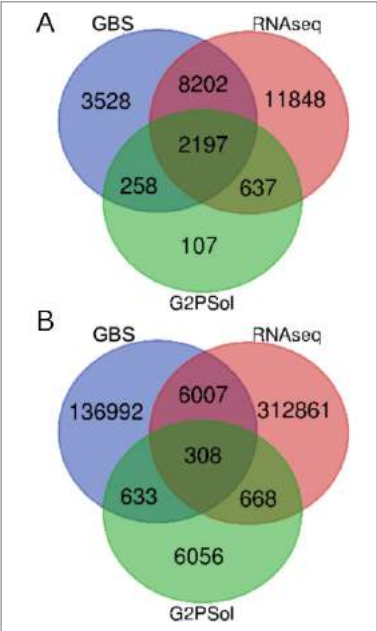
**DISCUSSION & CONCLUSION** Our aim was to obtain at least one polymorphic SNP for each gene. For 25% of pepper genes where no SNP was detected, we designed baits on the first or the last exon in order to catch UTR regions. In the candidate gene approach, we maximized the coverage of each gene to detect novel SNPs by designing baits on each exon taking into account their polymorphic sites. Moreover, to overcome the difficulty of designing specific baits on resistance genes that belong mainly to highly conserved NB-LRR families, we preferentially designed baits on the last exon close to the 3'UTR. Finally, a total of 60,000 baits were designed for bait sequencing capture and will be used further for genome wide association study and genetic mapping.

REFERENCES

[1] Kim S, Park J et al, 2017, Genome Biol, 18, 210. DOI 10.1186/s13059-017-1341-9  
[2] Holtz Y, Ardisson M et al, 2016, PLoS ONE, 11, e0154609. DOI: 10.1371/journal.pone.0154609

ACKNOWLEDGEMENTS

The G2P-SOL project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 677379



**Figure 1. (A)** Venn diagram showing genes with at least one SNP for the three datasets. **(B)** Venn diagram showing SNPs for the three datasets.

Type of GC	GC number	Pathogenes family
Resistance genes	57	Oomycetes
		Virus
		Nematodes
		Others
Susceptibility genes	15	Oomycetes
		Others
Interactants	304	Oomycetes
		Virus
		Others
Small RNA factors	14	Virus
ESCRT factors	12	Virus
TIR and non-TIR-NB-LRR analogous	80	NA
Temperature tolerance genes	238	NA
Photosynthesis genes	38	NA
Quantitative trait loci (QTL)	18	Nematodes

**Table 1.** Genes from Solanaceae, Rosaceae, Fabaceae, Brassicaceae and Poaceae family were identify from literature and sorted in function of their category. Resistance and susceptibility genes are known genes conferring resistance; interactants are plant proteins interacting with the pathogen or target of effectors; small RNA factors are dicer-like (DCL) proteins or targets of miRNA; ESCRT (Endosomal Sorting Complexes Required for Transport) factors are part of the membrane trafficking machinery.

# Development of a new highly efficient organogenesis protocol in eggplant (*Solanum melongena*) and ploidy level evaluation

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**BACKGROUND** Plant regeneration through in vitro culture is a very useful tool for many applications, such as the generation of transgenic or CRISPR/Cas9 edited plants, the handling of ploidy levels, or plant sanitation. The available protocols for the organogenesis induction in eggplant (*Solanum melongena*) generally have very low efficiency and are highly dependent on the genotype. Due to the great interest, both at commercial and research levels, the establishment of a stable and high-performance regeneration protocol in eggplant is needed. To reach this goal, we evaluated the effect of different combinations plant hormones, zeatin riboside (ZR) and indoleacetic acid (IAA), on two tissues, hypocotyl and cotyledon, in light and dark conditions. In addition, we developed a rooting protocol, by evaluating the effect of different concentrations of indole butyric acid (IBA) to promote the rooting and facilitate the subsequent acclimatization process.

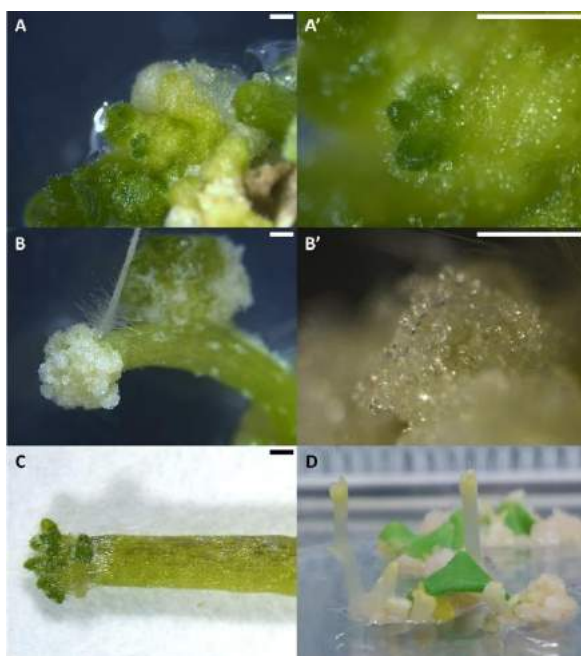
**MATERIALS & METHODS** Seeds of five *S. melongena* accessions and one of the close wild relative *S. insanum* were germinated in sterile conditions. The hypocotyl and cotyledon explants were subjected to two incubation treatments (light and dark) in eight culture media with seven different concentrations of ZR and IAA and a control without hormones. After 30 days of culture, the organogenic capability and the influence of the genotype were evaluated. The shoots were subcultured in six culture media with five concentrations of IBA and in a control medium to study the formation of roots. Finally, the ploidy of the acclimatized plants was analyzed by flow cytometry using the fluorescent dye DAPI.

**RESULTS** The analysis suggested that the highest rate of bud formation was for the cotyledon (Figure 1A, A'). The hypocotyl formed more biomass of callus (Figure 1B, B') however, shoot formation was also observed (Figure 1C). On the other hand, the light conditions significantly favored the formation of shoots, with less shoots and longer in the dark conditions (Figure 1D). Of the seven hormones combinations, four of them displayed very similar results, although the medium O6 with a concentration of ZR of 2 mg/L and no IAA showed a slightly higher average for the formation of shoots in both analyzed tissues (Table 1). Shoot formation was observed in all the different genotypes, however, there were noticeable differences in this biological process amongst the genotypes. The length of the main roots and the number of secondary ones decreased significantly, although its thickness increased with IBA concentrations, being the R2 medium, with 1 mg/L of IBA, the one that produced the largest number of secondary roots. Amongst the regenerated plants, different levels of ploidy were observed. The number of acclimatized plants was less than the number of shoots observed in the counts per month as shown in Table 1.

**DISCUSSION & CONCLUSION** In this study, we present an organogenesis protocol in which the genotype is not a limiting factor. The ZR is an effective hormone in the formation of buds in eggplant as in other works [1]. The cotyledon is the best tissue for techniques such as CRISPR/Cas9 editing due to its high organogenic capability. An optimal IBA concentration has been established for in vitro root induction. Due to a possible pattern of polysomaty present in the different plant tissues [2], tetraploid regenerants have been obtained, so this protocol can be an alternative to other methods in which harmful substances are used to obtain them.

## REFERENCES

- [1] Muktadir M.A. et al., 2016, Agriculture and Natural Resources, 50(1):38-42.
- [2] Dolezel J. and Bartos J., 2005, Annals of Botany 95: 99-110



**Figure 1.** Beginning of the formation of a bud in cotyledonary tissue of eggplant under light culture conditions (A); and close up, where it can be observed an organized structure and the formation of the first trichomes (A'); Callus formed in hypocotyl tissue under light culture conditions (B); which at further increases can be observed that it is disorganized cell structure (B'); Formation of shoots in hypocotyl tissue under light conditions (C); Appearance of the buds formed in cotyledonary tissue in dark culture conditions, elongated growth and absence of chlorophyll in the apex was observed (D). All the images were taken after a month of culture, bars (1mm).

Genotypes	Hypocotyl				
	Explants with shoots (%)	Acclimatized Plants	Acclimatized Plants /initial explants (%)	% of 2x regenerants	% of 4x regenerants
MEL1	20 ± 0,23	6	13,33 ± 0.05	77,78	22,22
MEL3	10 ± 0,12	4	8,88 ± 0.04	90,48	9,52
IVIA	86,66 ± 0,06	13	28,88 ± 0.06	65,00	35,00
BB	70 ± 0,08	21	46,76 ± 0.07	66,67	33,33
MM	86,66 ± 0,06	9	20,00 ± 0.06	76,92	23,08
INS	90 ± 0,05	17	37,77 ± 0.07	74,80	25,20
Genotypes	Cotyledon				
	Explants with shoots (%)	Acclimatized Plants	Acclimatized Plants /initial explants (%)	% of 2x regenerants	% of 4x regenerants
MEL1	7,5 ± 0,04	5	11,11 ± 0.04	75,00	25,00
MEL3	15 ± 0,05	7	15,55 ± 0.05	69,23	30,77
IVIA	96,66 ± 0,03	36	80,00 ± 0.06	50,00	50,00
BB	100 ± 0,00	15	33,33 ± 0.07	66,67	33,33
MM	69,23 ± 0,07	13	28,88 ± 0.06	64,71	35,29
INS	100 ± 0,00	24	53,33 ± 0.07	75,00	25,00

**Table 1.** Results of the number of acclimatized plants for each eggplant genotype using the 06 medium in light conditions from two different tissues, hypocotyl and cotyledon, as an initial explant using the R2 medium for the induction of roots, formation and analysis of the ploidy of regenerated plants, initial explants used (n=45).

# Colchicine-induced doubled-haploid eggplant (*Solanum melongena* L.) production

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**BACKGROUND** The efficiency of completely fertile doubled haploid eggplants production is restricted by the unsolved diploidisation. It can be established that the spontaneous diploidisation event is not effective. While treating of young haploid pepper regenerants with colchicine *in vitro*, the ratio of induced doubled haploids reached to 95%, in the case of eggplant this method resulted only low amount of doubled haploids. In addition, the survival rate of treated young eggplant haploid plants were very poor. To get fertile doubled haploid plants at higher rates, we studied the treatment days and colchicine doses effect for induced doubled haploid production.

**MATERIALS & METHODS** Doubled haploid plants were obtained through anther culture of three Hungarian eggplant cultivars. The donor plants were grown under greenhouse conditions. We selected mainly mid and late-uninuclear microspores containing anthers (1500 anthers/trial). Anthers were cultured on the modified induction medium according to [1], but using 120 g/l maltose in place of sucrose, 5 mg/l NAA and 5 mg/l kinetin and adding 20, 50, or 100 mg/l colchicine. We maintained the cultures at 30°C in the dark for 2, 3, 4 and 6 days and then transferred to the colchicine free medium in the growth chamber, maintained at 25°C, a 16/8-h (day/night) photoperiod. After 6 days, the anthers were placed in regeneration medium (on the basis of [1]) containing kinetin (0.1 mg/l) and 0.5 g/l activated charcoal. Regenerated shoots were rooted on rooting medium on the basis of [1] with 10 mg/l sucrose. The androgenic origin of the dihaploids was demonstrated by ploidy determination with flow cytometry. The protocol used for the flow cytometry analysis has been reported by [2].

**RESULTS** When we used colchicin-free as antimitotic agent-free induction medium the spontaneous diploidization rate of studied eggplant cultivars was 15 - 27 %. The four day colchicine (100 mg/l) treatment has led to the significant rise in the production of doubled haploid eggplant regenerants, resulting in 39 - 58% diploidisation respectively.

**DISCUSSION & CONCLUSION** Anther culture of eggplants is an attractive system for production of doubled haploid plantlets [3] for hybrid breeding. However, an efficient genotype-independent protocol and optimized diploidisation are still lacking. Plant breeders are increasingly using this system in their mainstream pure-line programs to reduce the number of years needed from crosses to commercial variety registration. Despite of its convenience only very few reports can be found in this field. If a sufficient quantity of doubled haploids has to be produced for use in breeding, the efficiency of *in vitro* techniques must be continuously improved.

## REFERENCES

- [1] Dumas de Vaulx R et al., 1981, *Agronomie*, 1:859–864
- [2] Gémes Juhász A et al., 2006, *Acta Hort.*, 725: 845–854
- [3] Salas P et al., 2012, *Euphytica*, 184: 235–250



# Antimicrobial peptides of *Capsicum annuum* L.: natural molecules to control fungi of agronomic importance

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**BACKGROUND** Antimicrobial peptides (AMPs) are low molecular weight molecules (usually less than 10 kDa) with amphipathic character, which confer a high level of antimicrobial activity against different microorganisms, such as bacteria, viruses, protozoa, filamentous fungi and yeasts. AMPs participate in the innate immune response of many species of organisms, including plants. The identification of such proteins with high ability to control human and plant pathogenic agents is the central target of this research. In this study we report extraction, purification, and characterization of AMPs present in fruits and seeds of *Capsicum annuum* L., accession UENFI 381, and we evaluate their antimicrobial activities on plant pathogenic fungi, causal agents of diseases in important crops.

**MATERIALS & METHODS** Initially, proteins from *C. annuum* seed and fruit powder were extracted in phosphate buffer, pH 5.4, for 3 h at 4°C [1]. AMPs were purified basically by chromatographic methods. Purification process was monitored by gel electrophoresis in tricine SDS-PAGE. Purified peptides were submitted to automated N-terminal amino acid sequencing. These peptides were investigated in their ability to interfere with fungi development *in vitro* and their mechanisms of action were analyzed through viability and plasma membrane permeabilization assays, analysis of endogenous reactive oxygen species (ROS) production, mitochondrial functionality assays, and caspase activation tests.

**RESULTS** Our results showed the presence of four different families of antimicrobial peptides, such as lipid transfer proteins (LTPs), trypsin inhibitors, thionins and plant defensins (Figure 1) [1, 2, 3]. The isolated peptides, members of these families, showed strong antimicrobial activity against the two tested phytopathogenic fungi species, *Fusarium* spp. and *Colletotrichum* spp., with the half maximal inhibitory concentration (IC<sub>50</sub>) ranging from 10 to 100 µg.mL<sup>-1</sup>. These peptides also caused plasma membrane permeabilization in the two fungi species tested, induced oxidative stress, phosphatidylserine externalization of the cell membrane and were able to cause dissipation of mitochondrial membrane potential and caspase activation. Some of these peptides acted synergistically with fluconazole inhibiting the two tested fungi species, reaching 100% inhibition, with concentrations below their IC<sub>50</sub>.

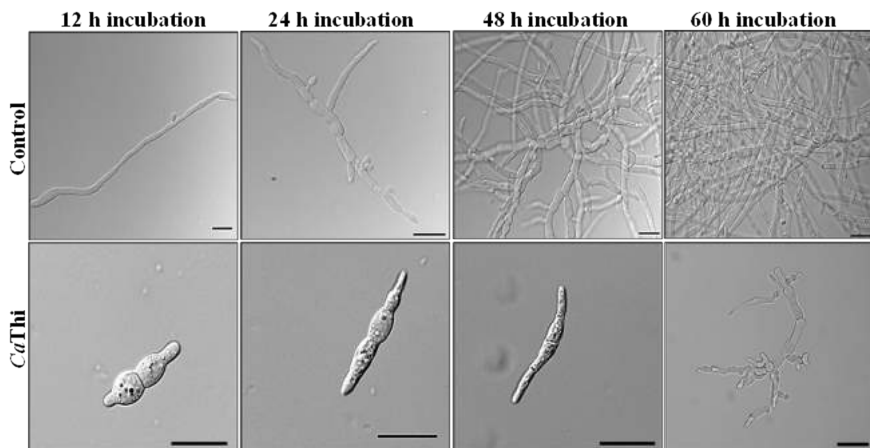
**DISCUSSION & CONCLUSION** Here, we demonstrated that different families of antimicrobial peptides from *C. annuum* have strong antifungal effect by *in vitro* inhibition of the growth, permeabilizing the membrane, inducing the oxidative stress response, activation of caspases and loss of viability of the fungi. Taken together, these results show the great potential that *Capsicum* extracts have, with their fractions rich in antimicrobial peptides, as a source of study and use in controlling diseases, especially those caused by phytopathogenic fungi. The results reported here may ultimately contribute to future efforts aiming to employ these plant-derived AMPs as new possibilities in pathogen control.

## REFERENCES

- [1] Taveira et al., 2017, Biopolymers Peptides Science, 108: 1-14. doi:10.1002/bip.23008
- [2] Silva et al., 2017, Journal of the Science of Food and Agriculture, 97: 3790-3801. doi:10.1002/jsfa.8243
- [3] Santos et al., 2017, Protein Expression and Purification, 132: 97-107. doi:10.1016/j.pep.2017.01.013

**A**

PEPTIDE FAMILIES	INHIBITORY ACTIVITY
Lipid transfer proteins (LTPs)	<i>Colletotrichum lindemuthianum</i>
Thionins	<i>Fusarium solani</i>
Serine protease inhibitors	<i>Colletotrichum lindemuthianum</i> and <i>Colletotrichum gloesporioides</i>
Defensins	<i>Colletotrichum gloesporioides</i>

**B****Figure 1.**

**(A)** Antimicrobial peptides from *Capsicum annuum* (accession UENF1381) and their inhibitory activities.

**(B)** Images of *Fusarium solani* cells by light microscopy after different incubation times with CaThi (50 µg.mL<sup>-1</sup>), a thionin from *C. annuum* fruits. Note the strong inhibitory effect on conidial germination and mycelial growth. Control cells, without CaThi. Bars = 20 µm.



# Development of a chitosan film-containing liposomal formulation of eggplant glycoalkaloids as a potential pharmaceutical

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**BACKGROUND** Solamargine and solasonine play roles in plant defense responses and are found in high concentrations in wild eggplant relatives. Although glycoalkaloids are potentially toxic to humans, they also have beneficial effects. Their antimicrobial, antifungal, antiviral, antiparasitic, antidiabetic, antibiotic, and anticancer activities have been reported [1]. Glycoalkaloids have been encapsulated in liposomes to eliminate their toxicity, improve their stability and bioavailability, and protect them from environmental conditions [2]. Although advantageous as nanocarriers, liposomes have drawbacks, namely their chemical and physical instability [3]. This problem can be overcome with a film layer from chitosan, a natural polymer. Embedding the liposomes into a chitosan film provides a physical barrier that prevents aggregation. In our study, we encapsulated the glycoalkaloids into liposomes which then were embedded in a chitosan film. Method parameters were optimized to increase the stability of the liposomes.

**MATERIALS & METHODS** Encapsulation was done using a thin film hydration method. Different lecithin types, pH, temperatures, cryoprotectants and chitosan-tripolyphosphate (TPP) crosslinking were evaluated to improve liposome stability and encapsulation. Characterization of liposomes was done including determination of size and polydispersity by dynamic light scattering (DLS); morphology by scanning electron microscopy; compatibility by FTIR spectroscopy; encapsulation efficiency by ultrafiltration and liquid chromatography and *in vitro* release by dialysis. Chemical and physical stability were determined by spectrophotometry and DLS, respectively.

**RESULTS** Experiments indicated that 10 mM NaCl (pH=5.5), 40°C, hydrogenated lecithin with 70% phosphatidylcholine were the best hydration solvent, temperature, and phospholipid, respectively. The size of the liposomes was quantified as 116 nm. The polydispersity index (PI) was 0.22. Liposomes were considered as monodisperse with PI < 0.50. The physical stability of the liposomes was evaluated at 4, 25, and 37 °C. Size and PI value did not change significantly after 3 months at 4°C. Liposomes stored at higher temperatures were not stable. Physical (size: 985 nm, PI: 0.52) and chemical stability (up to 35% lipid oxidation/degradation) decreased at the end of 2 months. Encapsulation efficiency was calculated as >80%. Liposomes and chitosan were found to be compatible. Chitosan-TPP crosslinking method was optimized and was shown by FTIR at the 1550-1500 and 1370-1290 regions as expected. In addition, cryoprotectant type and ratio were evaluated for lyophilization. Sucrose was found to be the best cryoprotectant among carbohydrates (glucose, maltose, sucrose, dextrose). The optimum mass ratio was 2:1, cryoprotectant:liposome. After three months storage, size and PI of liposomes remained the same.

**DISCUSSION & CONCLUSION** Liposomal formulations were optimized and the formulation was stable for 3 months. Chitosan film provided some advantages: (i) prevention of liposome aggregation and improved stability; (ii) controlled and sustained release; (iii) topical use, therefore, decreased systemic toxicity; (iv) easy to use patch film formulation that patients can apply themselves. Moreover, the natural antimicrobial effect of chitosan provides an additional advantage. This formulation can be used to treat skin diseases, especially skin cancer. Indeed, further studies are needed including *in vitro* cell culture studies and *in vivo* animal models to measure the efficacy of the formulation.

## REFERENCES

- [1] Al Sinani SSS, Eltayeb EA, 2017, S. Afr. J. Bot., 112:253-269.
- [2] Somiya M, et al. 2017, Bioeng., 4:73-92.
- [3] Goy RC, 2009, Polimeros, 19:241 - 247.

# MicroRNAs as potential post-transcriptional gene regulators in anthocyanin biosynthesis

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**BACKGROUND** Anthocyanin biosynthesis has been well studied in Solanaceous crops, although in the case of pepper (*Capsicum annuum* L.) conflicting results are available on the regulation of the pathway. Plant microRNAs (miRNAs) are 20-22 nt short sequences that target complementary mRNAs by either cleavage or transcriptional repression, thus playing a regulatory role in gene expression. Several miRNAs have been described to affect anthocyanin biosynthesis – either directly or indirectly - by altering the expression of R2R3-MYB transcription factors [1, 2, 3]. miR156 has been shown to negatively regulate the anthocyanin biosynthetic pathway in *Arabidopsis thaliana* by targeting the *SQUAMOSA PROMOTER BINDING LIKE* (SPL) genes. One member of the SPL family disrupts the MBW (MYB-bHLH-WD40) transcriptional complex which activates anthocyanin biosynthesis [1]. According to our hypothesis, the overexpression of miRNA156 would lead to an increase in anthocyanin accumulation of the pepper plants, thus catering to the interests of the market and shedding further light on its regulatory role on the pathway.

**MATERIALS & METHODS** Pepper plants were provided by PepGen Ltd. Besides the well-known Hungarian Cecei type peppers, for the preliminary studies *Petunia hybrida* was used as well. The plants were kept under greenhouse conditions. For the *in silico* studies, miRBase, psRNA Target and NCBI databases were used. The transient assay was carried out using the TRV (Tobacco rattle virus) vector-based system. As a negative control, an empty vector was used, for the positive control a TRV-PDS (PHYTOENE DESATURASE) vector was applied. The agroinfiltration was carried out with the GV3101 strain. The effect of miR156 overexpression was monitored by gene expression studies of its target genes.

**RESULTS** Computer analysis revealed that miR156 in pepper targets the SPL1 – with 100% complementarity - and SPL9 transcription factors – with only one nucleotide difference – therefore, overexpression of miR156 would lead to a cleavage of these native SPL transcription factors. A TRV-pre-mi156 construct was built using a 100 bp genomic sequence amplified from *Capsicum annuum* L., which was cloned into the TRV vector. Both the identity and the orientation of the insert were verified by sequencing. For the preliminary studies, the model plant *Petunia hybrida* was used. Agroinfiltration was carried out when the plants were 3 weeks old. Gene expression was measured 14 days and 4 weeks after the infiltration. In the case of pepper, after the agroinjection to the immature berries, we expect to experience anthocyanin build-up, since the MBW transcription complex may remain intact and thus initiate anthocyanin biosynthesis without being disrupted by the SPL transcription factors. Changes in the anthocyanin pattern are monitored both by gene expression and by analytical studies.

## REFERENCES

- [1] Gou JY et al. 2011, Plant Cell, 23(4):1512-22.
- [2] Jia X et al. 2015, Planta, 242: 283.
- [3] Yang F et al. 2013, Plant Cell Tiss Organ Cult, 115: 159.



# Implementation of cell and molecular tools to improve eggplant and pepper breeding

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**BACKGROUND** The increase in *Solanaceae* production worldwide is closely linked to the recent use of innovative biotechnology in plant breeding programmes. Improving pest resistance involves a wise use of cell biology and molecular tools and their efficient implementation in these processes. For conventional breeding, hybridization, including interspecific crosses, and speed are the key points. Cell biology provides information for pollen fertility management and techniques to ease hybridization (embryo rescue) or production of genetically fixed genotypes (double haploid production). Molecular markers allow accurate monitoring of favourable characters (marker-assisted selection) and protection of innovative genetic material. In this poster, we illustrate different approaches to improve the implementation efficiency of conventional breeding support technologies.

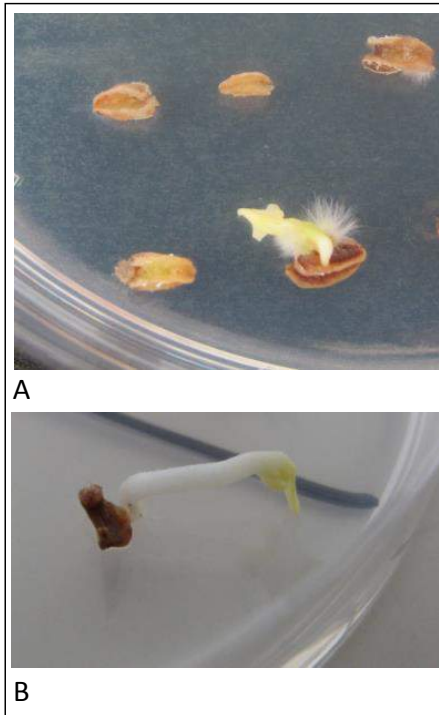
**MATERIALS & METHODS** Eggplant and pepper hybrid plants were grown in glasshouses in Vegenov. Eggplant hybrids were supplied by MC Daunay, INRA Montfavet and pepper hybrids were provided by a private company. Anther culture was employed for both species, following [1] procedure for eggplant, and [2] for pepper. Ploidy level of the obtained plantlets was controlled using CyFlow Space technology (Sysmex Partec). Doubling of haploid plants was provoked with a colchicine treatment (500 mg/l). Pollen viability of hybrids was measured using the automatized impedance flow cytometer AmphaZ32 (Amphasys) [3]. Fingerprinting was done using SSR technology.

**RESULTS** We validated that anther culture was successful for these two species (Figure 1), with variable yields according to genotype. Embryo yield and conversion to plant are the first bottlenecks, followed by return to diploidy. In some cases, chromosome doubling was spontaneous (around 37% of plants). After colchicine treatment, plants were carefully grown to maximise seed production despite chimaeras. About 68% and 32 % of these plants produced seeds, for pepper and eggplant respectively. Pollen viability measurement with impedance flow cytometry allowed us to determine various levels of fertility of interspecific eggplant hybrids. The results (Table 1) illustrate the great variability of hybrid fertility, in terms of quantity and viability of pollen. This constitute a useful tool to determine a more efficient strategy for further DH production, back-crossing or selfing. Genetic fingerprinting databases were developed to control seed lots and plantlet production and to protect new varieties. For eggplant, we tested 68 SSR markers and we selected a panel of 5 SSR markers that are the most polymorphic ones to optimize eggplant genotypes distinction. For pepper, we tested 13 SSR markers and we selected a panel of 8 polymorphic SSR markers to produce specific pepper genetic fingerprints.

**DISCUSSION & CONCLUSION** Conventional breeding tools are now well-described in bibliography, but still can lead to disappointing results. An accurate knowledge and characterisation of the specificity and needs of atypical genetic material can greatly improve the project achievement. Nevertheless, improvement is still possible strengthening staff know-how and integrating new technologies of cell analysis. Efficiency of integration of these approaches also depends on the interaction between the lab and the breeding and the seed production teams.

## REFERENCES

- [1] Dumas de Vaulx R & Chambonnet D, 1982, *Agronomie* 2 (10), pp.983-988
- [2] Olszewska D et al., 2014, *Türk J Biol*, 38: 118-124
- [3] Heidmann I et al., 2016, *PLoS ONE*, 11(11) e0165531



**Figure 1.** Anther culture and embryo development of pepper (A) and eggplant (B).

Plant code	number of cells /ml (x 1000)	% pollen viability
Hyb 1	35,1 (± 9,4)	5,16 (± 0,96)
Hyb 2	9,8 (± 1,4)	79,45 (± 12,02)
Hyb 3	112,1 (± 10,7)	17,95 (± 0,75)
Hyb 4	123,3 (± 21,7)	20,29 (± 2,48)
Hyb 5	106,2 (± 25,4)	6,48 (± 1,51)
Hyb 6	112,2 (± 5,5)	50,50 (± 1,40)
Hyb 7	42,7 (± 6,6)	9,15 (± 5,00)

**Table 1.** Pollen quantity and viability of eggplant interspecific hybrids.

# Rapid creation of parental pure lines for an interspecific hybrid of *Capsicum annuum* L. and *C. frutescens* L. using biotechnological approaches

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**BACKGROUND** The aim of this work was to create an interspecific hybrid of hot pepper with good taste and high decorative properties. Homozygous parent forms are necessary for a hybrid. The original population of *Capsicum frutescens* Cz-544-14 was heterogeneous. Classical methods for the selection of specimen with the required characteristics (early maturing, determinant type, compact, 25-30 cm tall, with a rounded shape of the fruit, violet colour in technical and red - in biological ripeness) were not successful, because undesirable phenotypes have been observed over generations. A microspore culture was used to obtain a 100% homozygous line.

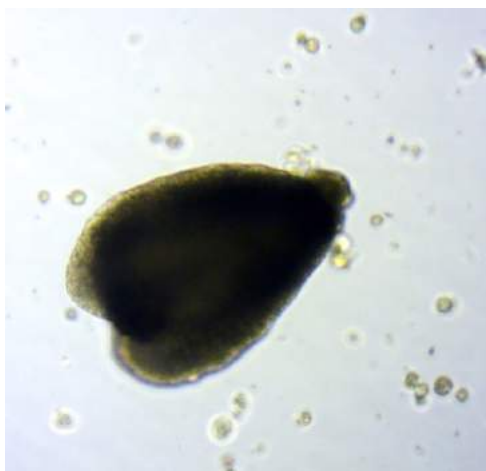
**MATERIALS & METHODS** Heterogeneous populations of hot pepper *C. frutescens* Cz-544-14 and the line *C. annuum* (Rb-551) were used as material for the research. Phenological observations were made in developmental phases during the growing season according to published methods [1] and [2]. Double haploid (DH) plants were regenerated according to the protocol described previously [3], with some modifications: sterilized anthers were crushed and microspores were obtained by filtration. Next, after washing, microspores were cultivated on a growing two-layer medium Nitsch and Nitsch with the addition of 13% sucrose and 2% maltose. Interspecific hybridization was carried out by pollination of the castrated buds of mother plants with paternal pollen.

**RESULTS** The original heterogeneous population of *C. frutescens* included the following variations: plant type (compact / spreading, determinate / indeterminate), height (25-120 cm), leaf colour (green, purple), flower colour (white, purple, with edging), fruit shape (rounded, triangular; heart-shaped), fruit colour in technical ripeness (green, purple), in biological ripeness (orange, red) and fruit weight (6-10 g). Plants with a given phenotype characteristics of the parent line were used as anther donors for the culture of microspores (fig. 1). The obtained DH plants regenerated were characterized. Dh-1 Line No. 4 (fig. 2) was the most interesting for further breeding. Dh-1 L-No. 4 is a mid-season (the period from germination to the start of technical ripeness of fruits is 125 days). The plant is bush, standard, compact, 25-30 cm high. The leaves are small, violet. Fruits are directed upwards, rounded, smooth, glossy, purple in technical ripeness and red - in biological. The wall thickness of the fruit is 1.2 mm. Fruit weight is 8 g. This line was used as parent in a cross with the *C. annuum* line (Rb-551) (fig. 3) and a hybrid of hot pepper «Christmas bouquet» was obtained (fig. 4).

**DISCUSSION & CONCLUSION** Microspore culture of pepper is not a widely used technology as far as there is a large dependence on the plant genotype and cultivation conditions. We developed the optimal conditions for obtaining DH plants from a heterogeneous *C. frutescens* population. The quick development of clean parental lines helps to create a new interspecific hybrid «Christmas bouquet» with the desired properties: plant height 25-30 cm, small, cone-shaped fruits, with different colours, up to 120 pieces per plant. The length of fruits is 3.8 cm, diameter is 1.2 cm, weight is 5.0 g, the taste is pungent, with a strong pepper flavour. This hybrid is highly decorative.

## REFERENCES

- [1] Pivovarov VF, 1997 Guidelines for breeding varieties and hybrids of pepper and eggplant for open and protected soil: 88 p.
- [2] Methods of testing for distinctness, uniformity and stability. *Capsicum annuum* L. . (<http://gossort.com/22-metodiki-ispytaniy-na-oos.html>).
- [3] Shmykova NA et al., 2014 Russian Agricultural Sciences, 40 (6): 417–421.



**Figure 1.** Embryoid development in microspore culture of *Capsicum frutescens* Cz-544-14



**Figure 2.** *C. frutescens* Dh-I Line No. 4, paternal parent of “Christmas bouquet” F1 hybrid



**Figure 3.** *C. Annuum* Rb-551 line, maternal parent of “Christmas bouquet” F1 hybrid



**Figure 4.** F1 hybrid «Christmas bouquet»



# Differential gene expression analysis during fruit development between a cultivated and a wild variety of chili (*Capsicum annuum* L.)

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**BACKGROUND** Chili pepper is one of the most important crops in the world. It is used as an ingredient in cuisines around the world and has pharmaceutical and industrial applications. Of the five domesticated species of chili, *Capsicum annuum* L. is the most important because of its widely distribution and metabolites produced in the fruit over the maturation process, like capsaicinoids and carotenoids. The chiltepin (*C. annuum* L. var. *glabrusculum*) is a wild variety of the *C. annuum* L. species that is representative of the Sonora state, and it plays a high socio-cultural and economic role in the region. Furthermore, it could be used as a genetic resource to improve the current chili varieties. The aim of this study is to analyze the differences on gene expression qualitatively and quantitatively during fruit development of two varieties of chili; a domesticated one (chile serrano, *C. annuum* L. var. "Tampiqueño 74") and a wild one (*C. annuum* L. var. *glabrusculum*; "Chiltepin").

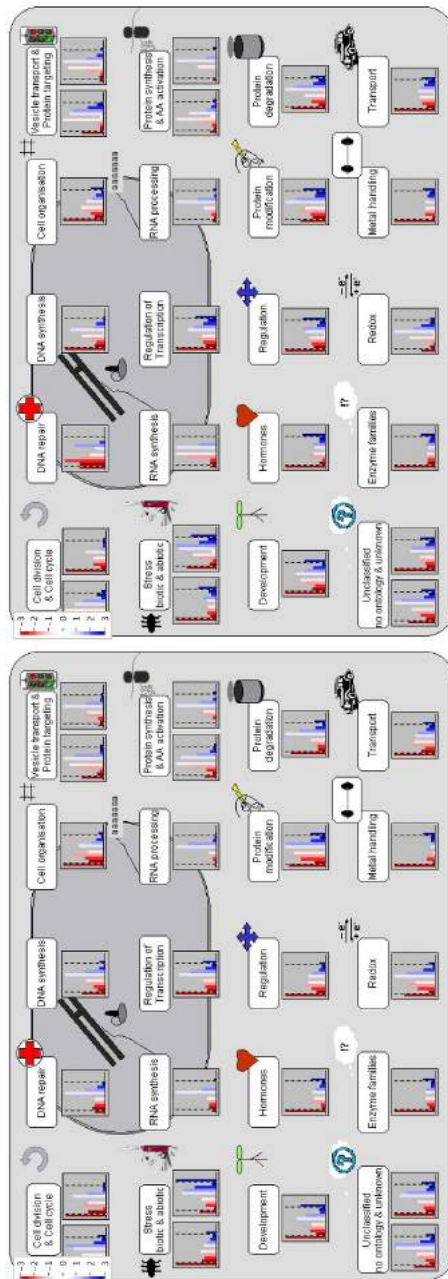
**MATERIALS & METHODS** Chiltepin seeds were germinated and cultivated under greenhouse conditions. Individual flowers were tagged at anthesis and fruits were collected at 20 Days Post Anthesis (DPA) (immature stage) and 68 DPA (mature stage), with 2 biological replicates per condition. For each, RNA was extracted using a NucleoSpin RNA Plant Kit, cDNA libraries were constructed using Illumina TruSeq RNA sample v2 and sequenced on a 2x150 bp mid output Illumina NextSeq 500 platform. Sequencing reads were trimmed using PRINSEQ. Trinity was used for *de novo* assembly of the transcriptome. Abundance was estimated using Express software and differential gene expression analysis was performed using EdgeR.

**RESULTS** Sequencing of 4 Chiltepin libraries produced 260,680,166 raw reads, after quality filtering 239,933,282 filtered reads were obtained and used for *de novo* assembly producing a transcriptome assembly of 383,476 contigs. More than 95% filtered reads mapped back to the assembly for each Chiltepin library. 7,289 Differentially Expressed Genes were identified for Chiltepin between 20DPA and 68DPA (43.3% down-regulated and 28.7% up-regulated), and 5,877 DEGs were detected for Serrano between 20DPA and 60DPA for Serrano (42.5% downregulated and 28.2% up-regulated), with a log fold change  $|\log_2| > 1$  and  $FDR < 0.01$ . Common contigs between Chiltepin and Serrano were selected using blastx against the tomato proteome (ITAG 3.2), both peppers share 14,794 genes expressed at 20DPA and 13,427 genes expressed at 60(68) DPA, and 3,855 DEGs between both maturation stages (2,587 down-regulated and 1,268 up-regulated). Mapman was used for a DE analysis overview (Figure 1) for both peppers. For example, 217 and 183 DEGs were found for Chiltepin and Serrano respectively, related to secondary metabolites production, further 583 and 487 DEGs were found for Chiltepin and Serrano respectively, related to development.

**DISCUSSION & CONCLUSION** There are differences and similarities in the gene expression patterns of Chiltepin and Serrano peppers during the fruit maturation process, in both peppers most DEGs are up-regulated at 20DPA and downregulated at 68 DPA, which is in agreement with results from previous studies [1]. We found changes in the gene expression pattern of a great amount of genes related to the synthesis of phytohormones of both peppers, previous studies have proven that pepper ripening is regulated by complex phytohormone mechanisms [2]. MyB transcription factors related to capsaicinoid production are down-regulated from 20 to 60 (68) DPA in both peppers which agrees with previous analyses [3].

## REFERENCES

- [1] Martínez-López L et al., 2014, BMC Genomics, 15 (1), doi:10.1186/1471-2164-15-143
- [2] Hou B Z et al., 2018, BMC plant biology, 18(1), 162, doi:10.1186/s12870-018-1377-3
- [3] Arce-Rodríguez ML & Ochoa-Alejo N, 2017, Plant physiology, 174(3), 1359–1370, doi:10.1104/pp.17.00506



**Figure 1.** Mapman visualization of Differentially Expressed Genes distribution by cellular function of Chiltepin (left) and Serrano (right) during the fruit maturation process from 20 to 68(60) Days Post Anthesis, respectively.

# Investigation of androgenesis capacity of Rwandan pili-pili variety (*Capsicum chinense* L.) in Turkey

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**BACKGROUND** Pepper is one of the most produced and consumed vegetables in the world. The consumption of cultivated species and types of these species may vary by habits and cultures of countries. Pili-Pili is one of well-known and popular pepper variety in Rwandan cuisine. This variety belongs to *Capsicum chinense*. There are many studies on anther culture of pepper [1]. However, the rapid progress of breeding projects carried out in pepper, a highly demanded crop in the world, justifies the continuation of studies aiming at the adaption of anther culture protocols to each high value genotype. In this study, anther culture performance of Pili-Pili was assessed in the Mediterranean Region of Turkey and compared to two Turkish *C. annuum* controls having low (A111 Kahramanmaraş type-green type) and high (Inan 3363 variety- Urfa type) androgenesis capacity, respectively.

**MATERIALS & METHODS** The anthers sampled were cultured in Murashige and Skoog (MS) medium including 30 g L<sup>-1</sup> sucrose, 2.5 g L<sup>-1</sup> activated charcoal, 15 mg L<sup>-1</sup> silver nitrate (AgNO<sub>3</sub>), 4 mg L<sup>-1</sup> 1-Naphthaleneacetic acid (NAA), 0.5 mg L<sup>-1</sup> 6-Benzylaminopurine (BAP), 6.5 g L<sup>-1</sup> agar in the first stage. The anthers were then transferred to MS medium containing 30 g L<sup>-1</sup> sucrose and 6.5 g L<sup>-1</sup> agar. The embryos obtained were cultured in the same nutrient medium.

**RESULTS** At the end of the study, 19.4 embryos per 100 anthers were obtained from the control Inan 3363 and 4.46 from A111 (Table 1). As expected, Inan 3363 showed better performance than A111 genotype. The performance of Inan3363 changed according to months and nutrient mediums in a study carried out in Turkey [1]. It varied from 0 to 66.36% and the best results were obtained in August. This study was performed in Alata Horticultural Research Institute conditions as ours. Although the climatic conditions change every year, we can compare the results of two studies for Inan 3363 variety. In spite of all efforts, no embryo could be obtained in the Pili-Pili variety, although proper anther culture stage was especially investigated for this Rwandan variety. Cytological investigations showed that classical pepper anther culture morphological markers did not work in Rwandan Pili-Pili variety.

**DISCUSSION & CONCLUSION** In fact, the negative anther culture response in Rwandan Pili-Pili did not surprise us. Because, Turkish *C. chinense* genotypes had also low androgenesis capacity in our previous studies. A possible explanation might be that Pili pili ecological requirements are not fulfilled in Turkey, although the study was carried out in a Mediterranean part of Turkey known as the hottest and humid place of the country. In order to ascertain that the anther culture response of this variety is weak, other trials should be repeated in Rwanda. If there is no response in Rwanda, it could be concluded that this variety does not respond to anther culture.

## REFERENCES

[1] Ata A et al., 2018, Turk. J. Agric For, 42. doi:10.3906/tar-1802-35

Table 1: Anther culture response of genotype and varieties used in this study

Genotype-Variety number	Total petri number	Total anther number	Total embryo number	Embryo number per 100 anthers
Pili-Pili genotype	139	695	0	0
Genotype A111	112	560	25	4.46
Inan 3363 variety	100	500	97	19.4

# Possible use of SSR markers to optimise DUS testing in *Capsicum annuum* L.

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**BACKGROUND** Chili pepper and sweet pepper (*Capsicum annuum* L.) are important vegetable crops with a significant economic value. The French Group for the study and evaluation of Varieties and Seeds (GEVES) is in charge of Distinctness, Uniformity and Stability (DUS) tests for varieties registration and/or protection. This examination process is exclusively based on phenotypic traits and mainly involves field observations. For pepper, a large number of morphological characters are recorded as well as some biochemical properties such as pungency. According to the size of the pepper reference collection and varieties of common knowledge (more than 2500 varieties in the reference collection), field trials may be time and space consuming. In order to enhance field assays and manage reference collection, the use of molecular markers have already been assessed and validated on different crop species, for example maize and barley [1]. The present study aimed to examine the potential use of DNA based techniques as a tool to optimise DUS test in *Capsicum*.

**MATERIALS & METHODS** DNA extraction was carried out on a sample of 30 seeds with 57 cultivars. These different varieties represented a diverse sample in terms of morphological characters specially fruit shape and pungent characteristic. A set of 38 SSR were first selected according to previous studies ([2] and [3]) on different *Capsicum* species. Currently, 13 SSR markers have been assessed. First analysis on molecular diversity were carried out in R (R core Team). Genetic indices were used to select the most informative markers and Rogers genetic distances were calculated to examine genetic structure via UPGMA tree and PCOA analysis.

**RESULTS** All 13 primers tested amplified. However, one primer showed inconsistency and was difficult to score and therefore discarded for further analysis in this study. The 12 SSR primers generated a total of 42 polymorphic fragments with an average of 3.5 alleles per locus. The number of alleles per locus ranged from 2 to 9. PIC (Polymorphism Index Content) was estimated from 0.018 to 0.476. Few markers had a low PIC value. The polymorphic SSR markers were not able to distinguish the 57 pepper varieties tested. Rogers genetic distance values ranged from 0 to 0.88. To analyse the genetic relationships among the varieties, a cluster analysis was performed. PCOA and UPGMA dendrogram gave similar results, one major cluster containing 41 non-pungent varieties was observed. For the 16 remaining varieties a grouping pattern according to the morphological traits tested, i.e., pungent and fruit shape was not observed.

**DISCUSSION & CONCLUSION** To improve molecular descriptions of varieties, clustering analysis results from the 25 remaining SSR will be analysed. If results are conclusive, the marker set selected will be used to calculate genetic distances among candidate varieties and varieties from the collection. These genetic distances could be used as complementary information for field comparisons. Moreover, UPOV models now consider positively the use of molecular markers directly linked to a phenotypic trait. Diagnostic markers should then be examined to assess potential benefits in the framework of DUS testing. In *Capsicum*, the pungent or non-pungent character is a good candidate to evaluate this approach.

## REFERENCES

- [1] UPOV. 2013. 'TGP/15/1 Guidance on the use of biochemical and molecular markers in the examination of distinctness, uniformity and stability (DUS)', Geneva.
- [2] Nicolai M et al., 2013, Genet. Resour. Crop. Evol, 60 (8): 2375-2390
- [3] Toledo-Aguilar R et al., 2016, Chilean J. Agric. Res, 76(1): 18-26

# Development of genome-wide KASPar markers from a comparative transcriptomic analysis for mapping QTLs associated with the pungent trait of pepper (*Capsicum annuum* L.)

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**BACKGROUND** Pepper (*Capsicum* spp.), belonging to the Solanaceae family, is an important vegetable crop worldwide owing to its nutritional value and flavor. There are five domesticated pepper species (*C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L., and *C. pubescens* Ruiz et Pav.) and more than 20 wild relative species. Global pepper production is approximately 34.5 million tons annually. Under the threat of a changing environment, new varieties with enhanced resistance to biotic and abiotic stresses are demanded by farmers. Marker-assisted selection (MAS) has been used to introduce new and desirable characters into plants, such as disease resistance. The lack of sufficient molecular markers for pepper has been a bottleneck for genetic and linkage studies [1].

**MATERIALS & METHODS** Transcriptomes from five *C. annuum* L. accessions (Yolo Wonder, CM334, Perennial, PM687 and PM217) were sequenced. Unigenes of CM334, Perennial, PM687, and PM217 were mapped to the reference transcriptome of Yolo wonder [1]. Positions of SNPs and their flanking sequences were extracted to design KASPar markers. A genetic map based on KASPars was constructed by using Joinmap 4 thanks to 113 F<sub>9</sub>-recombinant-inbred-lines (RIL) issued from the intraspecific cross 'Perennial × 83-58'. Capsaicin and dihydrocapsaicin were quantified in the RIL progeny by ultra-high performance liquid chromatography (UPLC, Waters, USA) and a QTL analysis was performed by using the software MapQTL3.0.

**RESULTS** Comparison of the Perennial transcriptome with the Yolo Wonder transcriptome revealed 9,037 SNPs corresponding to 4,333 unigenes. Comparison of the transcriptomes of CM334, PM687, and PM217 with the Perennial transcriptome revealed 2,077 SNPs corresponding to 1,674 unigenes. We developed KBioscience Competitive Allele-Specific PCR (KASPar) markers for a set of SNPs. Out of the 975 KASPars developed, 303 (31%) were polymorphic between Perennial and 83-58. We constructed a genetic linkage map with 372 markers (including 278 KASPars) and detected quantitative trait loci (QTL) for the pungent trait (capsaicin and dihydrocapsaicin content) using the F<sub>9</sub> RIL progeny. Four QTLs determining the capsaicin and dihydrocapsaicin content were identified. For traits related to the capsaicin content, one major QTL was located between marker BD76366 and *Pun1* on chromosome P2; it explained 29% to 41.5% of the phenotypic variation. Two minor QTLs located on chromosome P2 and P12 explained 8.0% and 11.0%. For the dihydrocapsaicin content, one major and two minor QTLs were located at the same positions as the QTLs of capsaicin content on chromosomes P2 and P12; an additional minor QTL located on chromosome P12 explained 9.9% of the phenotypic variation.

**DISCUSSION & CONCLUSION** This research identified thousands of polymorphic positions among the genomes of pepper accessions. We developed KASPar markers covering the whole-genome and that can be used to locate QTLs or genes at any position. 31% of 975 KASPar markers were polymorphic; they were used to construct a molecular linkage map and identified QTLs associated with the pungent trait. The KASPar markers we have developed have potential value for pepper crop improvement and breeding for altered capsaicin content.

## **REFERENCES**

[1] Nicolai et al., 2012, Genetics and Molecular Research, 11 (3): 2295-2300. DOI: 10.4238/2012.August.13.3

## **ACKNOWLEDGEMENTS**

Lihao Wang thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



# Development of fluidigm SNP-type assays for foreground selection in Chili pepper breeding

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**BACKGROUND** Chili pepper including sweet paprika and hot pepper is an important horticultural crop in Korea. Marker-assisted selection (MAS) is commonly used to improve the efficiency of selection in many crops as well as in pepper [1]. MAS involves foreground selection of a target trait or traits of interest and can reduce the breeding period and the number of generations needed in a breeding program compared to conventional phenotypic selection. Numbers of molecular markers have been developed in pepper for disease resistance, male sterility and quality related traits. Therefore, high-throughput screening methods are required for the simplified and cost-effective analysis of multiple molecular markers in a single reaction. Among the several high-throughput SNP genotyping platforms, we focused on the Fluidigm 192.24 dynamic arrays, a flexible PCR-based SNP platform that analyzes simultaneously 192 plants with 24 SNP markers. This study was carried out to apply the SNP markers linked to genes or QTLs controlling disease resistances or capsaicinoids content to the Fluidigm dynamic arrays.

**MATERIALS & METHODS** Plant materials consisted of three populations, PG (*Capsicum germplasms*), CHB (*C. annuum* 'AI' × '2620'), and JN (*C. annuum* 'NB1' × *C. chinense* 'Bhut Jolokia'). Target sequences were employed to design appropriate primers for SNP-type assays. Length of the target sequences had a minimum of 60 bp (including both upstream and downstream of the target SNP site) and a maximum of 250 bp. Only one SNP was present in each target sequence. For insertions/deletions (In/Dels) the length of the In/Del was shorter than 10 bp. The G/C content of the target sequence was < 65%. Primers and related information are listed in Table 1. Each SNP-type assay consisted of three types of primers: a specific target amplification (STA) primer; a locus-specific (LS) primer; and an allele-specific (AS) primer [2].

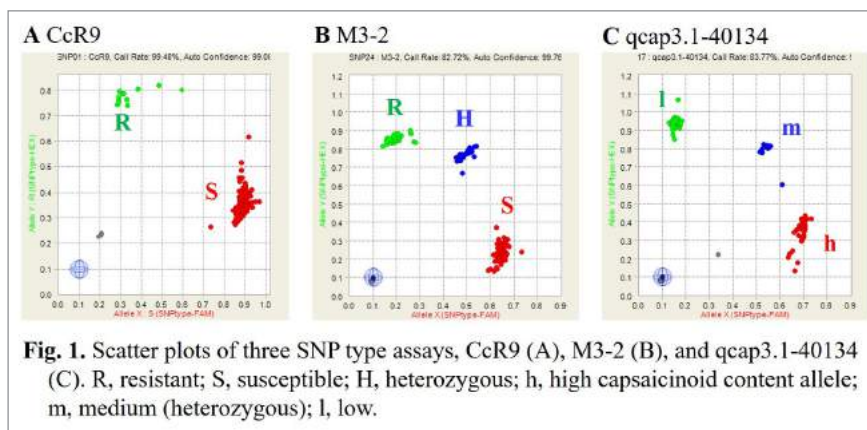
**RESULTS** A total of 43 primer sets were designed. Clearcut results were obtained for 20 SNP-type assays, which indicate a kind of SNP marker; yielding two or three phenotype groups (Figure 1). Red, green, and blue points indicate XX (fluorescence of only FAM dye), YY (only HEX dye), and XY (both FAM and HEX dyes) marker genotypes, respectively. Each genotype matched either resistant (R), heterozygous (H), or susceptible (S) phenotypes for disease resistance, and high (h), heterozygous (m for medium), or low (l) capsaicinoid contents (Figure 1 and Table 1). The 20 successful SNP-type assays are listed in Table 1, together with the original names of genes or QTLs for disease resistances (*Phytophthora* root rot – Phyto.5.2, anthracnose – CcR9 and CaR12.2, powdery mildew – Ltr4.1 and Ltr4.2, bacterial spot – Bs2, CMV – Cmr1, TMV – L4, and potyvirus – pvr1 and pvr2) and for capsaicinoids content (qcap3.1, qcap6.1, qdhc2.1, and qdhc2.2). The 20 successful SNP-type assays showed clear results that were polymorphic in at least one population (Figure 1 and Table 1). In addition, 11 SNP-type assays were compared with the corresponding original High-Resolution Melting (HRM) markers. In result, genotypes of SNP-type assays cosegregated with those of corresponding HRM markers.

**DISCUSSION & CONCLUSION** We have successfully applied 20 SNP-type assays to the Fluidigm dynamic array platform, which can analyze simultaneously 192 plants with 24 SNP-type assays [3]. This method was first used for foreground selection of targeted genes or QTLs in pepper. This system can save both resources and time by reducing the reaction volume and producing 4,608 data points at a time [2]. The accuracy of 11 SNP-type assays was confirmed by comparing with the corresponding original HRM markers. These results suggest that the newly developed SNP-type assays can be used in place of the original markers.

These SNP-type assays will be useful in molecular breeding programs for developing new pepper varieties resistant to multiple diseases and with increased pungency.

## REFERENCES

- [1] Collard BCY, Mackill DJ, 2008, Phil Trans R Soc B 363:557-572. doi:10.1098/rstb.2007.2170
- [2] Wang J et al., 2009, BMC Genomics 10:561. doi:10.1186/1471-2164-10-561
- [3] Kim H et al., 2017, Hortic Sci Technol 35:465-479. doi:10.12972/kjst.20170050



**Table 1.** List of Fluidigm SNP type assays used in this study and relevant information.

Assay No.	SNP type assay	Trait	Target gene or QTL	Position	SNP	SNP (color of dye*)
A1	M3-2	Phytophthora root rot resistance	<i>Phyto.5.2</i>	Chr.5	...GTA[C/T]GTA...	C(R):T(G)
A2	M3-3	Phytophthora root rot resistance	<i>Phyto.5.2</i>	Chr.5	...TGT[CAGA/GAGT] GAT...	CAGA(R):GAGT(G)
A3	CcR9	Anthracnose resistance	<i>CcR9</i>	Chr.9	...ACA[A/C]TTA...	A(R):C(G)
A4	CA09g12180	Anthracnose resistance	<i>CcR9</i>	Chr.9	...TAT[A/C]GTG...	A(R):C(G)
A5	CA09g19170	Anthracnose resistance	<i>CcR9</i>	Chr.9	...GGT[C/T]GTA...	C(R):T(G)
A6	CA12g17210	Anthracnose resistance	<i>CaR12.2</i>	Chr.12	...CAT[T/G]GAA...	T(R):G(G)
A7	CA12g19240	Anthracnose resistance	<i>CaR12.2</i>	Chr.12	...GAT[CGCGAA/AGCGAG]AAA...	CGCGAA(R):AGCGAG(G)
A8	Ltr4.1-40344	Powdery mildew resistance	<i>Ltr4.1</i>	Chr.4	...ATC[AAAAC/GAAAT]TTG...	AAAAC(R):GAAAT(G)
A9	Ltr4.2-56301	Powdery mildew resistance	<i>Ltr4.2</i>	Chr.4	...TTA[A/C]GAG...	A(R):C(G)
A10	Ltr4.2-585119	Powdery mildew resistance	<i>Ltr4.2</i>	Chr.4	...CGA[C/T]ATT...	C(R):T(G)
A11	Bs2	Bacterial spot resistance	<i>Bs2</i>	Chr.2	...CTC[AT]GTG...	A(R):T(G)
A12	Cmr1-2	CMV resistance	<i>Cmr1</i>	Chr.2	...GAA[G/C]GAG...	G(R):T(G)
A13	L4	TMV resistance	<i>L4</i>	Chr.11	...AAC[A/T]CTC...	A(R):T(G)
A14	pvr1	Potyvirus resistance	<i>pvr1</i>	Chr.4	...AAT[A/C]CAG...	A(R):C(G)
A15	pvr2-123457	Potyvirus resistance	<i>pvr2</i>	Chr.4	...CAG[T/A]GGC...	T(R):A(G)
A16	qcacp3.1-40134	Capsaicinoid content	<i>qcacp3.1</i>	Chr.3	...CTT[A/C]AGA...	A(R):C(G)
A17	qcacp6.1-299931	Capsaicinoid content	<i>qcacp6.1</i>	Chr.6	...CAG[G/A]AGG...	G(R):A(G)
A18	qcacp6.1-589160	Capsaicinoid content	<i>qcacp6.1</i>	Chr.6	...AGG[G/A]AAA...	G(R):A(G)
A19	qdhc2.1-1335057	Capsaicinoid content	<i>qdhc2.1</i>	Chr.2	...ATT[A/G]GCA...	A(R):G(G)
A20	qdhc2.2-43829	Capsaicinoid content	<i>qdhc2.2</i>	Chr.2	...CCG[G/A]ACC...	G(R):A(G)

zR, red (FAM dye); G, green (HEX dye).

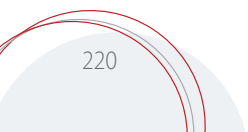
yR, resistant; S, susceptible; H, allele for high capsaicinoid content; L, allele for low capsaicinoid content; pvr2<sup>123457</sup> indicates pvr2<sup>1</sup>, pvr2<sup>2</sup>, pvr2<sup>3</sup>, pvr2<sup>4</sup>, pvr2<sup>5</sup>, or pvr2<sup>7</sup>.





# PRE-BREEDING AND BREEDING

SESSION 5



# Highlights of *Capsicum* breeding research over the past 20 years

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In this paper I will present an overview of some topics covered and methods developed in the breeding research on pepper (*Capsicum* spp.) since about 1998, the year when this Eucarpia working group last convened in Avignon. Breeding research is a wide and ill-defined topic. Treating this whole field would involve rather more time than available for this presentation. This is therefore necessarily a personal selection. I have split this overview in a part on tools and methods and one on specific plant traits.

**BREEDING (RESEARCH) TOOLS AND METHODS** Looking back, the progress in our field is most clearly seen in the development and use of new research and breeding tools, and sometimes replacement of older methods. These tools vary a lot, from male sterility, to new ways of obtaining plants such as through regeneration, haploid induction and GMOs, to molecular tools such as marker technologies and whole-genome sequences.

## **Anther culture and doubled haploids**

Successful anther culture for the induction of haploid and doubled haploid (DH) plants was first reported in 1981 by Robert Dumas de Vaulx, from the INRA here at Avignon. Although the technique was quickly taken up by many researchers and breeders over the following 20 years, it still has its challenges as it is quite genotype-specific and for recalcitrant genotypes all kinds of modifications are still being developed. In research DH populations have been used extensively in QTL mapping of various traits. The advantage of these populations, which can be obtained within 2-3 years after making the cross, is that they are immortal, like RIL populations. The disadvantage compared to F2 and RIL populations is that the amount of recombination is very limited: each individual is derived from the F1 plant through only one meiosis.

## **Male sterility**

Male sterility can obviously be of great use for producing F1 hybrid varieties. Both cytoplasmic and genic male sterility have been known and used for a long time. The most widely used system is a genic form of male sterility where a homozygous recessive *ms/ms* genotype is male sterile; several of these genes are known. In spite of its long use, research into this system continues today, often focused on fine mapping of the *Ms* gene and identification of candidate genes. Also a cytoplasmic-genic system is in use, mostly in hot pepper breeding, but less research seems to be devoted to this.

## **Interspecific hybridization**

Around the main cultivated species, *Capsicum annuum*, there are three more or less closely related species: *C. frutescens*, *C. chinense* and *C. baccatum*. In the past much research has been done on characterizing the interspecific barriers (or lack of them) among these and more distantly related species in a systematic way, and on overcoming these barriers, including the development of embryo culture for difficult combinations. This type of research has tailed off; not many new results have been published in the past twenty years. Needless to say, interspecific hybridization among these closely related species has been, and remains today, an important tool for the introgression of traits; mostly resistance but also quality traits. The potential for interspecific hybridization varies considerably among genotypes, even of species that are generally considered to be rather well compatible, which means that introgression is not always successful or straightforward.

## **Regeneration, GMO, CRISPR/Cas9**

Regeneration is a prerequisite for obtaining genetically modified (GM) plants. *Capsicum* species are not easy to regenerate, apart from anther culture and shoot development from pre-existing axillary buds. Nevertheless, over time many reports of plant regeneration from various explants have appeared. This mostly involved explants from young seedlings. However the described methods differ and probably need to be adapted for each genotype. These difficulties with regeneration have also limited the successes with genetic modification. Still several reports of successful transformation with reporter genes (*gus*, *nptII*) exist, and also reports of transformation with coat proteins of CMV to obtain resistance. So far, no reports on successful application of the gene-editing technique CRISPR/Cas9 have been published and alternative approaches for construct delivery may be needed.

## **VIGS**

After identification of a candidate gene for a phenotypic trait, one way to confirm its role is by transiently silencing its expression through VIGS (virus-induced gene silencing). The method was first demonstrated in pepper in 2004. It has not been widely used in *Capsicum* but there are reports from The Netherlands in 2013 where it was used to elucidate the role of *Mlo* gene analogs in susceptibility to powdery mildew, and by two Chinese groups for studying several genes involved in the anthocyanin coloring of leaves and in resistance to *Phytophthora capsici*.

## **Mutation breeding, TILLING populations**

Genetic modification (GM) in *Capsicum* is problematic, among others because most genotypes are recalcitrant to regeneration and also because of GMO legislation in Europe. For the purpose of testing whether a candidate gene indeed affects a trait under study a mutation approach offers an alternative, especially in an approach called TILLING (Target Induced Local Lesions IN Genomes). This method allows to efficiently select plants with a mutation in a target gene. If they show the expected (desired) phenotype they can also be used in breeding as non-GMO donors of the mutation.

Constructing such a TILLING population involves a considerable effort and I know of only a few examples in *Capsicum*. In France the INRA has a TILLING population which is not publicly available. Recently a Korean group has also constructed a TILLING population based on a Korean *C. annuum* landrace.

## **Molecular markers, mapping populations, linkage maps, QTL mapping**

Among all breeding tools, molecular marker technology shows probably the most pronounced development over the last 20 years. At the end of the last century multiple marker datasets had been accumulated in the *Capsicum* research community, including various marker types such as RFLPs, RAPDs, SSRs, AFLPs and also phenotypic markers and isozymes. Genetic linkage maps were being published based on various populations, mostly F2 or DH. In 2004 an integrated linkage map was published by researchers from several groups in different countries, aligning these linkage maps as well as possible.

Nowadays markers are a relatively low-cost commodity. SNPs can be readily identified by sequencing and genotyping can be performed using a variety of techniques. In combination with publicly available genome sequences it is possible to select a set of SNPs well distributed over the genome for QTL mapping and Genome-wide association (GWA) studies, or to zoom in on regions of interest and select extra SNPs to facilitate fine mapping of QTLs and Marker-assisted Selection (MAS). Also other marker-aided breeding approaches become feasible, such as genomic selection, although this is not yet seriously applied in *Capsicum* to my knowledge.

## **Genome sequences**

Over the past years several *Capsicum* genome sequences have become publicly available. These are very rich and useful resources. As indicated earlier they can be used to select markers for genome-wide QTL mapping and for fine mapping. Also, their annotation of genes can help select likely candidate genes in an identified

QTL region. Further they can be used to identify syntenic regions and chromosomal rearrangements between species, within the *Capsicum* genus but also more widely.

In 2014 the first whole-genome sequences in *Capsicum* were published, of three *C. annuum* accessions: Criollo de Morelos 334 (CM334), Zunla and the wild accession Chiltepin. This was followed in 2017 by the sequences of a *C. chinense* and a *C. baccatum* genome, and in 2018 by a 10X derived sequence of a *C. annuum* F1 hybrid with CM334 as one of the parents.

### **Phenotyping methods**

With the improvements in marker technology, phenotyping becomes the limiting factor in QTL analyses. Advances in automated phenotyping have been described in *Capsicum*, e.g. an automated system to image and measure plants in the EU-project SPICY. However we are still in the very early stages with this technology.

**PLANT TRAITS** Breeding research in *Capsicum* has focused on a myriad of different plant traits. Many traits have received more or less continuous attention while others only have been briefly investigated.

### **Disease resistance**

Resistance to pathogens are an example where research usually continues over long periods, whether because new sources of resistance are required when the old ones are overcome, or because of increasingly detailed studies into the mechanisms and genetic backgrounds of resistance. The diseases and pathogens on which studies were published in the past 20 years include many viruses (CMV, PVX, potyviruses, Pepino Mosaic Virus, TSWV), bacteria (bacterial spot, bacterial wilt), fungal diseases including anthracnose, *Rhizoctonia*, *Stemphylium*, *Verticillium* and *Phytophthora*. Resistance to insect pests has been studied over a long time but in recent years there is a marked increase of activities in this field, especially on thrips and aphid resistance. A clear trend over all these resistance studies is that genetic studies nowadays aim for fine mapping of QTLs and in some cases identification of target genes.

### **Fruit quality**

Another set of plant traits that have been well studied over the past 20 years can be grouped under the heading of fruit quality. Pungency is a very important trait and has been studied for a long time. Especially since about 2010 many studies have focused on the genetics of other aspects of fruit composition and taste, as well as fruit color.

### **Other plant traits**

Studies on many other plant traits have also been reported over the past 20 years. To name just a few: parthenocarpy, fruit size and shape, flowering time, plant architecture and yield. Often a particular trait was the subject of an isolated study or PhD project and then attention disappeared again. Reasons for this vary: for instance, the trait may be of limited commercial interest or only useful in a specific region, or it may be pursued mostly within private companies and just not be published.

**CONCLUSION** A short overview like this cannot hope to do justice to the entire field of *Capsicum* breeding research. I hope to have shown that our field is very much active and enjoys many exciting new developments and innovations, but that there are also unanswered challenges. *Capsicum* breeding and research is blooming and there will be no shortage of subjects for our Eucarpia group meetings in the foreseeable future.

### **ACKNOWLEDGEMENTS**

Roeland Voorrips thanks the Agropolis Fondation and the SFR Terys for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant.



# Embrapa's *Capsicum* breeding program: release of new cultivars

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**BACKGROUND** Embrapa's *Capsicum* program is considered to be the largest public investment in hot pepper breeding in Brazil. The germplasm bank established in the early 1980's has been efficient in providing germplasm with variability, adaptability, yield and characteristics demanded by the breeding program. Moreover, the strategy adopted by the program focuses in the development of specific products demanded by the market (about 80% of the total resources) and an allocation of about 20% of the breeding efforts to explore new opportunities [1]. The establishment of agreements with the private sector has been also crucial for the success of the program. In the last years, Embrapa has developed and made available around ten cultivars of different types and species: Brasilândia, BRS Sarakura, BRS Garça, BRS Seriema, BRS Moema, BRS Mari [2], BRS Juruti, BRS Nandaia, BRS Tui, and BRS Acará. Most recent efforts address the development of new, uniform, high yielding and quality, diseases resistant Habanero (*C. chinense*), Calabrian and Jalapeño type cultivars.

**MATERIALS & METHODS** Several breeding methods have been used to generate new cultivars to assist small-scale farmers as well as the medium and large farmers and agribusiness. Most recent efforts have included the development of a base population of *C. chinense*, from which new recombined genotypes are being obtained, such as uniform, high yielding, high quality, disease resistant Habaneros. Additionally, Jalapeños adapted to mechanical harvesting and Calabrian type cultivars are being developed. For Calabrian and Jalapeño peppers, the breeding method used was individual selection of plants with progeny test. All new cultivars and inbred lines have been registered in the National Cultivar Registry (RNC) and protected by the National Plant Variety Protection Service (SNPC) of the Ministry of Agriculture, Livestock and Supply (MAPA).

**RESULTS** Three Habanero lines selected from a base population with high variability for relevant traits such as fruit shape, fruit color and capsaicin content were selected for high yield, high pungency (> 300,000 SHU) and color (yellow and red mature fruit). Capsaicin content among the selected Habanero lines (Figure 1) varied from 2,000 (CNPH 15,744) to 500,000 + SHU (CNPH 15,749). Two Calabrian lines (Figure 2) for processing as dehydrated flakes were selected based on plant yield (0.6 - 1.0 kg/plant), earliness, absence of lateral shoots, and low pungency (~ 5,000 SHU). Jalapeño plant selection for mechanical harvesting has been mainly based on plant height (> 60 cm), height of first bifurcation (> 16 cm), erect plant growth habit, earliness, high yield (> 1 kg/plant), fruit pungency (> 30,000 SHU) and concentrated fruit set. Four lines (CNPH 30,375, 30,777, 30,607 and 30,609) were selected (Figure 3).

**DISCUSSION & CONCLUSION** The biggest challenge in Brazil nowadays is the lack of labor for harvesting, which has demanded the development of Jalapeño cultivars for mechanical harvest [3]. Selected Jalapeño lines (Figure) will be cultivated in Central region of Brazil for additional tests that will be undertaking using an Israeli mechanical harvester Moses 1000-Etgar. New tests in different locations and under field conditions will be carried out in order to determine yield, resistance to diseases, capsaicinoid and vitamin concentration in fruits of selected lines. The selected lines of Habanero, Calabrian and Jalapeño types have high potential to meet different Brazilian market demands.

## REFERENCES

- [1] Reifschneider FJB, Lopes CA, et al., 2016, Horticultura Brasileira, 34(2):155-160
- [2] Reifschneider FJB, Ribeiro CSC, et al., 2013, In: XV Eucarpia Meeting on Genetics and breeding of Capsicum and Eggplant, Turin: 71-77
- [3] Ribeiro CSC, Reifschneider FJB, et al., 2013, In: XV Eucarpia Meeting on Genetics and breeding of Capsicum and Eggplant, Turin: 435-437

## ACKNOWLEDGEMENTS

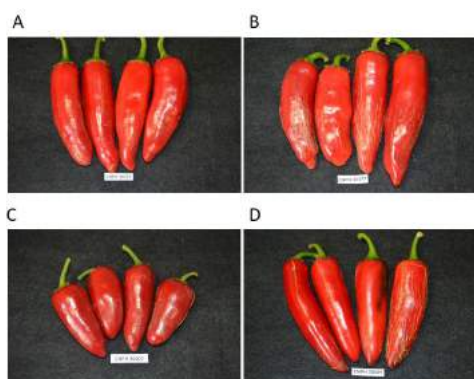
The authors wish to thank CNPq, the Brazilian National Research and Development Council and Sakura Nakaya Alimentos Ltda for their support, as well as the Brazilian network of pepper researchers (Grupo Capsicum) for their invaluable support.



**Figure 1.** Fruit of the selected Habanero lines CNPH 15,740 (A), CNPH 15,744 (B) and CNPH 15,749 (C) of Embrapa's breeding program



**Figure 2.** Fruit of the selected Calabrian lines CNPH 50,112 (A) and CNPH 50,116 (B) for processing as dehydrated flakes



**Figure 3.** Fruit of selected Jalapeño lines CNPH 30,375 (A), CNPH 30,377 (B), CNPH 30,607 (C) and CNPH 30,609 (D) of the Embrapa's breeding program for mechanical harvesting

# Fighting anthracnose in *Capsicum*: a multidisciplinary approach to elucidate plant resistance mechanisms and breeding for disease resistance

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**BACKGROUND** Anthracnose in *Capsicum* has posed a challenge to breeders. Despite recommended as the most efficient control alternative, the use of resistant cultivars is still restricted. This is mainly due to three factors: i) pathogen complexity, with many different species as causal agent; ii) difficulty in finding sources of resistance and transferring the alleles of the resistant accessions to cultivars, and iii) genetic control to pathogen reaction independent on unripe and ripe fruits. Resistance sources have been described in *C. chinense* and *C. baccatum*, but to date only one *C. annuum* accession has been reported as resistant to anthracnose [1,2]. In this work, we report a multidisciplinary approach focused on understanding *Capsicum-Colletotrichum* association. Our team, using the *C. annuum* resistance source UENF 1381, has integrated efforts to carry out inheritance studies, mapping of resistance loci, transfer of resistance alleles, research on the biochemical mechanisms involved in the expression of resistance, and pathogen characterization (Figure 1).

**MATERIALS & METHODS** A biparental cross (2285x1381) was used to study inheritance and generate segregant populations for mapping and to start a breeding program. Using pedigree and SSD, we were able to advance generations and estimate genetic parameters for plant selection. To study biochemical defense mechanisms, seed and fruit extracts were purified by reverse-phase chromatography [3]. The peptides were investigated in their ability to interfere with *Colletotrichum* *in vitro*. Secondary metabolites were quantified from unripe and ripe fruits inoculated, using ultra-high performance liquid chromatography. Morphological, cultural and molecular techniques were used to characterize *Colletotrichum* isolates.

**RESULTS** For both maturation stages, one major gene associated with genes with minor effect controlled anthracnose resistance. Plants highly promising were identified based on predictive genetic value for anthracnose resistance components. Genetic map is still being constructed and up to now, all molecular markers were distributed in eight linkage groups. Four different families of antimicrobial peptides such as lipid transfer proteins (LTPs), trypsin inhibitors, thionins and plant defensins were found related to resistant parent. Also, high concentrations of caffeic and chlorogenic acid were detected in quantification of secondary metabolites produced in pepper fruits during fungus infection, with different values according to fruit development stages (unripe and ripe) and time (1st and 8th day post-inoculation). Although both secondary metabolites were detected in resistance and susceptible plants, higher concentrations were observed in resistant plants after pathogen infection. These biochemical compounds are putatively involved in defense mechanism in response to anthracnose disease. Fungus characterization showed that all 12 tested isolates were *C. scovillei*, species reported in Brazil firstly in 2014.

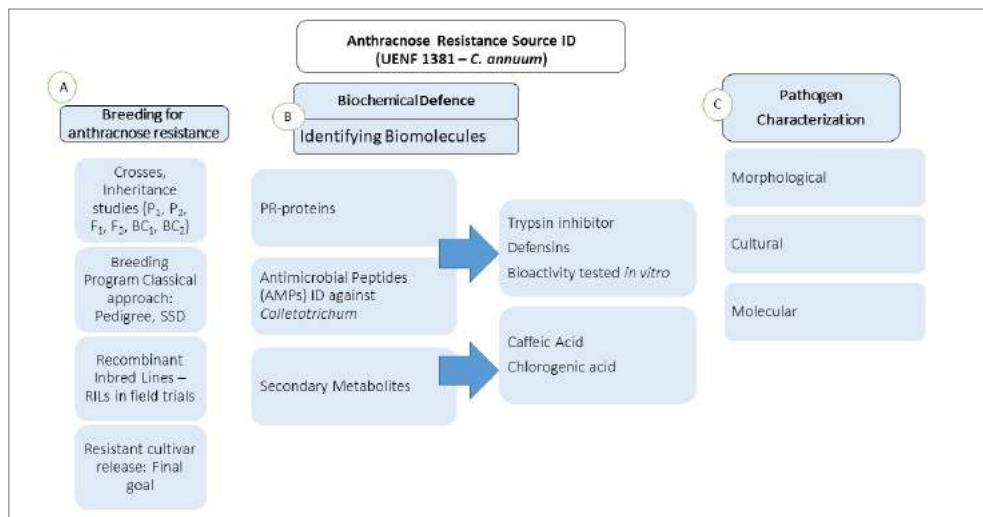
**DISCUSSION & CONCLUSION** The combination of phenotypic, genetic and biochemical information can assist plant breeders in developing resistant cultivars, along with better understanding of *Capsicum-Colletotrichum* interaction.

## REFERENCES

- [1] Bento CS et al., 2013, Genetics and Molecular Research, 12: 1074-1082.
- [2] Baba VY et al., 2019, Scientia Horticulturae, 246: 469-477.
- [3] Pereira LS et al., 2018, Acta Physiologia Plantarum, 40: 107-113

## ACKNOWLEDGEMENTS

Paola Alvares Bianchi thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.



**Figure 1.** Interdisciplinary strategy to approach *Capsicum-Colletotrichum* interaction. A) Breeding steps from crosses using resistant parental *C. annuum* UENF 1381; B) Biochemical investigation of defense mechanisms using UENF 1381 as resistant genotype; C) *Colletotrichum* isolate characterization.

# Predicting genetic potential of advanced pepper lines for resistance to anthracnose caused by *Colletotrichum gloeosporioides*

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**BACKGROUND** Anthracnose is one of the most devastating fungal disease in *Capsicum* worldwide. There are reports of losses of up to 100% in field grown susceptible cultivars. The high inter and intraspecific variability of the pathogen, associated with polygenic control of resistance, have been a challenge for breeders seeking stable and broad-spectrum resistance cultivars. At the present time, there are no resistant commercial genotypes available to farmers in Brazil, and high contamination of pepper fruits with pesticides has been frequently reported. Breeding programs to obtain anthracnose resistant peppers cultivars are of key importance for agribusiness scenario. Our team was able to identify a resistance source and started a breeding program based on the accession UENF1381 (*C. annuum* var. *annuum*), which is also resistant to bacterial spot [1]. In this work, we report the phenotyping of F3:4 plants, in a field trial, for anthracnose resistance and predicting genetic potential using a mixed model strategy, with the aim to select pepper progenies with resistance against anthracnose.

**MATERIALS & METHODS** Seventy-nine F3:4 progenies and parent lines UENF2285 (susceptible) and UENF1381 (resistant) were grown in the field. Ten plants represented each progeny, and 624 plants (595 F3:4 + 39 parents) in total were evaluated. Three unripe fruits of each plant were harvested, taken to the lab, disinfested and inoculated with *C. gloeosporioides* isolate. Variables Incubation Period (IP), Latent Period (LP), and Area Under Disease Progress Curve (AUDPC) were evaluated. Genetic parameters were estimated via Restricted Maximum Likelihood/Best Linear Unbiased Prediction. Mulamba & Mock index was used for ranking and selection purposes. SELEGEN-REML/BLUP software was used for data analysis [2].

**RESULTS** Genetic variance estimates among progenies were different from zero for all traits (Table 1). This meant that there was sufficient genetic variability among progenies to supply advance by selection for anthracnose resistance, especially for AUDPC, for which predominance of genetic over residual effects ( $\sigma_a^2 > \sigma_r^2$  and  $CV_{gi} > CV_e$ ) was observed. Genotypic variation for AUDPC was under additive genetic control and was confirmed to have high narrow-sense heritability ( $h^2_a = 0.55$ ) [2]. IP and LP presented medium  $h^2_a$  (0.32 and 0.40, respectively) indicating greater influence of environment. Due to high heritability on a progeny-mean basis ( $0.82 \leq h^2_{mp} \leq 0.92$ ), high correlations between predicted and true genotypic values were also observed ( $0.91 \leq \text{Acprog} \leq 0.96$ ). Phenotypic differences for AUDPC were large among F3:4 progenies ( $0 \leq \text{AUDPC} \leq 40$ ) (Table 2). The best 185 F3:4 plants scored an AUDPC value lower than 7.5, the highest AUDPC observed in the resistance control. The genetic parameters allowed selection of 168 anthracnose resistant plants, which include positive values of additive genetic effects for IP and LP and negative values of genetic additive effects for AUDPC. Fifty-five F3:4 plants were equally or more resistant than the highly resistant parent in terms of their observed phenotypes and predicted genetic values.

**DISCUSSION & CONCLUSION** Highly promising plants were identified based on their predictive genetic value for anthracnose resistance components. A total of 168 plants with resistance against anthracnose disease were selected for continuation in the pepper breeding program.

## REFERENCES

- [1] Bento et al., 2017, Genetics and molecular research, 16(3). Doi: 10.4238/gmr16039789
- [2] Resende, MD, 2016, Crop Breed. Appl. Biotechnol., 16(4). Doi: 10.1590/1984-70332016v16n4a49

Table 1: Genetic parameters for variables of response to anthracnose in F <sub>3:4</sub> progenies and parent lines of <i>Capsicum annum</i> var. <i>annuum</i>										
	$\sigma^2_a$	$\sigma^2_e$	$\sigma^2_p$	$h^2_a$	$h^2_{mp}$	$Ac_{prog}$	$h^2_{ad}$	$CV_g\%$	$CV_e\%$	$CV_t$
IP	0.604	1.288	1.892	0.319	0.824	0.908	0.234	22.54	32.92	0.68
LP	0.907	1.344	2.251	0.403	0.871	0.933	0.337	16.62	20.24	0.82
AUDPC	40.962	33.495	74.457	0.550	0.924	0.962	0.612	50.74	45.89	1.11
<sup>a</sup> Genetic variance among progenies ( $\sigma^2_a$ ), residual variance ( $\sigma^2_e$ ), phenotypic variance ( $\sigma^2_p$ ), narrow sense heritability ( $h^2_a$ ), progeny-mean heritability ( $h^2_{mp}$ ), additive heritability within the progeny ( $h^2_{ad}$ ), accuracy of progeny selection assuming a complete survival ( $Ac_{prog}$ ), individual additive coefficient of genetic variation ( $CV_g$ ), coefficient of residual variation ( $CV_e$ ), and overall average.										
Table 2: Minimum, average and maximum phenotypic values observed for response to <i>C. gloeosporioides</i> infection in the control parents and F <sub>3:4</sub> progenies.										
	F <sub>3:4</sub> Progenies			UENF 2285 - Susceptible			UENF 1381 - Resistant			
	AVE	MIN	MAX	AVE	MIN	MAX	AVE	MIN	MAX	
IP	3.45	1.00	8.00	2.59	2.00	3.00	5.11	3.00	8.00	
LP	5.73	2.00	8.00	3.40	3.00	4.00	7.23	6.00	8.00	
AUDPC	12.61	0.00	40.00	25.79	20.50	30.50	3.45	0.00	7.50	

# Development of advanced backcrosses of eggplant with three wild species for obtaining introgression lines sets

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**BACKGROUND** Eggplant (*Solanum melongena* L.) is related to many wild species growing in highly stressful environments. In consequence, introgression breeding could contribute to eggplant breeding, as well as to broaden the genetic base of this crop. Therefore, in 2008 we started the development of a set of introgression lines with *S. incanum* [1]. Subsequently, a programme for hybridization and introgression with wild species from the primary, secondary and tertiary genepool was initiated in 2013. Here we describe the process and status of development of three new sets of introgression lines (ILs).

**MATERIALS & METHODS** Three accessions from wild species from the primary (*S. insanum* INS1), secondary (*S. dasyphyllum* DAS1), and tertiary (*S. elaeagnifolium* ELE2) genepools were selected as donor parents for the development of three sets of introgression lines with three eggplant accessions (MEL5, MEL1 and MEL3, respectively). Interspecific hybridizations were performed and embryo rescue was used for the hybrid with *S. elaeagnifolium*. The three interspecific hybrids were backcrossed to the recurrent parents to obtain the BC1 generation. For the BC1 with INS1 a Sequenom platform with 40 markers was used for selection, while for the BC1 with DAS1 and ELE2 no genotyping was performed. The subsequent BC2 and BC3 generations for the three ILs programmes were genotyped with the 5k Single Primer Enrichment Technology (SPET) platform developed in the G2P-SOL project [2].

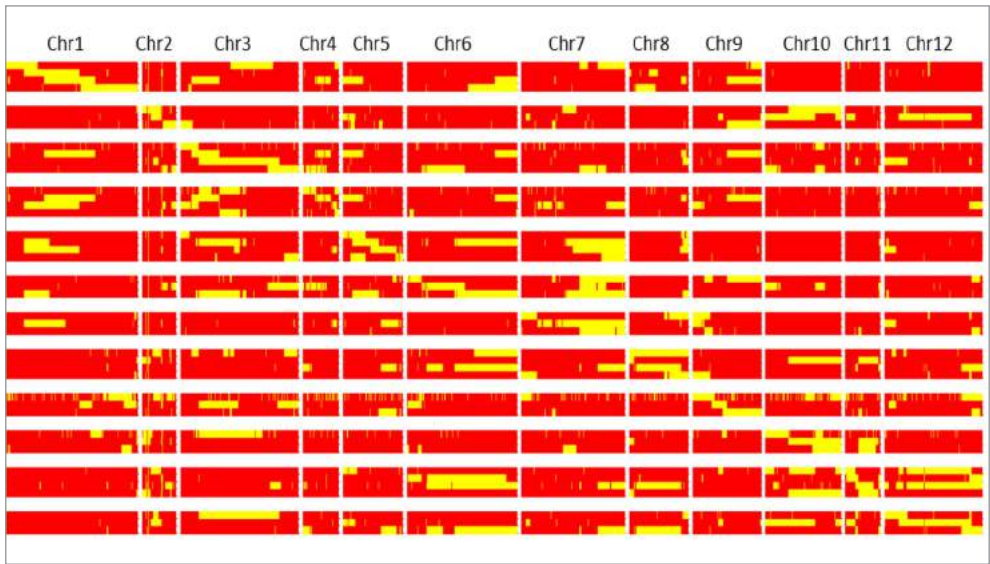
**RESULTS** Fruit set and the number of seeds/fruit were higher with *S. insanum*, intermediate with *S. dasyphyllum*, and lower with *S. elaeagnifolium*. With the latter species, viable hybrids were obtained via embryo rescue. A total of 181 plants of the BC1 with *S. insanum* were genotyped and 30 of them were selected for obtaining the BC2 generation. For the BC1 with *S. dasyphyllum* and *S. elaeagnifolium*, given the lower number of available plants, no genotyping was performed and 20 plants of each ILs programme were used for obtaining the BC2. Several individuals (typically between 2 and 5) for each of the BC2 progenies (between 16 and 28) were genotyped with the 5K probes SPET platform (Figure 1). A selection based on the genotype was performed in the BC2 and a set of selected plants were used to obtain BC3 generations. Following a similar scheme, the BC3 plants of the *S. insanum* and *S. dasyphyllum* programmes were genotyped with the same SPET platform and a selection of plants made for obtaining the BC4. Most of the genome of the donor species is conserved in the three ILs programmes.

**DISCUSSION & CONCLUSION** Advanced backcross generations in which most of the donor parent genome is represented have been obtained with three eggplant wild relatives. The use of the SPET genotyping platform [2] greatly facilitated marker-assisted selection of plants in the BC2 and subsequent generations. The materials obtained will allow the development of sets of ILs with the three wild species, which will be of great interest for eggplant breeding.

## REFERENCES

- [1] Gramazio P et al., 2017. Front. Plant Sci., 8: 1477.
- [2] Barchi et al., 2019. This same volume.





**Figure 1.** SPET genotyping profile for the plants selected (in rows) of the BC3 generation between *S. insanum* INS1 (donor parent) and *S. melongena* MEL5 (recurrent parent). Red cells represent the recurrent allele, while yellow cells are heterozygotes containing introgressions from the *S. insanum* donor parent. Each of the 12 vertical blocks represents one chromosome.



# Introgression breeding in eggplant from *S. tomentosum* and genetic mapping of novel key agronomical traits

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**BACKGROUND** Fruit shape and colour as well as plant architecture and resistance to diseases are main target traits in eggplant breeding programs. *Solanum tomentosum* L. is a wild relative of eggplant representing a valuable source for key breeding traits, like resistances to pathogens, plant/fruit features, and fruit biochemical composition. Its short internodes and red colored ripe fruits, as well as its high glycoalkaloids content at every fruit developmental stage and its resistance to several pests make this species suitable to be employed in breeding programs aimed at the genetic improvement of eggplant for agronomic performance, fruit quality, nutraceutical value or ornamental purposes. Final aim of the project is to develop a population of introgressed lines (IL) each presenting a unique fragment from the wild species inserted at homozygous level into the genome of eggplant line 67/3, representing a living library of the *S. tomentosum* genome introgressed into the genetic background of *S. melongena*. The IL population will be usefully exploited for both basic and applied researches.

**MATERIALS & METHODS** At every cycle of backcross, molecular analysis with 140 markers (CAPS, SSR and HRM-SNP) of known position into the intraspecific 305E40 x 67/3 eggplant map [1] was performed to identify in each BC plant the presence and chromosomal position of introgressed fragments from the allied species. Phenotypical analysis was performed in open field to evaluate fruit pigmentation, plant height and internode length traits. Resistance to fungi and nematodes was assessed by evaluation of plant symptoms and counting number of eggs/galls/larvae in replicate plants submitted to artificial inoculation and compared with resistant/susceptible control lines. Statistical analysis was performed with Jump and R software's.

**RESULTS** From the intraspecific cross between eggplant 67/3 x *S. tomentosum*, F1 plants were obtained and backcrossed with 67/3. At every cycle, the plants carrying the introgressed fragment of interest were crossed with the recurrent parent for cleaning the genome from residual undesired fragments. The IL population is currently composed by nearly 90 BC plants, covering 97% of the wild genome. Seventy-two plants from selfing of BC4 plants showing a different flesh (green vs white) fruit pigmentation with respect to eggplant 67/3 were field evaluated and genotyped leading to the discovery of a region spanning 17 cM on ch8 involved in the control of the trait, and two candidate markers associated. Three hundred and thirty-eight plants from 13 ILs selected for dwarf phenotype were field evaluated and genotyped. The progenies segregate for plant height, and the most severe phenotypes were observed in plants from selfed progenies having the associated marker at the homozygous level. Combining both phenotypical and molecular analysis led to the identification of a region of 13 cM on Ch3 involved in both plant height and internode length traits. A repositioning of some markers thanks to the availability of the genome sequence of 67/3 [2] allowed a more correct interpretation of data and identification of tightly linked molecular markers which can be exploited for MAS in breeding programs. *Solanum tomentosum*, following artificial inoculations, was resistant to *Fusarium*, highly tolerant to *Verticillium*; resistance to nematodes was confirmed by two independent laboratories.

**DISCUSSION & CONCLUSION** The suitability of this population for disclosing chromosomal regions associated to key breeding traits was evaluated by performing a deep molecular and field phenotypical

characterization of the progenies displaying segregation for two quality traits: fruit flesh color and a reduced plant height due to short internodes. The development of an IL population representing the entire genome of the wild species will allow the elucidation of the genetic basis of many other key breeding traits like resistance to fungi and nematodes or biochemical composition thus facilitating their employment in breeding programs aimed at the genetic improvement of cultivated eggplant.

## REFERENCES

- [1] Barchi L et al., 2012, Plos One, 7(8) E43740 doi:10.1371/Journal.Pone.0043740
- [2] Rotino GL et al., 2014, Proceedings of the Plant and Animal Genome XXIIInd Conference, 10-15 January PAG San Diego, California, USA



**Figure 1.** Segregating plants displaying different plant architecture and height in field: from the left: Wt phenotype (>50cm); intermediate phenotype (between 20 and 50 cm), and dwarf phenotype (below 20 cm). Right: detail of internode length in a Wt plant (4-5 cm each).

# Genetic and environmental variability in aubergine roots: traits for future breeding and stress adaptation?

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**BACKGROUND** Aubergine (*Solanum melongena* L.) is cultivated in various climates, from wet to dry conditions in tropical and temperate regions, mostly in Asia, Africa and the Mediterranean basin. The use of wild relatives in aubergine breeding has been hampered by poor knowledge of these species, limited availability in genebanks and the difficulty of obtaining fertile progeny from interspecific crosses. The characterization of genetic resources is key to future breeding programmes: phenotyping has focused on fruit characteristics although it should also include the root system because of its essential role in nutrition and tolerance to biotic and abiotic stresses. We present a study on phenotyping previously described root traits [1] and fresh and dry weight traits for 25 accessions representative of 9 *Solanum* species, including 16 accessions of *S. melongena*, 3 of the African cultivated eggplants *S. aethiopicum* and *S. macrocarpon*, and 6 of wild relatives (*S. incanum*, *S. insanum*, *S. linnaeanum*, *S. sisymbriifolium*, *S. torvum* and *S. viarum*) at two different sites in South-East France.

**MATERIALS & METHODS** Aubergine seeds were sown in April or May 2018 in a mixture of sieved compost and vermiculite (each 50% v/v) in 1 m high PVC tubes, 10 cm diameter. Four plants per accession were retained at each of the two experimental sites. One plant was kept per tube and roots and aerial parts were harvested after 4-5 weeks growth once the longest root had reached the bottom of the tube. Roots were carefully washed and samples taken for scanning of the largest roots (roots at the bottom of the tube) and fine roots (near to the crown). Root traits included: root depth and root growth rate, maximum and minimum diameter; ratio of lateral root diameter to maximum diameter; and root inter-lateral distance.

**RESULTS** We focused on three factors: (i) the intra- and inter-specific genetic variability (ii) trait stability between the two sites and which accessions showed the most trait stability and (iii) the correlations between root traits and biomass.

(i) The two root traits that showed the largest variability between accessions were maximum diameter and inter-lateral distance. Interestingly, the variability within the *S. melongena* group for these two traits was almost as large as the total variability when the other species were included.

(ii) Most of the traits were significantly affected by the experimental site except for total root length (fixed by the experimental method), root maximum diameter and shoot dry weight per day. Furthermore for most traits there was an interaction between genotype and site. The root inter-lateral distance (ILD) trait is strongly affected both by the site and by a genotype x site interaction. For this trait, it is notable that *S. linnaeanum* and *S. macrocarpon* accessions had stable ILD values at the two sites.

(iii) Our data indicate that maximum root diameter and the ratio of daughter to mother root diameter were not correlated with any other trait whereas for ILD, correlations were found with several traits including root fresh weight.

**DISCUSSION & CONCLUSION** The phenotyping of root traits is an important part of characterising genetic resource collections. The quantification of the genotypic and environmental contribution to individual root traits is also necessary to understand how the environment influences plant growth and resource allocation. We have confirmed that root maximum diameter is a stable trait under genetic control

and that root inter-lateral distance is a much more plastic trait affected by the environment [2]; its correlation with general allocation traits is to be confirmed in a wider study with a larger number of accessions.

#### **REFERENCES**

- [1] Pages L. 2014. *Annals of botany* 114, 591-598.
- [2] Pages L, Kervella J. 2018. *Annals of botany* 122, 107-115.

# Inheritance of resistance to chili anthracnose in two introgression populations of hot pepper

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**BACKGROUND** Chili pepper (*Capsicum* spp.) is an important horticultural crop and is consumed daily by approximately 25% of people worldwide. Chili anthracnose (caused by members of the genus *Colletotrichum*), is one of the most severe pepper diseases and can result in a yield reduction of over 50%. Management approaches for chili anthracnose include sowing certified seed, practicing crop rotation, minimizing fruit wounds by controlling insects, applying fungicides that contain mancozeb or copper, and planting resistant cultivars. The use of resistant cultivars is considered to be the most environmentally-friendly and economic strategy to reduce the losses associated with chili anthracnose.

**MATERIALS & METHODS** In this project, resistance in two F2 introgression populations was evaluated to *C. acutatum* isolate Coll-524 at two stages (green mature and red mature fruits) using the high-pressure spray inoculation technique. Resistance in the first population (2504: 1219-906 × AVPP9905) was derived from an introgression line 1219-906 (BC2F4) from the *C. baccatum* line PBC 80, while resistance in the second population (2501: AVPP0022 × 1219-906) was derived from two introgression lines, one (AVPP0022) derived *C. chinense* line PBC 932 and other 1219-906 derived from PBC 80.

**RESULTS** We found that the 2501 population had higher levels of resistance against chili anthracnose than the 2504 population at both green and red fruit stages. This was expected because 2501 population was derived from two resistant parents possessing resistance from two *Capsicum* species. The segregation ratio for anthracnose resistance in 2504 population did not significantly deviate from a 1:3 resistant to susceptible ratio at both green and red fruit stage, indicating the resistance at both stages of this population was inherited as monogenic recessive. However, segregation in the 2501 population did not significantly deviate from a 7:1 resistant to susceptible ratio at green fruit stage and a 3:1 resistant to susceptible ratio at red fruit stage, indicating the interaction between resistance genes from two resistance resources could have different mechanisms at different fruit stages. A segregation ratio of 7:1 is typically observed in polyploid organisms or in the presence of duplicate genes in diploid individuals. Our observation in diploid *Capsicum* in this study could be due to the presence of resistance genes in the introgression lines from two donor species. However, a comprehensive molecular marker analysis would be needed to confirm the presence of two resistance genes from two different species.

**DISCUSSION & CONCLUSION** This study suggests that pyramiding resistance genes from different species of *Capsicum* might be an effective strategy to develop cultivars with more durable resistance to anthracnose.



# Genetic potential of chili peppers for use as ornamental plants

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**BACKGROUND** The cultivation of *Capsicum* genus peppers in Brazil has been growing and developing in recent decades. Over the years the species of this genus has extended beyond its use in cooking and in the pharmaceutical industry. Due to the great genetic variability, the chili peppers have great potential of commercialization in the ornamental sector. The ornamental market of chili peppers aims at smaller species that provide a harmony of architecture and beauty in smaller vases used indoors [1]. Among the most attractive characteristics of chili peppers for the ornamental sector are the diversity of colors and shapes of their fruits, which, together with the height and diameter of the plant and the position of the fruits, are criteria used by consumers at the time of purchase. The objective of this work was to evaluate accessions of *Capsicum* spp. belonging to the germplasm bank of the Universidade Federal do Espírito Santo regarding ornamental potential, aiming at a new market alternative for the ornamental flowers producers of the region.

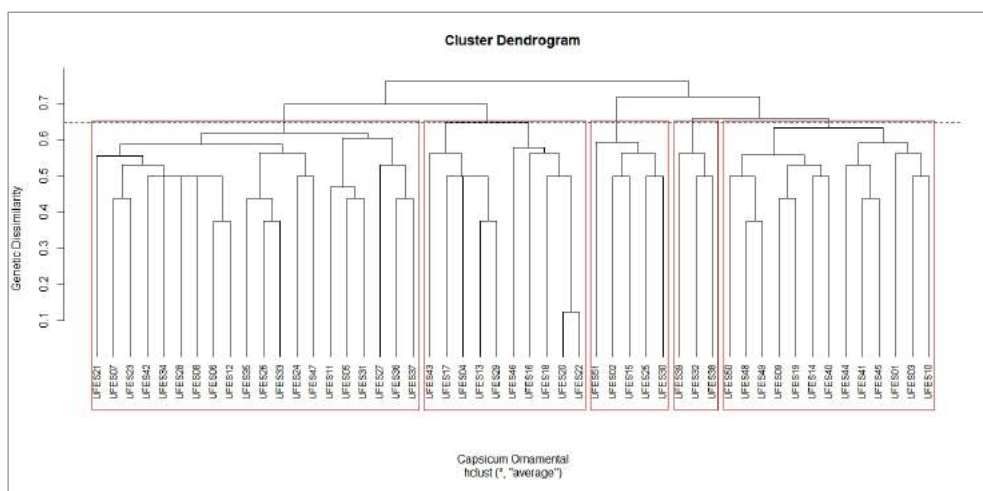
**MATERIALS & METHODS** Fifty-one accessions of *Capsicum* spp. were evaluated. The statistical design used was completely randomized, with three replications, in greenhouse. Four different qualitative characteristics (plant growth habit, stem color, flower and fruit position) and four quantitative characteristics (days for flowering and fruiting, height and plant diameter) were evaluated, as descriptors for *Capsicum* L. (Bioversity International). Genetic dissimilarity was estimated by the Gower method and for the dendrogram formation, the UPGMA method was used. To obtain the optimal number of groups formed in the dendrogram the Mojena Method was used. For all analyses the software R was used.

**RESULTS** Considering the obtained dendrogram (Figure 1) it was possible to identify five distinct groups with the accessions evaluated. The first group consisted of 20 accessions that were grouped by having similar characteristics as: the habit of growing intermediate to erect. All genotypes of this group have the green stem color and smaller days for flowering (137 days). Group II was composed of 10 accessions that were distinguished by having the highest value for fruiting days (202 days) and prostrate and intermediate growth habit. Group III was constituted by five accessions that have green stem color with purple stripes. Group IV consisted of three accessions, whose growth habit was intermediate and the position of the fruit pendant. The last group V was formed by 13 accessions, that had the smallest crown diameter (16 cm). All accessions regardless of the formation of the groups did not vary greatly in height, being in the range of 49 cm in height.

**DISCUSSION & CONCLUSION** Genotypes with intermediate growth habit, such as those of group III, have a differentiated architecture, being more harmonious in pots. The market of potted peppers also seeks cultivars with diameters and smaller heights [2], providing a more attractive aesthetic look to the consumer. In this way, of the 51 accessions considered, those of group V are the most promising for the ornamental market, since their plants are smaller in diameter and height. These accessions can be used as a new income alternative for flower producers in the southern region of the Espírito Santo state and in breeding programs for *Capsicum*.

## REFERENCES

- [1] Silva CQ et al., 2017, Hort. Brasileira, 35: 349-357, doi:10.1590/S0102-053620170306
- [2] Luz PB et al., 2018, Ornamental Hort., 24(1):7-11, doi:10.14295/oh.v24i1.1109



**Figure 1.** Genetic diversity among the 51 accessions of *Capsicum* spp. by means of the UPGMA dendrogram.



# Genotyping *Capsicum chinense* accessions using PCR-based molecular markers

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**BACKGROUND** Pepper fruit (*Capsicum* spp.) has a great importance in the world due to its high versatility and wide application in the industry, cooking and for decoration. *Capsicum chinense* species stands out with a wide diversity of sizes, colors, shapes, and levels of pungency, and is the most Brazilian chili pepper as the Amazon Basin is its center of domestication. Characterization of its diversity is essential for the success of the species conservation and breeding programs [1]. This work aims to genotype 55 accessions of *Capsicum chinense* of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) germplasm bank, using dominant (ISSR – Inter Simple Sequence Repeat) and codominant (SSR – Simple Sequence Repeats) molecular markers.

**MATERIALS & METHODS** For each *C. chinense* accession, leaves of five replicates were pooled and their DNA was extracted following the CTAB method. 35 ISSR primers and 47 SSR pairs developed for *C. annuum* were amplified by PCR and fragments were separated on agarose gel. For the SSR markers, estimates of the mean number of alleles per polymorphic locus, expected heterozygosity, observed heterozygosity and polymorphic information content were obtained using the Genes and GenAlex softwares. Bayesian model-based cluster analysis was performed to determine the optimal number of genetic clusters for the two sets of markers using the Evanno method for Structure 2.3.4 software.

**RESULTS** Among the 35 ISSR primers tested, 18 amplified and 15 detected polymorphisms in the accessions. It was verified the presence of a total of 97 amplified bands, being 46 polymorphic. No duplicates accessions were observed in the samples. Two groups were found by Bayesian clustering, with the group I composed of 50 accessions and group II composed of five accessions. It was not possible to discriminate the accessions according to their geographic origin but was possible distinguished them according to their genetic variability. Among the 47 pairs of SSR primers tested, 17 were amplified and nine were considered polymorphic, equivalent to a transferability rate of 36.17%. The cross-transferability of these markers indicate that there is a high level of sequence conservation within the species of the genus. The polymorphic information content (PIC) ranged from zero to 0.375. For the analyzed loci, the observed PIC varied between low and medium [2]. Using Bayesian clustering, the genotypes were divided in two groups. Group I had 41 accessions, most of which had a growth habit of the erect type and group II had 14 accessions, predominantly red when matures and pungent.

**DISCUSSION & CONCLUSION** There were neither duplicates between accessions when using the molecular markers nor correlation between genotype and place of acquisition. Although only 1/3 of the collection of *C. chinense* of the UENF had been characterized, a significant diversity was observed among the accessions of the same species with the potential to integrate several programs of genetic improvement of the genus *Capsicum*.

## REFERENCES

- [1] Moreira AFP et al., 2018, *Scientia Horticulturae*, 236, 1-9, doi: 10.1016/j.scienta.2018.03.012
- [2] Xie WG et al., 2010, *Biochemical Systematics and Ecology*, 38, 740–749, doi: 10.1016/j.bse.2010.06.009
- [3] Leite PSS et al., 2016, *Genetics and Molecular Research*, 15, doi: 10.4238/gmr.15048482

## ACKNOWLEDGEMENTS

Paola Alvares Bianchi thanks the Agropolis Fondation for covering the registration fees for the 17th EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.

# Postharvest fruit changes in kapa pepper genotypes

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**BACKGROUND** Pepper (*Capsicum annuum* L.) is a very popular vegetable in Serbia and other parts of Southeast Europe. Compared to other vegetable species (apart from potato) pepper has the first rank in Serbia with 17,386 ha of total vegetable growing area in 2017 [1]. According to the questionnaire which included over 400 people (Danojević, unpublished data), the preferred pepper type in Serbia is kapa. Kapa fruits are used for very diverse purposes in Serbian cuisine. Pepper fruits lose a lot of water after harvest, especially when stored at room temperature. Postharvest storage of sweet pepper fruits at low temperatures prolongs the storage of fruits, but also could impair their physical composition. Thus our aim was to determine the response of eight different kapa genotypes from the collection of the Institute of Field and Vegetable Crops in Novi Sad (Serbia) to postharvest storage during a period of 25 days at 8°C and relative humidity 90%. The results of this evaluation will be used for breeding purposes.

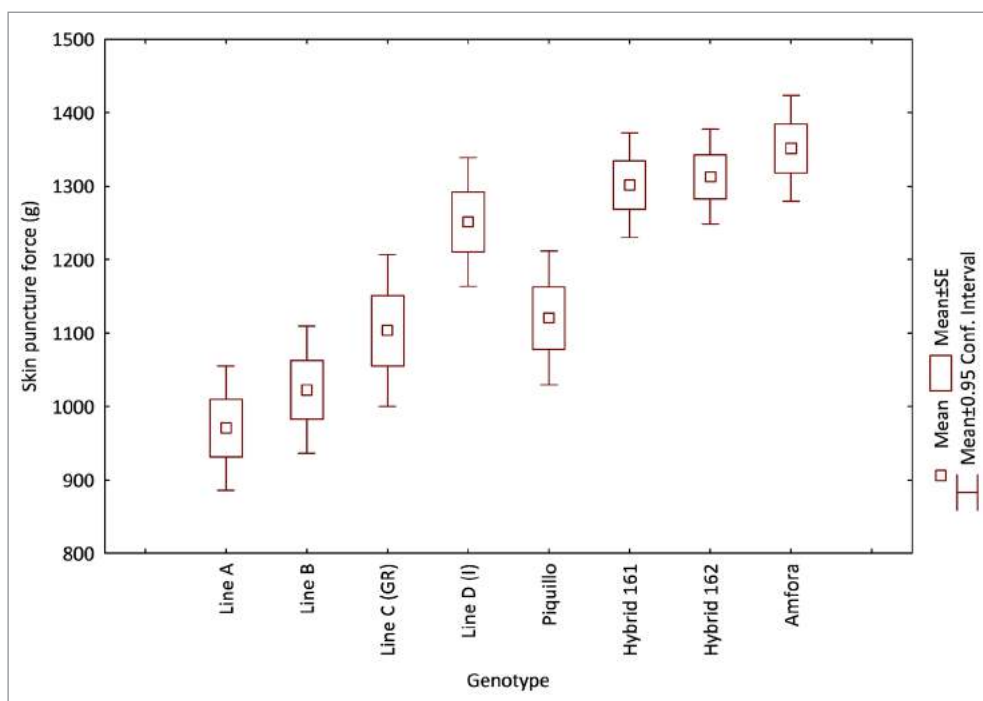
**MATERIALS & METHODS** The experiment was established in a randomized block design in 2015. There were 3 replications (rows) per genotype and 20 plants per row. Plants were transplanted into the open field at the end of May. The density of plants was 70 × 25 cm. Ten fruits per replicate were harvested in October at the technological maturity. Texture properties of freshly harvested fruits were measured at 25 days postharvest by penetration using a Texture Analyser. Colour of pepper fruits was determined with a Chroma Meter. Four fruits were chosen for storage, and used for evaluation of water loss. Colour and fruit weight were measured after harvest, then 14, 21 and 25 days postharvest.

**RESULTS** The highest skin puncture force was measured in Amfora, Hybrid 161, 162, and Line D, while the lowest puncture force was registered in Lines A and B. According to colour measurements, the highest lightness was noted in Line B in each post-harvest period. The lightness significantly increased in Lines A, B, and Piquillo from the beginning to the end of the trial. There were no significant differences in parameter a (-greenness to + redness) after harvest, while at the end of the trial (25 days postharvest) the highest increase was noted in Hybrids 161, 162 and Piquillo. Fourteen days postharvest, there were no significant differences in fruit weight loss among the evaluated genotypes, but 25 days postharvest significant differences were noted. Fruit water loss was ranged from 8.6–19.9% 25 days postharvest. The lowest fruit water loss was noted in fruits of Lines D, C, and Amfora (8.6%, 10.1%, and 10.3%, respectively). Hybrids 162 and 161 had the highest fruit water loss (19.9% and 18.9%) 25 days postharvest.

**DISCUSSION & CONCLUSION** Despite the relatively small number of tested genotypes, they differed in all the evaluated traits. According to our results, variety Amfora could be a good parent line because of high skin puncture force. Although Piquillo has very tough fruits [2], in our case this variety had a lower skin puncture force than four of the evaluated genotypes (Figure 1). High skin puncture force in some cases (Hybrid 161 and 162) did not indicate low fruit water loss. The Piquillo variety could be a good hybrid parent for a fast colour change, because of its ability for changing colour from greenness to redness.

## REFERENCES

- [1] Statistical Office of the Republic of Serbia, 2018, Database. RZS, Belgrade, <http://data.stat.gov.rs/Home/Result/130102?languageCode=sr-Cyrl>
- [2] Eggink PM et al., 2012, Food Chem, 132: 301–310



**Figure I.** Skin puncture force of evaluated kapya genotypes 25 days postharvest.

# Breeding of blocky type pepper varieties adapted for extended production periods in passive greenhouses and net-houses in mild winter regions

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**BACKGROUND** The production areas of pepper in passive greenhouses and net houses in regions with mild winter conditions are expanding very rapidly. The availability of rather simple plastic-covered structures facilitates protected pepper production during the winter. A typical crop is planted at the end of the summer and ripe fruits are harvested during the fall, winter and spring seasons. Paradoxically, the environmental conditions inside such structures are extreme. During the beginning and the end of the production season, the plants are exposed to high temperatures, up to 45°C. During the winter, minimum temperatures range from 4 to 10°C. The minimum night temperature for proper fruit set of blocky pepper was initially thought to be 18°C. The second limiting factor is vine storage which allows extending the harvesting period, thus improving significantly the income. Vine storage is terminated by either fruit softening, or fruit cracking. Objectives: to breed under low temperatures varieties with high-quality fruit set and firm fruit resistant to cracking.

**MATERIALS & METHODS** Wide genetic variation was repeatedly selected under the target conditions in the Arava Valley in Southern Israel and in Almeria, Spain. The genetic variation was obtained by testing diverse introductions and the progeny of many crosses that yielded segregating populations. Repeated selection against this disorder, for firmness and extended vine storage, was made for both parental lines and their hybrids in the Arava Valley and Almeria. Genotype by environmental interactions hindered selection and the data obtained in the diverse locations were taken into account.

**RESULTS** Selection led to the development of germplasm that set normally shaped blocky fruits under 8-10°C conditions. It also had extended vine storage when firmness on the vine was combined with resistance to cracking. Overcoming the genetic correlation between these two traits proved difficult but achievable. During the last 30 years, many blocky hybrids were obtained and commercialized. The hybrids that were game changers are listed hereby. In 2000 the variety "Cannon" (FAR158) was introduced in the Arava Valley. In 2001 "Melchor" (FAR181) which has similar characteristics was introduced in Almeria. These two varieties facilitated expanded harvest period and improved profitability. In the Arava Valley, it contributed to a 4 fold increase in the growing area. In Almeria, it created a very significant late planting segment. These varieties are still the market leaders in their segments. "Cannon" also contributed to the development of passive protected cultivation in Mexico. In 2013 "Merkava" was introduced in Almeria with even wider ecological additivity than "Melchor" and larger and firmer fruits. In 2014 "Tundra" was introduced. A yellow fruited hybrid that combines the traits above. It facilitated the development of the yellow-fruited, in the late planting segment in Almeria.

**DISCUSSION & CONCLUSION** Intensive and focused breeding facilitated the development of blocky type pepper hybrids with wide ecological adaptation. Combined with suitable agro-technological means, in this case, simple structures combined with growing knowhow, it provided farmers with sustainable production systems. This resulted in continuing growth over the last 20 years. This is a good example how breeding can replace expensive growing systems with simple ones, while maintaining the high quality and value of the product.



# Introgression of easy de-stemming to enable mechanical harvest of green pepper (*Capsicum annuum* L.)

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**BACKGROUND** Most peppers are currently hand-harvested, accounting for 20-50% of the total production costs. For processing, the pedicel must be removed (destemmed) prior to slicing, dicing, and/or sauce and salsa production. Mechanical harvesting of peppers is a top priority for California due to unavailability of labor which is threatening its green pepper industry. Co-development of pepper varieties and machines for harvesting red ripe peppers in New Mexico resulted in 80% adoption in 5 years, adding stability and increasing the size of the industry. Similar technology is currently not available for green chiles or blocky peppers. A unique pepper line conferring significantly lower green fruit de-stemming force has been identified which may enable the development of pepper varieties amenable to machine harvest of green fruit, lowering production costs.

**MATERIALS & METHODS** The low de-stemming force semi-domesticated *Capsicum annuum* L. accession UCD-14 was crossed with 'NuMex Garnet' and 'Maor' from which the F3 populations, GUC14 and MUC14 respectively, were generated. Field trials were carried out using a randomized complete block design with 2 or 3 replications. The F3 families were evaluated for straight pull de-stemming force in 2015 and both straight pull and torque force using a Torque Watch Gauge 651 (Honeywell, Columbus, OH) in 2016. Genetic maps were generated from F2:F3 genotype by sequencing markers. Regions co-segregating with de-stemming were detected by quantitative trait loci and genome wide association mapping. Low de-stemming force lines were advanced and evaluated along with commercial lines in 2016 and 2017. In 2018, selected families were evaluated using an Etgar<sup>tm</sup> stripper-header type mechanical harvester.

**RESULTS** The pepper line UCD-14 produced serrano-like fruits that were easily destemmed at the mature green stage. Green fruit de-stemming was observed in the GUC14 and MUC14 F1 individuals and F2 populations derived from this line. A novel method to measure the force of de-stemming using a torque meter was developed and evaluation of F3 families showed that several MUC14 lines had about one half (19-25 Newtons) the torque force as the next best commercial line over 2 years. The relative de-stemming force between lines was stable across environments and years. Lines evaluated using an Etgar stripper-header type mechanical harvester in 2018 performed very well with >50% of peppers destemmed cleanly from the plant with the harvester vs <2% of next best variety tested. In addition, de-stemming force measured with the torque meter was highly correlated with the yield of destemmed peppers using the Etgar harvester. Preliminary analyses of F3 families from both the GUC14 and MUC14 populations have shown the presence of de-stemming fruit was simply inherited, controlled by two loci, each explaining 15-22% of the variance. There were three additional loci associated with low de-stemming force.

**DISCUSSION & CONCLUSION** After 4 years of plant breeding and evaluation, the easy de-stemming trait from a semi-domesticated small fruited pepper was transferred to a jalapeño type pepper, improving machine harvestability. Notably, the harvester used in this study did not have a cleaning mechanism (shaker or fan) that could further improve the percentage of destemmed fruits recovered. Populations derived from UCD-14 have revealed five genomic locations that co-segregate with green fruit de-stemming ability and force. Further genetic and molecular studies may lead to an understanding of the molecular mechanisms underlying the regulation of fruit shedding, a process that plays a pivotal role in crop production.





# Evaluation of an F3 population for resistance to *Phytophthora capsici* in a single seed descent method for breeding a landrace of *Capsicum* pepper from Yeongyang

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**BACKGROUND** 'Subicho' is a land race of pepper (*Capsicum annuum* L.) from Yeongyang, Gyeongbuk province in Korea, characterized by a good mixture of hot and sweet taste, surviving among the commercial hybrid cultivars [1]. We have run a breeding program to introduce resistance to *Phytophthora capsici* and to a viral complex into 'Subicho', and bred a variety named 'Shinsubi' meaning a new Subicho [2, 3]. The new breed still has drawbacks of late maturity and relatively low productivity. 'Shinsubi' was crossed in 2016 to a breeding line (PRsR1), which was bred resistant to *P. capsici* and *Ralstonia solanacearum* and early in maturity, to breed a *Phytophthora-Ralstonia* resistant variety, while maintaining the good taste of the original 'Subicho' and 'Shinsubi'. Single seed descent method (SSD) of breeding was applied to F2 and F3 populations. In the F3 population, the plants were inoculated with *P. capsici* and highly resistant plants were selected for next generation.

**MATERIALS & METHODS** Two seeds each taken from 600 F2 plants of the cross, PRsR1 × Shinsubi, grown during winter 2017 and spring 2018 were composited to make an F3 population. The F3 seeds were sown and grown in 32-cell trays filled with commercial growing mix, 'No. 1 Mix' in summer 2018. For materials included as control, 20 plants each were grown and used in inoculation. The plants at age of 2 months after sowing were inoculated by pouring a sporangial suspension of *P. capsici* at soil-line of the stems. Disease on the base of stem and root systems was scored 3 weeks after inoculation on a scale of 1 (no symptom) to 4 (dead) for stem rot and 1 (no symptom) to 5 (complete root rot) for root rot. Plants free of stem or root rot were selected and planted in growing mix in plastic pots 12 cm in diameter and 8.5 cm high to grow for seed production.

**RESULTS** Only a few plants among nearly 1,200 F3 plants of the cross were infected with *P. capsici* with symptoms ranging from mild root rot to complete root rot to death (Table 1). The rest of them remained disease free. PRsR1 and Shinsubi, the parents of the cross, remained symptomless or only with traces of root rot. Materials bred for being used as rootstocks showed also good resistance. In contrast, Subicho, the original land race, was severely infected and most plants died.

**DISCUSSION & CONCLUSION** Limited infection in the F3 population was as expected because both parents of the cross were breeding lines bred for resistance. Disease-free plants were selected for F4 seed production. A composite of one F4 seed secured from each F3 plant was sown for selection for resistance to *P. capsici* and *Ralstonia solanacearum* in the SSD procedure.

## REFERENCES

- [1] Hwang JM & Chung KM, 1998, J. Korean Soc. Hort. Sci., 39(6):666-669
- [2] Kim BS et al, 1996, J. Korean Soc. Hort. Sci., 37(1):5-11
- [3] Siddique MI et al., 2017, Hort. Sci. Technol., 35(6). doi:10.12972/kjhst.20170080

Sowing No.	Description	Disease score <sup>z</sup>		Remark
		Stem	Root	
18Y601	F3(PRsR1 x Shinsubi)	~1.00	~1.00	Only a few plants infected, dead
18Y602	16G025-7MSx16G025-3	1.00	1.13	Phytophthora-Ralstonia R GMS
18Y606	PRsR1	1.00	1.13	PRsR1
18Y607	Shinsubi	1.00	1.00	Phytophthora resistant Subi
18Y616	YG3	1.00	1.00	Phytophthora-Ralstonia R Rootstock
18Y618	YG4	1.00	1.06	Phytophthora-Ralstonia R Rootstock
18Y619	YG5	1.00	1.00	Phytophthora-Ralstonia R Rootstock
18Y620	YG6	1.00	1.06	Phytophthora-Ralstonia R Rootstock
18Y621	PRsRCMS * YG5	1.00	1.00	Hybrid rootstock
18Y622	PRsRCMS * YG6	1.00	1.00	Hybrid rootstock
18Y623	PRsRGMS * YG5	1.00	1.00	Hybrid rootstock
18Y624	Subicho	4.00	5.00	Land race, the original

**Table 1.** Disease score of F3 population and controls 3 weeks after inoculation with *P. capsici*. Disease score 1=symptomless to 4=dead in stem; 1=root healthy to 5=whole root rot.

# Development of an improved set of introgression lines of *Solanum incanum* in the genetic background of *S. melongena* and identification of QTLs related to traits of interest

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**BACKGROUND** Creation of introgression lines (ILs) populations represent an excellent strategy to introduce natural variation and introgress traits of interest in modern plant breeding pipelines. ILs are generated through a marker-assisted-selection backcross scheme from an F1 between a selected donor parent, almost always an exotic or wild genotype, and a recipient cultivated parent, generally an elite material. In addition to the introduction of new variability in the crop, ILs have demonstrated a higher efficiency in QTLs estimation than other segregating population, such as F2 or double haploid lines [1]. In the case of eggplant (*Solanum melongena*), the first set of ILs consists of 25 fixed ILs covering altogether 61.7% of the donor parent genome, *Solanum incanum* [2]. *S. incanum* is a wild species that grows in desertic and semi-desertic areas and shows a considerable drought tolerance [3]. The analysis of the introgressed materials will be very useful for the genetic dissection of traits of interest and for developing new eggplant varieties with improved traits, including complex ones like drought tolerance. Here, we describe the progress in the development of the ILs population and the identification of QTLs related to the traits of interest.

**MATERIALS & METHODS** In order to develop new ILs with *S. incanum* (accession MM557) using *S. melongena* (accession AN-S-26) as recipient parent, plants selected for their genotype were hand-pollinated and crosses were performed between the recurrent parent and the selected progenies. The newly developed materials were genotyped using different markers and approaches and new ILs with single introgressions were developed. For QTLs identification, a subset of 16 ILs (Figure 1) was used from the first collection [2]. Five plants of each IL were grown in two different environments (tunnel and open field) and phenotyped using 17 morphological descriptors of plant, flower and fruit. ILs data were analysed using an analysis of variance (ANOVA). The mean of each IL in both environments was compared to the recipient parent using the Dunnett's test at  $p < 0.05$ . When the means of the ILs varied significantly compared to the recurrent parent in both environments we considered that a QTL was present.

**RESULTS** From the first set, 20 new fixed ILs were developed reaching a total of 45 ILs, covering altogether 71.7% of the *S. incanum* genome [2]. Among the 16 selected ILs, 10 QTLs were identified, dispersed over seven chromosomes (Table 1). Significant values were found mainly for QTLs corresponding to traits of plant, flower and fruit size. Four lines (SMI\_2.4, SMI\_3.1, SMI\_5.1 and SMI\_8.1) presented QTLs for plant height, stem diameter, stigma length and corolla prickles. Moreover, in five lines (SMI\_1.3, SMI\_2.4, SMI\_4.1, SMI\_8.1 and SMI\_12.6) the values for peduncle length, fruit pedicel length and total average weight were significantly lower than in the recipient parent.

**DISCUSSION & CONCLUSION** This results suggest that the introgression materials of *S. incanum* in the genetic background of *S. melongena* represent a useful source for dissecting the genetics of traits of interest in this crop.

## REFERENCES

- [1] Yin et al., 2016, Crop Systems Biology
- [2] Gramazio et al., 2017, Front. Plant Sci, 8:1477. doi: 10.3389/fpls.2017.01477
- [3] Knapp et al., 2013, PLOS ONE, 8:e57039. doi: 10.1371/journal.pone.0057039



**Figure 1.** Graphical genotypes of the selected ILs for QTL identification. The rows indicate the IL codes and the columns indicate the chromosomes. Homozygous introgressions of *S. incanum* are depicted in red, while the genetic background of the recipient parent is depicted in blue.

Trait	Environment	QTL_Name	Chromosome	Position (Mb)	Increase over the recipient parent (%)
Plant height	Open field	ph8	8	3 - 109	30,90
	Greenhouse	ph8	8	3 - 109	34,26
Stem diameter (5 months after transplanting)	Open field	sd5.2	2	75 - 81	40,83
	Greenhouse	sd5.2	2	75 - 81	25,56
Peduncle length	Open field	pn11	1	27 - 36	-35,77
	Greenhouse	pn11	1	27 - 36	-26,75
Stigma length	Open field	sl8	8	3 - 109	86,88
	Greenhouse	sl8	8	3 - 109	196,40
Corolla prickles	Greenhouse	cp3	3	7 - 86	180,00
	Open field	cp3	3	7 - 86	240,00
	Greenhouse	cp5	5	35 - 43	180,00
	Open field	cp5	5	35 - 43	300,00
Fruit pedicel length	Open field	fpl4	4	4 - 105	-35,87
	Greenhouse	fpl4	4	4 - 105	-34,26
	Open field	fpl8	8	3 - 109	-41,27
	Greenhouse	fpl8	8	3 - 109	-31,37
	Open field	fpl12	12	3 - 96	-38,40
	Greenhouse	fpl12	12	3 - 96	-41,60
Mean fruit weight	Open field	mfw2	2	75 - 81	-39,54
	Greenhouse	mfw2	2	75 - 81	-39,14

**Table 1.** QTLs identified in the ILs of *S. incanum* in the genetic background of *S. melongena*.

# Assessment of reconstitution of the recurrent parent (CMS A-lines) genome by using molecular markers in *Capsicum annuum* L.

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**BACKGROUND** The cytoplasmic male sterility (CMS) system is a cornerstone of F1 hybrid seed production in chilli pepper, and this reduces hybrid seed cost by up to 50%. Recently, we have transferred S-cytoplasm from the Gniffke et al. [1] source into elite breeding lines with diverse genetic background [2]. Backcross breeding is a common method of transferring a target trait and the aim of repeated backcrossing with the recipient parent is to increase the contribution of the recurrent parent genome in the progeny. Hence, the purpose of this study was to ascertain whether the genome of the recurrent parent has been fully recovered after 5 cycles of backcrossing (BC5F1) and selection.

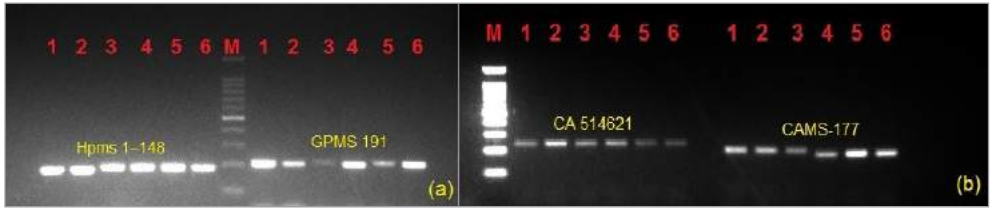
**MATERIALS & METHODS** The genome recovery of the recurrent parent in three selected CMS A-lines, 'CMS4611A', 'CMS4626A' and 'CMS463D13A', and their corresponding CMS B-lines (alloplasmic maintainer) was assessed by SSR marker analysis. For this purpose, 120 SSR markers (10 from each linkage group) covering the whole *Capsicum* genome were screened.

**RESULTS** Out of 120 markers screened, 84 markers (70.0%) were amplified in the 'CMS4611A' and 'CMS4611B' lines, 90 markers (75.0%) in the 'CMS4626A' and 'CMS4626B' lines, and 88 markers (73.3%) in the 'CMD463D13A' and 'CMD463D13B' lines. Almost all the amplified markers showed monomorphic bands between the A- and the B- lines. Between the 'CMS463D13A' and 'CMS463D13B' lines, polymorphic banding patterns were observed for two markers on chromosome 1 (GPMS 191 and CAMS 679), and for one marker on chromosome 2 (CAMS 177) (Figure 1). One marker showed polymorphic bands between the 'CMS4626A' and 'CMS4626B' lines (GPMS 191 on chromosome 1), and one marker (HpmsE 065 on chromosome 10) between the 'CMS4611A' and 'CMS4611B' lines, but the percentage of markers which showed polymorphic bands was negligible (1.2% in the CMS4611A and B-lines, 1.1% in the CMS4626A and B-lines, and 3.4% in the CMD463D13A and B-lines). The probable reasons for polymorphism between the A- and their corresponding B-lines could be attributed to either some linkage drag between lines or to a low number of molecular markers per chromosome. After five cycles of backcrossing (BC5F1) and selection, the genome recovery of the recurrent parent in 'CMS4611A', 'CMS4626A' and 'CMS463D13A' was estimated to be 98.8, 98.9 and 96.6%, respectively, measured by the percentage of the monomorphic marker ratios. Since the CMS lines are maintained by backcrossing with the respective maintainer lines, 100% genome the recurrent parent will be recovered with one or two additional backcrosses.

**DISCUSSION & CONCLUSION** Based on the proportion of amplified monomorphic and polymorphic bands, the genome recovery of the recurrent parent in three selected CMS A- and B-line pairs was more than 96 percent. The lines are, therefore, genetically stable and are ready for utilization in hybrid breeding programs.

## REFERENCES

- [1] Gniffke PA et al., 2009, The World Vegetable Center Publication No. 09-718. Shanhua, Taiwan.
- [2] Meena OP et al., 2018, Breed Sci, 68(4): 404-412. <https://doi.org/10.1270/jsbbs.17150>

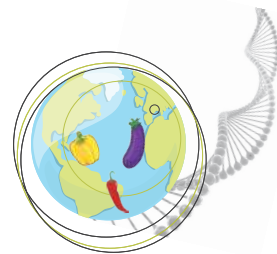


**Figure 1.** A sample of electrophoresis pattern for the recovery of RPG in BC5F1 by using SSR markers where, (a) monomorphic and polymorphic bands between the CMS A- and B- lines on chromosome 01; (b) monomorphic and polymorphic bands between the CMS A- and B- lines on chromosome 02; 1: CMS4611A; 2: CMS4611B; 3: CMS463D13A; 4: CMS463D13B; 5: CMS4626A; 6: CMS4626B; M: 100bp DNA ladder.



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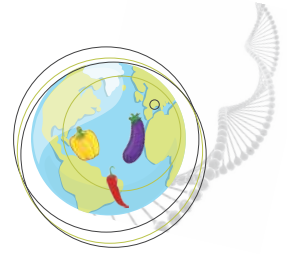
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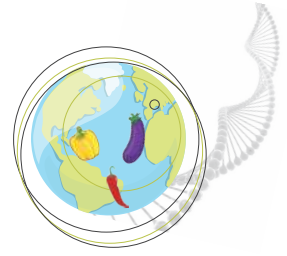
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**ISBN**  
2-7380-1429-1

**EAN**  
9782738014290

**Legal Deposit**  
September 2019



Innovations in Genetics and Breeding of Capsicum and Eggplant

24 €  
ISBN : 2-7380-1429-1

