

## GENETIC DISTANCE OF MAIZE INBREDS FOR PREDICTION OF HETEROSIS AND COMBINING ABILITY

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A panel of seven maize inbred lines belonging to Zemun Polje commercial pool were genotyped using SNPs bead chip. 21 hybrids, developed according to the half diallel mating design, were tested in the field together with inbred lines *per se*. The goal of the study was to determine the genetic distance among seven maize inbred lines and to establish whether there was a significant correlation between the genetic distance among parental inbreds and grain yield, specific combining abilities (SCA) and high-parent (HP) heterosis for the grain yield. The inbred lines ZPL2 and ZPL4 with the genetic distance of 0.487 were the most genetically distant parents, while inbred lines ZPL1 and ZPL2 with the genetic distance of 0.191 were the closest ones. Three subclusters of inbred lines were distinguished in the dendrogram. Inbred lines ZPL5, ZPL6, ZPL3 and ZPL7 were grouped into the first subcluster, while inbred lines ZPL1 and ZPL2, i.e. the inbred line ZPL4 were grouped in the second, i.e. the third subcluster, respectively. The values of the Spearman's rank correlation coefficient between the genetic distance among inbred lines based on the SNP markers, and grain yield, specific combining abilities (SCA) and high-parent (HP) heterosis were positive and statistically significant. The highest correlation coefficient was exhibited between the grain yield and high-parent (HP) heterosis (0.93), and then between the genetic distance and the grain yield (0.92) as well as between the genetic distance and high-parent (HP) heterosis (0.91).

*Key words:* inbred lines, maize, genetic distance, SNP markers, heterosis

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## INTRODUCTION

The utilisation of heterosis is of a great importance in agricultural production and is one of the most successful models in the cultivation of maize (DUVICK, 2001). The development of hybrids is based on the development of homozygous inbreds from different native populations and then on the evaluation of their traits *per se* as well as on various crosses (TROYER, 2004). Heterosis expresses a percentage gain of F<sub>1</sub> generation in relation to a better- and mid-parent but fails to identify the causes of the hybrid superiority (AMIRUZZAMAN *et al.*, 2013). The breeding practice has repeatedly shown that good potential in parents does not always result in high yields in F<sub>1</sub> progenies. High-yielding hybrids do not necessarily originate from elite parents (YU *et al.*, 2020) and gene interactions between the two genomes such as dominance, overdominance, and epistasis have been suggested to explain the increased biomass and yield (FUJIMOTO, 2018). One explanation of the occurrence of heterosis is that recessive alleles that otherwise restrict the growth, are in this case suppressed or complemented by dominant alleles, resulting in a heterozygous hybrid. Diallel crosses have been broadly used in plant breeding to study combining abilities of parental inbreds, and also to identify superior parents for the use in the hybrid development programmes. The diallel design was also applied in genetic research to determine both the inheritance of important traits among sets of genotypes and gene effects (MALIK *et al.*, 2005).

Combining abilities are used to assess inbred lines with high yield potential and significant expression of heterosis in progenies. The hybrid development is based on the ability to evaluate general combining abilities (GCA) in the early stages of breeding, as well as specific combining abilities (SCA) of certain combinations that result in commercialisation of hybrids (SPRAGUE and TATUM, 1942).

In order to classify maize inbred lines into heterotic groups, the information on their origin, as well as molecular marker techniques are mostly used.

The use of molecular markers to assess genetic distances has been proposed to overcome obstacles in breeding and development of inbred lines (NDHLELA *et al.*, 2015). The advantage of this use is the possibility of estimating only the appropriate crosses between the most distant inbreds. Single Nucleotide Polymorphism (SNP) molecular markers are a powerful tool used to characterise germplasm, as well as to estimate genetic diversity. These are biallelic, highly polymorphic and reproducible. They are also known as point mutations because changes occur in one location in one nucleotide. SNP markers attract much attention because they appear more frequently in the genome than SSR markers (VAN INGHELANDT *et al.*, 2010). Using a microchip, it is possible to genotype a large number of SNP loci.

The aim of this study was to observe genetic polymorphism among parental inbred lines using SNP molecular markers, as well as to determine the association between the genetic distance and high-parent heterosis and specific combining abilities for grain yield.

## MATERIAL AND METHODS

Seven maize inbred lines were used in the study. The inbred lines ZPL1 and ZPL2 belong to the *Lancaster Sure Crop* heterotic group, ZPL3, ZPL5 and ZPL6 belongs to the *BSSS (Iowa Stiff Stalk Synthetic)* heterotic group, while inbreds ZPL4 and ZPL7 have been derived from the

Independent source. The selected inbred lines were crossed according to the diallel design with no reciprocal combinations (Griffing model II), on the basis of and 1 single cross hybrids were developed. Inbred lines and single cross hybrids were studied in the trail set up according to the randomised complete-block design with two replications in three locations (Novi Sad, Zemun Polje and Školsko Dobro – near Belgrade) during the 2017-2018 period. Mechanical sowing was performed. After a certain time, thinning was done to the final number of plants that amounted to 52 plants per plot. The harvest was done by hand. Each elementary plot consisted of two 5-m rows, with the inter-row distance of 0.75m. The elementary plot size was 7.5m<sup>2</sup>.

The yield was determined as t ha<sup>-1</sup> and calculated to 14% moisture. Based on the yield, the values of specific combining abilities (SCA) and high parent heterosis (HPH) were calculated.

The analysis of specific combining abilities was estimated after GRIFFING (1956), Method 2, mathematical model I, without reciprocal crosses:

$$S_{ij} = X_{ij} \frac{1}{p+2} [(T_i + ii) + (T_j + jj)] + \frac{2}{(p+1)(p+2)} xGT$$

(T<sub>i</sub>+ jj) – total j<sup>th</sup> row plus a mean value of the parent j

High-parent heterosis (HPH) was calculated according to the following formula:

HPH= [(F1-HP)/HP] x 100 where:

F1- average value of the F1 generation

HP- average value of a better parent

Genotyping of observed inbreds was performed using the Maize 25K XT<sup>3</sup> evenly distributed across the entire maize genome. SNPs unsuccessfully called more than 5% of inbreds (miss rate > 5%), and those with the minor allele frequency below 5% (MAF< 5%) were excluded from further analyses. A total of 18889 highly informative and quality markers (SNPs) were used in the bioinformatics analysis, and for that purpose, the program for the analysis of genetic structure STRUCTURE 2.3.4 was used (PRITCHARD *et al.*, 2000). STRUCTURE is based on the application of Bayesian statistics using an algorithm that analyses the differences in allele frequencies and classifies observed genotypes on the basis of similar pattern of allelic variation.

The similarity matrix was used for cluster UPGMA (*Unweighted Pair Group Method with Arithmetic Mean*) analysis, calculated using NTSYSpc software version 2.1 (ROHLF, 2000).

The correlation between the genetic distance and specific combining abilities, as well as between the genetic distance and high-parent heterosis for the grain yield was calculated on the basis of the Spearman's rank correlation coefficient.

## RESULTS AND DISCUSSION

Early studies have indicated that low genetic diversity resulted in low heterosis and yield, suggesting that the genetic distance may be a good parameter of heterosis (FLINT-GARCIA *et al.*, 2009). The genetic distance between pairs of inbred lines ranged from 0.191 (ZPL1 and ZPL2) to 0.487 (ZPL2 and ZPL4), with an average value of 0.34 (Table 1). Ten out of 21 combinations had a genetic distance ranging from 0.44 to 0.48. This range proved to be optimal and these

combinations had the highest yield. The inbred lines ZPL1 and ZPL2 in the combination with the inbred lines ZPL3, ZPL4, ZPL5, ZPL6 and ZPL7 had the greatest genetic distance.

Table 1. Genetic distance among observed inbred lines

Genotype	ZPL1	ZPL2	ZPL3	ZPL4	ZPL5	ZPL6
ZPL2	0.191					
ZPL3	0.472	0.477				
ZPL4	0.482	0.487	0.408			
ZPL5	0.471	0.479	0.246	0.396		
ZPL6	0.439	0.473	0.275	0.420	0.196	
ZPL7	0.439	0.446	0.356	0.398	0.359	0.341

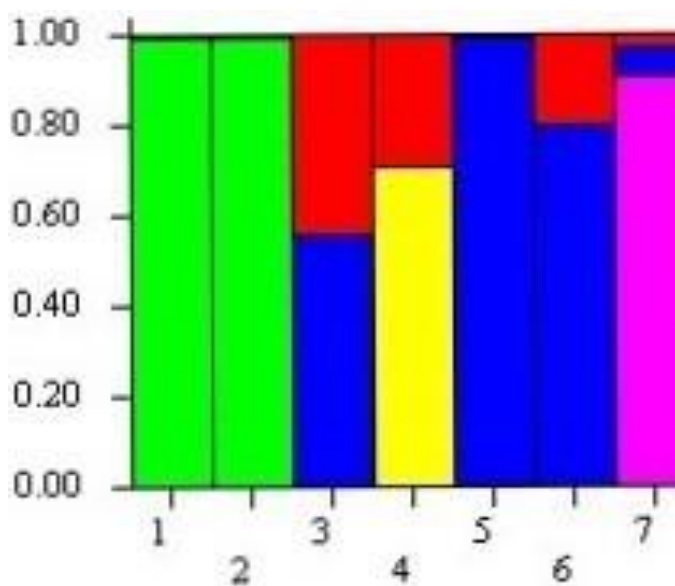


Figure1. Population structure of seven inbred lines based on SNP markers (bar plot)

Figure 1 shows the population structure of seven inbred lines based on SNP markers. The green colour refers to the *Lancaster Sure Crop* heterotic group; the blue colour refers to *BSSS* heterotic group; the colours of yellow, red and pink refer to the Independent source 1, 2 and 3. According to the colours inbred lines ZPL1 and ZPL2 and then ZPL5 and ZPL6 were the most closely related. TOMKOWIAK *et al.*, (2019) have also studied the similarity between parental components, determined on the basis of SNP markers.

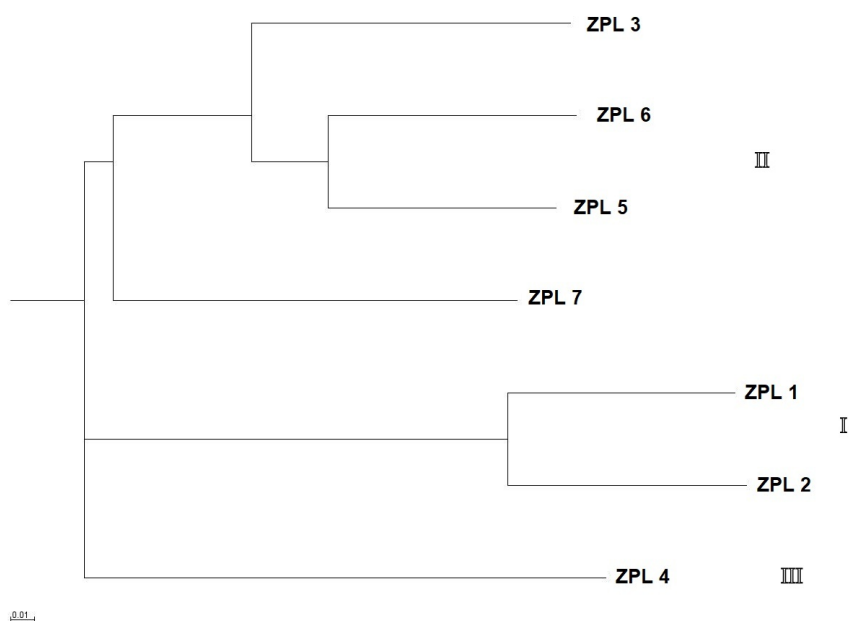


Figure 2. Dendrogram representing genetic distances among observed inbred lines

The dendrogram was constructed using the UPGMA clustering method (Figure 2). According to the dendrogram, the inbreds ZPL1 and ZPL2 are the most closely related, because they both belong to the Lancaster heterotic group, as well as the inbreds ZPL5 and ZPL6 that originate from the BSSS heterotic group. The inbred ZPL3 is most similar to inbreds ZPL5 and ZPL6, and then to the inbred ZPL7. The inbred ZPL4 formed the separate subcluster.

Based on the genetic distance, it is clear that the inbred lines ZPL1 and ZPL2 belonging to the *Lancaster* heterotic group combine well with remaining inbreds (ZPL3, ZPL4, ZPL5, ZPL6 and ZPL7) belonging to the *BSSS* and Independent source heterotic groups, which leads to the conclusion that inbred lines from distant sources exhibit good heterosis (Table 1). According to the dendrogram (Figure 2), it is observable that the inbred line ZPL4 is detached from remaining inbred lines and that in the combination with the inbred line ZPL2 it resulted in the greatest genetic distance (0.487). On the contrary, the smallest genetic distance (0.191) and the lowest yield (6.85 t/ha) was established in the ZPL1xZPL2 combination in which both inbred lines originated from the *Lancaster* heterotic group. Moreover, the genetic distance (0.196) and the grain yield (6.25 t/ha) were small in the ZPL5xZPL6 combination because both inbreds originated from the same heterotic group (*BSSS*). The genetic distance was smaller among inbred lines belonging to the *Lancaster* heterotic group than among inbred lines belonging to the *BSSS* heterotic group. These findings are in absolute accordance with findings of MLADENOVIĆ DRINIĆ *et al.* (2012), NDHLELA *et al.* (2015) concluded that crosses between individuals belonging to the

same heterotic group resulted in lower heterosis than heterosis resulting from crosses of individuals from different heterotic groups. The concept of heterotic groups is widely used to simplify the maize breeding process.

Average values of the grain yield over years, locations and replications and based on that, the specific combining abilities (SCA) and high-parent heterosis (HPH) are presented in Table 2. The analysis of variance showed a very significant difference among genotypes for observed parameters.

Table 2. Grain yield (t/ha), values of specific combining abilities (SCA) and % high-parent (HP) heterosis of observed genotypes

Genotype	GY	Genotype	GY	SCA	%HPH
ZPL1	3.66	ZPL1xZPL2	6.85	-0.78	87.16%**
ZPL2	3.62	ZPL1xZPL3	10.07	1.32**	167.82%**
ZPL3	3.76	ZPL1xZPL4	10.64	1.91**	159.51%**
ZPL4	4.10	ZPL1xZPL5	9.87	1.54**	169.67%**
ZPL5	3.34	ZPL1xZPL6	9.62	1.08*	132.37%**
ZPL6	4.14	ZPL1xZPL7	8.65	0.85*	136.34%**
ZPL7	3.62	ZPL2xZPL3	9.75	1.13**	159.31%**
		ZPL2xZPL4	11.10	2.06**	170.73%**
		ZPL2xZPL5	9.42	1.27**	160.22%**
		ZPL2xZPL6	9.61	0.99*	132.13%**
		ZPL2xZPL7	9.62	1.47**	165.75%**
		ZPL3xZPL4	8.98	0.35	119.02%**
		ZPL3xZPL5	6.42	0.14	70.74%**
		ZPL3xZPL6	7.57	0.69	82.85%**
		ZPL3xZPL7	7.87	0.62	109.31%**
		ZPL4xZPL5	8.77	0.65	113.90%**
		ZPL4xZPL6	8.74	0.66	111.11%**
		ZPL4xZPL7	8.49	-0.13	107.07%**
		ZPL5xZPL6	6.25	0.60	50.97%*
		ZPL5xZPL7	7.73	0.54	113.54%**
		ZPL6xZPL7	8.33	0.40	101.21%**

GY-grain yield (t/ha), SCA-specific combining abilities, %HPH-high-parent heterosis, \*, \*\* - significant at 0.05 and 0.01 probability level, respectively

The highest (4.14 t/ha), i.e. lowest (3.34 t/ha) grain yield of parental components was recorded in the inbred line ZPL6, i.e. ZPL5, respectively. The total average yield of inbred lines amounted to 3.75 t/ha (Table 2). On the other hand, the grain yield of hybrids varied from 6.25 t/ha (ZPL5xZPL6) to 11.10 t/ha (ZPL2xZPL4), while the total average value was 8.87 t/ha. The grain yield of the F1 generation was affected by the genetic distance: the combination with the greatest genetic distance had the highest yield. DEVI and SINGH (2011) established a positive correlation coefficient between the genetic distance and the grain yield.

The values of specific combining abilities (SCA) varied from -0.78 (ZPL1xZPL2) to 2.06 (ZPL2xZPL4) (Table 2). SCA values of two hybrid combinations were negative, while they were statistically significant and very significant for 10 hybrid combinations, which was also positively correlated with the genetic distance among inbred lines.

The values of high-parent heterosis ranged from 50.97% (ZPL5xZPL6) to 170.73% (ZPL2xZPL4) (Table 2). The same 10 combinations of inbred lines with the highest genetic distance also had the highest heterosis. STUPAR *et al.* (2008) monitored maize inbreds and hybrids to assess the levels of high-parent heterosis (HPH). As for the correlation between the yield and heterosis, the same 10 combinations had a high yield and high heterosis. Only the fourth combination (ZPL1xZPL5) differed, as heterosis was very high (169.67%) and the yield somewhat lower (9.87 t/ha) than expected. Many authors have established a significant positive correlation between the genetic distance and heterosis (SRDIĆ *et al.*, 2007; PAVLOV *et al.*, 2016; GEORGE *et al.*, 2011). GEORGE *et al.* (2011) stated that the relationship between the genetic distance and heterosis was complex and that the level of heterosis increased with the increasing of the genetic distance, but only to a certain point after which it decreased. FLINT-GARCIA *et al.* (2009) asserted that HPH was the most important parameter of economic viability for the development of high-yielding hybrids and that HPH was expressed for each trait in each hybrid. Furthermore, the same authors stated that related genotypes showed much lower heterosis than distant genotypes and that the occurrence of a positive or a negative correlation was largely dependent on the distance of genotypes. Early studies have indicated that low genetic diversity resulted in low heterosis and yield, suggesting that the genetic distance may be a good parameter of heterosis (FLINT-GARCIA *et al.*, 2009).

Table 3. Matrix of Spearman's rank correlation coefficients for genetic distance among inbred lines (GD), grain yield (GY), specific combining abilities (SCA) and high-parent heterosis (HPH)

	GY	SCA	HPH
GD	0.92**	0.85**	0.91**
GY		0.85**	0.93**
SCA			0.86**

GD-genetic distance, GY-grain yield (t/ha), SCA-specific combining abilities, HPH-high-parent heterosis

The values of the Spearman's rank correlation coefficient between the genetic distance among inbred lines based on the SNP markers and the grain yield, SCA and HPH were very statistically significant (0.92\*\*, 0.85\*\* and 0.91\*\*, respectively) (Table 3). However, in most cases genetic distance is positively correlated with heterosis (PATERNIANI *et al.*, 2008). Positive and statistically significant values between the genetic distance and specific combining abilities (SCA) were recorded by GRČIĆ *et al.* (2018); PAVLOV *et al.* (2016). The correlation coefficient for grain yield in relation to SCA and HPH amounted to 0.85\*\* and 0.93\*\*, respectively, and was statistically significant. The correlation coefficient between SCA and HPH was 0.86\*\* and was also statistically significant.

### CONCLUSION

The molecular marker technique is a reliable method for detecting favourable heterotic pairs of inbred lines. Statistically significant values of SCA and HPH were established in half of the total number of diallel crosses, which indicates that inbred lines from these crosses could be used in contemporary maize breeding programmes. The Spearman's rank correlation coefficient between the genetic distance among maize inbred lines and SCA and HPH was also statistically significant, which provides additional information in genetic studies of maize and the utilisation of examined maize inbred lines in commercial breeding. It can be concluded that the determination of genetic distance among maize parental inbred lines represents an effective approach for the assessment of their combining abilities and for the prediction of heterosis in maize hybrids.

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**GENETIČKA DISTANCA INBRED LINIJA KUKURUZA ZASNOVANA NA SNP  
MARKERIMA ZA PREDVIĐANJE HETEROZISA I KOMBINACIONIH  
SPOSOBNOSTI**

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Izvod

Panel od sedam inbred linija kukuruza koje pripadaju komercijalnom pulu Instituta za kukuruz u Zemun Polju je analiziran pomoću SNP čipa. Hibridi dobijeni po metodu nepotpunog dialela su ispitivani u polju zajedno sa inbred linijama *per se*. Cilj ovog istraživanja je bio da se utvrdi genetička distanca između inbred linija kukuruza i da se ispita da li postoji značajna korelacija između genetičke distance roditeljskih linija, posebnih kombinacionih sposobnosti (PKS) i heterozisa u odnosu na boljeg roditelja (HPH) za osobinu prinos zrna. Genetički najudaljenije linije su bile ZPL2 i ZPL4 sa genetičkom distancom 0.487, dok su najbliže bile inbred linije ZPL1 i ZPL2 sa genetičkom distancom 0.191, kao i inbred linije ZPL5 i ZPL6 sa genetičkom distancom 0.196. Dendrogram je podelio inbred linije u tri različita subklastera. Prvom subklasteru pripadaju inbred linije ZPL5, ZPL6, ZPL3 i ZPL7, drugom inbred linije ZPL1 i ZPL2 i trećem inbred linija ZPL4. Vrednosti Sperman-ovog koeficijenta korelacije ranga između genetičke distance zasnovane na SNP markerima, prinosa zrna, posebnih kombinacionih sposobnosti (PKS) i heterozisa u odnosu na boljeg roditelja (HPH) su bile pozitivne i statistički značajne. Najviši koeficijent korelacije je bio između prinosa zrna i HPH (0,93), zatim između genetičke distance i prinosa (0.92) i između genetičke distance i HPH (0.91).

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