Soybean intercropped with sunflower is an efficient solution to increase global grain production in low input systems

Laurent Bedoussac, David Champclou, Hélène Tribouillois, Grégory Vericel, Nathalie Landé, Eric E. Justes

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Carte blanche to…

… Vuk Djordjevic

Soybean, the legume queen

"Professor Ho, a historian at the University of Chicago, has aptly written (1955): It is foolish to believe that a certain plant can be introduced into a new area only once, and then only by a certain route. A new plant may score an immediate success in one region and remain neglected in another for a considerable time. Sometimes only through repeated trial and errors can a new plant strike root. Sometimes a new plant may actually be introduced more than once.

Soybean was quite common in Easter Asia for eons, while it reach Europe quite late, in 18 century. Timidly was grown in botanical gardens in Nederland, Paris and UK for curiosity, botanical and taxonomic purpose. First recorded agricultural production was in Dubrovnik, Romania, Czechoslovakia and Austria. And then, almost despair. In the same time, on the other part of the world, several interesting more or less successful stories were recorded about soybean introduction in US. By the late 1850s, soybeans were evaluated for forage potential by many farmers throughout the United States. And the story goes on with several (un) successful introduction of soybean. Today, Argentina and Brazil produce enormous amount of soybeans, mostly for fast growing population in the world. Ironically, one of the rain forests deforestation is consequence of high demand for soybean in the motherland. At the same time, the old countries fear to consume biotech food and aware of deforestation while river Danube crosses their mind with potential for self-sufficient soybean production. The plant Earth becomes too small for this marvelous plant and in the year 2002, soybean goes to the space. It is first-ever complete a major crop growth cycle at the International Space Station, from planting seeds to growing new seeds.

What is the first association when somebody mentions soybeans? For a middle age Asian, it is wide variety of food and beverages, for modern farmer it is profit and environmental effect of production, nutritionist thinks about desirable amino acids and other health promoting compounds, industrialist thinks about processing and product development, trader about buying and selling all that, I think that all those associations reflecting importance of soybeans for us and demonstrate permeation of this plant through our everyday life. This issue tries to present research on soybean around world, from well established US scientists, to the less famous but very interesting stories from all corners of our globe.

Institute of Field and Vegetable Crops, Novi Sad, Serbia (vuk.djordjevic@nsseme.com)

Origin of the word *soy*

by Aleksandar MIKIĆ[1]*, Vuk ĐORĐEVIĆ[1] and Vesna PERIĆ[2]

Abstract: Soya bean (*Glycine max* (L.) Merr.) originated in the Chinese-Japanese centre of origin. Modern Chinese language belongs to the Sino-Tibetan language family that is considered a part of a larger language superfamily called Déne-Caucasian. The Modern Chinese word, sù, exported together with the crop first to Japan and then to Europe and further, has its origin in the Proto-Chinese **shok*, denoting grain or seed. On the most ancient level, the the Proto-Sino-Tibetan root **sŏk* was derived from the Proto-Déne-Caucasian **ṣtHweḳÉ (~ -k-)*, denoting chaff, around 15,000–10,000 years ago.

Key words: *Glycine max*, palaeolinguistics, Proto-Déne-Caucasian language, soya bean

Soya bean (*Glycine max* (L.) Merr.) originated in the Chinese-Japanese centre of origin (6). It is assumed that soya bean was one of the most important crops in eastern Asia long before written records and has remained a major grain legume in China, Japan and Korea. Soya bean had a status of a sacred crop due to its beneficial effects in crop rotation, where it was mostly ploughed under to clear the field of food crops. It was first introduced to Europe in the early 18th century and to British colonies in North America in 1765, where it was first grown for hay. For instance, it is mentioned that it soya bean was cultivated by the Serbian border guards in the present region of the Serbian province of Vojvodina in early 19th century (2). However, soya bean did not become an important crop outside of Asia until about 1910. In USA, soya bean was considered an industrial product only, and was not used as a food before to the 1920s. It was introduced to Africa from China in the late 19th century.

Modern Chinese language belongs to the Sino-Tibetan language family that is considered a part of a larger language superfamily called Déne-Caucasian (Ruhlen). Many see Dené-Caucasian as a group of remains of the older Paleolithic inhabitants of Eurasia that in many cases, such as the speakers of Basque, Caucasian and Burushaski, retreated to isolated pockets difficult to access and therefore easy to defend, remaining surrounded by Nostratic newcomers who bore the Neolithic agricultural revolution when the last Ice Age ended some 11,000 year ago (3). In comparison to Nostratic/Eurasiatic, Dené–Caucasian is supported by weaker and less clear evidence, indicating that the spread of Dené-Daic/Dené–Caucasian occurred before that of Nostratic/Eurasiatic (4). Recent genetic research shows that the Basque people have the most ancestral phylogeny in Europe for the rare mitochondrial subhaplogroup U8a, situating their origin in the Upper Paleolithic and in West Asia, with two expansion periods, with the second one from Central Europe around 15,000–10,000 years ago (1). This could suggest that the starting point of the internal differentiation of the Dené-Daic or at least the Dené-Caucasian macrofamily was in West-Central Asia.

The Modern Chinese word 粟, *sù*, exported together with the crop first to Japan and then to Europe and further, has its origin in the Proto-Chinese **shok*, denoting grain or seed (6). It had rather complex evolution (Fig. 1), but retained its original meaning and, since the importance of soya bean, began to denote it as well. The Proto-Chinese word **shok* owes its origin to the Proto-Sino-Tibetan root **sŏk*, also denoting grain and seed, together with other members of the language family, such as Proto-Kiranti, with *sV̀k-c₁ə̀*, denoting seed, and producing modern words denoting lentil (*Lens culinaris* Medik.) in its modern descendants, such as Limbu, Yamphu and Yulung.

On the most ancient level, the the Proto-Sino-Tibetan root **sŏk* was derived from the Proto-Déne-Caucasian **ṣtHweḳÉ (~ -k-)*, denoting chaff. It is estimated that the Proto-Déne-Caucasian language was spoken around 15,000–10,000 years ago (6). This could suggest that the starting point of the internal differentiation of the Dené-Daic or at least the Dené-Caucasian macrofamily was in West-Central Asia. This Proto-Déne-Caucasian root also gave the Proto-Basque **a-hoc*, denoting husk and chaff of wheat, the Proto-North Caucasian **c̣HweḳĔ (~ -k-)*, denoting straw and chaff, and the Proto-Yenisseian **TVKV*, denoting husk (6).

It may be concluded that the modern Chinese word denoting soya bean, as well as its derived forms in neighbouring Japanese and Korean and most European languages, from times immemorial was connected with grain and seed, subsequently becoming a synonym for the most important pulse in eastern Asia: soya bean. ■

Acknowldgements

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[1] Institute of Field and Vegetable Crops, Novi Sad Serbia (aleksandar.mikic@nsseme.com)
[3] Maize Research Institute Zemun Polje, Belgrade, Serbia

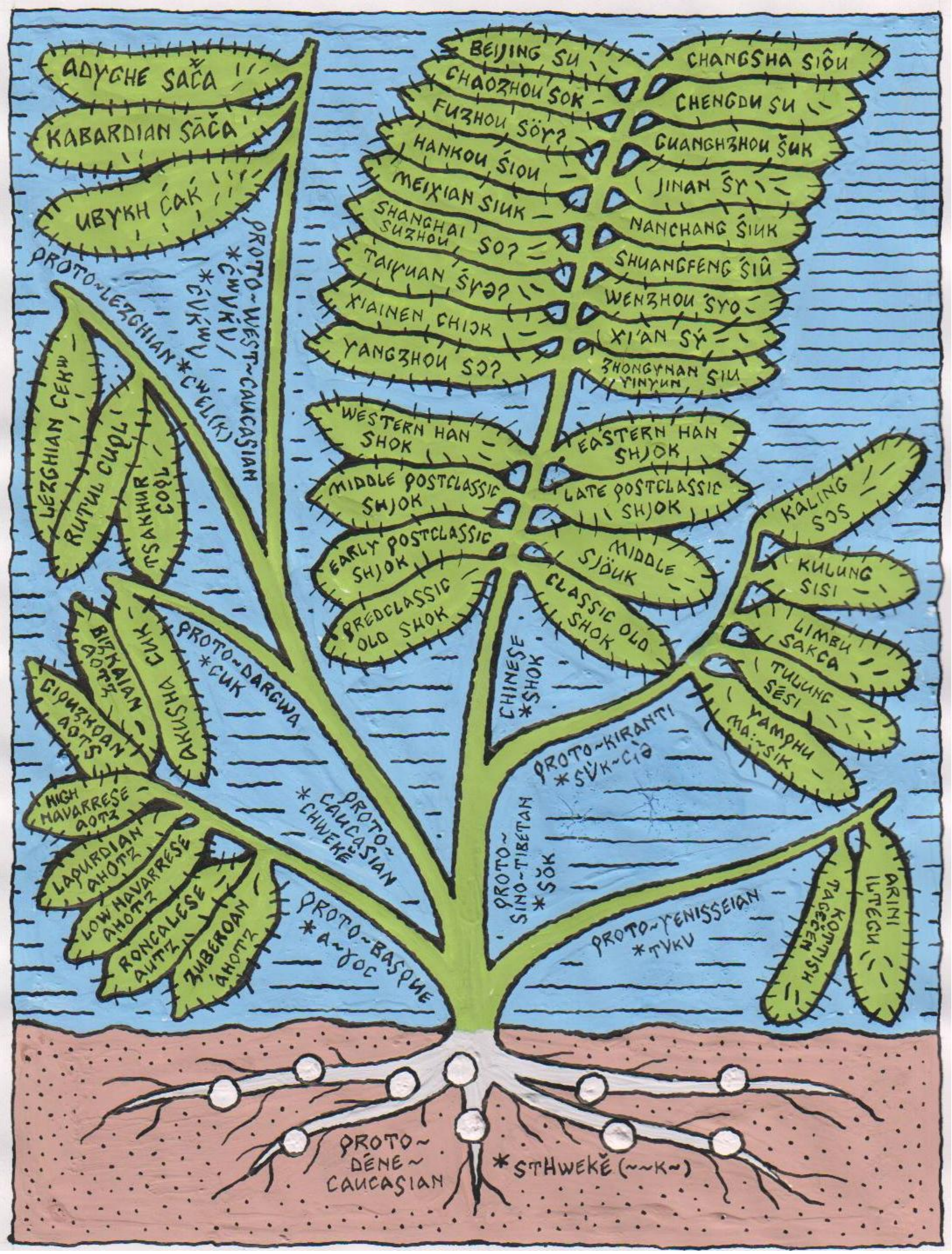

Figure 1. Evolution of the Proto-Déne-Caucasian root into its primary and modern descendants

Soybean genetic resources for the production in the Non-Chernozem zone of the Russian Federation

by Margarita VISHNYAKOVA* and I. SEFEROVA

Abstract: Vavilov Institute houses the greatest in Europe collection of soybean genetic resources.Since N. I. Vavilov's times one of the essential tasks of the Vavilov Institute is broadening the agronomic areas of the crops to the non-chernozem belt. Exploration of early matured soybean gene pool has been carried out in the location northern from the border of modern production area. 1238 accessions classified as very early and early matured originated from 35 countries have been evaluated. This gene pool is characterized by cold tolerance in early stages of ontogenesis, weak response to photoperiod during blooming, and seed yield less dependent on high summer temperatures.

Key words: early maturity, genetic resources, *Glycine max*, non-chernozem soil, soybean

The history of soybean promotion to the north

Soybean (*Glycine max* (L.) Merr.) is traditionally considered as the crop of warm season (7). The first domestication of soybean has been traced to the eastern half of North China between 17th and 11th century BC in the geographic interval 10º - 40º n. l. (5). From there the crop had been distributed during centuries. In the 16th century it had been planted in Manchuria and in the South of Far East region along the bed of Amur river. So, well in advance of the beginning of scientific breeding the crop expanded to the North till the 45°–48° north latitudes. Since the period when scientific breeding of the crop began in different countries mainly in China, Korea, Japan, USA and Russia the distribution of soybean acquired global scale.

Today the area of soybean production represents 100,000,000 ha, locating in the belt between 54-56° n. l. and 40-42 s. l. and we can tell, that the history of soybean is the history of crop adaptation to various environment: day length, temperatures, precipitation, soils etc. It surmises the occurrence in its gene pool the great variability of genotypes of diverse time of maturity, photoperiod response, temperature demand etc.

The existent classifications of soybean by maturity in Russia have 9 groups (10), in USA – 13 (12). The first three groups in both systems (1-3 in Russian and 0-000 in American) include very early and early matured varieties which could be promising to expand their production to the North. It is well known, that both temperature and photoperiod influence soy plant development (2, 6). To advance the crop to the north in short-season production areas the varieties would be tolerant to low temperatures during the early stages, to have weak photoperiod response during flowering (3) and capable of forming seed yield at a relatively low amount of active temperature.

The first varieties able to form seeds in the northern latitudes (58°36') had been bred by Swen Holmberg in Swedish experimental station Fiskeby. The varieties Bravalla, Ugra, Fiskeby formed the relatively high yield fed down the sum of active temperatures (higher 10° C) 1600-1700° C (4).

Vavilov's soybean collection – the source of adopted varieties

Since N. I. Vavilov's times one of the essential tasks of the Vavilov Institute is broadening the agronomic areas of the crops to the North – to the non-chernozem belt, characterized by podzolic, leached, and boggy soils. Common features of this area is a short growing period, a significant number of summer and winter precipitation, a relatively small amount of heat (13).

Vavilov Institute houses the greatest in Europe collection of soybean genetic resources - about 7000 accessions. For ninety years it has been the source for national breeding. The collection contains nearly 2000 accessions originated from various countries which are characterized as early matured in the southern regions of the Russia. In the traditional zone of soybean production they have period of maturation 75-110 days.

All-Russian N. I. Vavilov Institute of Plant Industry, Saint-Petersburg, Russian Federation
(m.vishnyakova@vir.nw.ru)

Soybean in Non-Chernozem zone of Russia

Up to day there are 102 commercial varieties in the National List of registered varieties in Russian Federation and 88 of them are of Russian breeding (10). Before seventies of 20th century soybean production in Russia had been located in southern regions of European part and of Far East not spreading much more to the north as 50-51° n. l. The main producing soybean area in Russia today is still situated in these regions. Nevertheless for the latest 30 years soybean production in European part made step to the north on 300-400 km. Today the northern boundary of experienced and, in part, the commercial cultivation of soybean in the European part of Russia is the central non-chernozem zone (1).

Breeding of early maturated varieties gained special currency and today they represent about a half of the list of varieties approved for the production. Many of them came from Holmberg's varieties and breeding lines. In eightieth of 20th century in Russia in Ryazan region varieties Mageva (1991), Okskaya (1995), Svetlaya (1999) had been bred and recommended to the production in the Central Region of Non-Chernozem zone of Russian Federation Today 10 varieties are approved for production in the Central Region of Non-Chernozem zone of Russian Federation: 8 ones of Russian breeding and two of Belorussian. Two of these varieties – Svetlaya and Pripyat are recommended for the more northern regions (10).

The searching new genotypes adopted to northern regions in Vavilov's collection

With the aim of searching new initial material for breeding very early-maturated varieties adopted to the northern regions of Russian Federation a screening of soybean collection has been done for some years (1998-2008). Exploration of early maturated soybean gene pool has been carried out in the location situated much more to the North from the border of modern agronomic area of the crop. For today it is the northernmost point of soybean management – the North-West of European part of Russian Federation – near Saint-Petersburg (59°44' n. l., 30° 23' e.l.). This environment is extreme for soybean. Average sum of active temperatures required for soybean maturation in this region does not exceed 2000. The typical sum of active temperatures (above 10ºC) during the crop season varied from 1742º C to 1970º C. Day length runs in June up to 18 h 52 min. The optimal dates of sawing have been stated – May 20-25th, when soil temperature is above 6º C. Plants were grown at a small plots with the density – 22 plants/m^2. Seeds were inoculated with *Rhizobium japonicum* active strains bred in All-Russian Institute of Agricultural Microbiology. The date of harvesting with artificial ripening at room temperature – the third decade of September.

Phenology, length of the main stem, number of productive nodes, number of pods, seed productivity and mass of 100 seeds, seed protein and oil content have been characterized. Variability of the traits of seed and dry matter productivity, their dependence on the environment (temperature and humidity), photoperiod response, tolerance to planting density as well as response to bacterium inoculation have been studied to reveal the most productive genotypes.

1238 accessions classified as very early and early maturated originated from 35 countries have been evaluated. From them 224 have been selected as forming well germinated seeds. These accessions originated from 17 countries, and 66 of Russian breeding. They have been divided into groups depending the level of ripening. The most early maturated varieties having yellow and falling foliage at harvest originated from 1) Fiskeby station (lines Fiskeby 345, 1274-26-17-7, 1285-6-4, 1285-53-6, 1289-4-6, 1292-7-8, 1312-13-6) and 2) Moscow breeding enterprises (M-31, M-70, M-134, M-140) (8). The most productive but having a little more prolonged maturity genotypes were: Fiskeby 1040-4-2 and Fiskeby 840-7-3 (Sweden); Mageva, Svetlaya (Russia, Ryazan region) and PEP-27 and PEP-29 (Petersburg's experimental populations) bred in Vavilov Institute. They had an average yield 2.7 t/ha, seed content of protein – 47% and oil – 18% (table) (9).

The accessions adopted to the North-West of Russian Federation have been represented by the commercial varieties and breeding lines but lacked landraces. All early-maturated landraces housing in the collection have been bred in the southern regions and have much more prolonged maturation period when are planted in northern environment. It is noteworthy that all early-maturated varieties are originated mainly from the regions bred soybean varieties with the aim to advance them to the north.

Yield response from inoculating with *Rhizobium japonicum*

Pre-sowing inoculation of seeds by commercial strains of *Rhizobium japonicum* is indispensable processing for soybean production in the North West of Russia. In this environment there is no indigenous symbiotrophic soil microorganisms for soybean able to compete with the commercial strains. That why inoculated plants in our experiment are significantly increased all parameters of productivity compared to unrefined control. The mass of green matter increased by 200-300% or more (66.6 g/plant in control and 231.1 in inoculated plants). This have been determined by greater plant height and foliage, the increased number of branches, productive nodes and pods per plant, weight of seeds per plant and weight of 100 seeds. Seed productivity increased up to 150-300%. The protein content in dry matter increased by 4.8-6.2%, in the seeds to 6.2-7.2%. Variety Mageva (Russia) have been distinguished by all characters studied. The protein content in seeds of this variety, depending on environment and applied strain of *Rhizobium* reached 43.4-49.1% (14).

Conclusion

Taking everything into account it is possible to tell that today a significant gene pool of northern soybean exists. For promotion of soybean agronomic area to the north in Russian Federation the set of adopted accessions is formed selected from the collection of Vavilov Institute. This gene pool is characterized by tolerance to cool temperature in early stages of ontogenesis, weak response to photoperiod during blooming, and seed yield not very much dependent on high summer temperatures. ■

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Table 1. Yield, 100 seed mass, seed protein and oil content of early maturated soybean varieties evaluated in North-West of Russia (Leningrad region 2003-2008)

Variety	Origin	Yield, t/ha	100 seed mass , g	Seed protein, %	Seed oil, %
Fiskeby 1040-4-2	Sweden	2.7	21.1	39,4	18,1
Mageva	Russia, Ryazan' region	2.5	15.9	42,5	16,2
Fiskeby 840-7-3	Sweden	2.5	20.3	37,5	16,7
PEP 28	Russia, Leningrad region	2.3	18.5	40,7	17,1
Svetlaya	Russia, Ryazan' region	2.3	15.5	41,9	17,0
PEP 27	Russia, Leningrad region	2.2	17.8	32,4	18,1
SibNIIK 15/83	Russia, Novosibirsk region	2.1	16.7	44,9	17,1
Altom	Russia, Altay region	1.8	20.4	39,8	16,3
Stepnaya 85	Russia, Kemerovo region	1.8	15.0	43,6	17,0
KG 20	Canada	1.6	14.7	46,6	15,2
SOER 4	Russia, Saratov region	1.4	16.8	42,6	15,3
USHI 6	Russia, Ul'yanovsk region.	1.3	14.7	43,4	15,6

A perspective on soybean genetic resources in relation to vegetable soybean

by Ramakrishnan NAIR and Warwick EASDOWN

Abstract: The immature pods of vegetable soybean usedfor human consumption are popularly known as edamame in Japan, maodou in China or green soybean in North America. On a dry weight basis, vegetable soybean has a protein and oil content similar to grain soybean, but contains more provitamin A, vitamin C, starch and sucrose. AVRDC- The World Vegetable Center's genebank houses 15,316 Glycine accessions and vegetable soybean account for 13% of the Glycine max collection. The breeding program employed selections from local landraces and the transfer of desirable traits from grain soybean. Efforts are in progress to promote its cultivation in South, Central Asia and Africa.

Key words: edamame, genetic resources, *Glycine max*, green soybean, maodou

The immature pods of vegetable soybean are harvested and the shelled green beans are consumed after cooking or steaming (Fig. 1). Sold as a fresh or frozen vegetable, it is popularly known as *edamame* in Japan, *maodou* in China or green soybean in North America. Compared to grain soybean, vegetable soybean seeds are larger (over 30g/100 seeds), have a milder flavor, nuttier texture and are easier to cook. On a dry weight basis, vegetable soybean has a protein and oil content similar to grain soybean, but contains more provitamin A and vitamin C, starch, and sucrose. It also contains health-promoting isoflavones and tocopherol (Shanmugasundaram and Yan, 1999). China, Japan, Taiwan and Thailand are the main producers (Fig. 2), while Japan is the main consumer, importing about half of its annual requirements.

Breeders are interested in traits related to high pod yield, pod size and colour, seed size and colour, seed number per pod, seed appearance, high sugar content and flavour, resistance to downy mildew and pod borer, early maturity, high nodulation, and ease of mechanical harvesting.

AVRDC- The World Vegetable Center began developing improved vegetable soybean lines in 1981. The Center's genebank houses 15,316 *Glycine* accessions and vegetable soybean types account for about 13% of the *Glycine max* collection characterized so far. Over 3000 breeding lines have been distributed to researchers worldwide. The breeding program employed selections from local landraces and the transfer of desirable traits from grain soybean. Breeders also use lines which are less sensitive to photoperiod and temperature to extend adaptability to more tropical zones.

As quality is of paramount importance, breeders tend to cross between parents which may share elite pedigrees. Mimura *et al* (2007) recently studied the genetic diversity of 130 vegetable soybean cultivars and landraces from Japan, China and the US and found that Japanese cultivars had a narrow genetic base compared to those of other countries. Germplasm from China, US, Canada, and Korea could be good sources for broadening the genetic base and disease tolerance of future Japanese varieties.

In order to enhance the taste of vegetable soybean and to broaden its market appeal beyond Japan, breeders have successfully utilized the fragrance genes from Japanese cultivars Dadachamame and Chakaori that confer a 'basmati' flavor to beans. Molecular markers for the fragrance trait have been developed (Juwattanasomran *et al* 2010) which would facilitate the selection for the fragrance trait in breeding programs.

AVRDC- The World Vegetable Center is promoting the cultivation of vegetable soybean world wide. Efforts are in progress to promote vegetable soybean cultivation in South Asia, Central Asia and Africa, and production has recently expanded in India, Bangladesh, Vietnam Mauritius and Sudan. Asian production is not only for domestic consumption but also for export. ■

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Figure 1. Pod and grains of vegetable soybean

Figure 2. A line for the vegetable soybean production

AVRDC-The World Vegetable Center
Regional office for South Asia, Hyderabad, India
(ramakrishnan.nair@worldveg.org)

Accelerated Yield Technology™ in Soybean: Marker assisted selection for simple and complex traits

by Scott A. SEBASTIAN*, Lizhi FENG and Les C. KUHLMAN

Abstract: Marker assisted selection (MAS) strategies known as 'forward selection' have been used effectively in soybean since the mid 1990's to pre-screen breeding populations for simply-inherited traits. Current experiments within multiple populations and across years are being conducted to accurately quantify and optimize the efficiency gains of CSM over pure phenotypic selection. But given the importance of yield and experience to date, we submit that CSM for yield is already both technically feasible and very cost-effective when applied judiciously. Efficiency gains are expected to improve with the ever-decreasing cost of genotyping and further optimization of the process.

Key words: breeding, *Glycine max*, marker assisted selection, soybean, traits

Marker assisted selection (MAS) strategies known as 'forward selection' have been used effectively in soybean since the mid 1990's to pre-screen breeding populations for simply-inherited traits (3). But many complex traits have not been amenable to forward selection because quantitative trait loci (QTL) detected within one genetic context have not been sufficiently predictive of other genetic contexts (1, 5, 6, 9). This has prompted us to investigate a 'Context Specific MAS' (CSM) approach for complex traits (Sebastian et al., 2010). For essential traits such as high grain yield potential, CSM has already demonstrated both technical feasibility and commercial success. The efficiency gains will only improve with experience and with increasingly affordable genome-wide markers. The combination of forward selection for simple traits followed by CSM for grain yield and other complex traits is now a key product development strategy known commercially as Accelerated Yield Technology™ or AYT™. Since simple trait mapping and forward selection techniques are already covered extensively in the literature, this report focuses on CSM for grain yield potential per unit land area - herein referred to as 'yield'.

The impetus for context specific MAS (CSM)

A conventional soybean breeding program can sample up to 20,000 unique inbred lines (e.g. 100 progeny from each of 200 populations) and consume 5 years of yield testing to derive even one new cultivar. This inefficiency is largely a consequence of Type I (false positive) and Type II (false negative) measurement errors – especially during the early years of yield testing when the number of lines is high but replication of each line is low. Even when good populations are sampled, they may not be sampled sufficiently to find the rare transgressive segregants that have commercial potential; and when these rare progeny are sampled, they are often discarded due to Type II errors that can occur at any stage of yield testing. Hence, any MAS strategy that can help to identify genotypes with high yield potential before or during the first year of yield testing would be of great value. This would improve efficiency and genetic gain by allowing breeders to focus the subsequent highly-replicated field trials on fewer selections – i.e. selections that are less likely to be artifacts of measurement error.

Pioneer Hi-Bred International
(scott.sebastian@pioneer.com)

A typical CSM protocol

Plant breeders are well aware of field test designs used to control experimental error and statistical methods used to detect and predict the effects of QTL on trait expression. In fact, the same algorithms that are used for genome-wide prediction across more complex population structures (Meuwissen et al., 2001; Bernardo, 2008; Dudley and Johnson, 2009) can be quite suitable for CSM. However, the critical feature of CSM is to focus the power of genome-wide genetic prediction within a narrow and well-defined genetic by environmental (GxE) context. Reducing the complexity of the GxE context may actually be essential for effective MAS of traits like yield that do not comply with assumptions made for MAS of simpler traits.

With the above considerations, selection within 'true biparental' populations - i.e. recombinant inbred lines (RILs) derived from homozygous and homogeneous parent lines – is an ideal application for CSM. Genetic purity of the parent lines is important because it insures the segregation of only two haplotypes at any given locus. In cases where the genetic purity of the parents is in question, one can achieve the same goal by generating the entire RIL population from a single F1 seed. In addition to simplifying the genetic space for prediction, true biparental populations have inherently high linkage disequilibrium (LD). This increases the effective size of linkage blocks and reduces the number of markers needed for genome-wide coverage. Within a true biparental population, genomic spacing of even one marker per 25 cm region (~100 genome-wide markers in soybean) can be adequate for building a reliable genetic prediction model for yield potential of RILs from said population. This sparse density may seem counter-intuitive to geneticists accustomed to fine-mapping specific gene(s) that define a given QTL. But empirical evidence from internal studies indicates that marker spacing of less than 50 cm quickly results in diminishing returns in terms of the predictive power for yield. This implies that estimating the net effect of large haplotype blocks across the entire genome is more important than determining the exact genomic location and yield effects of individual QTL.

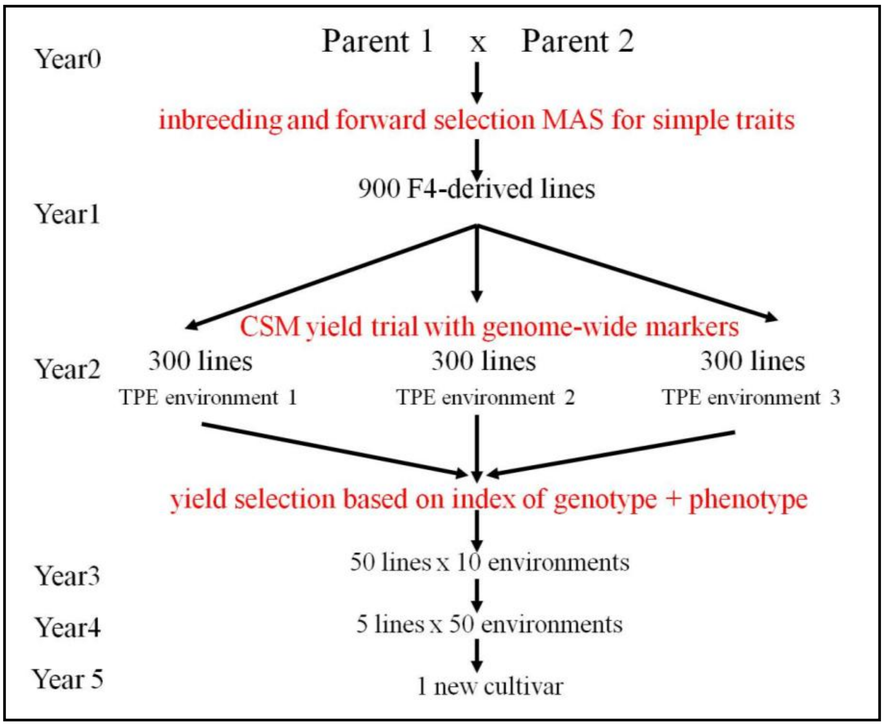

Figure 1. Example of AYT (forward selection + CSM) within one population

Since the specific combining ability of parents is difficult to predict based on the per se performance and/or the general combining ability of parents, it is recommended to focus CSM resources on biparental populations that have already demonstrated evidence of specific combining ability for both yield and key defensive traits. This information is typically available from previous phenotypic trials but often ignored in favor of previously untested populations from the 'latest and greatest' parents. But given the need to focus resources on fewer but larger populations with CSM, choosing populations based on prior empirical data can dramatically improve the odds of commercial success.

A specific example of the resources needed for CSM is shown in Figure 1, but the basic principles can be tailored to accommodate plant breeding programs that vary greatly in scope and budget. 500 to 1000 observations (i.e. RIL progeny) per population during the first year of yield testing are recommended to build a reliable genetic model for a complex trait like yield (2). In addition to adequate progeny numbers, more than one TPE environment should be sampled to increase the odds of detecting QTL that impart broad adaption as opposed to QTL that may be artifacts of any single environment. In the example shown (Figure 1), 900 F4-derived lines where divided across three TPE environments. In this sample, an average of 394 lines (7/16th of 900) are expected to be homozygous for each parental haplotype at each locus; and these progeny will be randomized within and across the sampled TPE environments. This gives great statistical power to determine which haplotypes are associated with higher yield across the entire range of micro and macro environments that are sampled. So, the genetic prediction essentially 'averages out' much of the experimental error and GxE interactions that are confounded with poorly-replicated phenotypic measurements (Figure 2).

The ideal weight of genotype vs. phenotype in the yield selection index is currently being investigated. But this determination requires highly-replicated field trials of different populations across multiple years and environments. As expected, the quality of the genetic prediction increases with better genome coverage, more progeny, and more environments sampled. In practice, the best prediction index is one that is weighted to reflect the relative quality of the genetic and phenotypic components. For example, in the CSM protocol shown in Figure 1, where individual progeny observations are not replicated, the genetic component of the selection index is typically weighted much more heavily than the phenotypic component for best results.

Of course, the predictive power of any genetic model is limited by the quality of the phenotypic data used to generate the model. So, efforts to improve phenotypic data quality will also improve the quality of the genetic prediction model. These efforts can include: 1) irrigation and other agronomic practices that promote maximum expression of yield potential at each environment, 2) experimental designs and statistical methods that are commonly used to correct for spatial trends within each field environment, 3) bordered plots to reduce the effect of plant height, relative maturity, and stand ability differences on adjacent plots, and 4) exclusion of flooded or damaged field plots from the genetic analysis.

Regardless of whether bordered plots are used, visual scores of plant height, relative maturity, and standability of each plot should be recorded before harvest and used as covariates in the genetic prediction process to correct for possible confounding effects on yield. The differential weighting of data from specific environments that are deemed to be more or less predictive of the TPE can also improve the quality of the genetic prediction. Once the genetic model is generated from high quality data, it can also be used to predict the yield of untested but genotyped RILs from the same population and/or tested RILs that were excluded from the genetic analysis due to poor phenotypic data quality.

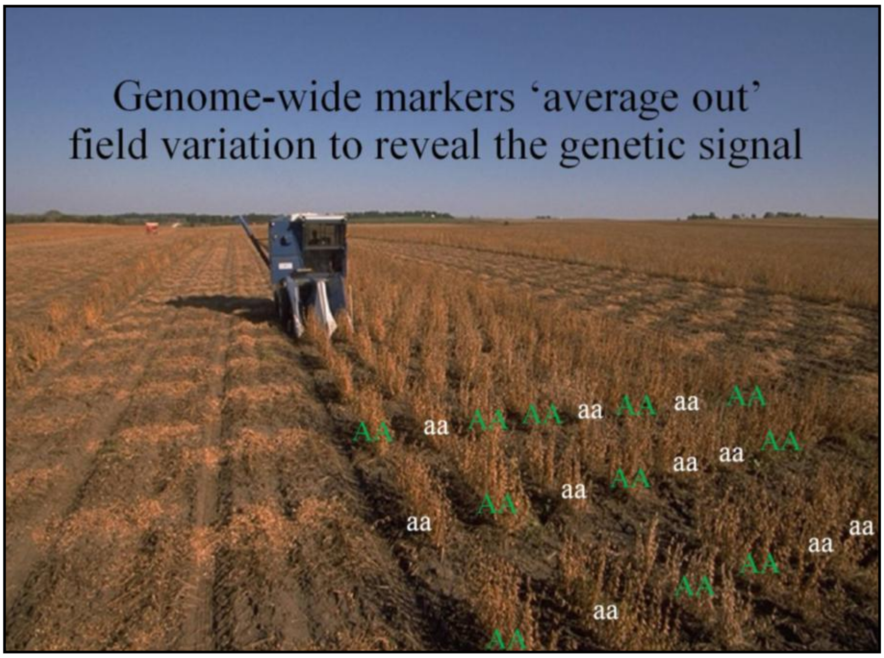

Figure 2. Genetic markers as heritable covariates to reduce experimental error

As expected, results for each breeding population can vary greatly depending on many factors including the combining ability of the parents, the quality of testing environments, genome coverage, and the predictive power of various genetic modeling methods. Current experiments within multiple populations and across years are being conducted to accurately quantify and optimize the efficiency gains of CSM over pure phenotypic selection. But given the importance of yield and experience to date, we submit that CSM for yield is already both technically feasible and very cost-effective when applied judiciously. Efficiency gains are expected to improve with the ever-decreasing cost of genotyping and further optimization of the process. ■

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Hybrid soybean

by Reid G. PALMER[1]*, Allison L. PAPPAS[1] and Evelyn ORTIZ-PEREZ[2]

Abstract: CMS systems that have adequate maintainer genes and restorer genes and that are stable across environments have been identified by several Chinese groups. Adequate levels of heterosis have been reported, but heterotic groups or associations are not known. Soybean can be moved from a highly autogamous species to an allogamous one using insect pollinators, coupled with phenotypic recurrent selection. The various components to commercialize hybrid soybean are being assembled. In addition to the anticipated benefits from heterosis, hybrids are an excellent mechanism to 'stack traits', because many are dominant genetic traits and can come from either the female or the male parent.

Key words: Cytoplasmic male-sterility, *Glycine max*, heterosis, hybrids, soybean

In a list of 40 important events and changes in agriculture in the past 50 years prepared by the North American Agricultural Journalists, hybridization and improvement of crop plants was noted by this organization as the most important change in agriculture (Plant Breeding News Edition 138, 5 May 2003).

Hybrid vigor or heterosis is the superior performance of the heterozygous hybrid. High-parent heterosis is the superior performance of the hybrid over the better parent, while mid-parent heterosis is the superior performance of the hybrid over the mid-parent value of the two parents.

Requirements for soybean hybrids (3).

1. Parental combinations that produce heterosis levels superior to the best pure-line cultivars.
2. A stable male-sterile, female-fertile system(s).
3. A selection system to obtain 100% female (pod parent) plants that set seed normally and can be harvested mechanically.
4. An efficient pollen transfer mechanism from pollen parent to pod parent.
5. An economical level of seed increase for seed companies and growers that ultimately benefits the consumer.

Heterosis

Soybean is an autogamous plant; however, soybean flowers possess most, if not all, of the anatomical characteristics of an entomophilous plant species (1). Heterosis levels above the better parent have been as high as 77%. Care needs to be taken when interpreting heterosis studies. Hybrid yield trials need to be conducted with replicated plots in several environments, preferably in multiple years (3). The parent performance is important. Many reports of very high percentage heterosis come from crossing diverse parents, each with average yield. That is, the starting yield, or base, is low. The best agronomic performance tests also include the highest yielding commercial cultivars as checks.

Stable sterility systems

A number of stable nuclear male-sterile, female-fertile mutant lines are available and have proved valuable in heterosis studies. Cytoplasmic male-sterile (CMS) systems in soybean initially have been reported as unstable; however, in certain genetic backgrounds, male sterility has been stable after vigorous selection (5).

Table 1. Seed-set from fertile-female soybean parents-derived five-way crosses compared in percent relative to their female fertile parent (Texas 2005)*

Fertile female parent	Mean no. seed/fertile female parent	Fertile female parents – derived five-way crosses**	Mean no. seed/ male-sterile line	% Seed-set relative to fertile female parent
A00-41 *Ms2*	219	A00-41 *ms2* x A00-73 (*Ms9*)	217	99
A00-68 *Ms3*	287	A00-68 *ms3* x A00-41 (*Ms2*)	234	81
A00-73 *Ms9*	384	A00-73 *ms9* x PI360.844	242	63

***With kind permission from Springer Science and Business Media: Euphytica 170:35-52. 2009. Table 4.**
****Male 1, DSR Experimental 202b; Male2; GH4190; Male3, DSR Experimental 202c.**

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[1] USDA-ARS-CICGR, Iowa State University, Ames, USA (rpalmer@iastate.edu)
[2] Dairyland Seed Co. Inc., Otterbein, USA

Selection systems and harvest

Selection systems with nuclear male-sterile genes include: seed size differential between self-pollinated seed and hybrid seed, linkage between gene(s) controlling an easily selected trait and the fertility/sterility locus, and linkage between a chemical resistant locus and the male-sterile, female-fertile locus. Several of these systems have provided hybrid seed for research studies, but not for large scale agronomic performance studies.

Mechanical harvest is necessary for commercialization of hybrid soybean. CMS systems would seem to offer the most efficient way to have mechanical harvest because all plants per unit area would be male-sterile, female-fertile. The male parents would be harvested separately. The application of a desiccantto the female parent might be necessary to ensure high seed quality.

Pollen transfer

An efficient pollen transfer system is probably the limiting factor in the commercialization of hybrid soybean. Phenotypic recurrent selection has been used with native pollinators to identify soybean genotypes that have up to 99% of normal seed-set when compared to the fertile version of the female parent (Table 1 and 2, 4). The soybean traits that contribute to insect-pollinators are not well known (6). The reason why an insect pollinator forages on a particular genotype can partly be attributed to differences in floral design and floral display. Floral display describes the number of flowers open at one time and their arrangement in inflorescences, whereas floral design refers to characteristics of individual flowers including their morphology, color, scent, nectar quantity and composition, and pollen production. But first, the pollinator needs to 'discover' the plant and ultimately be rewarded. Repeat visits to particular genotypes ensure high levels of out-crossing, i.e. hybrid seed (Figure 1). ■

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Figure 1. Green plants have low pod set (not insect pollinator attractive) whereas mature plants with brown pods have high pod set (very insect pollinator attractive; Texas 2005)

The future of soybean genomics is here

by Randy SHOEMAKER[1]*, Andrew SEVERIN[2], Jenna WOODY[1], Steven CANNON[1] and Michelle GRAHAM[1]

Abstract: Randy Shoemaker, Andrew Severin, Jenna Woody, Steven Cannon and Michelle Graham
For many years the size and complexity of the soybean genome was considered to be an unwieldy impediment to whole-genome sequencing and analysis. Successful assembly of the genome following a `shot-gun' sequencing strategy seemed out of the question. But that is exactly how the soybean genome was assembled (1). The sequence was generated by the U.S. Department of Energy's Joint Genome Institute (JGI) and was assembled by a team that included JGI, the United States Department of Agriculture's Agricultural Research Service, and several universities. A portion of the funding used to accomplish this task was provided by a grant from U.S. soybean producers.

Key words: genome database, genomics, *Glycine max*, sequencing, soybean

For many years the size and complexity of the soybean genome was considered to be an unwieldy impediment to whole-genome sequencing and analysis. Successful assembly of the genome following a `shot-gun' sequencing strategy seemed out of the question. But that is exactly how the soybean genome was assembled (2). The sequence was generated by the U.S. Department of Energy's Joint Genome Institute (JGI) and was assembled by a team that included JGI, the United States Department of Agriculture's Agricultural Research Service, and several universities. A portion of the funding used to accomplish this task was provided by a grant from U.S. soybean producers.

Genome composition

A striking feature of the genome is the repeat-rich, low recombination heterochromatic DNA that makes up 57% of the genome (generally comprising the central ~two-thirds of most chromosomes) (Figure 1). Not all of the genome is repetitive. Forty-six thousand four hundred thirty high-confidence genes were predicted within the 1.1 gigabase genome. More than three-quarters of those genes are clustered near the ends of the chromosomes. Among those genes are scattered more than 5,600 transcription factors, representing 63 gene families. More than 38,000 transposable elements were also identified that are representative of almost all known plant transposable elements (2).

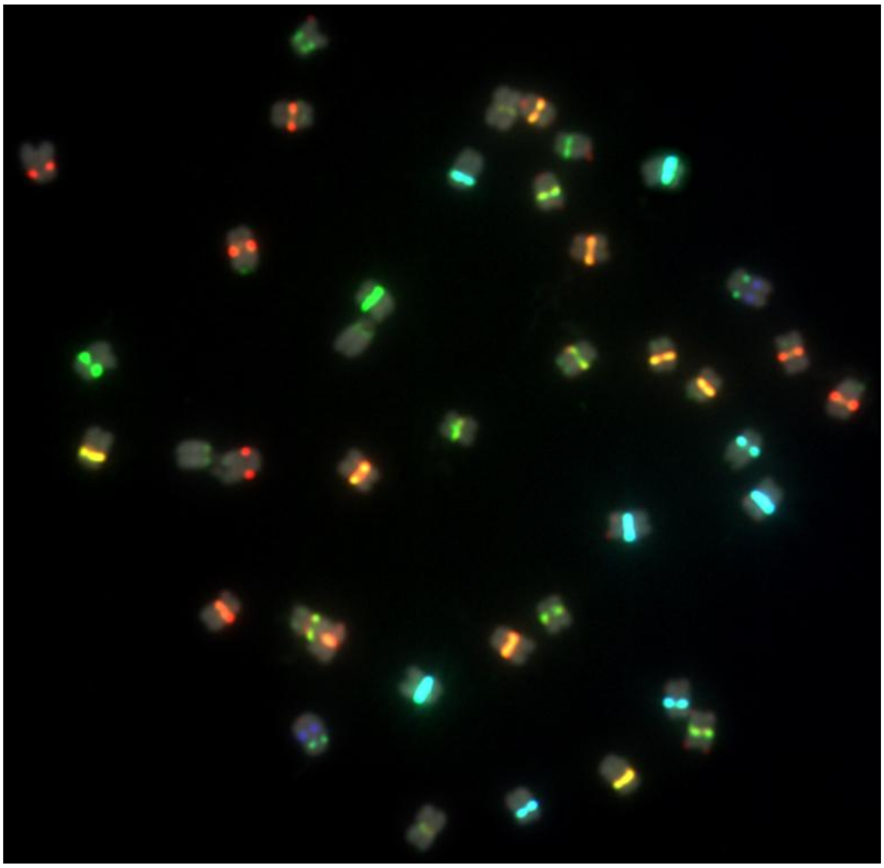

Figure 1. Soybean's 20 chromosomes (two copies of each). Fluorescent probes highlight different repetetive sequences near the centromeres. Figure courtesy Seth Findley and Gary Stacey; methods described in Findley et al. (2010) Genetics 185:727-744.

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[1] United States Department of Agriculture, Agricultural Research Service, Ames, USA (rcsshoe@iastate.edu)
[2] Iowa State University, Ames, USA

Paleopolyploidy

Early studies in soybean using RFLP markers suggested the genome had undergone two or more large-scale duplication events. This conclusion was supported using data from expressed sequence tags (ESTs). Since then, an analysis of the whole-genome sequence has shown that soybean has in fact experienced three major polyploidy events; a whole genome triplication roughly 100 million years ago (mya) (long before the legumes originated) and two whole genome duplication events that occurred early in the legumes, roughly 60 mya, and another that occurred within the *Glycine* genus, about 13 mya (2). Despite the high level of repetitive genomic sequence in the soybean genome, due to high retention of genetic information after three polyploidy events, approximately two thirds of short read sequences generated from next generation sequencing align to unique locations in the genome.

Functional genomics

Information about gene expression patterns, primarily from short-read next generation sequencing (NGS) sequences provides fascinating insights into gene functions and interactions. Unlike hybridization techniques (Northern blots, Affymetrix GeneChips, microarrays, etc.), NGS does not require prior knowledge of the genomic sequence. All measurable RNA present in a tissue sample should also be present in the data. Nevertheless, NGS "RNA-Seq" data is more powerful when combined with genomic sequence, as NGS reads can be counted with respect to predicted genes. Despite the high level of repetitive genomic sequence in the soybean genome, approximately two thirds of short read sequences generated from NGS align to unique locations in the genome (3). Analyzing expression data along with genomic data has told us much about how genomic structure might affect expression (Table 1). We have learned that depth and breadth of gene expression is closely associated with structural features of genes and intergenic regions (4). Additionally, RNA-Seq analysis has the advantage of identifying SNPs and indels that can be used to understand the relatedness of lines, identify candidate genes responsible for observed phenotypic differences between lines, and characterize genetic diversity in domestic lines and in wild relatives.

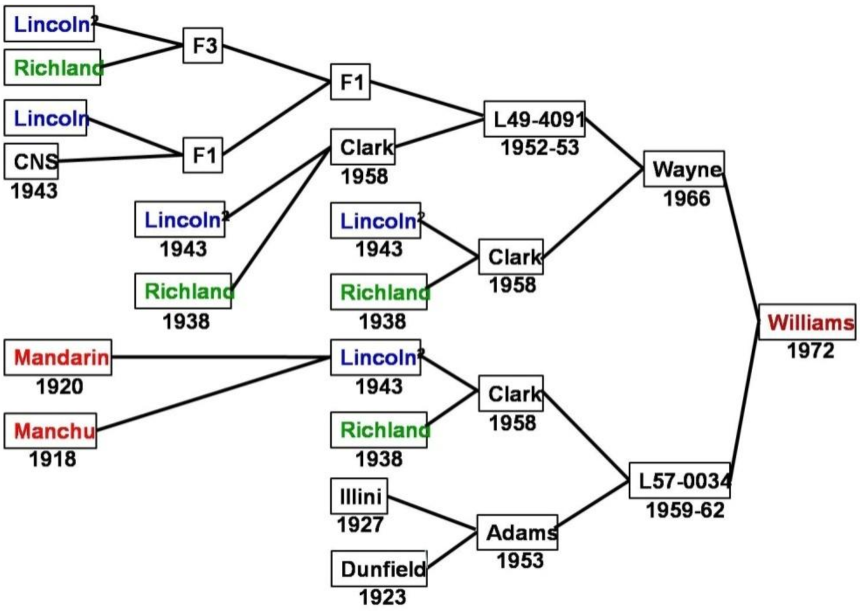

Figure 2. Pedigree of the soybean cultivar `Williams' showing a typical sequential development of new cultivars from crosses between early cultivars. Figure is courtesy of James Specht.

Genome database

The soybean research community is fortunate to have several high quality genomic databases. The largest is SoyBase (www.SoyBase.org), a genome database supported by the United States Department of Agriculture and possessing long-term financial support. Not only do these databases provide access to vast amounts of information, but they bring together agronomic data, 20 years of QTL mapping data involving over 80 agronomic traits, structural and functional genomic data. Resources are currently being developed that allow the integration of all of these data types overlaid onto metabolic pathways. Soybean genomics is on the verge of a `holy grail' in crop genomics; efficient association of genotype and phenotype.

The future

What about the future of soybean genomics? A recent Strategic Planning White Paper on soybean genomics identified resequencing of selected genotypes as a high priority for soybean advancement. These genotypes included the original land races brought into the U.S. and grown in the 1920's, and subsequent `milestone' cultivars released during the 80 years that followed. The rationale behind this is simple. The first crosses used to develop the first generation of improved cultivars were between high yielding land races. Soybean cultivar development proceeded sequentially with crosses between high yielding cultivars producing the following generation of cultivars, and so forth (see example in Figure 2). This developmental series produced a yield increase of approximately 0.4 bu/acre/year. Sequencing technologies have now made it possible for efficient and affordable resequencing of the genomes of the land races as well as the milestone cultivars. This will permit scientists to follow yield improvements with selection for specific chromosomal segments and specific alleles.

Table 1. Number of genes in low, intermediate and high expression categories. Data is based on Severin et al. (2010) and Woody et al. (2011)

Number of tissues in which genes are expressed	Low expression (a)	Intermediate expression (b)	High expression (c)
1	3071 (d)	3056	979
2	1800	1309	318
3	1530	876	174
4	1328	732	157
5	1101	654	132
6	1304	523	86
7	1237	433	61
8	1140	361	52
9	1183	329	50
10	1159	337	48
11	1062	271	49
12	973	257	42
13	979	196	47
14	723	105	63

(a) Number of genes expressed with a transcript count of nine or under; (b) Number of genes expressed with a transcript count of ten to 49; (c) Number of genes expressed with a transcript count of fifty or over; (d) Number of genes in the expression group

Knowing which regions of chromosomes have recombined during the breeding process is only one dimension of the story behind soybean yield. During breeding programs chromosomal segments were selected for because of the genes they contain. The function(s) of the vast majority of the genes in the soybean genome remains unknown. By coupling resequencing with whole-genome transcriptome analyses we will be able to a) monitor changes in expression of individual genes, b) changes in specific metabolic pathways, and c) identify base pair changes in key genes that will be used to develop selectable markers to enhance breeding programs.

We are learning that many changes in gene expression are caused by chemical modifications to the genome itself, and not necessarily mutation in the base pairs comprising the gene sequence. These modifications are called `epigenetic' changes. One of the common changes is the addition of a small `methyl' group to the DNA sequence. Methylation results in changes in gene expression without actually changing the nucleic acid sequence. The role of methylation in soybean productivity has never been examined. Patterns of methylation may provide insight into the quantitative nature of many important agronomic and developmental traits. Evidence of methylation patterns associated with components of soybean yield will change the way we think about soybean improvement strategies.

The release of the soybean genome sequence has ushered in a new era of scientific discovery combining molecular, computational and traditional agronomic approaches. We are now able to examine gene expression on a whole genome level, learning when and how genes are turned on or off during development, in specific tissues or genotypes and in response to environmental stimuli. Transcriptomics combined with the genome sequence and genetic data is being used to understand the basis of Quantitative Trait Loci (5). While soybean transformation is still difficult and time consuming the function of genes involved in traits or pathways of interest can now be studied using new virus induced gene silencing technology (VIGS). Targeted resequencing of genotypes of interest can be used to develop new markers to facilitate cloning of resistance genes and to aid in marker assisted selection (5). ■

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Diasease resistance in soybean

by Kristina PETROVIĆ* and Miloš VIDIĆ

Abstract: Several parasites on soybean appear athigh intensities in the agroecological regions of Europe, while others are either not present or occur sporadically. Climatic factors are those which primarily determine the dominant parasite in a particular region. It has been found that the most important parasites were *Peronosporamanshurica* and *Pseudomonas syringae*pv. *glycinea* on leaves; *Diaporthephaseolorum* var. *caulivora* and *Sclerotiniasclerotiorum* on stems; *Macrophominaphaseolina* on root, and *Diaporthe/Phomopsis* species which are the main causes of seed decay. The development and cultivation of resistant soybean genotypes is the most effective control measures for all these diseases.

Key words: *Glycine max*, parasites, resistance, soybean, symptoms

A large number of phytopathogenic microorganisms are parasites on soybean (*Glycine max* (L.) Merr.). They cause various pathological changes in all organs of the plant. Soybean diseases can significantly affect yield, quality and stability of this industrial crop. In addition, epiphytotic outbreaks may threaten the profitability of soybean production. More than 135 pathogenic microorganisms on soybean were described. However, only about 30 species belong to the group of economically important pathogens (19). Soybean crop can be successfully protected with a combination of measures, among which the development and utilization of resistant cultivars is most efficient, economical and ecologically most acceptable. This paper provides a brief overview of the sources of resistance to economically important soybean pathogens in Europe, as well as the possibility of their incorporation into commercial cultivars.

Institute of Field and Vegetable Crops, Novi Sad, Serbia (kristina.petrovic@nsseme.com)

Downy mildew (*Peronospora manshurica*)

Downy mildew is the most common foliar diseases of soybean, but is seldom of economic importance in terms of yield lost. However, infected pods and seeds may lead to reduced seed quality. If extensive defoliation occurs, yields can be severely reduced. The initial symptoms are small pale green or yellowish spots, which necrose and merge with time (Fig. 1). A tan to gray cover forms on the underside of the leaf, especially under wet and humid conditions. Pods may be infected without any symptom, and seeds are partly or completely encrusted with white mycelia and oospores (Fig. 1).

High variation in pathogenicity has been observed within the population of *P. manshurica*. 32 physiological races were identified and the gene *Rpm* was shown to impart resistance to all of these races (2). It was incorporated into cultivar Union, but resistance was overcome by the new race 33 (12). The gene, *Rpm2*, conditioned resistance to race 33 and segregated independently of *Rpm* (11). In Poland, 11 races were characterized, and seven were described for the first time, numbered from 34 to 40 (14).

Soybean genotypes reaction to downy mildew ranges from susceptible to resistant to specific races of the pathogen, but there is no genotype resistant to all races of *P. manshurica*. The large number of physiological races and steady recurrence of new races make soybean breeding a continuous process since resistant cultivars become more or less susceptible with time. Soybean genotypes Colfax and Burlison, and high-protein lines Barc-6, Barc-8, and Barc-9 have a satisfactory level of resistance to *P. manshurica*. These genotypes were included in the breeding program of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. A collection of 52 less susceptible soybean genotypes was established. Based on the results obtained in the last three years, 36 lines were identified that exhibit a high level of resistance to *P. manshurica*.

Figure 1. Downy mildew: (a) symptoms on leaf; (b, c) symptoms on seed

Bacterial blight (*Pseudomonas syringae* pv. *glycinea*)

The bacterial blight can cause large economic damage, especially in regions with cool and rainy climate. Disease causes leaf shed resulting in reduction assimilation area, which ultimately reduces the yield. The bacterium attack all above-ground plant parts, but symptoms on infected leaves and pods are most apparent. The typical symptoms - small, angular, translucent, water-soaked and yellow to light brown spots (Fig. 2) - occur on the bottom and middle leaves of adult plants. Within the spot, on the underside of the leaf, a dense and sticky bacterial exudate covers the spot giving it a glossy appearance. The same symptoms occur on pods, while seeds from diseased pods become infected.

It has been found that *P. syringae* pv. *glycinea* has 12 physiological races, but the race 4 is dominant on soybean worldwide (7). Soybean cultivars vary in degree of suscebtility to all races. Molecular studies have identified six different avirulence (*avr*) genes in seven different races (0, 1, 2, 3, 4, 6 and 8) of *P. syringae* pv. *glycinea*. The genetic analysis determined that soybean carries six resistance genes, *Rpg*1, *Rpg*2, *Rpg*3, *Rpg*5, *Rpg*6, and *Rpg*7 that correspond to *avr*B, *avr*A, *avr*C, *avr*E, *avr*F, and *avr*G, respectively (5). They also determined that soybean carries *Rpg*4 resistance gene for the race 4, which correspond to *avr*D, cloned from *P. syringae* pv. *tomato*. All known races of *P. syringae* pv. *glycinea* contain non-functional *avr*D alleles. These alleles contain mutations, and it was supposed that the gene mutated to escape defense surveillance in soybean plants containing resistance gene *Rpg*4. There is no genotype completely resistant to race 4, but there is a significant differences in the rate of susceptibility. The genotype P9241 has a satisfactory level of resistance to *P. syringae* pv. *glycinea* and it was used in breeding programs at Institute of Field and Vegetable Crops. A collection of 24 less susceptible genotypes has been established and in the last three years, allocated the seven lines that exhibit a satisfactory level of field resistance to *P. syringae* pv. *glycinea.*

Figure 2. Symptoms of bacterial blight on soybean leaves

Figure 3. Typical symptoms of northern stem canker

Northern stem canker (*Diaporthe phaseolorum* var. *caulivora*)

The northern stem canker has the greatest economic importance because it causes wilt and drying of plants during pod development and grain filling. A significant epidemic of stem canker broke out in Europe in the 1980s. Prematurely wilted plants yielded 50-62% (depending on genotype) less than healthy plants. First symptoms can be seen at the flowering stage. Early symptoms are reddish-brown lesions that appear on one or more basal nodes of soybean stem. These small lesions can develop into elongated, sunken, dark brown cankers that spread up and down along the stem (Fig. 3). The earlier occurrence of symptom cause more damage, whereas the late infections cause considerably less damage. Pods of infected plants dry up and remain empty, or small and insufficiently filled grains are formed.

Examination of different genotypes has shown large differences in suscepbility to *D. phaseolorum* var. *caulivora* (22). It was found that the reaction of soybean genotypes depends on their maturity groups. Early-maturing genotypes were less susceptible and usually respond with mild symptoms (stem blight), while the late-maturing genotypes were much more susceptible and the symptoms are manifested in the form of premature wilting of plants. Early-maturing genotypes avoid the infection.

Studies of the pathogenicity of a large number of *D. phaseolorum* var. *caulivora* isolates, observed variability in the parasite and assumed the existence of more races. There were significant differences in the reaction of some cultivars to isolates originating from the northern parts of the USA compared to isolates from the southern parts. Six physiological races were differentiated based on the reaction of six soybean cultivars (8). Cultivar Tracy-M exhibited resistance to the southern, and susceptibility to the northern f isolates, while other cultivars exhibited minor differences in susceptibility. Based on this, stem canker was divided into northern and southern stem canker were named *caulivora* and *meridionalis*, respectively; first as *formae speciales* (17) and then as *varietas* (6) at the subspecific level of *D. phaseolorum*. It was found that southern stem canker was controlled by four major genes (*Rdm*1, *Rdm*2, *Rdm*3, and *Rdm*4), but these genes did not confer resistance to var. *caulivora* (18). In Europe, var. *meridionalis* was not detected. Regarding var. *caulivora,* differences in the susceptibility were established among genotypes, but genes responsible for resistance have still not be identified.

White mold (*Sclerotinia sclerotiorum*)

White mold generally occurs in humid regions and in irrigated soybeans. This is potentially the most dangerous soybean disease, as it can cause wilt and rot of developing plants. Damage is especially extensive if the infection occurs in the phenophases of flowering and pod forming. Initial lesions usually develop at stem nodes during or after flowering. The infected stem tissue becomes soft and watery, and the whole plant rots in moist conditions. The infected plant parts become covered with cottony mycelium and black sclerotia develop on or inside infected stems and pods. During harvest, sclerotia fall to the ground or mix with seeds.

There are no sgenotypes resistant to *S. sclerotiorum*, and the situation is similar with the other host plants of this parasite. However, differences in the susceptibility have been observed in the field. The presence of partial resistance in cultivars Dassel, Corsoy 79, DSR137, S19-90, and PI194639 controlled by several genes were reported (1, 3). Also, it is assumed that physiological resistance and avoidance mechanisms are responsible for different reactions of soybean cultivars to *S. sclerotiorum*. Three loci (QTL) were mapped, two of which control avoidance mechanism and one locus is most probably responsible for partial physiological resistance (9).

Pathogenesis of *S. sclerotiorum* has been associated with fungal toxic secretion of oxalic acid (OA). There are three classes of known enzymes that can catabolize OA, namely oxalate oxidase (OXO), oxalate decarboxylase (OXDC), and bacterial oxalyl-CoA decarboxylase. The genes, which control OXO and OXDC enzymes, have been utilized to produce soybean transgenic resistant plants. The plants which transformed with the OXO *gf*-2.8 (germin) gene from wheat and decarboxylase (*oxdc*) gene from a macrofungi *Flammulina* sp. showed high resistance to *S. sclerotiorum* (4, 13).

Charcoal rot (*Macrophomina phaseolina*)

The disease is economically important in regions with the warm and dry climate. The charcoal rot is a disease of the root and basal part of the stems. The typical symptoms of disease appear during or after flowering. The symptoms occur firstly on plant roots as light brown spots, which latter spread to the entire root system, basal part of stem, lateral branches, and in favorable conditions cover a larger part of the plant. The surface of the root and stem become light grey (silvery discoloration), while numerous black microsclerotia develop beneath the epidermis, which give them a charcoal-sprinkled appearance (Fig. 4).

Specific resistance to *M. phaseolina* in soybean have not been identified, but few soybean genotypes, including DeltaPineland 3478, Hamilton, Jackson II, Davis, and Asgrow 3715 possess either moderate resistance or tolerance (20, 21). The drought-tolerance mechanism is often equated with charcoal rot resistance. Therefore, it is recommended to avoid of cultivation of the genotypes with late reproductive stage that coincides with periods of drought stress. Identification of resistant genotypes has been limited because of the lack of an efficient disease assessment method and the lack of a consistent classification scheme across experiments and years (15). A new classification system for soybean genotypic reactions to *M. phaseolina* based on a colony-forming unit index (CFUI) was develop and evaluate. It was also suggested that the growth stage R7 is the optimum stage for assessing disease using CFU (16). Four out of 24 genotypes were identified to be moderately resistant using these parameters.

Phomopsis seed decay (*Diaporthe/Phomopsis* complex)

Phomopsis seed decay (PSD) is the major cause of poor seed quality in most soybean-growing countries. The disease is caused primarily by the fungal pathogen, *Phomopsis longicolla*, along with other members of the *Diaporthe/Phomopsis* complex. In addition to *P. longicolla*, this complex consist of *Diaporthe sojae* which causes pod and stem blight and two varieties of *Diaporthe phaseolorum*, in which var. *caulivora* and var. *meridionalis* (present only in the southern hemisphere) cause stem canker. However, recent research shows that other species of the genus *Diaporthe/Phomopsis* also present in soybean seeds and usually are associated with PSD (23).

The agents of PSD favour long rainy and warm periods during soybean maturation and harvest. Infected seed are usually small, shrunken, with cracked seed coats and often appear chalky-white (Fig. 5). The less infected seeds have a normal appearance, without symptoms (the latent infection). They have reduced germinability, vitality and quality.

Figure 4. Charcoal rot: (a) symptom on root; (b) symptom on stem; (c, d) microsclerotia

Figure 5. Phomopsis seed decay

Screening soybean lines for resistance to PSD is the first step toward developing PSD-resistant cultivars. Seeds were tested for *Phomopsis* spp. infection, percentage of germinated seeds and the quality (seed wrinkling, molding, mottling, and discoloration). Resistance to the agents of PSD was found in several plant introductions (PI), but inheritance of most reported sources of resistance is still not clear. Resistance was characterized as qualitative, controlled by one or two pairs of major dominant genes (10). This means that the resistance of different genotypes is not always controlled by the same genes, and probably for this reason gene symbol has not been assigned yet. ■

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Trypsin inhibitors in soybean

by Vesna PERIĆ*, Mirjana SREBRIĆ and Snežana MLADENOVIĆ-DRINIĆ

Abstract: The main protease inhibitors in soybean - Kunitz trypsin inhibitor (KTI) and the Bowman–Birk inhibitor (BBI) constitute the main anti-nutritional factors of soybeans. The major advancements in lowering TI activity of raw soybean are achieved by crossing parent donor of desirable character (titi line) with high yielding varieties (Ti line) resulting in releasing of two varieties lacking KTI – Lana and Laura.The daily weight gain and food conversion of the group of pigs fed raw KTI-free soybean were lower, suggesting that nutritional value of raw soybean, although higher than that of the standard variety, is still too low to sustain normal growth and development of animals.

Key words: breeding, *Glycine max*, kunitz trypsin inhibitor, pigs, soybean

The main protease inhibitors in soybean - Kunitz trypsin inhibitor (KTI) and the Bowman–Birk inhibitor (BBI) constitute at least 6% of the protein present in soybean seed. Trypsin inhibitors are responsible for the reduced digestibility of seed proteins, and for this reason, constitute the main anti-nutritional factors of soybeans. Kunitz trypsin inhibitor accounts for almost a half of trypsin inhibitory activity in raw soybean grain. Grain of conventional soybean varieties requires heat processing to break down trypsin inhibitor's activity before using as food or animal feed. The excessive heat treatments (uncontrolled temperature; long period of time) may decrease protein solubility and lower amino acid availability. Soybean lines with reduced protease inhibitor content could reduce or eliminate the need for expensive heat treatments and lessen the chance of lowering amino acid availability.

Five electrophoretic forms of KTI have been discovered. The genetic control of four forms, *Ti a, Ti b, Ti c*, and *Ti d*, has been reported as a codominant multiple allelic series at a single locus (3). The same authors found that the fifth form does not exhibit a soybean trypsin inhibitor and is inherited as a recessive allele designated *ti*. The Ti locus has been located on linkage group 9 in the classical linkage map of soybean. Recessive form was found in soybean germplasm samples PI 157440 and PI 196168, lacking KTI. The germlasm PI 157440 was a parent of the first released Kunitz-free genotype named Kunitz. This variety was developed by backcrossing and is the progeny of an F_2 plant selected from the fifth backcross, Williams 82 and PI 157440 (1).

The major advancements in lowering TI activity of raw soybean in the breeding program of Maize Research Institute »Zemun Polje« are achived by crossing parent donor of desirable character (*titi* line) with high yielding varieties (*Ti* line). Identification of lines lacking KTI was done by protein electrophoresis of mature seed from the individual plants in several segregating generations. After field trials and yield testing, two varieties lacking KTI – Lana and Laura, were released (6). Trypsin inhibitor content in this varieties ranges from and 15,01 mgg^{-1} for Laura to 15,35 mgg^{-1} for Lana which was about 50% reduced as compared with the genotypes of standard grain type (3).

In order to determine what kind of effects has early generation selection for seed yield on proportion of the lines with presence or absence of KTI, we made a following study. Two hundred plants from F_2 population derived from a cross between soybean varieties Kunitz (lacking KTI) and Kador (standard grain type) were chosen for analysis. Identification of the genotypes lacking KTI among 200 selected F_2 plants was made by gel-electrophoresis. Segregation ratio was 3:1 for the presence or absence of KTI, as it was expected. The same ratio was found in 100 high-yielding and 100 less-yielding plants, as well as in 48 highest-yielding lines chosen for the seed yield trials. Seed yield of 48 highest-yielding F_2 plants was tested in the trials conducted in two locations. The results showed that same ratio of the lines with presence or absence of KTI was maintained also in progenies that outyielded the highest-yielding parent. Early generation selection for seed yield didn't eliminate lines lacking KTI.

Recently, indirect selection based on DNA marker tightly linked to *Ti* locus became an easier and more efficient method than protein electrophoresis for detecting genotypes of interest. An F_2 population derived from a cross between Kador and Kunitz variety was analyzed by SDS-PAGE and with specific PCR primer to select seeds missing KTI protein. The analysis resulted in 78 genotypes with KTI protein band and 15 with no KTI protein band. Comparison of gel electrophoresis for Kunitz trypsin inhibitor protein and banding pattern amplified by Satt228 marker showed there was a strong agreement between protein band for KTI protein and banding pattern by Satt228 marker (2). The both methods allow identification of KTI and will be useful in early generation detection of the genotypes homozygous for recessive allele (*titi* lines).

Maize Research Institute Zemun Polje, Belgrade, Serbia (vperic@mrizp.rs)

Numerous studies investigated effect of soybean variety and processing on growth performance of pigs. Inclusion of raw Kunitz-free soybean in diet was beneficial in terms of better growth compared with conventional cultivars, but still inferior to the performance obtained by soybean meal, where the KTI and other anti nutritional factors are inactivated by heating. Our study was carried out to estimate the nutritional value of new cultivars in feeding trials with pigs and determine whether raw Kunitz-free soybean cultivars could be fed successfully to pigs in different stages of development. The daily weight gain and food conversion of the group of pigs fed raw KTI-free soybean were lower, suggesting that nutritional value of raw soybean, although higher than that of the standard variety, is still too low to sustain normal growth and development of animals (5).

Nevertheless, KTI free soybean cultivars can offer nutritional advantages and reduce the processing costs, since they need a shorter heating time and lower temperature for inactivation of trypsin inhibitors. These varieties could possibly solve the problem of direct livestock nutrition in extensive farm systems with own feed production and animal growing. ■

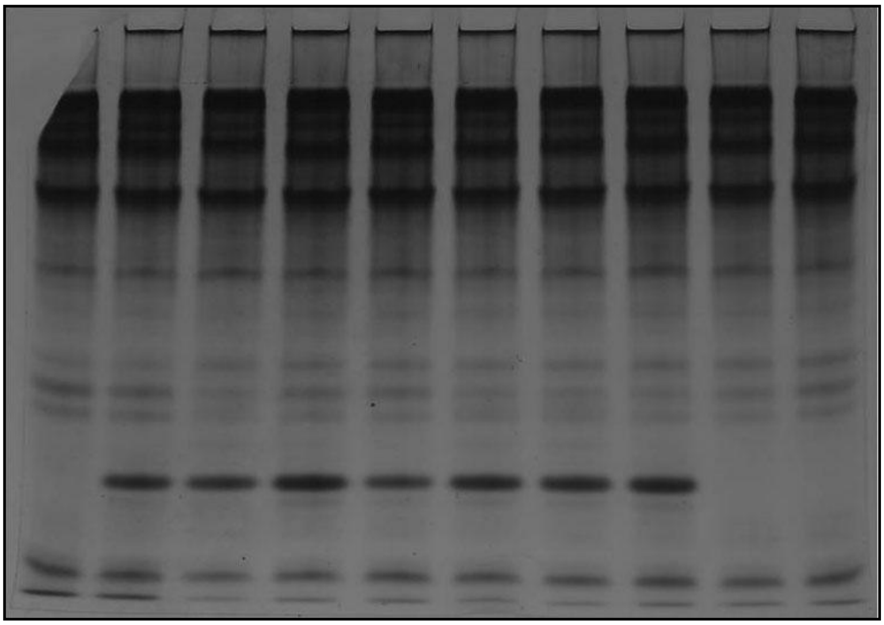

Figure 1. Polyacrylamide gel of protein extracted from parents and F2 seeds. Line 1. Kunitz, 2. Kador, 3-10 F2 plants, arrow points to the Kunitz trypsin inhibitor band (21.5 KDa)

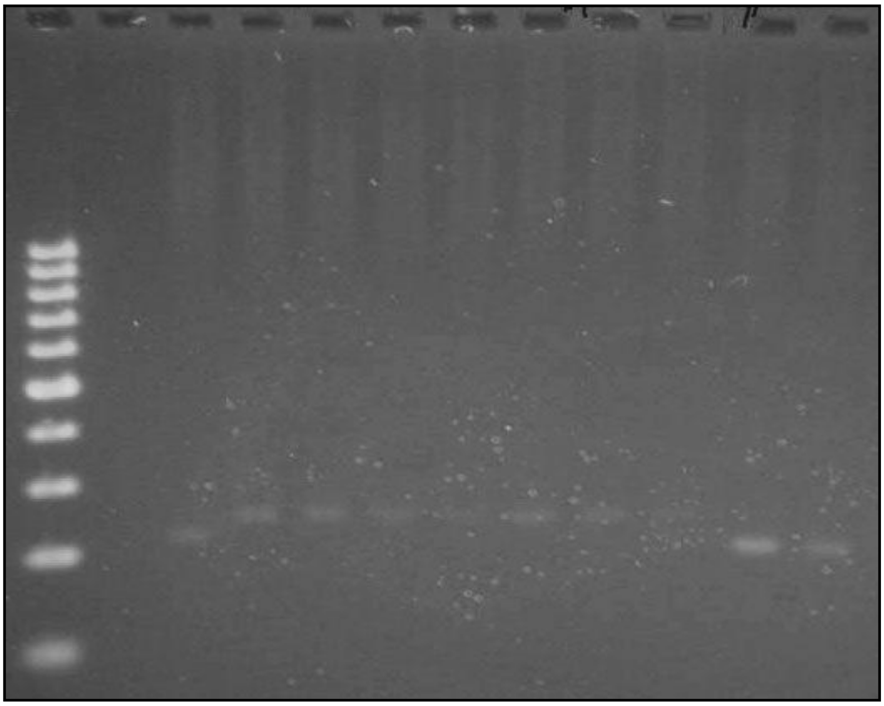

Figure 2. Sat228 marker analysis of parents and F2 seeds. Line 1. Kunitz, 2. Kador, 3-10 F2 plants

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Health benefits of soybean consumption

by Anna ARNOLDI

Abstract: Soybean has a high protein content and is rich in lipids, in particular polyunsaturated fatty acids, a-linolenic acid included. It also contains vitamins, minerals and phospholipids, such as lecithin, which facilitate the metabolism of cholesterol. Being almost devoid of starch and purines, it is particularly suitable for diabetics. The cholesterol reducing effect of soy proteins became the basis for the US Food and Drug Administration approval of the health claim for the role of soy protein consumption in coronary disease risk. In addition, ancillary benefits of soy, i.e. blood pressure reduction and possibly reduced body weight, are of considerable therapeutic interest.

Key words: anti-cholesterol activity, *Glycine max*, health benefit, hypotensive activity, soybean

Introduction

Soybean (*Glycine max*) is a herbaceous plant belonging to the family of *Leguminosae.* It is native of Eastern Asia and cultivated for food purposes. The leaves of the plant vary in color between green and yellow, the flowers are small and in clusters and the fruits are pods about 50 cm long with seeds used to produce oil and flour. In respect to other legumes, soybean is more digestible and richer in protein, as well as associated with fewer problems of flatulence. It has a high protein content (about 35-40%) and is rich in lipids (15-20%), in particular polyunsaturated fatty acids, α-linolenic acid included. It also contains vitamins (A, B, B2, D, and E), minerals (calcium, iron, and potassium) and phospholipids, such as lecithin, which have emulsifying properties and facilitate the metabolism of cholesterol. Being almost devoid of starch and purines (metabolized in the body to uric acid), it is particularly suitable for diabetics.

Anti-cholesterol activity

In many countries soy foods are appreciated for their potential role in atherosclerosis prevention. In fact, soy protein has been shown to successfully reduce cholesterolemia in experimental animals, as well as in humans with cholesterol elevations of genetic or non-genetic origin. In the earliest studies in the 70s, a soy protein preparation, given to hospitalized highly hypercholesterolemic patients, was found to be highly effective for cholesterol lowering and well tolerated. In this six-week controlled crossover investigation (17), there was a 20-22% reduction in total cholesterol (TC) level and a 22-25% reduction in LDL-C, without significant changes of triglyceridemia. The cholesterol reduction was inversely related to baseline cholesterolemia and not modified by the addition of dietary cholesterol (17). The numerous ensuing clinical studies were summarized in a meta-analysis (1) of 38 studies up to 1995, in both hypercholesterolemic and normolipidemic individuals. This meta-analysis confirmed that serum LDL-C concentrations are modified, depending on the baseline cholesterolemia, from a minimum of -7.7% in subjects with total cholesterol in the normal range (<200 mg/dl), up to -24% in subjects with a clear-cut hyper-cholesterolemia.

The cholesterol reducing effect of soy proteins, potentially leading to a reduced cardiovascular risk, became the basis for the US Food and Drug Administration (FDA) approval of the health claim for the role of soy protein consumption in coronary disease risk reduction (6).

A meta-analysis published in 2006 (14) criticized these results, since the studies published in the following years apparently did not confirm the powerful cholesterol reducing effect of soy proteins. This apparent incongruence may be explained by the fact that clear-cut hyperlipidemics are always excluded in recent trials for ethical reasons (17), since today to treat these subjects with hypolipidemic drugs is compulsory. Figure 1 compares the cholesterol reductions observed in the studies included in the Anderson meta-analysis (white points) and more recent investigations (black points) *vs.* the average baseline cholesterol level of each study. This chart demonstrates very clearly that both in old and recent studies, the ranges of cholesterol responses of the groups of patients belonging to the same quartiles of baseline cholesterol are essentially identical (5). It confirms also that about 25% of old studies were based on severely hypercholesterolemic individuals, whereas in recent years patients with cholesterolemia in the very high range (>335 mg/dl) have never been selected for dietary treatment.

The efficacy of soy protein in cardiovascular prevention has been recently confirmed by the 20-year follow-up of the Nurses Health Study, which indicated a significant correlation between vegetable protein intake and reduced cardiovascular risk (9).

University of Milano, Department of Endocrinology, Physiopathology and Applied Biology, Milan, Italy (anna.arnoldi@unimi.it)

Rodent and in vitro studies have attempted to establish a link between the hypocholesterolemic effects of soy and the activation/depression of liver low density lipoprotein receptors (LDL-R): animals on cholesterol/cholic acid dietary regimens with casein have a dramatic down-regulation of liver LDL-R and this effect is reversed in the presence of soy proteins. Two studies have addressed the potential of soy protein preparations to increase LDL-R expression in human beings. In the former (12), FH patients were treated with animal protein or textured soy protein (with the addition of cholesterol to balance the two diets). Both plasma lipids and LDL degradation by circulating lympho-monocytes (used as mirror images of hepatocytes) were monitored. After the animal protein diet there were minimal changes in LDL-C levels or LDL-R activity, whereas during the soy protein diet, in addition to a marked LDL-C reduction, an increase of around 8-fold in LDL degradation was observed. This study, clearly suggesting that some soy protein components are able to up-regulate LDL-R mediated LDL degradation, was subsequently confirmed in individuals with lesser cholesterol elevations (4).

Studies on the mechanism whereby soy proteins may reduce cholesterolemia have clearly indicated that the intact soy protein *per se* is effective for cholesterol reduction, not a mixture of soy amino acids. The identification of soybean components responsible for the hypocholesterolemic effect has received a significant contribution from the early clinical studies where soy protein products contained less than 0.15 mg/g isoflavones (16) versus contents of 2-3 mg/g very frequently encountered in most commercial soybean products. While initially the responsibility of these phytoestrogens in cholesterol reduction was suggested primarily on the base of studies in monkeys (2), a number of more recent reports have definitely concluded that dietary isoflavones make no contribution to the hypocholesterolemic action (8), including a clinical study performed on pure genistein (3).

A full understanding of the mechanism of action of soy protein has become vital for the selection of the most appropriate forms of soy for treating hypercholesterolemia. This selection can only be made once the active component/s and their mechanism of action have been fully elucidated. Proteomics may be a powerful tool to achieve this objective (7). The major storage proteins of soybeans are 7S and 11S globulins: from early studies the 7S globulin appeared to be primarily responsible for the hypocholesterolemic effects of soy protein preparations, whereas the 11S component appeared essentially inactive. Very recently a hypocholesterolemic protein sub-component has been pinpointed more precisely, i.e. by showing that the isolated 7S globulin α' subunit given to cholesterol-fed rats leads to a strong up-regulation of liver LDL-R activity as well as to dramatic plasma cholesterol/TG reductions (5).

Hypotensive activity

Biologically active peptides or functional peptides are food derived peptides that exert also a physiological effect in the body. They are inactive in the original protein but, once released, function as regulatory compounds with hormone–like activity (18). They may be released from the parent protein during gastrointestinal digestion or during food processing. Active peptides in general contain from 2 to 20 amino acid residues and are partially or totally resistant to hydrolysis. They may be absorbable and thus exert systemic effect. Activities may be very different, including angiotensin I converting enzyme (ACE) inhibition, anti-thrombotic and anti-microbial activities.

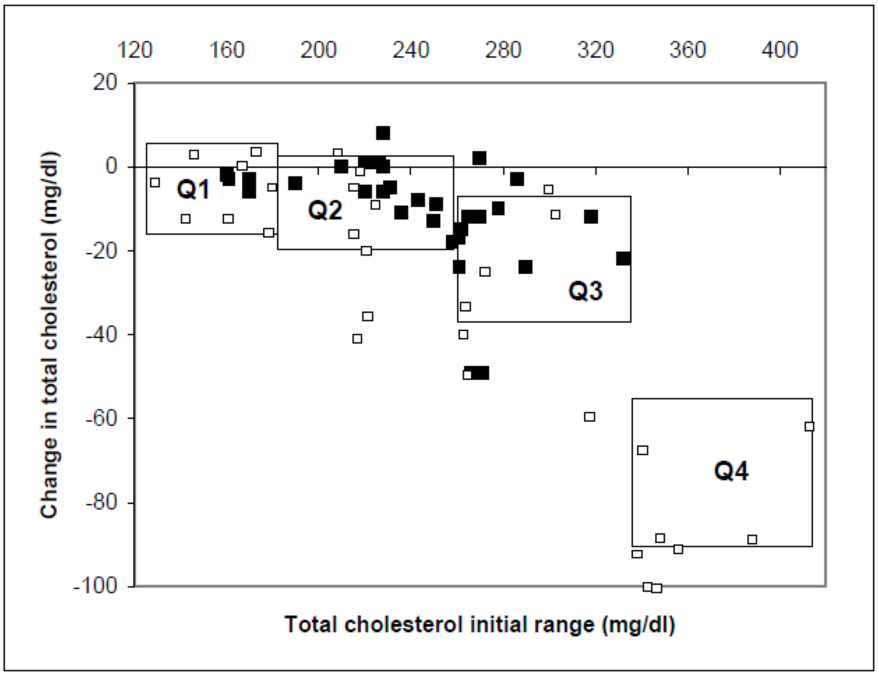

Figure 1. Initial cholesterol values and net cholesterol changes after soy protein based diets in the studies evaluated in the meta-analysis by Anderson et al. [54] (□) and in later studies [56] (■). Boxes indicate the "Anderson normogram", i.e. the initial ranges of cholesterol values and 95% confidence intervals of cholesterol changes for each quartile considered in the Anderson meta-analysis (Reproduced from Future Lipidology 2007, 2, 313-339 with permission of Future Medicine Ltd)

ACE plays an important role in the renin-angiotensin system. It regulates arterial blood pressure as well as salt and water balance. ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor, and degrades bradykinin, a vasodilator. Therefore, inhibition of ACE results in an anti-hypertensive effect in humans and animals. Although the anti-ACE activity has been initially reported in casein peptides, this activity is not only restricted to animal proteins. In particular, very recently a remarkable activity of soy nuts (containing 25 g of soy proteins) on systolic and diastolic blood pressure has been shown in hypertensive (less so in normotensive) post-menopausal women (19). It seems reasonable to hypothesize that the hypotensive activity may be related to bioactive fragments within the sequence of soy proteins, released by hydrolysis during digestion. In fact, recent investigations have suggested that specific peptides obtained by hydrolysis of soy protein are effective in reducing blood pressure by way of ACE inhibition (10, 11, 13, 20).

Conclusion

Soy proteins can selectively reduce LDL-C in experimental animals and in humans with cholesterol elevations of genetic or non genetic origin. The extent of cholesterol reduction appears to be related to baseline cholesterolemia. In addition, ancillary benefits of soy, i.e. blood pressure reduction and possibly reduced body weight, are of considerable therapeutic interest. ■

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Soybean breeding at the Institute of Field and Vegetable Crops

by Jegor MILADINOVIĆ*, Vuk ĐORĐEVIĆ, Miloš VIDIĆ, Svetlana BALEŠEVIĆ-TUBIĆ, Vojin ĐUKIĆ

Abstract: At the Institute of Field and Vegetable Crops in Novi Sad, single seed descent method has been used successfully for more than twenty years resulting in 110 varieties registered in Serbia and 54 abroad. Soybean selection has so far focused on the increase of yield, yield stability and developing varieties adaptable to different growing conditions. Italso takes into account the customerspreferences and the processing industry. This is reflected most notably in our work on increasing and balancing oil and protein content.Recent investigations are aimed to further improvement of nutritional and medicinal properties by researching the activity of secondary metabolites, particularly isoflavone and phytoestrogene.

Key words: breeding, soybean, *Glycine max*, protein, secondary metabolites, yield

Soybean (*Glycine max* (L.) Merr.) breeding, as well as growing, has not a long tradition in Serbia, in spite of favorable agroecological conditions, and soybeans took significant acreage in Serbia not before the mid-1970s. Since successful crop production and management of problems associated with crop growing cannot be carried out without the support of good and versatile research work, Soybean Department has been founded at the Institute of Field and Vegetable Crops in Novi Sad. Researchers at than newly founded department carried out work on identifying and resolving problems occurring in the cultivation of soy. The soybean breeders faced the most important, and the most demanding challenge – to create the first domestic soybean varieties that will have high yielding genetic potential and be well adapted for the domestic growing conditions.

Soybean breeding, like breeding of other crops, is a process involving the development of variability for desired traits, identification of superior genotypes and multiplication of their seed for commercial production. Variability is obtained by crossing parents that possess specific traits intended for transfer into new or improved varieties. Progenies of these crosses segregate genetically in the course of successive generations of selfing and new genotypes are thus formed. Various selection methods are used for identification of those progenies which possess the most useful combinations of the desired traits. The choice of the selection method depends on breeding objective as well as on other important factors such as the available variability, availability of agricultural machines and greenhouse, size and skill of breeding team, etc. In its turn, breeding objective depends on the local agroecological conditions, available acreage, intensity of production, market demand and the economy of production. At the beginning of work on soybean breeding at the Institute of Field and Vegetable Crops, pedigree selection method was chosen. But it was soon replaced by the single seed descent method (SSD), method that has been used successfully and most often in soybean breeding worldwide (21).

The single seed descent method was proposed by Brim (1) and the procedure has been the predominant method of soybean selection in the U.S. since. Single seed descent makes it possible to produce three generations of self-pollination in a single year using winter nurseries or greenhouses, thus accelerating the development of homozygous lines for the testing of yield in replicated trials. However, due to the unavailability of a winter nursery and a lack of sufficient greenhouse space that would accommodate all of the breeding materials, breeders at the Institute has had to adapt the method to make it suitable for such conditions and is making use of only those aspects that involve the reduction of space and labor while at the same time maintaining a satisfactory level of variability up to the F5 generation (20).

The single seed descent method is usually not applied until a certain level of homozygosity is reached in the F_4 or F_5 generation. Selection in the earlier generations can still be done, but on a smaller scale, i.e. it is performed in the sense that pods are not taken from plants that are diseased or lodged or prone to pod splitting and so on (Fig.1) .

Institute of Field and Vegetable Crops, Novi Sad, Serbia (jegor.miladinovic@nsseme.com)

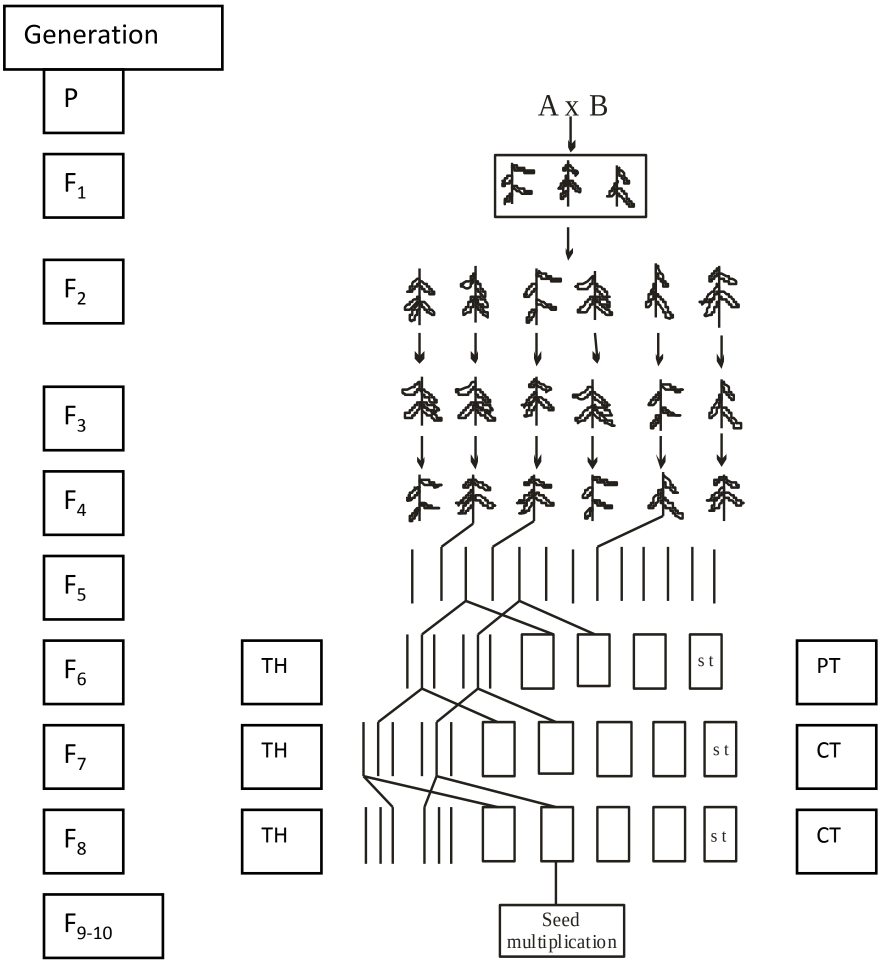

Figure 1. Single Seed Descent Method

Single seed descent requires that only the most basic data be taken down, such as the designation of the cross and what generation it is in. Also, minimal space is required to grow successive generations of individual plants when compared to the rows of progenies characteristic of pedigree selection. Another advantage of the single seed descent method is the presence of full variability in each generation. With no selection in the early generations, the amount of variability present among the F_5 plants is similar to that found in the F_2 generation. Finally, the number of recessive homozygotes increases in successive generations – with the postponement of selection for a recessive trait until the F_5 generation, nearly 47% of the plants will be homozygous for such a trait (25).

It is obvious that the soybean breeding is a complex and difficult task that takes a long time. It takes six years for a genotype to progress from the initial cross to a line that can be considered pure (F_5). Another three years are needed for preliminary and comparative trials. Then, even if no multi-site or large-plot trials are carried out, three more years are needed for the official variety trials. Therefore, it takes a minimum of 12 years for a soybean variety to be developed. Of course, testing can also be done in the early phase of breeding and it is still perfectly possible for an insufficiently tested variety to be put through the official trials and even outperform the standard variety.

At the Institute of Field and Vegetable Crops in Novi Sad, SSD method of selection has been used successfully for more than twenty years, and it resulted with 110 varieties registered in home country, and 54 in other European and Asian countries. Soybean selection at the Institute has so far focused the most on the increase of yield and yield stability (14, 16, 17) and on developing varieties adaptable to different growing conditions (4, 18, 19). However, the Institute's soybean program also makes sure to take into account the preferences of its customers and the processing industry and to adapt to the demands of the market. This is reflected most notably in our work on increasing and balancing oil and protein content of our varietis by conducting studies in field and laboratory conditions. In spite of its high heritability, the major difficulty in breeding programs for increased protein content of soybean seed has been the usually negative genetic correlations between seed protein and seed yield as well as the high negative correlations between seed protein and seed oil (12). In human nutrition, the ratio between oil and protein in the soybeans is important as well, for obtaining a high-quality final product. Improvement in chemical composition of soybean grain could be achieved by increasing both protein and oil content on the account of carbohydrates. Carbohydrates are not abundant in soybean grains but can be a limiting factor in the nutrition of certain animals. The amount of stachyose and raffinose in soybeans and products limits the digestibility and usability of soybean oil and protein in nonruminants. Breeding for grain carbohydrate composition is aimed at reducing the levels of stachyose and raffinose and increasing the sucrose content of soybean grain. Breeding programs on this are still in the early stages, so it is too early to talk about their results.

In addition to this, the balance between the levels of oligosaccharides and polysaccharides for the purposes of fish food production were studied (4, 24), as well as problems regarding nitrogen metabolism (5, 6, 13), and the antioxidative properties of soybean (7, 8, 9, 10, 11), which should furher emphasize the importance of soybean in human diet.

Recent investigations are aimed to further improvement of nutritional and medicinal properties of soybeans by researching the activity of secondary metabolites, particularly isoflavone and phytoestrogene (2, 3, 22, 23). Enriched isoflavone and phytoestrogene composition is not important just in direct use of soybean as a food, but even more in its use as a feedstock for pharmaceutical products. ■

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Swedish soya bean cropping – introduction of a hot crop to a cool climate

by Fredrik FOGELBERG[1]* and Charlotte LAGERBERG FOGELBERG[2]

Abstract: In Sweden, soya is an important feed for milking cows, poultry and pig production. However, all soya is imported. In order to find suitable cultivars and production systems for domestic soya, a pioneer research program started in 2010 to investigate basic issues to promote Swedish soya cropping. Cultivars of the 000-group have been used and proved to function well in southern and central Sweden. We have performed field trials with different seeding times, row distances and performance of a handful soya. We conclude that selected cultivars can be cropped up to the 59th latitude if warm, early soils are chosen and suitable field are used.

Key words: cool systems, cropping systems, *Glycine max*, soybean

In Sweden, soya is an important feed for milking cows, poultry and pig production. However, all soya is imported, mainly from South America, which has raised questions on environmental issues and GM-contamination.

In order to find suitable cultivars and production systems for domestic soya, a pioneer research program started in 2010 to investigate basic issues to promote Swedish soya cropping.

There is no breeding of Swedish soya cultivars, why we have chosen cultivars from Czech Republic, Austria, Swiss and partly Canada. In all cases cv. of the 000-group have been used and they have proved to function well in southern as well as central Sweden.

We have during 2010-2012 performed field trials with different seeding times, row distances and performance of a handful soya cultivars such as 'Bohemians' and 'Silesia' from Czech, 'Merlin' from Austria and 'Annoushka' from Ukraine.

In combination with our field trials, farmers have cropped soya using their own production system from about 1 ha to maximum 14 ha. A wide range of seeding techniques, fertilisation levels and soils have been used, and in 2012 about 40 ha of soya have been cropped.

The yields of the commercial farms have amounted to 1.5 – 2.4 t ha^{-1} measured as dry marketable yield. Protein content have typical been in the range of 39 - 41 % of DM..

Protein content in the trials have been high, typical 40-41 % of DM, but in some cases we have recorded protein levels of 42 - 44% of DM and oil content of 17%.

Early maturity is crucial for Swedish conditions since night frost may occur in late September. At this point the pods must be in their late stage of maturity.

We conclude that selected soya beans cultivars of the 000-group can be cropped in Sweden up to the 59th latitude if warm, early soils are chosen and suitable field are used.

Row distance have a minor affect on yields, but 25 or 50 cm seems to give a somewhat earlier emergence than 12.5 cm although this doesn't result in significant yield increase. However, early emergence is beneficial since it decreases risk of damage caused by birds. A wider row distance makes physical weeding possible.

The soya bean has quite a long period when it can be seeded and still result in acceptable yields. We have noticed that seeding can take place as early as May 5th and proceed until June 10th with quite small effects on yield. Our recommendation is however, that seeding should take place in May 15th to 25th so secure rapid germination and emergence.

Harvest is carried out in early October until mid of November. There has so far been little, if any, problem with plant lodging and/or seed dispersal before harvest. Flexible cutterbars will be introduced and tested in 2013. ■

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[1] JTI – Swedish Institute of Agricultural and Environmental Engineering, Uppsala, Sweden (fredrik.fogelberg@jti.se)
[2] Ideon Agro Food Foundation, Lund, Sweden

Figure 1. A wide range of European soya cultivars are tested in the south part of Sweden. The Ukrainian cv. 'Annoushka' to the left, has a different canopy compared to other cultivars, and has 2012 proven to be one of the more promising cv.

Soya beans – experience from a project in Denmark

by Søren S. PEDERSEN* and Ove Ø. EDLEFSEN

Abstract: Demand for organically and locally grown soya beans have shown an increasing interest in Denmark, mainly as an alternative to dairy products for human consumption, but also as a source of valuable protein fodder for organic husbandry farming. In the period 2008-2010 a number of trials to investigate the potentials of organicsoya production in Denmark were carried out. The aim of all trials was to find suitable varieties,investigate various cultivation systems and give information to a “grower’s manual” for Danish farmers.The trials indicated that it is possible to grow soya beans in Denmark, at least in the southern part of Denmark.

Key words: Denmark, *Glycine max*, organic farming, soybean, variety trials

Introduction

Demand for organically and locally grown soya beans have shown an increasing interest in Denmark, mainly as an alternative to dairy products for human consumption, but also as a source of valuable protein fodder for organic husbandry farming. The main objectives of the project were to examine if it would be possible to grow organic soya beans as far North as Southern Scandinavia.

Field trials

In the period 2008-2010 a number of trials to investigate the potentials of soya production in Denmark were carried out, at the Faculty of Agricultural Sciences (Aarhus University, AU), at the experimental station, ST. Jyndevad (54^0 54’ N, 9^0 07’ E) on a sandy soil. The aim of all trials were to find suitable varieties and to investigate various cultivation systems, as sowing time, method of seeding, handling after harvest, and the need for irrigation etc. Finally the trials should give information to a “grower’s manual” for Danish farmers.

Variety trials

According to Jens Petersen (1, 2) soya bean production has been tried in Denmark from time to time. The first time was in 1881; and during the Second World War, soya protein was difficult to import. Later initiatives on cultivation however never showed satisfactory results. The main obstacle has been late harvest, low yield and poor weed control. A warmer climate, better weed control and development of new varieties could alter the growing possibilities.

Different breeding companies were contacted to find suitable varieties. From NS Seme in Serbia and from Saatbau Linz in Austria we received some varieties. During two years we also tested two varieties from Canada (seed multiplied in Sweden). From Nordic Gene bank some material was received, developed by Sven A. Holmberg during his work in 1940’ies – 70’ies. We multiplied this material, and the seed was used in the variety test for one year. The material from Nordic Gene Bank showed a very short growing period (they were ready for harvest one month before the variety Merlin), but with poor yield. Furthermore these varieties were very short and not suitable for combine harvester. Some other varieties just kept on growing, and stayed green until the frost stopped the growth.

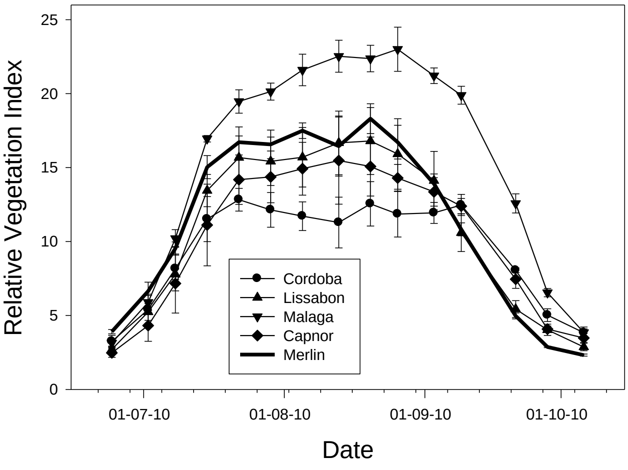

Figure 1. RVI for five Austrian varieties, and with Merlin as reference

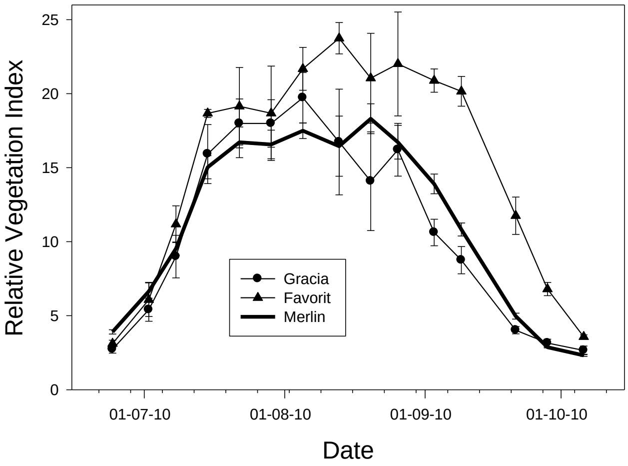

Figure 2. RVI for two Serbian varieties, with Merlin as reference

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Aarhus University, Faculty of Agricultural Sciences, Experimental Station St. Jyndevad, Tinglev, Denmark (soren.o.petersen@agrsci.dk)

Figure 3. Field trials with soya beans in Denmark

Figure 4. Weed control in the field trials with soya beans in Denmark

On a weekly basis during the growing season the solar reflection was measured, expressed as Relative Vegetation Index (RVI). The two figures below shows measurements for 2010 (Figs. 1 and 2). A high RVI for a long period means good potentials for caching sunlight. This indicates that the area below the curves can be related to harvested yield.

The 3 years of variety trials (Fig. 3) showed some promising varieties, with a yield up to more than 2000 kg dry matter seed. However the yield is very sensitive to the climate during the growing season. In 2010 the spring and early summer had a lower mean temperature than average (May was 1.4 ^{0}C lower). The soya showed slow growth and weed was difficult to control. Meanwhile soya seems rather resistance to night frost which occurred one night after germination, however the crop was just irrigated, which probably prevented severe frost damage.

Cultivation

To know more about how to cultivate the crop and to control weed, trials were conducted with various row distances. Row distance of 12.5 cm, 25 cm, 50 cm and 75 cm was tested, however a row distance of 50 cm showed to be the optimum for weed control and yield (Fig. 4). Sowing the soya on ridges was also tested, but germination and yield were lower than soya sown on flat soil, probably due to very dry conditions in the ridge.

Time of sowing had been an importing issue; and 3 different sowing times were tried every year. The earliest sowing time was medium of April followed by start of May, and the late May. The late sowing did not result in reduced yield, but did delay time of harvest. The result from these trials indicated that the time of sowing depends more on how the actually climate is, than the time of year (day length).

The trials were carried out on a sandy soil (Humic Psammentic Dystrudept, USDA) which has a very low water capacity (50 mm. in root zone). Parts of the trials were not irrigated, and yields in the non irrigated plots were cut with more than 50%, this clearly indicates that irrigation is essential.

Diseases and pest

There were not observed any major problems with diseases. However pigeons were the only major problem mainly in trials with small plots. On the other hand the problem might be minor if soya is grown on larger scale.

Conclusions

The trials indicated that it is possible to grow soya beans in Denmark, at least in the southern part of Denmark. However if grown under conventional growing conditions the production will probably not be able to compete with imported soya. As a niche production for organic farmers or as alternative to dairy products it may be possible to obtain a satisfactory production. However it is still not a reliably crop, if cultivation method or climate is not optimal. ■

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Soybean breeding in Belarus

by Denis V. Goloenko*, Vladimir E. Rosenzweig* and O. G. Davydenko

Abstract: CMS systems that have adequate maintainer genes and restorer genes and that are stable across environments have been identified by several Chinese groups. Adequate levels of heterosis have been reported, but heterotic groups or associations are not known. Soybean can be moved from a highly autogamous species to an allogamous one using insect pollinators, coupled with phenotypic recurrent selection. The various components to commercialize hybrid soybean are being assembled. In addition to the anticipated benefits from heterosis, hybrids are an excellent mechanism to 'stack traits', because many are dominant genetic traits and can come from either the female or the male parent.

Key words: breeding, cultivar model, genetic variation, *Glycine max*, soybean

Plant breeder faces three major blocks of problems: model of variety ('what should be selected'), initial material ('of what shall we select'), and selection procedure ('how to select'). Certain level of understanding these problems makes effective breeding process.

In Belarus, soybean cultivars of maturity group (MG) 00 are cultivated. Southern zone of the country ('Polesie') is characterized by 2400-2700 CHU and is most suitable for soybean growing. Sandy low-humus soils predominate in this region while precipitation in July-August is somewhat instable (about 30% of years with local droughts during soybean reproductive period).

Cultivar model

Investigations carried by 'Soya-North Co.' allowed substantiating a model of indeterminate cultivar with 13-14 main stem nodes, good branching ability, early flowering and extended reproductive stage, defined lateral racemes. Opposite to later MGs, within MG 00, node number appears to be tightly correlated with yield ($r = 0.44$, sufficient at a < 0.001) and makes a marked input to plant productivity. Thus, indeterminate plant habit is preferable in breeding for high yield. Determinate cultivars yield sufficiently lower in MGs 00 and 0 (3).

In particular, indeterminate growth type is promising for developing intensive soybean cultivars if lodging resistance provided. Indeterminate genotypes are more responsive to environment improvement because node number is a governing factor for yield plasticity in MG 00. Each additional mainstem node results in 4.6 (poor environment, 1 t ha^{-1}) to 5.9% (rich environment, 3 t ha^{-1}) seed yield increase (7, Fig. 1).

Semideterminate type is somewhat more prospective in breeding for yield stability in difficult environmental conditions, e.g. drought. Tall determinate varieties possess extremely high variance of specific adaptability in environmental gradient and demonstrate highly instable yields. This phenomenon is conditioned by their late flowering causing coincidence of critical stage (seed formation and filling) with moisture deficiency period. Reduction of number of days to flowering combined with extended reproductive stage contributes to the increase of drought resistance without declining yield potential.

Patterns of soybean plant types' reaction for different plant populations were revealed. Well branching cultivars provide stable yields in a wide range of stand densities (30 to 60 plants m^{-2}). Single-stem cultivars are unable to repair stand that leads to sufficient yield losses in case of thinning. Low bottom pod set adversely affects harvesting varieties with limited branching, and high optimal stand density results in excessive sowing rates (6).

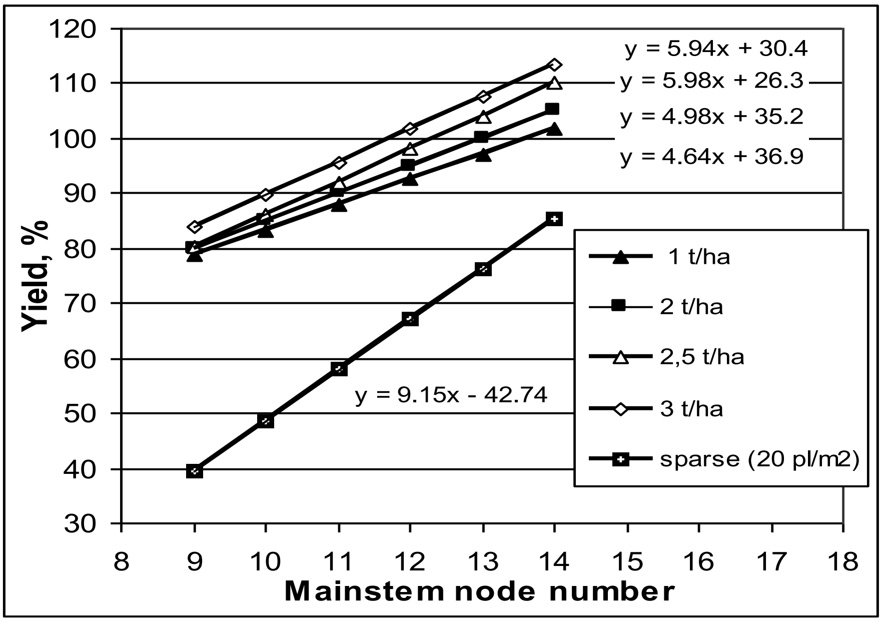

Figure 1. Stratified regression of soybean yield on main stem node number at different levels of environmental index and in sparse crop

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Soya-North Co., Ltd., Minsk, Belarus
(select@sever.by)

In general, a proposed model of early soybean plant architecture does not agree with a concept of 'green revolution' suggesting short-stem determinate habit with limited branching (2). In case of grain cereals, where reproductive sphere is apically concentrated in spike, it is possible to reduce stem height to prevent lodging without sufficient yield potential decrease. In soybean, generative organs are evenly distributed between crop strata, that's why stem truncation results in yield decline.

Early soybean cultivar may possess neutral photoperiod response but recently described weak gene E_7 (1) promotes yield increase by 9% (near-isolines treated) while delaying flowering and maturity by 4-5 days. As this allele effect may be hardly distinguished under the field conditions, screening of germplasm and E_7 genotypes selection are fulfilled using SSR-markers (3).

Photoperiodism studies allowed distinguishing a new genetic factor (tests for allelism unfinished yet). Preliminarily, it is linked with E_3 though its effect clearly differs from any of known loci. It protracts reproductive stage under the natural and 24-h day length by 7-8 and 30-40 days, respectively, while possessing no effect upon flowering induction. Under field conditions, presence of this allele increases yield by 9-10% and causes early flowering combined with extended reproductive stage. Due to optimal vegetation period structure, it promotes increasing not yield potential only but drought resistance and yield stability as well.

Additional lateral racemes in stem nodes controlled by two independent loci provide second wave of flowering delayed by 7-10 days (5). This trait may be exploited as a mode of escaping from environmental stresses (drought, low night temperatures) causing flower abortion. According to our data, presence and extent of lateral racemes correlate with seed yield (r=0.42, a<0.01).

Approaches to creating genetic variation

Analysis of pedigrees has allowed elaborating modes of parental pair selection to produce promising hybrid populations. Including in crosses late maturing, and even non-maturing in Belarus parental forms leads to progeny lines yield increase reaching 13% compared to hybrid combinations where both parents are early maturing. Maximal yield increase was obtained using MG I parents (Fig. 2). At the same time, involving late maturing germplasm into crosses does not prevent obtaining early cultivars (3).

Effectiveness of selection

Yield estimations may be considered reliable only at final breeding stages organized as replicated trials with multi-row plots. Elite plant selection when genotype performance is obscured by numerous kinds of useless variance ('noise') is the most complicated problem. Decreasing stand density to 20 plants m^{-2} in individual selection nursery reduces ecological competitive variance s^2_{ecom} about 1.5 times. Nevertheless, genotypic differences in competitive ability may cause G E interaction resulting in ranks shuffle in sparse crop. Currently, this problem is under study. At least, relative differentiative capacity of environment S_{ek} for both yield and node number increases by 1.5-1.6 times in sparse crop (20 plants m^{-2}). Therefore, low stand density increases probability of selection of genotypes producing greater amount of mainstem nodes, thus indirectly promoting productive genotypes release (7, Fig. 1).

Yield estimations in single row plots (breeding nursery) are also distorted by competitive genetic variance s^2_{gcom}. Empirical correction factors were proposed to reduce border effects caused by plant height or stand density differences, lodging etc. Application of correction coefficients increases reliability of yield evaluation making it more corresponding to multi-row trial.

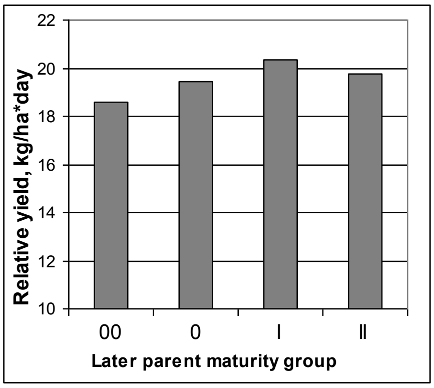

Figure 2. Dependence of soybean cultivars relative yield (expressed as yield / maturity ratio) from parental forms

One-sided breeding for yield potential often results in increased G E interaction and waste of stability (4). That's why preliminary and competitive trials should be organized in several localities differing by soil factors and management level. Thus, final stages of breeding process should be aimed at identification of stable genotypes among lines previously selected for general adaptability. Under the difficult environmental conditions (drought, low agricultural background) selection of lines guiding by traits of plant architecture is recommended. ■

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Soybean: State and perspective of the development in the Ukraine

by V. PETRYCHENCKO, A. BABYCH, S. IVANYUK, S. KOLISNYK and Viktor ZADOROZHNYI*

Abstract: The Ukraine is among nine world's biggest countries producing soybean and it has a great perspective of the extension of its seeding areas for more than 2 mln ha by 2015 producing more than 3,0mln t of seed. Nowadays in the Register of Plant Varieties suitable for cultivation in Ukraine there are more than 100 varieties. The main variety assessment is its productivity. Realization of the genetic potential requires the development and use of appropriate modern cultivation models, research on the regime of mineral nutrition by macro- and microelements, the optimum seeding terms of cold resistant varieties, pre-sowing seed treatment and the problem of drought resistance.

Key words: cold resistant varieties, *Glycine max*, quality, soybean, yield

World market conditions are quite favourable and the demand for soybean products is growing. Such a tendency is typical for Ukraine. At the same time there is sufficient domestic market of soya beans and products of their processing to meet the demands of animal husbandry and food industry. In 2006 Ukraine hold the first rank in Europe. Now it is among world's biggest producers and has a great perspective of the extension of its seeding areas for more than 2 mln ha by 2015 and more than 3,0 mln t of seed. Sowing areas of this crop were extended from 73 thousand ha in 2001 up to 1038 thousand has in 2010. Potential natural, organizational and economic resources of Ukraine are sufficient.

According to natural potential variety policy of soybean cultivation has been formed taking into account bioclimatic potential of Ukrainian regions. Thus, "soybean belt" of Ukraine has been scientifically substantiated by Feed Research Institute, where the zones of the stable and unstable production of the crop on non-irrigated soils have been selected (1).

Nowadays in the Register of Plant Varieties suitable for cultivation in Ukraine there are more than 100 varieties, among them 80% are breed in 8 Institutes of the National Academy of Agrarian Sciences. Due to intensive wok of the breeders from 9 breeding establishments of Ukraine varieties with the productivity level of 4,0-5,0 t ha^{-1}, ultra early varieties of up to 85 days, cold resistant, heat resistant and having better seed quality characteristics: protein content is 43% more, fat content is 24% more, reduced content of trypsin inhibitors and low urease activity and the like have been bred.

The main variety assessment is its productivity. Since yield capacity is a complex index, the components of which are the elements of individual productivity that substantially depend on it, has enabled to breed highly productive varieties due to increase of the tassel size and number of its beans. The sources of the tassel with numerous flowers and stem fasciations gen (f) have been used in breeding program, as a result of which varieties KyVin, Omega Vinnytska (Fig. 1) have been bred; the researchers are Feed Research Institute and the Institute of Soil Management.

Realization of the genetic potential of the above-mentioned varieties requires the development and use of appropriate modern cultivation models. For varieties forming tassel of 12-14 beans and more it is necessary to apply differentiated approach to the regime of mineral nutrition by macro- and microelements taking into account the phase of growth and development of plants (2).

It is offered by the researchers to arrange optimum seeding terms of cold resistant varieties Podilska 1, Podilska 416, Monada 10-14 days earlier towards early varieties that is very important for the rational use of soil moisture. On the basis of long-term researches carried out by Feed Research Institute has been developed a complex composition of preparations for pre-sowing seed treatment using highly effective strains of bulb bacteria, fungicides and biological growth stimulators (3).

The problem of drought resistance of varieties is solved by the strategy of passive drought resistance, particularly due to ultra early varieties and varieties with earlier florescence (researchers are Breeding and Genetic Institute, the Institute of Soil Management, Feed Research Institute and others).

To get high quality soybean seed with the yield increase of 0.5-0.6 t ha^{-1} in sowing late varieties the effect of non-root nutrition and desiccation is studied. ■

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Figure 1. Pod formation in variety Omega: top (left) and axil (right) tassel

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Feed Research Institute, Vinnitsa, the Ukraine
(fri@mail.vinnica.ua)

Soybean in Nigeria: Introduction, production and utilization

by S.R. AKANDE[1], P.O. OYEKAN[1] and Adenubi I. ADESOYE[2]*

Abstract: Soybean (*Glycine max* (L.)) was introduced to Nigeria in 1908. The production and export increased steadily until the civil war. Efforts to revive cultivation of soybean began in 1978 by the International Institute of Tropical Agriculture resulting in the formation of the National Soybean Improvement Cooperative.There has been steady increase in the production to satisfy the need as a protein source in human diet, poultry feed and vegetable oil industry, leadingto the establishment of local foodsindustries and oil mills. The major problem today is producing enough good quality soybeans for numerous industries and at affordable price to household consumers.

Key words: *Glycine max*, soybean, Nigeria, production, use

Soybean (*Glycine max* (L.) Merrill) was first introduced to Nigeria in 1908. The initial cultivation at Moor Plantation, Ibadan, in the humid South West Nigeria was not successful. Later introduction of the crop at Samaru and other less humid Savanna areas of Northern Nigeria from 1937 led to selection of the cultivar "Malayan" for commercial cultivation in the Tiv area of Benue State. The crop was grown exclusively for export with the first export of 9 (nine) tonnes in 1947. The production and export of the soybean cultivar Malayan increased steadily until the period of the civil war in Nigeria when its export was disrupted. As there was no local consumption of soybean, its production sharply declined when the export market for the crop collapsed during the civil war from 1966-1970. Soybean is usually planted in June in the north and July in the southern parts of Nigeria and harvested in October or November. Soybean grain yields of 1.5 to 3.0 tonnes/ha is obtainable with improved varieties. Subsistence farmers grow soybean in intercrop with maize or sorghum. Some of the initial problems against soybean acceptability in Nigeria include late maturity, pod shattering, poor seed storability under farmers' conditions, low yields, restricted market and low pricing.

Efforts to revive cultivation of soybean in Nigeria began in 1978 by the International Institute of Tropical Agriculture (IITA) in collaboration with National Scientists at the Institute of Agricultural Research and Training, (IAR&T), Ibadan, Institute for Agricultural Research (IAR), Samaru and National Cereal Research Institute (NCRI) Badeggi resulting in the formation of the National Soybean Improvement Cooperative Trials in 1980 which later became the Nationally Co-ordinated Research Project (NCRP) on Soybean when NCRPs were set up for a number of crops and commodities.

Multi-locational testing of soybean varieties developed by IITA and other Research Institutes all over the country led to the identification of varieties with desirable traits such as free nodulation with indigenous *Bradyrhizobium,* high yielding, resistance to prevailing various diseases and pests, early to medium maturity and high grain yield. This accomplishment made commercial cultivation of soybean possible across different agro-ecologies of throughout Nigeria with suitable varieties identified for different environments through multi-location trials (1, 2, 5, 7). Although, most of the soybean production in the country is still from Benue State (North Central part of the country). The limitation observed by medium scale farmer now is inadequate processing facilities and lack of mechanical services (6). About 17 (seventeen) improved soybean varieties have been officially released in Nigeria between 1983-2010. Production trend of soybean in Nigeria between 1961 and 2009 is shown in Table 1 (3). The monumental increase in soybean production between 1981 and 1990 (190.67% growth) attested to the efforts of IITA and other National Research Institutes at improving soybean production and local consumption in Nigeria during this period. Since then, there has been steady increase in the production of the crop to satisfy its growing need as a protein in human diet, poultry feed, infant formula and in vegetable oil industry. Soybean is also being recognized for its ability to enhance soil fertility and for its hull providing fodder for livestock.

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[1] Obafemi Awolowo University, Institute of Agricultural Research and Training, Ibadan, Nigeria
[2] University of Ibadan, Department of Botany, Ibadan, Nigeria (aadesoye@yahoo.com)

Table 1. Production trend of soybean in Nigeria between 1961 and 2009. (FAO, 2011)

	Production		Land area	
Year	Mean value (t)	Growth (%)	Mean value (t)	Growth (%)
1961 -1970	62,111		178.700	
1971 –1980	68,556	29.31	220,000	23.11
1981 – 1990	118,889	190.67	371,000	68.64
1991 – 2000	310,000	96.78	535,850	44.43
2001 – 2009	541,464	42.19	589,889	10.09

Table 2. Proximate chemical composition of some grain legumes in Nigeria (Fasoyiro *et al.,* 2006)

Samples	Protein	Fat	Fiber	Carbohydrate
Bambara groundnut	22.06e	2.02gh	2.31g	55.63a
African yam bean 1	24.19cd	5.04c	7.20a	50.02bc
Lima bean 1	25.01c	3.05d	1.98h	51.64b
African yam bean 2	23.97cd	2.48f	6.69b	50.30bc
Pigeon pea 1	25.98b	1.91h	4.62d	48.31cd
Lima bean 2	24.90c	2.92e	2.07h	50.44bc
Pigeon pea 2	26.06b	2.36g	4.06e	49.40bc
Cowpea	23.87cd	1.50i	3.93f	55.93a
Soybean	37.27a	17.79b	5.05b	25.19e
Groundnut	27.01b	45.81a	3.03f	13.74f

The nutrient contents of soybean are as follow: protein 37.27%, Fat 17.79%, carbohydrate 25.19% and fiber 5.05% (4). Comparatively, soybean is richer in protein content than any other grain legume cultivated in Nigeria (Table 2). It has a good balance of the essential amino acids and excellent sources of vitamins and minerals. Soybean is also rich in good quality oil with low level of cholesterol. The rising cost of animal sources of protein like meat, egg, fish and milk has led to inadequate supply of protein in human diet thus search for alternative source for inexpensive protein. In view of the high nutrient content of soybean, the Nationally Coordinated Research Project on soybean also undertakes research and promotion of local utilization of soybean in Nigeria and has come up with several local uses of soybean. This led to the establishment of cottage industries producing local foods and small to medium size soybean oil mills producing vegetable oil and soybean meal.

Soybean is particularly utilized as diary analogs (e.g. soymilk, yogurt, soy-cheese). It is processed into soy-ogi, a high protein gruel for healing malnourished children and a fermented paste used as flavouring in soups and stew (dawadawa or soy-iru). Soygari, a cassava and soybean mix that tastes the same as gari (traditionally made from fermented cassava only) and has 10-20 times more protein than traditional gari has also been developed., Hence, soybean that was once a minor localized export crop and known only in Benue State is now widely cultivated and consumed in Nigeria. The major problem of the crop in Nigeria today is producing enough good quality soybeans to meet raw materials needs of the numerous soybeans processing industries and making it abundantly available at affordable price to household consumers. ■

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Broadening environmental adaptation of soybean in Australia

by Robert J. LAWN[1]* and Andrew T. JAMES[2]*

Abstract: Breeding resources for soybean in Australia are limited compared with more widely grown crops and there has been strategic interest in broadening the environmental range of individual varieties.Breeding efforts to improve adaptation across regions and seasonsare focused on the use of the long-juvenile trait to develop short duration, less photo-sensitive cultivars and backcrossing the trait into temperate cultivars for (sub)tropical adaptation. Current research is exploring possibilities of molecular markers to help select potentially drought tolerant genotypescombining strong expression of three physiological traits. Saturated soil culture technique has been adapted to enable rice-soybean intercrops using a bed-furrow system in lowland rice areas in south-east Asia.

Key words: adaptation, drought, *Glycine max*, saturated soils, soybean

Commercially significant soybean production in Australia began in the eastern subtropics in 1969-70. Production subsequently expanded north into the tropics (lat. 14° S) and south into warm temperate regions (lat. 36° S). Today, soybean is cropped in both dryland and irrigated systems, usually in rotation with winter cereals, but occasionally in rotation with sugarcane. Where irrigation is available in the tropics, temperatures are warm enough to enable dry season or 'winter' crops. Despite the range of environments, the annual production area has never exceeded 100,000 ha nationally. Breeding resources for soybean are therefore limited compared with more widely grown crops and there has been strategic interest in broadening the environmental range of individual varieties.

Improving adaptation across regions and seasons

Initial attempts to grow soybeans in Australia, using varieties and agronomy from the southern USA, were characterised by variable biomass production, generally low seed yields, poor seed quality, and large variety x environment interactions. Much of the initial research in Australia therefore focused on understanding environmental constraints to adaptation of soybean in the subtropics and tropics, and applying this knowledge to develop appropriate management and to breed better adapted varieties (1). This early research showed that the time to flowering of this short-day plant, and thus yield potential per plant, varied depending on latitude, sowing date and varietal maturity, because of differences in response to photoperiod and temperature.

In general, at any given latitude and sowing date, time to maturity of early flowering genotypes was less sensitive than later flowering genotypes to the prevailing photoperiods. Movement of varieties from higher to lower latitude made them earlier flowering because midsummer days were shorter. Likewise, sowing varieties later in summer caused them to flower sooner because they encountered shorter photoperiods sooner after sowing than when sown in spring. Sowing date was often necessarily delayed either by harvest of the preceding winter crop, or by late sowing rains. While harvest index (HI) was enhanced where photothermal conditions promoted rapid flowering, the increases were often not enough to offset the reduction in vegetative growth per plant(2). Somewhat narrower rows and higher plant populations were therefore used than were common in the USA.

Five breeding programs were initially established in Australia to respond to the challenge of balancing the need for local adaptation to latitude and planting window with sufficiently short duration to permit rotation with a winter cereal. In part, this was an importation of the narrow-adaptation concept from the USA, albeit based on shorter duration and somewhat narrower row spacing than the vegetatively-vigorous, full season (150 d) varieties used in the USA. However, it enabled soybean production to be established in Australia and laid the foundation for the recent consolidation of the breeding effort into a single program based on the use of the long-juvenile (LJ) trait to develop short duration, less photo-sensitive cultivars suited to high plant populations. Ordinarily, in the short days of the tropics, temperate soybean varieties are relatively photoperiod-insensitive and flower precociously. However, when the LJ trait is backcrossed into temperate genotypes, their time to flowering is delayed by an average of two weeks while their relative insensitivity to subtropical photoperiods remains unchanged. This discovery opened up the potential for exploiting quantitative breeding advances in temperate regions, by backcrossing the LJ trait into temperate cultivars to convert them to subtropical / tropical phenological adaptation.

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[1]James Cook University & CSIRO Plant Industry, ATSIP, Townsville, Australia
(robert.lawn@jcu.edu.au)
[2]CSIRO Plant Industry, QBP, St Lucia, Australia
(andrew.james@csiro.au)

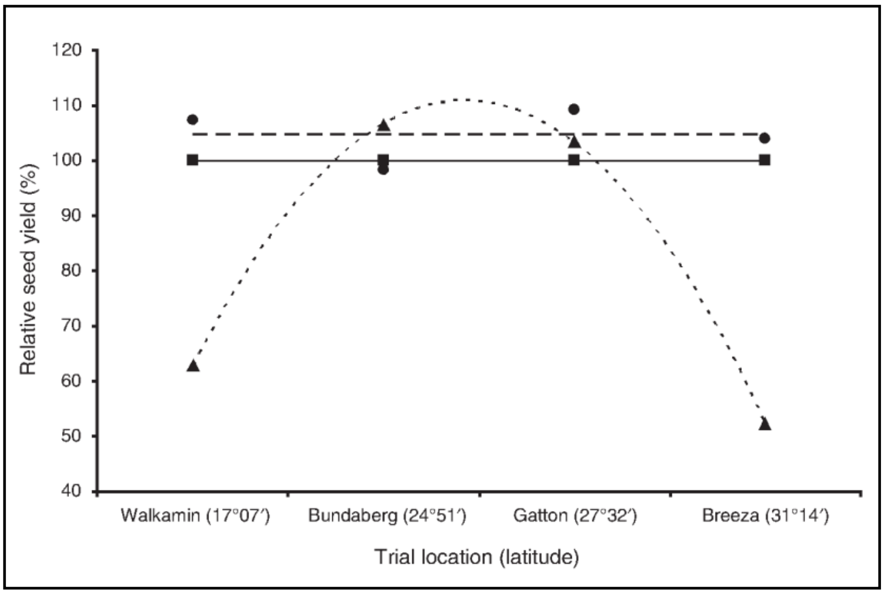

Figure 1. Seed yield of LJ semi-dwarf cv. Bunya (•) and photoperiod-sensitive cv. Fraser (▲),relative to a high-yielding adapted local cultivar (■) at four locations in eastern Australia (Adapted from James & Lawn 2011)

Proof-of-concept was achieved by converting high yielding, semi-dwarf, lodging resistant cultivars from Ohio in the USA to genotypes adapted to the Australian subtropics (4). The same strategy was used to adapt traditional culinary varieties from east Asia to subtropical Australian environments and several locally-adapted, LJ culinary soybean varieties are now grown in subtropical Australia. The LJ varieties are generally earlier maturing (105-115 d) than previous photoperiod-sensitive cultivars (> 120 d). As such, optimal plant populations are higher. However, because of their reduced photoperiod sensitivity, they can be grown across a wider range of latitudes (e.g. see Figure 1) and sowing dates. Only two or three cultivars from the single national breeding program are now needed to span the wide latitudinal range over which soybeans are grown in Australia.

Drought tolerance

Australia is a generally dry continent and drought is a major constraint to soybean production in rainfed areas. Therefore, there has been some research on physiological traits for tolerance to drought stress (3). Comparative studies showed that, as drought progresses, tolerant tropical grain legumes like pigeonpea and cowpea retain more green leaf area for longer than soybean. As a result, they are able to recover more effectively when the drought is broken. Three physiological traits have been shown to affect leaf survival during drought: (i) epidermal conductance, which is a measure of the rate of epidermal water loss after stomata have closed; (ii) active osmotic adjustment, which enhances the capacity of the roots to draw water from drying soil; and (iii) lethal relative water content (RWC), which is a measure of the minimal water content that leaves can tolerate.

Genetic studies indicate moderate to high heritability so all three traits may be susceptible to breeding and selection pressure (3). Epidermal conductance is the most stable, and can be measured on well-watered plants. However, measurement of osmotic adjustment and lethal RWC requires that the plants be stressed. Moreover, the expression of both traits is affected by the severity of the drought. Physiological studies have shown that soybean genotypes combining strong expression of all three traits recover more effectively after the relief of very severe water stress (5). However, these traits are time-consuming to measure and difficult to handle in a breeding program. Current research is therefore exploring whether molecular markers can be developed to help select prospective genotypes.

Saturated soil culture (SSC)

At the other extreme, research in Australia has highlighted ability of soybeans to acclimate to and grow well in saturated soils (2). The main requirements for acclimation to SSC are that the seedlings plants are not exposed to SSC until about two weeks after emergence and the water table is maintained constant at 5-10 cm below the soil surface. In practice, this is achieved by growing the crop on soil beds separated by water-filled furrows. After an initial acclimation period of 5-15 days, the plants recover and grow faster than well-watered controls. The leaf water status of acclimated SSC plants, as indicated by RWC and leaf water potential during the heat of the day, remains more favourable than in normally irrigated crops. Leaf area duration is also greater, while active nodule function and nitrogen assimilation continue for longer into pod filling.

In variety trials, the seed yields of SSC soybeans have averaged around 20% higher (range 2-49%) than fully irrigated controls. Because water is limited in Australia, SSC has not been commercially adopted. However, there is interest in lowland rice areas in south-east Asia, where the SSC technique has been adapted to enable rice-soybean intercrops using a bed-furrow system (see Figure 2). SSC soybeans have also been used as waste water treatment crops in the USA. The ability to acclimate to SSC also occurs in the wild annual progenitor of soybean, *Glycine soja* which grows wild on rice bunds in eastern Asia, but not in the Australian native perennial *Glycine* species. This suggests that the trait is ancestral and was retained during the domestication of soybean in rice-based agriculture of eastern Asia. ■

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Figure 2. Soybeans in saturated soil culture being intercropped with paddy rice in south-east Asia)

Forage soybeans

by Thomas E. Devine[1]* and Adrian CARD[2]

Abstract: Since the early 1900's the USDA conducted a successful research to develop better adapted forage soybean lines with resistances to pests, acceptable seed shattering, tall growth, lodging resistance, rapid drying,high biomass production, as well as dual purpose soybeans.Forage soybean program aimed at developing lines that would produce seed in a more southern location and the forage in a more northern location, considering the balance between quality and biomass yield at the different time of harvest and cutting height.The additional value of the crop is its capacity to make productive growth during the summer heat and its straw to provide protect the soil from erosion.

Key words: biomass, forage, *Glycine max*, quality, soybean

Domesticated Soybeans, as a large seeded legume, have provided nutritional protein for millions of people in China, Korea, and Japan for many centuries. In the late 1800's and early 1900's soybeans were used in the United States principally as forage for livestock. As many as 1,000,000 acres were planted for this purpose. Only in 1941 did the acreage grown for grain exceed that grown for forage in the US. In the 1900's the USDA conducted a successful research and development program to develop soybean as a grain crop to provide oil and protein. These breeding efforts resulted in better adapted lines with resistances to the biological pests encountered in the US. The use of soybean for forage declined due to difficulties in drying the forage for hay. Technology has provided machinery for crimping forage to promote more rapid drying and better methods of ensiling forage thus reducing the degree of drying needed to preserve the forage. Prompted by these developments and a request from a dairy farmer, Thomas Williams, in Pennsylvania, in the mid 1970's the research arm of the USDA initiated a breeding program to breed soybeans for forage at Beltsville, Maryland.

Four lines were selected for use as parents to start this breeding program: Forest, Wilson 6, Perry and a breeding line from Illinois L76-0523. These lines were intercrossed by conventional hybridization to produce a population for selection. The selection procedure was early generation phenotypic selection. Several lines emerged from the initial round of selection, but when tested in the field were found to have unacceptable seed shattering. This required a second round of hybridization to grain type cultivars from the mid western region of the US and subsequent selection among the progeny. From this program emerged lines with exceptional biomass production. After testing for production at several locations in the US three lines were released by the USDA as forage cultivars. These were 'Donegal' with maturity group V, 'Derry' with maturity group VI, and 'Tyrone' with maturity group VII. Soybeans are photoperiodic and the maturity groups indicate the time of flowering and seed maturation. Donegal was released for use in the northeastern US while Derry was released for the Midwestern region and Tyrone for the southeastern region (1). These cultivars were each characterized by tall growth (5 to 6 feet, Fig. 1), lodging resistance in the locations of adaptation, and high biomass production. Rather that waste the potential for forage production late in the season, when grain type soybeans would cease growth, drop leaves and dry down for mechanical harvest, the intention in the forage soybean program was to produce lines that would produce seed in a more southern location and produce the forage in a more northern location. This would allow a longer season for forage production in the northern location.

Later, after additional hybridization and selection, another cultivar named Tara, with maturity group V, was released for use as a dual purpose soybean which could be used for both grain production and forage production (2). Later, still, another cultivar named Mooncake was released for use as an edamame vegetable soybean particularly useful for organic vegetable production because of its exceptionally tall growth and ability to compete with late season weeds. These soybean cultivars have been licensed by the USDA office of technology transfer to seed producers and used by farmers. Tyrone has been of particular value in the southeastern US where plant protein has been difficult to obtain with traditional perennial crops such as alfalfa because of their susceptibility to plant diseases in the warm humid climate.

In addition to the use of these high biomass producing cultivars as forage, there has been interest in their use for summer green manure production. Field tests in Boulder County, Colorado in 2009, under summer irrigation, indicated that experimental lines of the high biomass forage soybeans provided 192 lbs of nitrogen per acre, substantially more than the cowpea and Lab-lab lines tested. Extension specialist suggested that the forage soybeans could be used to provide green manure nitrogen in rotation with crops such as spinach, turnips, kale, garlic, winter wheat or triticale in Colorado.

The tall growing cultivar Mooncake can provide the high value product edamame pods for human consumption. Edamame pods are increasingly desired by elegant restaurants in the US. After the green pods have been stripped from the plants the remaining green leaves and stems can be used as forage for cows, sheep, goats, rabbits, etc. or as green manure to enrich the soil. Currently, seed of Mooncake has been increased by seedsmen for large scale planting to produce sufficient edamame for processing in cans by a food products company in the northeastern US.

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[1] USDA, ARS, Sustainable Agricultural Systems Laboratory, Beltsville, USA
(thomasdevine@live.com)
[2] Colorado State University Extension, USA

As with other crops, the forage yields produced by the forage cultivars vary with weather conditions, soil fertility and length of the growing season. Soybeans are a warm season plant. In many areas of the US perennial forage legumes, such as alfalfa and red clover, typically produce their highest forage yields in the spring when temperatures are mild. During the moisture stress associated with the heat of the summer, yields of these perennials are reduced. In the autumn, forage production recovers somewhat. In contrast, soybeans have the capacity to make productive growth during the heat of the summer, thus distributing legume production more evenly over the season.

Many dairy farmers rely on alfalfa as an important source of legume forage for their cows. However, severe winter conditions can result in stand loss and unplanned deficits of high protein legume forage. Planting a large seeded heat tolerant legume such as forage soybeans can compensate for these deficits.

Questions arise as to when forage soybeans should be harvested. Several factors influence this decision. As with other forage crops, earlier harvest provides higher quality forage (higher protein and higher digestibility). In the case of lactating dairy cows a higher premium is placed on forage quality than in the case of beef cattle. Earlier harvest will usually result in lower biomass yield. Thus, the balance between quality and biomass yield is a consideration. In the case of lactating dairy cows, harvesting a week or two after the start of flowering seems a good point of compromise. The quality of the forage harvested can be controlled by the farmer not only by selecting the stage of growth for harvest but also by determining height at which the cutter bar is set. Setting the cutter bar closer to the ground will result in higher biomass yield, but will also result in the harvest of more lower quality stems, that are more lignified and subarized, than growth higher on the plant. Setting the cutter bar higher will result in harvest of higher quality forage with a higher proportion of leaves and lower portion of stem. In addition, leaving more of the lower quality stem stubble on the soil surface provides more organic matter for the soil and reduces the vulnerability to soil erosion due to wind and rain.

In practical terms the farmer will be motivated to harvest forage soybeans when he needs the forage and when he has time to do so. In the US, maize is the high priority crop and farmers will harvest soybeans when they are not occupied with maize operations. If a farmer encounters a drought situation, he may need to harvest promptly to provide forage for his livestock. In such circumstances the farmer may graze the forage.

It is well recognized that the conventional soybean cultivars presently grown for grain production do not provide adequate crop residue to protect the soil from erosion by wind and/or rain (3). In this respect soybean is a weak link in the corn soybean rotation. The high biomass cultivar Tara was released by the USDA as a multipurpose cultivar that could be utilized for both forage and grain production. When used for grain production the high biomass straw produced provides more crop residue to protect the soil surface from erosion. In addition, the pods are set higher on the plant thus permitting the cutting bar to be set higher. As a result the upright stubble remaining on the soil surface impedes the horizontal movement of wind and water across the surface. ■

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Figure 1. T.E. Devine by a typical forage soybean cultivar

Soybean intercropped with sunflower is an efficient solution to increase global grain production in low input systems

by Laurent BEDOUSSSAC[1,2]*, David CHAMPCLOU[2], Hélène TRIBOUILLOIS[2], Grégory VERICEL[2], Nathalie LANDE[3] and Eric JUSTES[2]

Abstract: Intercropping (IC) could be efficient and lead to eco-functional intensification. The main objective was to evaluate the potential advantages of soybean-sunflower IC for global grain yield. Based on recent experiments, we demontrated that the LER could be significantly higher than 1 when the cover pattern is adapted, i.e. for 2 sunflower rows combined with 4 soybean rows pattern (LER = 1.2). Finally, we confirm that IC is efficient only in low input systems, even for summer crops.

Key words: intercrop, land equivalent ratio, low input systems, N niche complementarity, soybean, sunflower, yield

Intercropping (IC), i.e. growing them simultaneously in the same field during a significant part of the time, could be efficient for grain production based on eco-functional intensification (1, 7). IC efficiency is based on species complementarity in resource use (light, C, N, nutrients and water) due to differences in aerial and root system architecture, growth dynamics and niche complementarity, thanks to the ability of the legume to fix atmospheric N_2 in legume-cereal IC (2, 5). Many studies have shown advantages of legume-cereal IC in comparison to their sole crops (SC) in low input systems (2, 5), while few papers are available about sunflower-soybean IC (4, 6). The main objective of our study was to evaluate the potential advantages of soybean-sunflower IC for global yield in a wide range of conditions.

Three experiments have been carried out at CETIOM and INRA (SW France) in 2010 and 2011 with cultivars of different precocities to modify species maturity gap (both species sown at the same time). Two spatial patterns were sown, alternating 2 sunflower rows with 2 or 4 soybean rows (named 2/2 and 2/4 respectively). Grain yield and accumulated N were measured together with the percentage of N derived from N_2 fixation (%Ndfa). The land equivalent ratio (LER) was calculated as a simple and relevant indicator of IC efficiency (3, 5, 7).

The IC best performances were obtained with no or low amounts of fertilizer-N and without irrigation. This was partially due to the significant high %Ndfa of the soybean in IC (Fig. 1) leaving most of the soil mineral N available to sunflower. The 2/4 pattern was more efficient than sole crops (LER=1.16 on average; significantly > 1; Fig 2) and particularly when combining the latest soybean and the earliest sunflower (LER=1.21) because it allowed to improve specie complementarities in time and space (at the cover level). Conversely, for the 2/2 pattern or the high N input conditions, LER was lower or equal to 1 due to strong interspecific competition of sunflower on soybean, in particular for light and water.

These results confirm that IC is particularly suited to low N input systems due to the complementary use of N sources by the two species. However, before proposing optimised cropping systems further research is still needed on genotype x environment interactions in order to analyse the effects of: i) precocity, ii) aerial architecture, iii) row structure and iv) N management, on IC performances. ■

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[1] ENFA, F-31320 Castanet-Tolosan, France (Laurent.Bedoussac@toulouse.inra.fr)
[2] INRA, UMR1248 AGIR, F-31320 Castanet-Tolosan, France (Eric.Justes@toulouse.inra.fr)
[3] CETIOM, F-78850 Thiverval Grignon, France

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Figure 1. The '2 sunflowers /4 soybeans' intercrop pattern and the 'low input' farming system seem to be an economically suitable combination for farmers

Intercropping soya bean with other annual legumes for forage production

by Branko ĆUPINA[1]*, Aleksandar MIKIĆ[2], Vuk ĐORĐEVIĆ[2], Vesna PERIĆ[3], Mirjana SREBRIĆ[3], Vojislav MIHAILOVIĆ[2], Đorđe KRSTIĆ[1], Svetlana ANTANASOVIĆ[1]

Abstract: A common idea of intercropping annual legumes with each other for forage production was jointly developed by the Institute of Field and Vegetable Crops and the Faculty of Agriculture in Novi Sad. In such intercrop models, soya bean plays the role of supporting crop. Intercropping soya bean with supported warm-season annual legumes successfully solves the problems with weeds. At the same time, most combinations produce 8-10 t ha^{-1} of forage dry matter.

Key words: annua;l legumes, forage, *Glycine max*, intrecropping, LER

Soya bean (*Glycine max* (L.) Merr.) is a warm-season multi-purpose annual legume crop, primarily used for the extraction of oil and protein and with a considerable potential for forage production.

A common idea of intercropping annual legumes with each other for forage production was jointly developed by the Institute of Field and Vegetable Crops and the Faculty of Agriculture in Novi Sad. Such intercrops are carefully designed in respect to four basic principles: i) the same time of sowing, ii) the similar growth habit, iii) the similar time of maturing for either cutting the forage or harvesting the grains and iv) one component has a good standing ability and plays the role of supporting crop, while another one is lodging susceptible and is considered supported crop (1).

A series of trials have been carried out with diverse combinations of both cool and warm season annual legumes since 2008 in Novi Sad and Belgrade.

In such intercrop models, soya bean plays the role of supporting crop. Early genotypes, belonging to 00 maturity group (MG), are intercropped with mung bean (*Vigna radiata* (L.) R. Wilczek, Fig. 1), adzuki bean (V. *angularis* (Willd.) Ohwi & H. Ohashi) and black gram (*V. mungo* (L.) Hepper), while late ones such as I MG are intercropped with cowpea (V. *unguiculata* (L.) Walp.) and lablab bean (*Lablab purpureus* (L.) Sweet, Fig. 2).

Intercropping soya bean with supported warm-season annual legumes successfully solves the problems with weeds. At the same time, most combinations produce 8-10 t ha^{-1} of forage dry matter and are economically justified with the Land Equivalent Ratio (LER) values higher than 1. ■

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Figure 1. Intercropping 00 MG soya bean (cv. Gracia) with mung bean

Figure 2. Intercropping I MG soya bean (cv. Laura) with lablab bean

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[1] University of Novi Sad, Faculty of Agriculture, Department of Field and Vegetable Crops, Novi Sad, Serbia (cupinab@polj.uns.ac.rs)

[2] Institute of Field and Vegetable Crops, Novi Sad Serbia

[3] Maize Research Institute Zemun Polje, Belgrade, Serbia

Organic soybean production

by Svetlana BALEŠEVIĆ-TUBIĆ* and Vojin ĐUKIĆ

Abstract: Seed production in organic agriculture is significantly different from conventional production, given that certain segments of the technology of growing such as fertilization, crop protection, pesticide seed treatment are paid special attention. Seed is inoculated by microbiological fertilizer NITRAGIN containing nitrogen fixing bacteria *Bradyrhizobiumjaponicum*prior to sowing in the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS) to ensure the higher yield and the quality of grain protein. Long-term selection in the IFVCNS has led to the development of high-yielding varieties differing in maturity and resistance to diseases, contributingto the pesticides-free production and the proper selection of varieties.

Key words: *Glycine max*, nitragin, organic production, seed production, soybean

Institute of Field and Vegetable Crops, Novi Sad, Serbia (svetlana.tubic@nsseme.com)

In the light of global climate changes on the Earth, organic production and organic products are gaining more and more popularity, while conventional production must comply with consumer safety and environmental protection. In contemporary programs and agricultural development strategies, the special attention is paid to the protection of biodiversity i.e. organic agriculture, based on sustainable management of natural resources, products of special quality, and environmental and consumer protection.

Each crop production, including organic starts with breeding, or development of cultivars. Modern breeding methods use biotechnology methods, such as modification of DNA, protoplast fusion and similar techniques that are not acceptable in organic production. Across the globe more and more attention is paid to techniques that are not based on genetic engineering, but on mutations similar to those that occur in nature, so called "green gene" technology.

The introduction of the above mentioned technologies creates conditions for the use of a wider range of varieties and hybrids in organic production. Interest in new approaches to the selection occurs with the aim to broaden the genetic base of varieties, which are: significant expansion of genetic base developed in selection programs; reduction of genetic erosion using the population approach to the selection; increase in the frequency of gene with a minimum loss of genetic variability through the feedback selection.

Seed production in organic agriculture is significantly different in comparison to conventional production, given that certain segments of the technology of growing such as fertilization, crop protection, pesticide application in the treatment of seeds are paid special attention.

Figure 1. Microbiological fertilizer Nitragin

Table 1. Yield and quality of soybean seed under conventional and organic production

Variety	Yield (t ha^{-1})		Protein content (%)		Oil content (%)	
	Conventional	Organic	Conventional	Organic	Conventional	Organic
Galina	4.81	4.55	38.65	38.76	20.83	20.78
Sava	4.63	4.15	39.31	38.86	21.95	22.16

Soil quality is the main factor of the crop quality and land must have adequate levels of available nitrogen for production of high quality protein. The high content of organic matter and efficiency of inoculants are the result of high nitrogen fixation and high protein content. Soybean in organic production has many advantages over other field crops. When it comes to seeds for planting, it can be said that the soybean seed is not treated during processing, and that is the basic prerequisite for organic production.

Fertilization is one of the critical moments in organic agriculture, especially in seed production, when in addition to high yield, the high quality of produced seed should be ensured. Due to the high content of protein soybean has a high demand for nitrogen. The major part of nitrogen in soybean as a leguminous plant is provided by nitrogen fixation.

Soybean seed is inoculated by microbiological fertilizer NITRAGIN containing nitrogen fixing bacteria *Bradyrhizobium japonicum* just prior to sowing in the Institute of Field and Vegetable Crops in Novi Sad. Regular use of biological fertilizers, i.e. the provision of nitrogen fixation by nodule bacteria, and the plowing of the crop residues immediately after crop harvest, contributes to the establishment of a positive N balance in the soil. Numerous studies confirmed that the inoculation of seeds with Nitragin significantly increases the yield (up to 20-50%), and the quality of grain protein (2).

Long-term selection in the Institute of Field and Vegetable Crops in Novi Sad has led to development of the high-yielding varieties differing in maturity, resistance to disease, which has contributed to the practice of growing without the use of pesticides and provided the proper selection of varieties. Problems with diseases and pests in soybean, in our growing regions, are not very pronounced but the crop rotation must be taken into account. This is one of the main preconditions for the possibility of producing seeds without the use of pesticides for protection against diseases and pests. Weeds are the major problem in organic production of soybean seed. However, by a proper and timely application of cultivation practices, tillage and seedbed preparation and optimal planting density, the weed can be maintained at the level that makes no significantly impact on the soybean yield.

Obtained results relating to the yield and protein content, as one of the important chemical properties of soybean, showed no statistically significant differences in organic production, compared to soybean seed under conventional production (1). This can certainly be achieved only if cultivation practices, recommended for organic agriculture, is followed. ■

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Soybean

SOYBEAN, second edition

Editors: Jegor Miladinović, Milica Hrustić, Miloš Vidić

Published by: Institute of Field and Vegetable Crops, Novi Sad and Sojaprotein, Bečej, Serbia

This is an English edition of the book entitled SOJA published in Serbian language, which displayed the accumulate knowledge on fundamental and applied soybean research, as well as soybean breeding effort at Institute of field and vegetable crops, Novi Sad. Simultaneously with increased in growing this plant species, scientific research on soybean has also been enhance, especially regarding fundamental research. For the most part, this book deals with achievements of Serbian researchers. Therefore, the authors would particularly recommended to foreign readership the chapters dealing with quantitative and qualitative genetics of soybean prepared for this edition by leading experts and professors from North Carolina State university, Iowa State University and University of Minnesota. First edition of this book was published in 1998, only on Serbian language. Updating knowledge on different aspects of soybean, second edition was published 2008. This is unique book in Europe, where 23 experts present most recent scientific knowledge of different aspects on soybean, classified in 14 chapters.

Hence we believe that this edition will be useful to everyone involved in soybean production, especially to students and scientist conducting research on soybean. ■

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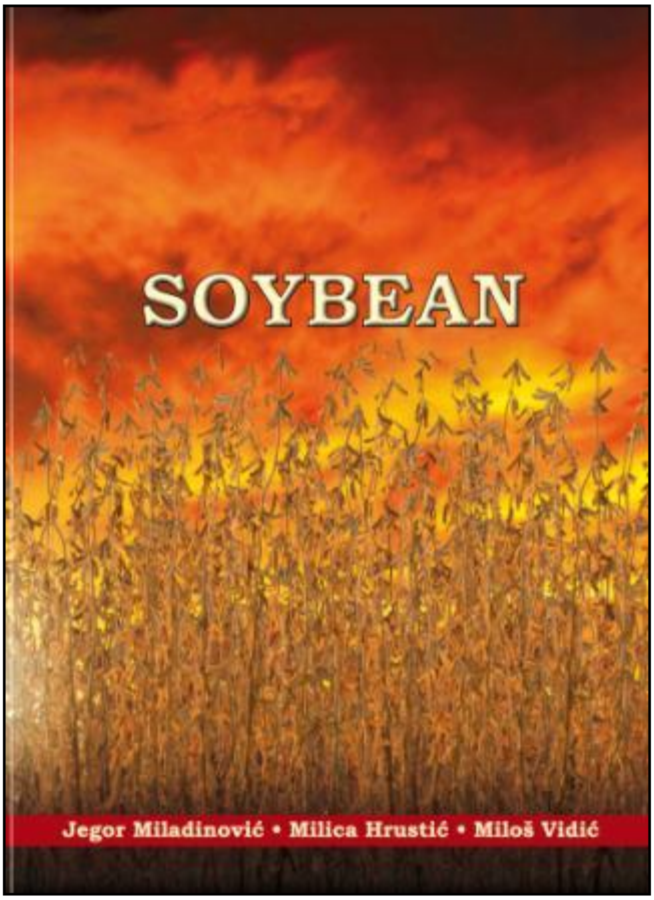

First Legume Society Conference *2013: A Legume Odyssey*
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We are cordially inviting you to participate in the First Legume Society Conference (LSC1), scheduled from May 9 to 11, 2013 in Novi Sad, Serbia, and organised by the Legume Society and the Institute of Field and Vegetable Crops in Novi Sad.

In the rich world of global agriculture, diverse legumes can play key roles to develop environment-friendly production, supplying humans and animals with the products of high nutritional value.

The Legume Society was founded in 2011 with two primary missions. One of them was to treasure the rich legume research tradition of the European Association for Grain Legume Research (AEP), with emphasis on carrying out its the triennial legume-devoted conferences. Another one is to fulfill a long-term strategy of linking together the research on all legumes worldwide, from grain and forage legumes pharmaceutical and ornamental ones and from the Old World to the Americas.

We do anticipate that your participation will be a unique and genuine contribution to our common goals: to promote the legume research and all its benefits into all spheres of the society, linking science with stakeholders and decision-makers, and to demonstrate how an efficient, useful and firm network of the legume researchers of the world is possible and sustainable.

Please include this event in your busy agenda and share this information with all your colleagues dealing with legumes. If you have not already joined the Legume Society, please do it for a free membership in 2013 by visiting the LSC1 web site at http://lsc1.nsseme.com.

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