NEW TRENDS IN PLANT BREEDING – EXAMPLE OF SOYBEAN

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Soybean breeding and selection is a continual process designed to increase yield levels and improve resistance to biotic and abiotic stresses. Soybean breeders have been successful in producing a large number of varieties using conventional breeding methods, the Single Seed Descent method in particular. In recent decades, with the increased use of genetic transformations, backcrossing is more frequent though the only trait that has been commercialized is glyphosate tolerance. Physiological breeding poses a particular challenge, as well as phenotyping and development of useful criteria and techniques suitable for plant breeding. Using modern remote sensing techniques provides great opportunity for collecting a large amount of physiological data in real environment, which is necessary for physiological breeding. Molecular based plant breeding methods and techniques are a conceptual part of any serious breeding program. Among those methods, the most extensively used is marker-assisted selection, as a supplement to conventional breeding methods.
Key words: breeding methods, crop phisiology, molecular markers, soybean

INTRODUCTION

Soybean breeding and selection is a continual process by which yield levels are increased and resistance to biotic and abiotic stresses is improved. Soybean is a self-pollinated legume with natural outcrossing of less than 0.5 to approximately 1% (CARLSON and LERSTEN, 1987). As a result of its self-pollinating reproductive behavior, conventional plant-breeding procedures such as backcrossing, single pod descent, pedigree breeding, and bulk population breeding are some of the more common procedures used in order to develop improved varieties of soybean (POEHLMAN and SLEPER, 1995). All of these procedures involve making crosses or hybrids by hand pollination followed by selection, testing, and ultimately release of a superior soybean variety. Soybean yield has increased over time in response to improved genetics and agronomic practices. In side-by-side comparisons, genetic gain was estimated to be 19 to 23 kg ha–1 yr–1 for cultivars released before

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1977 (SPECHT and WILLIAMS, 1984). SPECHT *et al.* (1999) summarized a number of previous genetic gain studies, and based on these studies, reported that the average annual increase in soybean yield due to genetic improvements ranged from 10 to 30 kg ha−1 yr−1 and today the yield potential of many modern cultivars is greater than 6700 kg ha–1 (COOPER, 2003).

Various studies have shown that genetic improvement for yield potential of soybean has been achieved through increased lodging resistance, increased stability across a wide range of environments, increased tolerance to water stress, adaptation of improved cultivars and production methods, increase in athmospheric CO_2 concentration, greater nitrogen fixation, supplying more assimilates during seed filling period, better tolerance to stress of high plant populations, and increased resistance to major pathogens (PATHAN and SLEPER, 2008), or even adjusted planting date (ROWNTREE *et al.,* 2013).

Conventional breeding methods

In general, each breeding method that leads to genetic improvement begins with choosing the parents or starting material which will be used to create segregating populations. In the following stages, those populations are advanced toward homozygosity without selection or with selection which may involve various techniques applied in order to produce relatively homozygous lines that are further subject to yield and other trait evaluations. The breeding method (or cycle) is complete when the best line(s) are released as improved pure-line cultivar(s) or improved germplasm. A pure-line cultivar is the one grown by the farmer (ORF, 2011). There are many different breeding methods that soybean breeders and geneticists use for cultivar and/or germplasm development. The choice of the selection method depends on the goal of the breeding program as well as on other important factors, such as variability one has at their disposal, availability of agricultural machinery and greenhouses, the number of personnel and their level of training, and so on. Soybean breeding employs methods that are also used in the selection of other self-pollinated crop species. These include pedigree selection, single seed descent, the bulk method, the early generation testing procedure, and backcrossing. With the discovery of genetic male sterility, recurrent selection has become another useful tool for developing new soybean varieties (HRUSTIĆ *et al*., 1997; WILCOX, 1998; ORF *et al*., 2004). Recurrent selection is a cyclic method of population improvement but does not directly lead to the release of cultivars; thus further breeding efforts are needed in order to release a cultivar from a recurrent selection population.

The backcross method is most often used in cases when a certain trait, such as disease, resistance needs to be incorporated into a good standard variety that is widely grown for commercial purposes, so that the said variety would be able to maintain its share on the market. The goal, therefore, is not to develop a new cultivar but to improve a pre-existing one that is of good quality. The term backcrossing, refers to the repeated crossing of hybrid progeny back to the recurrent parent.

The method of early generation testing is similar. This method is used when a cultivar containing a particular trait needs to be developed fast. Testing is conducted as early as the F2 generation and a large number of potentially good genotypes are discarded early (COOPER, 1990).

The bulk method is the most economical way of obtaining homozygous lines after hybridization. The method involves obtaining the next generation by planting a large number of seeds, harvesting the plants in bulk, and planting a sample of the seed the following year. The advantage of this approach is that it enables growing a larger number of crosses without the need

for a lot of labor, observation, and selection. Two major disadvantages of this type of selection are loss of genetic variability in each subsequent generation due to the use of an inadequate sample, and the possibility that natural selection occurring in the population may take an undesirable turn (EMPIG and FEHR, 1971).

The pedigree method of soybean selection consists of growing progenies of crosses through generations of self-pollination by growing rows of progenies of plants selected in each generation. The selection is based on phenotypic traits, with the pedigree of each line maintained in the subsequent generations. This method is useful for evaluating progenies of crosses between phenotypically different parents, since it enables identification and removal of a large number of undesirable progenies in the early generations, leaving a high frequency of superior lines for final selection in the later generations. Unlike bulk selection, the pedigree method reduces competition among different genotypes to a minimum, and eliminates its relevance for the success of the selection process. The greatest disadvantage is that this method is labor-intensive and requires a lot of manpower, which makes it highly unsuitable for handling a larger number of crosses. In addition, pedigree selection involves constant selection of a certain number of heterozygotes that would have become homozygous in the later generations even without the breeder's intervention.

The single seed descent method was proposed by BRIM (1966) 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. Modified version of SSD method is also used in the soybean program of the Institute of Field and Vegetable Crops in Novi Sad.

Single seed descent requires that only the basic data are recorded, such as the designation of the cross and what generation it belongs to. Moreover, minimum amount of space is required to grow successive generations of individual plants compared to the rows of progenies characteristic to the 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 F5 plants is similar to that found in the F2 generation. Finally, the number of recessive homozygotes increases in successive generations – with the postponement of selection for a recessive trait until the F5 generation, nearly 47% of the plants will be homozygous for such a trait (WILCOX, 1998).

Perhaps the biggest disadvantage of single seed descent is expressed in the irreversible loss of identity of superior plants from earlier generations. Besides that, a superior plant observed in the F2 generation will be represented by no more than a single plant in the subsequent generations, preventing the selection of a larger number of lines from superior plants. Moreover, plants that would otherwise be discarded will remain in the population up until the F5 generation.

Each method, therefore, has its advantages and drawbacks. The challenge posed on the breeders is to choose and apply the most effective method for the achievement of their breeding goals. Comparisons among the different selection procedures (MILADINOVIĆ, 1999; MILADINOVIĆ *et al.*, 2000; MILADINOVIĆ *et al*., 2011) have shown that the modified version of the Single Seed Descent Method that is in use in soybean breeding at the Institute of Field and Vegetable Crops is more effective than the other methods.

Soybean selection at the Novi Sad Institute has been focused the most in the increase of yield and on developing varieties adaptable to different growing conditions (MILADINOVIĆ *et al.,* 2003; 2006). However, preferences of the processing industry are taken into the account, so

breeding program is adapted to the demands of the market, by increasing protein content (MILADINOVIĆ *et al*., 1996a; 2001; 2004), increasing the quality of soybean oil (MILADINOVIĆ *et al*. 1996b; HRUSTIĆ *et al*., 1998), studying the antioxidative properties of soybean (MALENČIĆ *et al*., 2007; 2008; 2010; 2012), balancing the levels of oligosaccharides and polysaccharides (VUCELIĆ-RADOVIĆ *et al*., 2005; HOLLUNG *et al*., 2005) and improving nutritional and medicinal properties of soybean (CVEJIĆ *et al*; 2009; 2011).

Physiology of high yielding soybean

It seems that soybean yield is somehow connected to the production of photosynthate during the season, but the physiological connection between photosynthesis and soybean yield is not straightforward. It has been estimated that about 5% of the incident solar energy is captured by plants and the most important part of spectrum is in the approximate range of 400-700 nm (photosynthetically active radiation, PAR). At the field, light saturation of individual leaves is usually achieved at one-third or one-fourth of full sunlight. In fact, canopy photosynthesis is limited by concentration of carbon dioxide around leaves. Capacity of canopy to produce organic matter is determined by leaf area (LAI - leaf area index) and specific leaf photosynthetic efficiency. Managing and breeding for these two components makes significant contribution to final soybean yield. But, there are some limitations: maximum canopy photosynthesis is usually achieved when LAI is higher than 4, and after that point it reaches plateau. Maximum canopy photosynthesis is closely related to maximum crop growth rate. Those two physiological maxima are coincident with flowering and pod set, and then decrease during grain filling. Source-sink manipulation experiments suggest that reduce photosynthesis during reproductive development significantly decreases seed number, as one of the major yield components (EGLI and ZHEN-WEN, 1991). Seed number directly depends on canopy growth rate during flowering and pod set, and is very sensitive to photosynthesis supply. Some compensation with seed size is possible, due to increment of embryo sucrose uptake during photosynthesis reduction. This compensation between seed number and seed size has a very limiting effect in filed condition. Actually the most promising strategy for achieving high yield in soybean is to increase seasonal canopy photosynthesis which should result in high crop growth rate during flowering and pod formation, and lead to a higher seed number (CHRISTY and WILLIAMSON, 1985).

Comparison of soybean varieties released during the last century, indicates significant changes in crop physiology of modern varieties. Modern varieties are more efficient in light interception, conversion of light energy to biomass and partitioning biomass to seed. As consequence of soybean breeding and greater efficiencies (KOESTER *et al*., 2014), modern varieties have higher net photosynthesis, chlorophyll content, specific leaf weight, transpiration and lower leaf area and water use efficiency, compared to the old varieties. Higher net photosynthesis and chlorophyll content are related to light interception and conversion of energy to biomass, while lowering of the leaf area is related to efficiently partitioned biomass into seed. In modern soybean breeding transpiration was more increased than photosynthesis. GUONING *et al.* (2012) found a 15% increase of net photosynthesis, while transpiration increased by more than 50% during 82 years of soybean breeding for yield in China.

Another important point of soybean breeding for high yield is transpiration and water use efficiency (WUE). Water regime and photosynthesis are in fact closely related. It is estimated that at leaf stoma 1 molecule of CO_2 is exchanged for 400 molecules of water, which is equivalent to 6.1 g of CO_2 per 1000g of transpirable water (NOBEL, 2009). This is an illustrative relation

between photosynthesis and transpiration in soybean plants. Concerning soybean water use, drought tolerance is the main focus. In well watered environments, carbon assimilation is not limited by water losses through transpiration, but in water limiting environments it is the key limiting factor of organic matter production. Drought stress is a complicated agronomic problem conditioned not only by lack of rain, but also by evapotranspiration from the soil/plant system, rooting depth and proliferation, and the amount of rainfall which enters and maintains in the rooting zone (LOOMIS and CONNOR, 1992). According to PASSIOURA (1977) economic crop yield can be explained by three factors: WUE, transpiration and harvest index. When water is the limiting resource, soybean genotype will have a linear yield-to-water response, and the slope of regression line is WUE. Value of WUE will initially be constant, but will eventually decline when it approaches yield maximum (plateau), and decrease as the water ceases to be a limiting factor. There are genetic variations in WUE among soybean genotypes, which is promising for the future breeding progress. Certainly, one of the most promising strategies for yield improvement is alerting transpiration. Breeders selecting for greater yield have generated a variety with stoma which are generally more open during the day. This finding is consistent with the fact that plants must exchange water for carbon dioxide, thus linking high yields with open stomates as long as enough water is available for transpiration when the pores are open (SPECHT, 2014). To increase crop biomass production, more water must be used in transpiration (SINCLAIR *et al.,* 1984).

Important progress on drought tolerant soybean was made by a group of US scientists, who introduced slow wilting as a trait in adapted germplasm. Slow-wilting trait was found in several exotic germplasms and it is related to sensitivity of stoma closure to higher vapor pressure deficit (VPD). The risk of using this trait in breeding programs is potentially yield penalty. Earlier stoma closure will provide less water losses through transpiration (water conservers), but it allows less CO_2 for photosynthesis thus causing slower crop growth. Breeding effort was made for introducing this trait in adapted germplasm, and there are several lines which cause no yield penalty in drought-free environments, but have higher yields in water limiting environments. Slow-wilting trait would be particularly desirable in low humidity environments where water deficits commonly develop in the later part of the season. Restricted transpiration rate during the middle of the day with high vapor pressure deficit would result in water conservation, allowing both increased yield and water use efficiency (FLETCHER *et al*., 2007).

 The aboveground area also plays a very important role in drought avoidance. Crop transpiration can be enhanced through improvements of root characteristics. Fast rooting, fibrous root and deeper root penetration are promising traits for better water uptake and maintain high transpiration rate. One of the most drought sensitive traits in soybean is biological nitrogen fixation (BNF). Genotypic differences exist in maintaining BNF during drought condition. Compared to other varieties, cultivar Jackson possesses a certain level of BNF drought tolerance (VADEZ and SINCLAIR, 2001).

Breeding strategies and tools

Breeding for drought tolerance is probably one of the most challenging goals in soybean breeding. One of the oldest breeding strategies for drought tolerance is drought index. Breeding goal is to minimize yield differences between stress and non-stressed environments. But the problem with stress index is negative correlation between mean performance (stress and non-stress yield average) and stress index (stress and non-stress yield difference), therefore breeding for stress tolerance can actually lead to yield decrease.

A more physiological approach to drought breeding includes incorporation of several desirable trials in high yielding variety. The "ideal genotype" should possess a fast-growing deep (or fibrous) root system, the ability to maintain BNF in water limiting environments, stoma sensitivity to high VPD coupled with higher photosynthetic efficiency (CHEN, 2014). Deep or fibrous root system provides efficient water uptake, both in shallow and deeper soil layers, while BNF provides enough nitrogen for the metabolic process. During the day with high VPD, "ideal genotype" should close stoma, but at the same time, it needs to compensate CO_2 shortage with more efficient photosynthesis and to provide optimal crop growth rate. The development of such genotype seems as very difficult task, but surprisingly there are exotic germplasm with several drought tolerant, and the incorporation of these traits in high-yielding germplasm should not be an unattainable task.

It is clear that a soybean breeder should use different tools than the ordinary for breeding on a physiological trait. High thought phenotyping equipment, suitable for field research, provides measurements of desirable physiological traits in real environments. High quality phenotypic characterization of germplasm and breeding material under field conditions is required for greater breeding progress and better understanding of genotype-phenotype associations.

Accurate measurement of some physiological parameters in large plant populations in field conditions is impractical or even impossible. So, a phenotyping tool should provide sufficient accuracy and at the same time express the capability of processing a large amount of samples. There are several techniques of remote sensing for crop condition assessment, and the most promising are crop reflectance and canopy temperature. Crop reflectance relies on physical properties of light scattering on leaf anatomical structures. Healthy photosynthetically active leaves reflect more NIR lights compared to unhealthy or senescent leaves, and the reflectance spectrum provides information about crop condition. Typical and widely used is Normalization Difference Vegetation Index (NDVI). Earth satellites carry instruments for remote sensing and use NDVI for vegetation observation and other research purposes. The typical NDVI uses two channels (wave bands) and precise phenotyping requires more information from VIS-NIR spectrum (crop chemometrics). Calculating different indexes from the reflectance spectrum enables estimation of crop biomass, chlorophyll content, light capture efficiency, LAI, vegetation cover, and plant water status, all of which are highly correlated with soybean yield. If reflectance measurements are taken in time intervals, they display the dynamic of crop growth rate and other important parameters. Canopy temperature (CT) measurements are based on using thermal infrared radiation related to evaporation or transpiration from a plant leaf. It is a fast and accurate way to study stomatal conductance and leaf transpiration. When a plant stops transpiration and closes the stomata, the temperature of the leaf increases. MCKINNEY et al. 1989, found a positive relation between CT and soybean yield. Also, CHRISTENSON (2011) developed a model for soybean yield prediction based on crop reflectance and CT.

The most challenging task is to collect field data in a relatively short period of time during variable weather conditions. There are two solutions already established by different research groups. Light vehicle with measuring equipment, designed to move easily through plots, where data acquisition is on a plot-by-plot basis. High platform or flying drones provide imaging of the whole or a part of the experimental field, and the analysis is based on extrapolation of data from the image. Both of these two techniques have specific benefits and drawbacks so the technique should be adjusted according to a specific breeding goal. The most difficult task is

breeding for aboveground traits and it still remains very difficult to analyze a large number of genotypes in target environment

Physiological tools in soybean breeding programs enable a more precise and sophisticated way in soybean yield improvement. It can be used for early yield predictions and monitoring the key physiological processes that are essential for high yields. Knowledge of physiological processes that lead to yield increase and manipulation of the desired characteristics can ensure the sustainability of breeding in the changing environments.

Molecular breeding

Molecular based plant breeding methods and techniques are a conceptual part of any serious breeding program. Among those methods, the most extensively used is marker-assisted selection, as an addition to conventional breeding methods. Integration of molecular based techniques into breeding programs is possible in several ways, using different procedures in a soybean breeding scheme.

There are a lot of marker techniques that can be used in soybean breeding programs, but the most suitable are markers based on the single nucleotide polymorphism (SNP). Single nucleotide polymorphism is a type of genetic variation that can be found anywhere in genome, from coding, regulator and non-coding regions. It is estimated that frequencies of SNP in soybean genome is 1 SNP per 2 kbp for coding regions, while 1 SNP per 0.2 kbb can be found in non-coding regions of soybean genome (VAN *et al.,* 2005). For illustration purposes, based on sequencing of the whole genome (17 *Glycine soja* and 14 *Glycine max* genotypes), 5.9 million SNPs were found in wild soybean, and 4.1 million SNPs in cultivated soybean (LAM *et al.,* 2010). There are several techniques for SNP genotyping three of which are most extensively used: allele specific hybridization, oligo-nucleotide ligation and primer extension based techniques. The actual technique should be adjusted in order to obtain optimal results under specific conditions (number of samples, analysis time, analysis cost, equipment, staff….).

Molecular markers have significant implementation in breeding for a so-called defensive trait (BURTON and MIRANDA, 2013). Disease resistance is usually controlled by a single gene and marker assisted selection has great advantages over conventional methods. Screening a large soybean population for disease resistance can be time consuming. Instead of large inoculation experiments, breeders prefer fast and accurate information of presence or absence of desirable gene in breeding material. Successes of this type of strategy depend on several factors: genetic nature of disease resistance, type of marker, distance of marker and gene, and availability of resources. A lot of breeding programs uses markers in resistance breeding for *Phytophthora sojae*, *Heterodera glycines*, *Soybean mosaic virus*, soybean sudden-death syndrome, etc.

Implementation of markers in breeding programs when dealing with true quantitative traits is still a challenging task. For multigenic traits, such as yield, a widely accepted application of markers in breeding programs does not yet exist. Quantitative trait loci (QTL) have been identified for many traits and mapped in diverse populations, but there is no application in breeding programs. Identification of QTLs is useful information for plant breeders, and there is still a gap between the fundamental and applicative research (BURTON and MIRANDA, 2013).

The most common strategy for QTL analysis in soybean is linkage mapping using recombinant inbred lines (RIL). This type of analysis has high power of detection but low resolution and estimate effect of single QTL depending on population size (it is usually over-estimated). Also, for multigenic traits, the results are highly population specific. Another approach

is association mapping which includes wider genetic variability, and after removing the population structure, QTL can be found in collection of divergent genotypes. Association mapping has high resolution and allelic richness, but poor power of detection. Large efforts have also been made in combining these two methods with the development of soybean Nested Association Mapping population (NAM). Soybean NAM population is developed by crossing "hub parent" IA3032 (high yielding variety) with 40 other parents (high yielding, diverse ancestry lines and plant introductions), and development of RIL population for each family (DIERS, 2014). Parents and RIL families are genotyped and re-sequenced, and tested across a wide range of environments. This research should provide more reliable information about QTLs for yield and other agronomical important traits.

Based on previously mapped QTLs, several strategies of molecular breeding can be implemented. F_2-enrichment is the procedure that increases probability of favourable allele fixation. In F_2 population, plants that are homozygous for the unfavourable allele are eliminated, while plants heterozygous and homozygous for the favourable allele are advanced for inbred development. On that way, frequencies of favourable alleles increase while during inbred development the probability of fixation of all or the majority of favourable alleles increase. Power of F_2 enrichment procedure decrees, if favourable and unfavourable QTLs are in close linkage and if working with large number of QTLs (more than 10). Actually, F_2 enrichment has advantages when working with oligogenic traits (BERNARDO, 2010).

Context-specific marker-assisted selection, developed by SEBASTIAN *et al*. (2010), was adopted by DuPont Pioneer in the so-called Accelerated Yield Technology. They found QTL hotspots that have shifted allele frequencies as a consequence of long term soybean breeding. For any F_2 population, set of markers and QTL are available and by testing in target environment it is possible to find the favorable QTLs. All these techniques of marker implementation in soybean breeding include previous knowledge of marker trait association. The relationship between QTL and marker are established and partly based on marker score, in which the "best" genotype is selected. The potential problem is that sometimes it is not easy to transfer the effect of single QTL to a new genetic background and the existence of epistatic interactions can also complicate the breeding procedure.

Genomewide selection (genomic selection) implies the selection without prior knowledge of marker QTL relation and do not implement any significant test. The idea behind genomic selection is using a lot of random markers, evenly distributed over the genome, and genomewide selection predicts a continuum of effects across a set of markers (BERNARDO, 2010). Some markers have a large effect and some have effect close to zero, but still all markers are used in selection. It is almost a fundamental concept of genetic control of yield - a lot of genes with small effect. Genomewide selection is a relatively new concept which needs more research and theoretical development. Several simulation studies confirm significant genetic gain, but there is still no empirical evidence for usefulness of this procedure in soybean breeding.

Soybean genetic transformation is widely used technique for introducing genes from unrelated organisms. Up to date, only tolerance to total herbicide glyphosate has commercial use. There is public concern about the use of these technologies in soybean breeding in different parts of the world and several sensitive techniques were developed for detection of this type of varieties and products (NIKOLIĆ *et al*., 2008.)

Molecular markers are an important part of soybean breeding program. At this moment, the most reliable application in forward selection is disease resistance breeding. There is still a gap

in breeding for quantitative traits, such as yield and other agronomical important traits. With powerful genetic technologies, and more theoretical and empirical research, advancement in selection efficiency can be expected.

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NOVI PRAVCI U OPLEMENJIVANJU BILAJAKA – PREMER SOJE

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Izvod

Oplemenjivanje soje je kontinuiran proces, čiji je cilj povećanje prinosa i poboljšanje otpornosti prema biotičkom i abiotičkom stresu. Oplemenjivači soje su konvencionalnim metodama stvorili veliki broj sorti, a najčešće korišćena metoda oplemenjivanja je potomstvo jednog zrna. U prethodnom periodu razvijene su različite metode transformacija biljnog genoma. Otpornost prema glifosatu je metodom povratnih ukrštanja unešena u sorte soje i ovo je jedina komercijalno prihvaćena transformacija kod soje. Oplemenjivanje fizioloških osobina predstavlja svojvrstan izazov, počevši od uspostavljanja ciljeva oplemenjivanja pa sve do razvoja pogodnih tehnika. Upotrebom modernih tehnika daljisnke detekcije fizioloških procesa, otvaraju se široke mogućnosti prikupljanja velike količine podataka u realnim uslovima životne sredine. Tehnike molekularne genetike su konceptualni deo ozbiljnih oplemenjivačkih programa. Kao najčešće primenjivana tehnika je marker asistirana selekcija, kao dodatak konvencionalnim metodama oplemenjivanja.