

The conventional and contemporary technologies in maize (*Zea mays* L) breeding at Maize Research Institut Zemun Polje

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Abstract

Broad genetic variability of starting biological material is crucial prerequisite for the successful breeding program. Maize Research Institute, owning Gene bank with more than 6,000 accessions consisted of the local and introduced collection, has the opportunity for the investigation both fundamental genetic processes, pre-breeding and breeding for commercial purposes. To search for new sources of maize drought tolerance Gene bank accessions were scored visually on stay-green phenotype and total appearance. More than 50 genotypes have been identified as a potential source for drought tolerance. Besides breeding on improved storage protein quality decreasing of phytate content in kernel is also under the consideration, because the most phosphorous is bound in phytate. In breeding program it is desirable to have genotypes with higher content of available phosphorous (P) not bound in phytate. Among 60 analyzed populations from Gene bank only one has been determined to have very low phytate content and will be used in maize breeding program on low phytate in kernel. Process of maize breeding, to get high yielding hybrids, begins by the genetic variability determination of starting breeding material, either populations or selected inbred lines. Genetic divergence of parental inbred lines is main step to get high heterotic effect in yield after crossing. Use of different molecular markers allowed cluster analysis by use of UPGMA methods and select high number of genotypes to be included in crossing process.

Keywords: drought, grain quality, maize, molecular markers, yield

Introduction

A modern maize breeding program based on inbreeding-hybridization concept has established at Maize Research Institute (MRI) in 1953. From that time to present, the main objective in the breeding program was the development of maize hybrids with a superior potential for high yields and good grain quality. The naturally high genetic variability of maize is considerably reduced through the selection process and the modern production methods. The development of high-yield hybrids has had significant economic benefits. However, such crops due to their genetic uniformity are extremely sensitive to unpredicted environmental factors. One of the ways to reduce this risk is by developing and making accessible to the market a greater number of genetically different hybrids. Modern science requires from breeders to develop new highly yielding hybrids tolerant to various stress factors in as shorter as possible period. These requirements resulted in needs for the development and the application of contemporary and efficient techniques and methods in plant breeding.

Assigning of maize genotypes into heterotic groups has been the key to the economic success of the crop because it allows the exploitation of heterosis, particularly for grain yield (Reif et al, 2005). Molecular markers have shown to be useful for classifying unrelated inbred lines into heterotic groups (Li et

al, 2002). Based on this information, the integration of molecular markers in maize-breeding programs can increase their efficiency. However, because of the complexity of multi-trait and multi-stage selection as well as the complexity in the genetics of economically important traits molecular markers still do not have a prominent role in breeding programs (Hallauer, 1999).

Methods of molecular biotechnology applied at the Maize Research Institute are used for genetic characterisation and the determination of genetic diversity of germplasm, classification of the breeding material into heterotic groups, observation of the heterosis phenomenon and its prediction, tests of quantitative traits, detection of QTL for yield, tolerance to drought, increased oil content, MAS and genetic modification of maize genotypes.

Genetic characterisation by molecular markers encompasses the characterisation of the source material, populations, varieties, inbred lines and hybrids. The aim of the characterisation is a formation of a genetic identity card for each accession, providing an identifier that will in any time make possible checking identities of genotypes, their purity and it is also important for intellectual property protection. New sources of resistance to stress factors, as well as, new sources of cytoplasmic male sterility and potential new heterotic groups, can be identified in autochthonous, local germplasm by the application of molecular markers

Beside yield, breeding for grain quality traits add economic value to the crop. The utilization of maize for ethanol, biodegradable polymers and nutritional products demands a re-direction of breeding programs to modifying and increasing the kernel composition of starch, protein and oil, as well as their efficient extractability and fermentability (Mazur et al, 1999; Bothast and Schilicher, 2005). Development of maize with unique grain quality traits has not received the same emphasis in genetics, breeding and economics as higher yield and agronomic performance (Scott et al, 2006).

Worldwide genebanks were established to preserve genetic diversity in order to be used as a source of genetic variability for breeding and research. Despite their wealth only 14% of the maize breeders use genebanks (Teixeira et al, 2010) and in temperate area breeding programs have used less than 5% of the available maize germplasm (Hallauer and Carena, 2009). This makes a gap between the genetic resources and breeding and prevents genetic diversity from the genebanks to reach elite working collections of the breeder. Prebreeding includes all activities related to introduction, adaptation, evaluation and improvement of germplasm resources for use in breeding. This concept requires long-term goals which are not popular, because immediate, short-term results are often difficult to measure and/or do not lead to development of commercial products.

The paper will present some of results of studies performed at the Maize Research Institute. The aim of the studies was to apply conventional and modern methods in order to developed hybrids of standard and modified grain quality, of high yield potential, that better response to conditions of greater densities, as well as, to conditions of biotic and abiotic stress.

ZP maize hybrids development

At the beginning of maize breeding and selection, landraces were used as source materials. ZP lines from the three landraces (Ruma Golden Dent, Šid dent and Vukovar dent) were used for the development of the first DC hybrids with dent type of kernel, which represent first generation of ZP hybrids widely grown between 1965 and 1970. The yield potential of this generation of ZP maize inbred lines, indicated the possibility to use them as female parents of single cross hybrids so development of SC hybrids started. Better yield potential and uniformity of single cross comparing with double cross hybrids lead to very rapid introducing of the most yielding single cross hybrids to the production. From the end of 60' to the end 70's first generation of single cross hybrids were developed and designated as second generation ZP maize hybrids. Each of following generations of ZP hybrids was characterized by introduction of the new potentially higher yielding hybrids and with better other agronomics characteristics than previously widely grown hybrids. The main cause of these changes was

driven by changes in genetic background. The third generation represents hybrids with a much better resistance to lodging and tolerance of a higher planting density compared to the second generation. The genetic background of those ZP maize hybrids consisted of inbred lines derived from crosses of public inbred lines from Iowa Stiff Stalk Synthetic Stiff Stalk x first generations inbred lines developed from local open pollinated cultivars and Lancaster Sure Crop. At the beginning of 90's the hybrids with better yield potential, and stalk strength were developed. Genetic background of fourth generation of ZP hybrids consist mainly the changes in genetic background in Non Stiff Stalk parents which in the process of recycling using lines from local adapted genetic pool resulted in a new inbred lines generation. The fifth generation of ZP maize hybrids is characterized with especially fast dry down rate and tolerance on drought growing conditions. Genetic background of this generation of hybrids consist inbreds from recycled Stiff Stalk and Non Stiff Stalk genetic pools with already incorporated local adapted materials and introduced exotic germplasm. Finally, from the beginning of first decade of 21st century new ZP maize hybrids have been developed.

Bauer et al (2007) studied temporal changes in genetic diversity among ZP maize hybrids realized from different periods during 1953 to 2001 by RAPD markers. The number of alleles increased from the Period B (1966 - 1975) to the Period E (1994 - 2001), except insignificant decrease of allele number in the Period D (1986 - 1993) comparing with the Period C (1976 - 1985) due to higher loss of alleles compared to the occurrence of new alleles. Hybrids from the Period E have the highest number of alleles as well as the highest number of new alleles. The average GD between hybrids within Periods increase from Period B to E (0.179, 0.186, 0.205, 0.266, respectively). Higher genetic diversity was obtained between hybrids from different periods than among hybrids within periods (0.31 compared to 0.21). The higher genetic distance among hybrids in each subsequent period can be explained by reduced use of identical parental components in different hybrids and introduction of novel germplasm in each period. The analyzed ZP hybrids revealed diversity in time.

Cluster analysis of genetic distances, Wards method, clearly distinguishes ZP hybrids from different selection cycles into five clusters, which is in contrast to the UPGMA cluster method which does not make such a clear distinction (Babić et al, 2009). The hybrids from fifth generation formed one cluster, and hybrids from the other generations second one (Figure 1). The hybrids of the third and fourth generation represent sub-clusters of a bigger cluster, meaning that they have the biggest similarities among them. Then, they are joined by hybrids of the second, and then the first generation, while the fifth joins at the end, indicating its greatest genetic distance. The

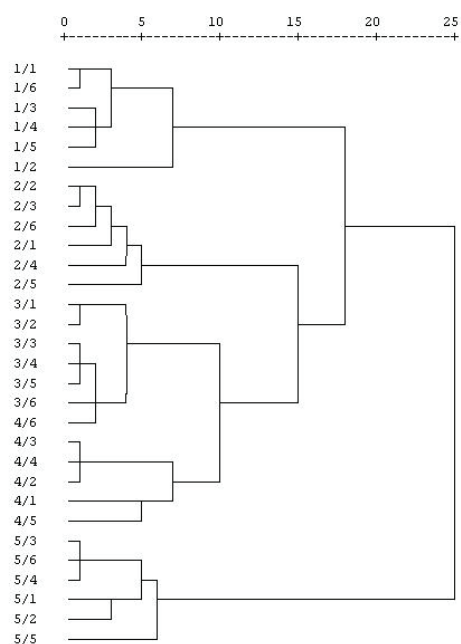


Figure 1 - Dendrogram of cluster analysis of ZP hybrids from different selection cycles, Ward's method (Babic et al, 2009)

results of correspondence analysis of genetic similarities also indicate that the first dimension clearly separates the fifth generation from the rest, while the second dimension separates the first from the second generation. The third and fourth generation can not be differentiated either by introducing the third dimension into the analysis. Such grouping results coincide with the information regarding pedigree data of tested hybrids. A total of 665 ZP maize hybrids (582 with standard kernel quality and 83 with specific traits) has been released in the country during the last 65 years. The majority of registered hybrids belongs to FAO 500, 600 and 700 maturity groups (42%), 22% to FAO 100-200 and 19 % to FAO 300-400. Maize breeding for modified grain quality was initiated at the Maize Research Institute in 1959, while the hybrid ZP 730b was the first released hybrid of this group. Until nowadays, 34 sweet corn, 13 pop corn, 13 white kernel, eight high oil, eight tolerant to Focus ultra, five opaque and two waxy ZP hybrids were released.

Continued improvements in maize breeding programs had significant impact on maize grain production in Serbia. According statistical data from Statistical Yearbook of Serbia 2010, from 1947 to 2010 maize production in Serbia have increased 54 kg ha⁻¹ year⁻¹ (Figure 2).

During this time-span four periods of different rate of increase grain production in Serbia have been recognized. First one, from 1947 to 1958, characterized by growing landraces following US imported double cross hybrids had no increase in maize production. Grain production during the period from 1959 to 1986, represented by domestic SC maize hybrids and im-

proved agricultural practice has been increased 111 kg ha⁻¹ year⁻¹. Due to mainly economic difficulties in agricultural production during the last decade of XX century, grain production varies a lot. At the beginning of XXI century, introduction of new modern type of hybrids, with good adaptability to high plant densities and tolerance to abiotic and biotic stresses, contributed to grain yield increase by 173 kg ha⁻¹ year⁻¹.

Maize is grown on approximately 60% of arable land (1,200,000 ha) in Serbia with average yield of 4.9 t ha⁻¹ during last five years. At the same time, the most recent developed ZP hybrids achieved average yields over 11 t ha⁻¹ in pre-official, official and macro trials. This indicates that genetic potential of the yield was used less than 45% in the production, which was conditioned by the inadequate application of cropping practices.

Breeding of ZP maize hybrids for grain yield

The development of maize hybrids with high grain yielding potential is one of the most important tasks of breeding. Grain yield depends on the genetic constitution of hybrids, i.e. on the frequency of favourable alleles controlling the yield and even more on the capacity of hybrids to resist limiting environmental factors.

Early maturity hybrids have a shorter growing season and significantly lower grain moisture content at harvest which is a great advantage in maize storing. On the other side, late maturity hybrids have higher grain yield potential, but they need better agroecological conditions to release their high potential. Medium early maturity maize hybrids (FAO 300-400) can successfully be grown in regions with both, longer and shorter growing seasons. A lesser habitat provides growing of these hybrids in greater densities due to which higher yields are often obtained in arid years in relation to maize hybrids with a longer growing season. The 28 hybrids, FAO 300-400, was analysed with aim to establish which hybrids, in respect to the kernel type, are more suitable for regions with a shorter growing season and which hybrids are more suitable for regions with a longer growing season. Eight unrelated ZP maize inbred lines, four dent inbreds and four flint inbreds were crossed by diallel crossing. As a result of this crossing six dent, six flints and 16 dent x flint hybrids were derived. Field trial was carried out in two locations, one typical for a region of a long growing season and second typical for hilly mountainous regions during two years with density of 72,400 plant ha⁻¹. Results obtained in a location with a longer growing season show that medium early maturity dent hybrids overyielded and had better grain dry-down than flint and semi-dent-semi-flint hybrids. On the other hand, in locations with a shorter growing season, there were no significant differences either in yields or in grain dry-down in any of this hybrids. Currently, hybrids from FAO 600 maturity group are most distributed in Serbia.

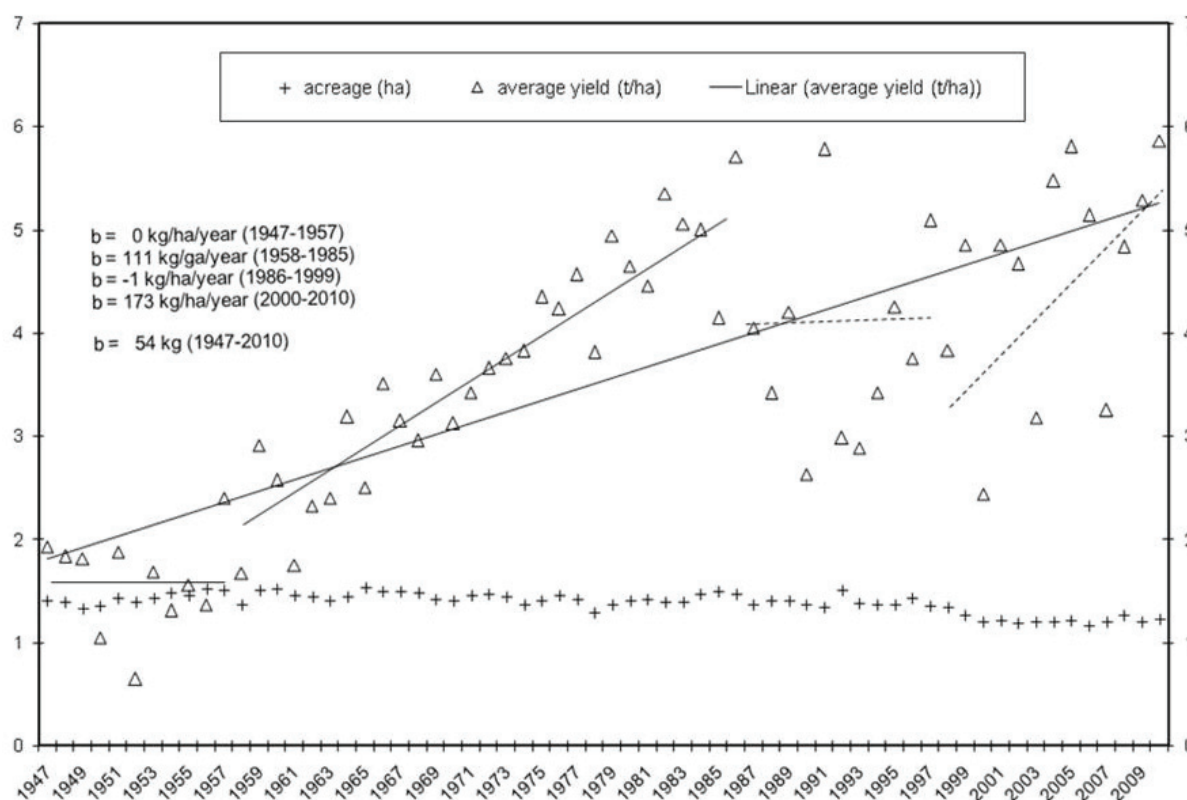


Figure 2 - Average maize grain yield ($t\ ha^{-1}$) and acreage under maize (ha) in Serbia, 1947-2010 (Babić et al, 2009)

One of the most important issues in plant breeding is the determination of the interrelationships of traits for which selection is done. Different factors can affect associations among yield components, including the germplasm used, various environments and the statistical analysis employed to define associations among observed variables. The two-year four-replicate trials were set up with 15 ZP hybrids of different FAO maturity (FAO 400-700) according to the RCB design in two densities ($D1 = 54,900\ plants\ ha^{-1}$ and $D2 = 64,900\ plants\ ha^{-1}$) at six locations (Babić et al, 2011). Grain yield ($kg\ ha^{-1}$), yield components and morphological traits were analysed. Plant height, ear height, ear length, ear diameter, cob diameter, number of kernel rows and number of kernels per row are statistically highly significantly correlated with yield ($p < 0.01$), while grain moisture is statistically significantly correlated with yield ($p < 0.05$). In lower density plant height, ear height, ear length and the number of kernels per row had a greater effect, while ear and cob diameter, as well as, grain moisture had a crucial effect in the trial with a higher sowing density. As plant and ear height equally participate in the formation of both factors their significance as a breeding criterion for the selection of genotypes to be grown in higher densities is reduced. The correlations of number of kernel rows with both factors over both densities are low and it points out that this trait does not share the common structure with the remaining yield

components.

The selection of locations, different soil and climatic conditions and long-term studies completely provide the adequate regional distribution of hybrids for different agroecological areas in Serbia. In order to make maize production more stable and to improve it, it is necessary to adequately select maize hybrids of certain locations.

The biological basis of the regional distribution of hybrids is established on the specific genotype \times environment interaction, which is complex and has been an object of interest of science and practice for many decades. Stevanović et al (2011) conducted study with the aim to determine via production trials the highest and most stable yields of ZP hybrids in different agroecological maize growing regions of Serbia. Five maize hybrids of different FAO maturity groups (ZP-341: FAO 350, ZP-434: FAO 400, ZP-505: FAO 500, ZP-544: FAO 550, ZP-684: FAO 650) were tested in macro trials in 14 locations during the two-year period. Two medium maturity hybrids ZP-341 and ZP-434 had very good grain yields, better than late maturity hybrid ZP-684 and medium maturity hybrid ZP-544. It could be explained by their shorter vegetation period. These hybrids go earlier through critical periods of their growth and are able to escape the periods of drought. Values of the regression coefficient b_i indicate that the highest performing hybrids (ZP-505 and ZP-434) not express highest stability but

have a high level of adaptation to favourable growing conditions. Trial results show that the average yields varied over production regions and a recommendation for an appropriate choice of hybrids should be given for each area. The application of statistical models for assessing yield stability can be useful when giving recommendations for growing certain hybrids.

Genetic diversity analysis

Information regarding genetic diversity of maize inbred lines derived from different origins will maximize the efficiency in hybrid combinations and the development of new inbreds. Genetic diversity evaluation is frequently used by the breeders as an alternative germplasm selection method and allows lines to be arranged into groups that, when intercrossed, provide the most promising results, reduce expenses and the time required to perform the hybrid combinations, which would then be unnecessary. Three independent studies were conducted to evaluate genetic diversity of maize genotypes. In the first study, 96 maize inbred lines of different origin were analyzed by molecular markers (Figure 3). All inbred lines were grouped in one of three heterotic groups, BSSS, Lancaster and European group. Forty one inbred lines belonging to BSSS heterotic group were clustered into four subclusters (19 in A, 11 in B, 3 in C, and 8 in D). Seven ZP inbred lines grouped with B73 in subcluster A. Subcluster B formed six ZP inbred lines and A680. Three ZP inbred lines independently form subcluster C and six ZP inbred lines grouped with L300 inbred lines. Thirty four inbred lines belonging to Lancaster heterotic group was placed into 3 subcluster. Subcluster E formed 14 inbred lines form which five ZP inbred lines. Three ZP lines grouped with B97 and two with L155 in subcluster F. Subcluster G formed 13 inbred lines. The third subcluster consists of 20 inbred lines from European heterotic group. Thirteen inbred lines including three ZP formed subcluster H and seven inbred lines formed another subcluster.

In the second study, six popcorn inbred line was genetically characterized by RAPD markers (Pajic et al, 2010). Eighteen random 10-mer primers were used to amplify fragments from the DNA templates of six inbreds. A total of 108 alleles were scored across analyzed genotypes, out of which 52.7% were polymorphic. The number of alleles per locus varied from 5 to 9, with a mean allele number of 6.0. The lowest genetic distance was calculated for combination ZPPL2 x ZPPL1 (0.086) and the highest (0.212) for combination ZPPL2 x ZPPL5, with an average value of 0.153. The cluster analysis based on genetic distance computed from RAPD data classifies each of six inbreds into one of two principal heterotic groups (Figure 4). The first group encompasses two inbreds developed from synthetic populations. In the second group inbreds ZPPL4 and ZPPL5, belonging to South American germplasm, form subgroup and in-

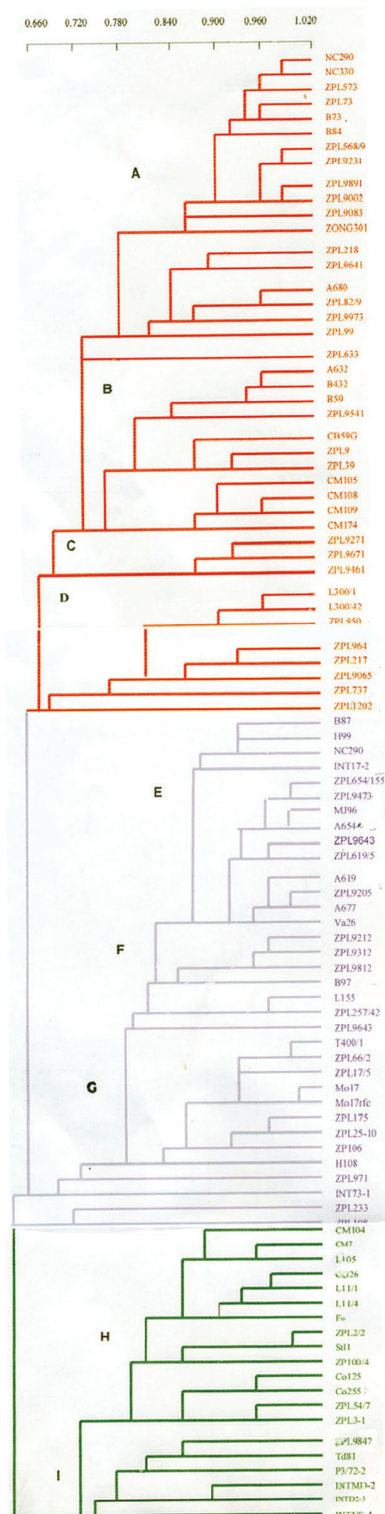


Figure 3 - Cluster analysis of 96 maize inbred lines

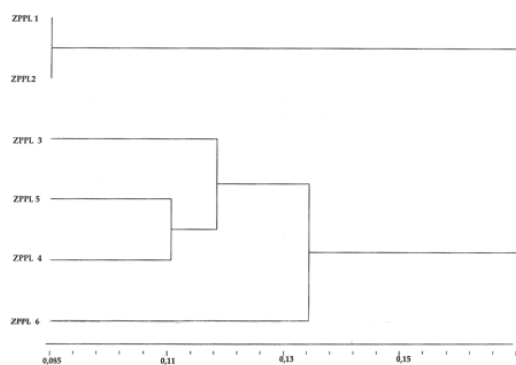


Figure 4 - Cluster of 6 popcorn lines obtained by RAPD markers (Pajic et al, 2010)

bred ZPPL 6, from crosses Supergold x Amber Pearl, is loosely linked to this subgroup.

Application of molecular markers becomes almost a necessity for the breeding of sweet corn, especially because this is the type of maize in which still no definitive heterotic patterns have been determined. So getting to know genetic divergence of the sweet corn inbred lines is of great importance for its breeding. The third study evaluated genetic similarity of six sweet corn inbred based on SSR markers (Srdic et al, 2008). Their origin was from introduced varieties from Mexico, Iran, populations of sweet corn made in Maize Research Institute Zemun Polje, and F2 population of hybrid Jubilee. Results of the polymorphism of SSR markers in this study showed that each of the analyzed genotype had specific profile. From the 40 primers applied 32 were polymorphic. The genetic similarity calculated for 15 combinations of six sweet corn inbred lines, based on SSR markers ranged from 0.381 in the combination ZPLsu6 x ZPLsu2, to 0.744 between ZPLsu6 and ZPLsu5. Cluster analysis for estimates of genetic similarity of six sweet corn inbreds showed clear grouping of lines into two subclusters. The first subcluster encompassed four inbreds. ZPLsu5 and ZPLsu6 that had the highest estimate of GS were most closely attached in this subcluster. This subcluster had also ZPLsu1 and ZPLsu3 which was loosely attached to other three lines. The second cluster consisted of lines ZPLsu2 and ZPLsu4.

Those studies have shown that by assessment of genetic diversity using molecular markers a larger number of lines could be classified into heterotic groups, with reduction of the number of unnecessary crossing, and increasing the efficiency of breeding programs.

Prediction of hybrid performance has been of primary interest to essentially all hybrid breeding programs. Breeders have always been interested in choosing the parental lines, which would result in heterotic combination without necessarily making all possible crosses among the potential parents. Advances in genome research have generated considerable interest in predicting hybrid performance

using DNA markers. The study aimed to investigate the relationship between heterosis and genetic distance determined by RAPD and SSR markers was conducted. Six maize inbred lines of different origin, three inbred lines related with BSSS (ZPL142, ZPL680, ZPL357) and three with non-BSSS genetic background (ZPL255, ZPI17, ZPL173) were crossed to generate diallel set of progenies with reciprocal. Parent inbred lines and F1 crosses were arranged in a randomized block design with four replications in two densities (44,640 and 64,935 plants ha⁻¹) in three years. Parental genomic DNA was analyzed with 10 RAPD and 29 SSR markers. The highest genetic distance was established between inbred lines ZPL142 and ZPL17 and lowest between ZPL17 and ZPL173, as expected due to their pedigree (Table 1).

Table 1 - Genetic distance among maize inbred lines based on RAPD and SSR markers

	ZP142	ZP255	ZP680	ZP17	ZP357
ZP255	0.70				
ZP680	0.62	0.71			
ZP17	0.73	0.59	0.68		
ZP357	0.54	0.71	0.66	0.63	
ZP173	0.67	0.57	0.69	0.50	0.67

Genetic distance was positively correlated with MPH in all environments. The correlation coefficient between averaged midparent heterosis across all environments and genetic distance was positive and significant (0.81). The obtained results are in agreement with results of other studies which obtained middle strong positive correlation between heterosis for grain yield and genetic distance based on molecular markers (Drinic Mladenovic et al, 2002; Pajic et al, 2010). The application of molecular markers could provide breeders with essential information about potentially useful hybrid combination and therefore improve effectiveness of breeding programs.

Grain quality of ZP lines and hybrids

The genetic background undoubtedly influence chemical quality of maize hybrids and may be modified in profit of the chemical constitution and so achieve new germplasm with excellent attributes related to industrialization and nutritional value. Development of maize with unique grain quality traits, however, has not received the same emphasis as higher yield and agronomic performance (Hallauer, 2001; Scott et al, 2006). Fifteen hybrids from Maize Research Institute Zemun Polje, belonged to the various maturity groups from FAO 300 (ZP341, ZP362), FAO 400 (ZP427, ZP471), FAO 500 (ZP505, ZP555, ZP560), FAO 600 (ZP600, ZP606, ZP666, ZP677, ZP684), FAO700 (ZP718b, ZP730 ZP789) as well as 100 maize inbred lines was studied. The protein content of ZP hybrids ranged from 9.37 (ZP362) to 11.5% d.m, (ZP666), and starch content ranged from

79.26% d.m (ZP341) to 81.22% d.m (ZP677), (Stevanovic et al, 2011). Hybrids had oil content between 3.98% (ZP600) and 5.12% (ZP505), averaged 4.51% d.m. Obtained results is agreement with findings of Corcuera et al (2004), that protein content of 72 maize hybrids varied from 8.2 to 12.6%, average 10.5%.

Protein content in inbreds ranged from 9.7% to 14.07 % and oil content ranged from 2.78 to 5.07%. The starch percent in inbreds varied from 59.8 to 73.1%.

With aim to identify maize hybrids for enhanced ethanol production a set of 20 ZP hybrids was analysed. Maize hybrids differ in ethanol yield production. The highest ethanol yield (in percent of the theoretical yield) achieved after 34 hours of the fermentation, was obtained with ZP 434 and ZP243. Those hybrids are suitable for the production of bioethanol and starch, as they had the highest ethanol yield, as well as, starch recovery.

Approaches to resolve problems of the bad nutritive quality of grain phytate include breeding maize with reduced levels of phytate. Genetic variability in phytate contents among 40 ZP maize inbred lines and 22 hybrids was observed, with values ranging from 2.64 to 4.19, averaged 3.29 g kg⁻¹, (Drinic et al, 2010). Three groups of inbred lines and hybrids were identified as having low, intermediate, and high phytate content. The low phytate concentration was measured in 15 inbred lines and seven hybrids, intermediate in 14 inbred lines and eight hybrids and high in 11 inbred lines and seven hybrids. Hybrid ZP 296 was determined to have the low phytate concentration, the highest Pi concentration but lower than average protein content. Inbred lines ZPL 29 have low phytate, high Pi and average protein content. Inbred lines with low phytate will be used in further breeding programs.

Prebreeding

Prebreeding does not provide new cultivars directly, rather develops germplasm resources that are used to develop new cultivars. Original maize populations in the former Yugoslavia developed through a complex adaptation process of the different original genotypes, introduced over previous centuries, to the diverse climatic and soil conditions. The MRI collection comprises 2217 landraces collected from all agro-ecological regions of former Yugoslavia. Usually the size of germplasm collections limits their utilization in plant breeding research. To resolve this situation it has been proposed to select a set of accessions from a collection, containing as much genetic diversity as possible. Such a set of selection were defined as core collection (Frankel, 1984). The material that should be represented by the core depends on the available material and the purpose of the further research. At MRI Genebank two approaches of core collections formation were applied (Radovic et al, 2000). The first approach, designated as eco-core,

was based on eco-geographical variability of local populations. Twenty percent of accessions were selected from each of the 18 agro-ecological groups with the aim to use group typical traits. The second approach is designated as the elite-core, according to combining ability of populations. Combining abilities of 900 medium- and late-maturing local populations, from temperate regions, were tested with four inbred testers. As a result of testing with three inbred lines, additional core subsets were created (Radovic and Jelovac, 1994).

The search for superior genotypes regarding yielding ability, disease and pest resistance, stress tolerance or better nutritional quality is very hard, competitive and expensive. Landraces are natural source of alleles for tolerance to abiotic stress, low antinutritional components content as phytate or a large grains content of proteins, oil or starch that can be used for the infusion with genes of adaptability to particular environment conditions. The phytate and Pi content was analysed in a set of 54 local populations belonging to one of 18 ecogeographical groups, from Maize Research Institute Genebank (Drinic et al, 2009). Populations from group Derivate flint have the lowest phytate content (1.95) while populations from group Flinty dents have the highest (3.43). Populations from group Mediterranean flint have the lowest Pi content (0.46) and populations from group Dent type of southeast USA, (0.82) the highest. Dent type landraces have a little bit lower phytate content (3.0) and higher Pi content (0.66) than flint type landraces 3.03 and 0.63, respectively. Population 216 was determined to have the lowest phytate concentration of 1.14 g kg⁻¹, a Pi concentration 30 % greater than Pi mean. Population 216 will be used for further breeding genotypes with low phytate content and good agronomic traits.

The collection of the MRI Genebank was exposed to controlled water stress in Egypt, at Sids Agricultural Research Station (150 km south of Cairo). There is no precipitation at the location during growing season. The material was divided into five groups, according to the duration of the growing season: extra early, early, medium, medium late and late. Groups were sown separately and have been irrigated until the appearance of the first tassels (within each group). A total of 672 accessions (10.5%) were selected on the basis of ASI, percent of plants with seed set, percent of seed set and percent of grain filling for further experiments. Screening for both ASI and grain filling is desirable for any maize breeding program developing drought tolerant genotypes (O'Neill et al, 2004). Chosen genotypes were tested in Egypt next season, as well as at two locations in temperate zones (Zemun Polje and Macedonia, Andjelkovic et al, 2010). According to the field test 53 genotypes were selected on the basis of previously observed traits, as well as for ear, cob and kernel weight. The formation of core collection for drought tolerance re-

quires, also, information about combining ability and heterotic patterns. Selected genotypes were crossed to three elite inbred testers. Testcrosses were field tested in two years at three locations. Based on good general combining ability, 41 genotypes were selected (14 local and 12 introduced populations and 15 inbred lines). Genotypes usually combine well when crossed to lines from only one opposite specific heterotic group (Melchinger, 1999). However, six accessions have shown the flexibility to produce successful crosses with three lines representing different heterotic groups (Andjelkovic, unpublished data). They enable broadening of genetic variability and these accessions can make a completely different source of favourable germplasm.

Development of prebreeding program for drought tolerance and further multidisciplinary approaches will broaden the genetic base of the elite breeding material. Selected genotypes could be used for breeders' working collections and creation of new ZP hybrids of higher yielding potential and adaptability.

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