

## GENETIC DISTANCE OF MAIZE INBRED LINES BASED ON SSR MARKERS FOR PREDICTION OF HETEROSIS AND COMBINING ABILITY

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Genetic distance among six elite maize inbred lines was analyzed using SSR markers. Hybrid progeny obtained by crossing inbred lines according an incomplete diallel design was tested in field trials together with inbred lines per se. The objective of this study was to determine genetic distance of inbred maize lines and to examine if a significant correlation exist between the genetic distance of parental lines and the exhibited high parent heterosis (HPH) and specific combining abilities (SCA) for grain yield, ear length, kernel row number and number of kernels per row. Twenty one SSR primers were used for genetic assesment of inbreds with detected 92 alleles. Genetically most distant lines were ZPL1 and ZPL5 and ZPL6 with the GD value of 0.549, while the closest one were ZPL2 and ZPL3 with GD value of 0.11. The dendrogram distinguished two main groups of inbreds: ZPL5 and ZPL6 grouped in a smaller cluster and ZPL1, ZPL2, ZPL3 and ZPL4 forming the second cluster. Values of the Spearman's rank correlation coefficient between genetic distance among inbred lines based on SSR markers and SCA for all analyzed traits were positive and significant with the exception of rows per ear. Highest correlation was exhibited between the genetic distance and SCA for number of kernels per row (0.643). Spearman's rank correlation coefficient between GD and high parent heterosis was positive and significant for ear length and kernel number in row with coefficient values of 0.554 and 0.611, respectively.

*Keywords:* inbred lines, maize, SSR markers, genetic distance, heterosis

## INTRODUCTION

Maize breeding programs are mostly based and designed following the idea of the heterosis exploitation although the genetic basis of that phenomenon is not completely understood (DUVICK, 2001; TROYER, 2006; GARCIA *et al.*, 2008; LARIEPE *et al.*, 2012). The magnitude of heterosis depends on the difference in the gene frequency of two parental lines at all loci affecting the concerned trait (FALCONER and MACKAY, 1996) therefore the understanding of genetic relationship among inbreds is essential for germplasm organisation and exploitation (MELCHINGER *et al.*, 1991; BERNARDO, 2002; VANČETOVIĆ *et al.*, 2015). In that way inbred lines involved in breeding programs could be grouped and assigned to heterotic pools allowing breeders to choose appropriate parental lines to be crossed according to their genetic distance (GD). The performance of a hybrid and expressed heterosis is related to the general (GCA) and specific (SCA) combining abilities of the inbred lines involved in the cross with SCA being more important in elite breeding material due to a greater importance of dominance and epistasis (HALLAUER and MIRANDA, 1988; VASAL *et al.*, 1992; GAMA *et al.*, 1995; NASS *et al.*, 2000). Various methods for maize germplasm characterization and assignment were developed like coancestry coefficient calculated from the pedigree records (MESSMER *et al.*, 1993), morphological markers, diallel analysis and topcrosses to testers (FALCONER and MACKAY, 1996; BARATA and CARENA, 2006). The common feature of those estimations was that they were conducted in field conditions which demanded extensive labor work, was time consuming and exposed to considerable environment influence.

Today use of molecular genetic methods is the preferable option for assessment of genetic background of breeding material (DUBREUIL *et al.*, 1996; MLADENOVIĆ-DRINIĆ *et al.*, 2012; FRASCAROLI *et al.*, 2013; NIKOLIĆ *et al.*, 2015).

Simple sequence repeat (SSR) markers are DNA marker type used very widely to characterize germplasm collections of crops because of their easy use, relatively low price, and high degree of polymorphism provided by the large number of alleles per locus (VIGNAL *et al.*, 2002). Simple sequences are stretches of DNA which consist of only one, or a few tandemly repeated nucleotides – these types of simple sequence have been shown to be repetitive and interspersed in many eukaryotic genomes. (TAUTZ and RENZ, 1984).

Although newer markers like the single nucleotide polymorphism (SNP) markers are used for germplasm characterisation, SSRs exhibit a higher average number of alleles per locus and a greater discriminating power (HAMBLIN *et al.*, 2007; VAN INGHELANDT *et al.*, 2010).

The correlation between genetic divergence of parental inbreds based on SSRs and hybrid performance was the object of numerous studies (XIA *et al.*, 2004; SRDIĆ *et al.*, 2008; SINGH, 2010; MLADENOVIĆ-DRINIĆ *et al.*, 2012; PAVLOV *et al.*, 2016; ZHONG *et al.*, 2017).

The objective of this study was to estimate the genetic polymorphism of investigated elite inbred maize lines already assumed to belong to specific heterotic groups and to determine if a significant correlation exists between the genetic distance (GD) of parental lines and the exhibited high parent heterosis and SCA for grain yield, ear length, kernel row number and number of kernels per row.

## MATERIALS AND METHODS

Six maize inbred lines belonging to different heterotic patterns were included in the experiment. Inbred lines ZPL5 and ZPL6 are assumed to belong to a Lancaster Sure Crop (LSC) heterotic group, line ZPL2, ZPL3 and ZPL4 are sharing a common BSSS origin, while ZPL1 is of

unknown heterotic origin. Crossings according to the incomplete diallel design (Griffing model II) were performed among the inbred lines in order to obtain 15 of their F<sub>1</sub> progenies. The field trials were set at 3 locations (Zemun Polje, Becej and Pancevo) during two years (2011 and 2012). The trials were sown according to a RCB experimental design in 4 repetitions. The size of the elementary plot was 7.5m<sup>2</sup> and the sowing was done mechanically with a 0.75m spacing between rows.

Traits that were investigated in this trial are grain yield (t/ha), ear length (cm), kernel row number and number of kernels per row.

Estimates of specific combining abilities (SCA) for the investigated traits were evaluated according to method 2 by GRIFFING (1956).

The following formula was used for calculation of specific combining abilities (SCA) for crosses:

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

$T_j + jj = \text{total } j\text{-row} + \text{average value of parent } j.$

High parent heterosis (HPH) was calculated using the following formula:

$$HPH = \frac{F_1 - BP}{BP}$$

$F_1$  - mean value of the F1 progeny

$BP$  - mean value of the better parent

Characterization and genetic similarity assessment of the six inbred lines using SSR markers was done utilizing 21 SSR primers. List of SSR primer names, their chromosome positions and sequences are presented in the table 1.

The modified protocol according to SAGHAI and MAROOF (1984) was applied for the DNA extraction. Simple matching coefficient (SM) was used for the determination of genetic distance between inbreds. DNA bands presence or absence was transformed into binary data (1 and 0 respectively) and genetic similarity matrices were generated. 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). For a statistical association between GD on one side and SCA and HPH values for each trait on the other side, Spearman's rank correlation coefficient was calculated according to a method by HADZIVUKOVIC (1973).

Table 1. List of SSR primers names, bin and sequences

Name	Bin	Primer sequence
<b>bnlg 1643</b>	1.08	5'-ACCACCGTCCACCTCCAC-3' 5'-ATTGACCCCGTGACCCTC-3'
<b>phi 033</b>	9.01	5'-ATCGAAATGCAGGCGATGGTTCTC-3' 5'-ATCGAGATGTTCTACGCCCTGAAGT-3'
<b>umc 1526</b>	2.08	5'-TTTTACAAGCGTGAGAGCAAGAAA-3' 5'-AACTGCTGGAACAAGAAACCGAG-3'
<b>bnlg 1350</b>	3.08	5'-TGCTTCAGCGCATTAAACTG-3' 5'-TGCTCGTGTGAGTTCCTACG-3'
<b>umc 1140</b>	3.08	5'-CGAGCAAAGAGAGGGGAGAGAGA-3' 5'-GCCTCTACCACCTCGTCCATC-3'
<b>umc 1265</b>	2.02	5'-GCCTAGTCGCCTACCCCTACCAAT-3' 5'-TGTGTTCTTGATTGGGTGAGACAT-3'
<b>phi 087</b>	5.06	5'-GAGAGGAGGTGTTGTTTGACACAC-3' 5'-ACAACCGGACAAGTCAGCAGATTG-3'
<b>umc 1019</b>	5.06	5'-CCAGCCATGTCTTCTCGTTCTT-3' 5'-AAACAAAGCACCATCAATTCGG-3'
<b>umc 1126</b>	2.08	5'-CAACAGGGTGAACCCTCTGTACTT-3' 5'-AATATGGTGTGTGATTTGCATCG-3'
<b>umc 1394</b>	3.01	5'-CCCGAGTCAGAAAAACATTCACTT-3' 5'-CCTAACCTGAAGAAGGGAGGTCAT-3'
<b>bnlg 1443</b>	6.05	5'-TACCGGAATCCTCTTTGGTG-3' 5'-TTTGACAACCTCTCCAGGG-3'
<b>umc 1859</b>	6.06	5'-ATATACATGTGAGCTGGTTGCCCT-3' 5'-GCATGCTATTACCAATCTCCAGGT-3'
<b>umc 1695</b>	7.00	5'-CAGGTAATAACGACGACGAGCAA-3' 5'-GTCCTAGGTTACATGCGTTGCTCT-3'
<b>umc 1400</b>	3.05	5'-TTACCAATTGTATCCATCACACCG-3' 5'-ACAACATAGCAGCCATCCTACTCG-3'
<b>umc 1799</b>	7.06	5'-GTGATGAATAATGTCCCAATTCC-3' 5'-GGACAGATGTCTGGAGATTGCTTT-3'
<b>umc 1782</b>	7.04	5'-CGTCAACTACCTGGCGAAGAA-3' 5'-TCGCATACCATGATCACTAGCTTC-3'
<b>umc 1040</b>	9.01	5'-CATTCACTCTCTTGCCAACTGA-3' 5'-AGTAAGAGTGGGATATTCTGGGAGTT-3'
<b>umc 1492</b>	9.04	5'-CTGCTGCAGACCATTTGAAATAAC-3' 5'-GAGACCCAACCAAACTAATAATCTCTT-3'
<b>umc 1957</b>	9	5'-CATGATCGCCGGGATTAATACTAC-3' 5'-GTCCAAGGACGACGATTACGAC-3'
<b>umc 2047</b>	1.09	5'-GACAGACATTCTCGCTACCTGAT-3' 5'-CTGCTAGCTACCAAACATTCCGAT-3'
<b>bnlg 2235</b>	8.02	5'-ATCCGGAGACACATTCTTGG-3' 5'-CTGCAAGCAACTCTCATCGA-3'

## RESULTS AND DISCUSSION

The genetic divergence among parental maize inbred lines was determined using 21 SSR primers which detected 92 alleles with a mean of 4.38 per locus. There were 87 polymorphic alleles which accounted to 94.6% of detected alleles. Similar results were reported by LU and BERNARDO (2001), who made characterization of 80 inbred lines and detected 4,9 alleles per locus. The greatest number of detected alleles per primer was six and it was detected using primers *bnlg1443* and *umc1695*. Values of genetic distance (GD) indicated considerable level of genetic diversity among the investigated inbred lines ranging between 0.110 and 0.549.

The smallest GD value of 0.110 was detected between ZPL2 and ZPL3 which share a common heterotic background. Genetically most distant lines were ZPL1 and ZPL5 and ZPL6 with the GD value of 0.549.

Table 2. Genetic distance (GD) among the analysed maize inbred lines

	ZPL1	ZPL2	ZPL3	ZPL4	ZPL5
ZPL2	0.488				
ZPL3	0.488	0.110			
ZPL4	0.427	0.256	0.220		
ZPL5	0.549	0.439	0.451	0.402	
ZPL6	0.549	0.537	0.537	0.463	0.354

Using UPGMA clustering method a dendrogram was constructed (Figure1). The dendrogram distinguished two main groups of inbreds: ZPL5 and ZPL6 grouped in a smaller cluster and ZPL1, ZPL2, ZPL3 and ZPL4 forming the second cluster. This separation follows the heterotic pattern of the analysed inbreds line, ZPL5 and ZPL6 being the only LSC type lines in this study. Analysis of the bigger cluster reveals the existence of two subcluster, one grouping together all the BSSS inbreds (ZPL2, ZPL3 and ZPL4) leaving ZPL1 isolated in the second subcluster. Furthermore, in the BSSS subcluster ZPL2 and ZPL3 are strongly linked one to each other.

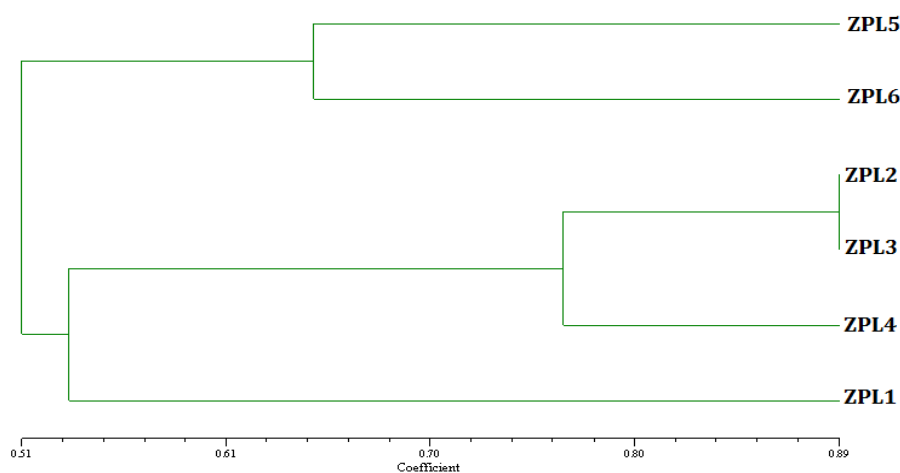


Figure 1. Dendrogram according to the genetic distance of the analysed inbred lines

Values of grain yield (GY), ear length(EL), kernel row number (KRN) and number of kernels per row (NKPR) for inbred lines and their crosses, as well as estimations of SCA and HPH for mentioned traits are presented in Table 3.

Table 3. Grain yield (t/ha), ear length (cm), kernel row number and number of kernels per row, specific combining abilities (SCA) and high parent heterosis for investigated traits

	SCA				HETEROSIS							
	GY	EL	KRN	NKPR	GY	EL	KRN	NKPR	GY	EL	KRN	NKPR
ZPL 1 X ZPL 2	10.62	18.10	15.97	34.96	2.00**	1.38**	0.80**	3.29**	1.35**	0.34**	0.03	0.45**
ZPL 1 X ZPL 3	10.15	18.74	15.53	34.99	2.01**	2.19**	-0.03	3.97**	1.32**	0.38**	-0.09**	0.45**
ZPL 1 X ZPL 4	10.40	17.87	15.61	32.44	1.59**	2.14**	-0.10	3.93**	1.26**	0.32**	-0.09**	0.34**
ZPL 1 X ZPL 5	11.86	20.07	14.78	39.01	2.54**	1.48**	0.58*	3.74**	1.47**	0.24**	0.08**	0.33**
ZPL 1 X ZPL 6	11.56	19.43	13.17	41.13	1.63**	1.09**	0.04	3.66**	0.81**	0.13**	-0.01	0.22**
ZPL 2 X ZPL 3	5.67	14.05	16.53	25.89	-1.41**	-1.54**	-0.28	-3.35**	0.25	0.04	-0.04*	0.08
ZPL 2 X ZPL 4	8.56	15.15	17.33	28.32	0.39	0.57	0.37*	1.59*	0.87**	0.12**	0.01	0.18**
ZPL 2 X ZPL 5	11.56	19.88	15.95	38.32	2.89**	2.26**	0.49*	4.82**	1.41**	0.23**	0.03	0.30**
ZPL 2 X ZPL 6	12.30	19.70	14.85	42.11	3.03**	1.83**	0.46*	5.37**	0.93**	0.15**	-0.04*	0.25**
ZPL 3 X ZPL 4	7.84	14.08	17.52	26.03	0.16	-0.52	0.16	-0.30	0.70**	0.06	0.02	0.11*
ZPL 3 X ZPL 5	11.40	19.84	16.05	37.14	3.21**	2.37**	0.21	4.29**	1.37**	0.22**	-0.06**	0.26**
ZPL 3 X ZPL 6	11.10	19.48	14.82	41.02	2.30**	1.76**	0.03	4.94**	0.74**	0.13**	-0.14**	0.40**
ZPL 4 X ZPL 5	11.05	17.68	16.37	33.01	2.19**	1.04**	0.37*	2.67**	1.30**	0.09*	-0.04**	0.12*
ZPL 4 X ZPL 6	12.61	18.44	14.92	37.74	3.15**	1.56**	-0.02	4.16**	0.98**	0.07*	-0.13**	0.12*
ZPL 5 X ZPL 6	8.25	19.14	13.28	40.25	-1.72**	-0.61	-0.14	-0.09	0.29*	0.11**	-0.03	0.19**
ZPL1	4.38	13.55	13.26	24.15								
ZPL2	4.52	13.50	15.51	24.03								
ZPL3	3.89	13.30	17.16	23.47								
ZPL4	4.61	11.37	17.12	17.56								
ZPL5	4.80	16.22	13.73	29.38								
ZPL6	6.38	17.17	12.18	33.80								

GY- grain yield, EL-ear length, KRN-kernel row number, NKPR -number of kernels per row

\*,\*\* Significant at the 0.05 and 0.01 probability levels, respectively

Grain yield for parental inbred lines ranged from 3.89 t/ha (ZPL3) to 6.38 t/ha (ZPL6). Yield of crosses were in range from 5.67 t/ha for the cross ZPL2xZPL3 to 12.61 t/ha which is recorded in the cross between inbred lines ZPL4 and ZPL6. Values of ear length for inbred lines were in range from 11.37 (ZPL4) to 17.17 (ZPL6), while for crosses varied from 14.05 cm (ZPL2xZPL3) to 20.07 cm for ZPL1xZPL5. Kernel row number for inbred lines ranged from 12.18 (ZPL 6) to 17.16 (ZPL 3) and for crosses between 13.17 (ZPL1xZPL6) and 17.52 (ZPL3xZPL4). Lowest value of number of kernels per row was detected for inbred line ZPL4 (17.56), while the highest value had inbred line ZPL6 (33.80). For crosses, values of number of kernels per row were in range from 25.89 (ZPL2xZPL3) to 42.11 (ZPL2xZPL6).

Estimates of specific combining abilities for grain yield were statistically significant for all crosses, except ZPL2xZPL4 and ZPL3xZPL4. SCA values of grain yield were in the range between -1.72\*\* (ZPL5xZPL6) and 3.21\*\* (ZPL3xZPL5). For three crosses negative SCA values of ear length were recorded, with 12 crosses overall having statistically significant values. The highest value of ear length SCA estimate (2.37\*\*) was obtained for the cross ZPL3xZPL5. Only 6 crosses were having statistically significant SCA values for kernel row number. SCA estimates for that trait were in range from to -0.28 (ZPL2xZPL3) to 0.80\*\* (ZPL1xZPL2). SCA estimates for number of kernels per row were statistically significant for all crosses with the exception of ZPL3xZPL4 and ZPL5xZPL6. Highest value was recorded for the

ZPL2xZPL6 cross (5.37\*\*). Significant SCA values for observed traits were also reported by PAVLOV (2013).

High parent heterosis for grain yield was positive for all crosses and statistically significant except for ZPL2xZPL3. Highest heterosis for grain yield was recorded in crosses ZPL1xZPL5 and ZPL2xZPL5 (1.47 and 1.41 respectively). The highest values of HPH were recorded in crosses between genetically distant inbred lines, which is in accordance with the results published by REIF *et al.* (2003). Values of HPH for ear length were significant in all crosses, except in ZPL2xZPL3 and ZPL3xZPL4. Kernel row number heterosis was significant in 9 crosses with its values ranging from -0.14 in ZPL3xZPL6 to 0.08 in ZPL1xZPL5. HPH heterosis for number of kernels per row was significant in 14 crosses. Only for cross ZPL2xZPL3 value of HPH was below statistical significance.

Values of the Spearman's rank correlation coefficient between genetic distance among inbred lines based on SSR markers and specific combining abilities (SCA) for all analyzed traits were positive and significant with the exception of kernel row number (Table 4). The highest correlation was exhibited between genetic distance and SCA for number of kernels per row (0.643). Spearman's rank correlation coefficient between GD and high parent heterosis (HPH) was positive and significant for ear length and number of kernels per row with coefficient values of 0.554 and 0.611, respectively. Positive correlation between genetic distance and SCA and HPH were reported by several authors (PAVLOV *et al.*, 2016, MLADENović-DRINIĆ *et al.*, 2012; BETRAN *et al.*, 2003).

Table 4. Spearman's rank correlation coefficient between genetic distance among inbreds and specific combining abilities (SCA) and high parent heterosis (HPH) for analyzed traits

	GY	EL	KRN	NKPR
SCA	0.585*	0.514*	0.36	0.643**
HFP	0.443	0.554*	-0.05	0.611*

GY- grain yield, EL-ear length, KRN-kernel row number, NKPR-number of kernels per row

\*,\*\* Significant at the 0.05 and 0.01 probability levels, respectively

## CONCLUSIONS

Genetic characterization using SSR markers discriminated investigated maize inbred lines in accordance with their heterotic pattern and pedigree making SSR markers a reliable and relevant tool for genetic diversity studies in maize. Statistically significant values of SCA and HPH for investigated traits were recorded in the majority of crosses indicating that inbred lines from this study could be used efficiently in maize breeding programs having desirable alleles for important agronomic traits. Spearman's rank correlation coefficient between genetic distance among inbred lines and SCA and HPH was statistically significant for some traits confirming a certain amount of predictability that could be used as an additional information in maize genetic studies.

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**PREDVIĐANJE HETEROZISA I KOMBINACIONIH SPOSOBNOSTI INBRED LINIJA KUKURUZA NA OSNOVU GENETSKE DISTANCE ODREĐENE SSR MARKERIMA**

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**IZVOD**

Genetička distanca šest elitnih linija kukuruza je određena upotrebom SSR markera. Hibridno potomstvo dobijeno ukrštanjem ispitivanih inbred linija prema šemi nepotpunog dialela je ispitano u poljskim ogledima zajedno sa roditeljskim linijama *per se*. Cilj ovog istraživanja je da utvrdi genetička distanca inbred linija i da se utvrdi značajnost korelacije između distance roditeljskih linija i ispoljenog heterozisa (HPH) i specifičnih kombinacionih sposobnosti (SCA) u potomstvu za prinos zrna, dužinu klipa, broj redova zrna i broj zrna u redu. Upotrebjeno je 21 prajmera u genetičkoj analizi linija što je omogućilo da se detektuje 92 alela. Genetički najudaljenije linije su bile ZPL 1 i ZPL5, odnosno ZPL6 sa vrednošću GD od 0.549, dok su najsirodnije ZPL2 i ZPL3 sa vrednošću GD od 0.11. Dendrogram je inbred linije razvrstao u dva glavna klastera sa ZPL5 i ZPL6 u manjem, odnosno ZPL1, ZPL2, ZPL3, ZPL4 grupisanim u većem klasteru. Vrednosti Spirmanovog koeficijenta korelacije ranga između GD i specifičnih kombinacionih sposobnosti su bile pozitivne i značajne za sve osobine osim broja redova zrna. Najveća korelacija ranga je utvrđena između GD i SCA za broj zrna u redu (0.643). Spirmanovov koeficijent korelacije ranga između GD i ispoljenog heterozisa je bio pozitivan i značajan za dužinu klipa i broj zrna u redu sa izračunatim vrednostima koeficijenta od 0.554, odnosno 0.611.

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