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CORRELATION OF YIELD AND HETEROSIS OF MAIZE HYBRIDS AND THEIR PARENTAL LINES WITH GENETIC DISTANCE BASED ON SSR MARKERS

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The yield, grain yield heterosis and genetic distance based on SSR markers were analyzed in eight maize hybrids and their parental lines. The mean grain yield of all F1 hybrids was 11.37 tha^{-1}. The F1 hybrid from the crosses between L4xL6 gave the highest grain yield of 12.12 tha^{-1}. For the mid parent heterosis (MPH) grain yields of the F1 hybrids, the data showed the average value of 164.25%, and ranged from 136.72% (L4xL6) to 218.07% (L8xL2), and for better parent heterosis (BPH) from 100.70% (H4) to 212.60% (H2), averaged 137.36%. The average genetic distance among parental inbred lines of analyzed hybrids was 0.58 with a range from

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0.55 to 0.61. The GD showed a positive correlation with the grain yield of the F1 hybrids (0.22), as well as with MPH and BPH, with the values of 0.12 and 0.45, respectively.

Key words: grain yield, heterosis, maize, SSR markers

INTRODUCTION

Information on germplasm diversity and relationships among elite materials is of great importance in crop improvement. Genetic diversity studies using molecular markers reveal patterns of diversity in crops that are obscured by the complexities of pedigree records. Various kinds of markers can be used to estimate genetic diversity in maize germplasm. Among them PCR-based markers such as microsatellites have been widely used (SENIOR *et al.*, 1998, ENOKI *et al*., 2002, PINTO *et al*., 2003, ADETIMIRIN *et al*, 2008; KARANJA *et al,* 2009, PABENDON *et al*, 2009). Microsatellites or SSR (Simple Sequence Repeats) correspond to the sequences from 2 to 6 base pairs repeated in tandem, and are broadly used because they are codominant, highly polymorphic, multi-allelic and have a high polymorphism information content.

Heterosis has been extensively exploited but, despite a century of investigations, its genetic basis is not completely understood, yet. For maize breeders of particular interest is to identify genetic factors contributing to heterosis as well as a suitable method that could predict heterosis with some accuracy before field evaluation of test hybrids. In maize, the main strategy is based on the `distance' model: heterosis, defined and measured as the superiority of the hybrid over the midparent is related to the genetic divergence between its parental lines. The relationship between DNA marker-based GD and single-cross grain yields and grain yield heterosis in temperate and tropical maize cultivars were studied, (DRINIC *et al*., 2002, REIF *et al*, 2003; BARBOSA *et al*., 2003; BETRAN *et al*., 2003; XU *et al*., 2004, PHUMICHAI *et al*, 2008, KUMARI *et al*, 2008, BALESTRE *et al*, 2008, PONSGAI *et al.* 2009, SRDIC *et al.,* 2011). The general tendency was that the prediction efficiency of the "distance" model is high when hybrids between related lines and hybrids between both related and unrelated lines are considered. However, correlations between genetic distances of unrelated lines only and their respective interheterotic crosses were of low practical predictive value. The goal of this study was to investigate the relationship between yield, yield heterosis and genetic distance determined with simple sequence repeat (SSR) markers in maize hybrids and their parental lines.

MATERIALS AND METHODS

In this study a set of 16 maize genotypes, 8 parental lines and 8 F1 hybrids was used. Hybrids H1 (L1×L7) belonged to FAO maturity group 300; H2 ((L8×L2) belonged to FAO maturity group 400, H3 (L3×L7) and H4 (L4×L8) belonged to FAO maturity group 500, H5 (L4×L6); H6 (L3×L6), H7 (L3×L8) and H8 (L6×L5) belonged to FAO maturity group 600. All hybrids were yellow-seeded dent type.

From parental inbred lines L2 and L4 were from independent source germplasm, L1, L3 and L5 from BSSS heterotic group, and L6, L7 and L8 from Lancaster germplasm. An experiment set up as randomized block design with two replications at two experimental fields of Maize Research Instiute „Zemun Polje“, during 2011. Four rows were planted for each hybrid, with 20 plants per row, resulting in a plant density of 67,000 plants per ha. The distance between rows amounted to 0.75 m, and the plot size was 12 m^2 (4 x 3 m). Two middle rows within the plot were used for data collection. The plot size for inbreeds was 3 m^2. The identical cropping practices were applied for all genotypes at both locations. Yield of each plot was used for calculation of grain yield per hectare (t ha^{-1}) with 14% moisture. Heterosis was determined as follows:

Mid-parent heterosis (MPH) (%) = ((F1-MP)/MP)*100
Better-parent heterosis (BPH) (%) = ((F1-BP)/BP)*100

where, F1 is the hybrid performance, MP = (P1+P2)/2 in which P1 and P2 are the performances of inbred parents and BP is the better parent value.

The SSR analysis was conducted with bulked samples of five plants per genotypes. Harvested leaves were freeze-dried and ground to powder. Genomic DNA was extracted using a CTAB method (SAGAI-MAROOF, 1989). Screening the Maize DataBase (www.agron.missouri. edu), 20 primers from the bnlg/umc/phi set were assayed using the sample of 16 genotypes. Primers were excluded from the study when banding patterns were difficult to score accurately on agarose gels, or when they failed to amplify consistently in all genotypes. A final set of 15 SSR primers were applied in the analysis of the genotypes according to the method of SENIOR *et al*. (1998). Sequences for these primers, type of repeat amplified, and map position are presented in Table 1.

The amplification reaction was carried out in 25 µl reaction volume containing 1x enzyme buffer, 2.4 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM primers, 1xBSA, 1U *Taq* polymerase and 200 ng of DNA. The amplification profiles followed were: an initial denaturation at 95°C/5min, followed by 15 cycles each of denaturation at 95°C/30sec, annealing at 63.5°C/1min (-0.5°C/cycle) and extension at 72°C/1min; another 22 cycles of 95°C/30sec, 56°C/1min and 72°C/1min were performed. DNA banding patterns from SSR gels were converted into binary form, where a ‘one’ indicates the presence of a specific allele and a ‘zero’ indicates the absence of that allele. Pairwise comparisons of samples were done to estimate Jaccards coefficient of similarity.

The similarity matrix was submitted for hierarchical cluster analyses of unweighted pair group using arithmetical average (UPGMA) method and necessary computation were performed using NTSYS-pc program (ROHLF, 2000).

Table1. SSR markers, primer sequences, map location, number of fragment and polymorphism level

primers	sequences	bin	Total number of fragment	Number of polymer. fragment	Polymor. (%)
phi087	5'-GAGAGGAGGTGTTGTTTGACACAC-3' 5'- ACAACCGGACAAGTCAGCAGATTG-3'	5.06	10	9	90
umc126	5'-CAACAGGGTGAACCCTCTGTACTT-3' 5'-AATATGGTGTTGTGATTTGCATCG-3'	5.06	5	4	80
bnlg2235	5'-ATCCGGAGACACATTCTTGG-3' 5'CTGCAAGCAACTCTCATCGA-3'	8.02	9	9	100
bnlg1443	5'- TACCGGAATCCTCTTTGGTG-3' 5'- TTTGACAACCTCTTCCAGGG-3'	6.05	10	6	60
umc1040	5'-CATTCACTCTCTTGCCAACTTGA-3' 5'-AGTAAGAGTGGGATATTCTGGGAGTT-3'	9.01	7	7	100
umc1827	5'- GCAAGTCAGGGAGTCCAAGAGAG -3' 5'- CCACCTCACAGGTGTTCTACGAC -3'	10.04	2	1	50
umc1109	5'-GCAACACAGGACCAAATCATCTCT-3' 5'-GTTCGGTCCGTAGAAGAACTCTCA-3'	4.10	6	6	100
phi033	5'- ATCGAAATGCAGGCGATGGTTCTC-3' 5'- ATCGAGATGTTCTACGCCCTGAAGT -3'	9.01	6	5	83.3
bnlg1350	5'-TGCTTCAGCGCATTAAACTG-3' 5'-TGCTCGTGTGAGTTCCTACG-3'	3.08	10	10	100
umc1506	5'-AAAAGAAACATGTTCAGTCGAGCG-3' 5'-ATAAAGGTTGGCAAAACGTAGCCT-3'	10.05	5	5	100
umc1695	5'-CAGGTAATAACGACGCAGCAGAA-3' 5'-GTCCTAGGTTACATGCGTTGCTCT-3'	7.00	7	6	85.7
phi112	5'- TGCCCTGCAGGTTCACATTGAGT -3' 5'- AGGAGTACGCTTGGATGCTCTTC -3'	7.01	8	8	100
bnlg1633	5'- GTACCTCCAGGTTTACGCCA -3' 5'- TCAACTTCTCATGCACCCAT -3'	2.07	16	16	100
umc2129	5'- ACGTGGTCATCACTCACCGC -3' 5'- AAGGAGGAGCGTTCTCGTGG -3'	2.07	9	9	100
bnlg1526	5'-ACGAGCGAGTGGAGAATAGG-3' 5'-AGCCCAGTACGTGGGGTC-3'	10.04	11	9	82
total			**121**	**110**	**89.4**

RESULTS AND DISCUSSION

Hybrids yielded an overall mean of 11.37 tha^{-1}, with range from 10.91 to12.12 tha^{-1} (Table 2). The highest yield in both locations had H5 (L4xL6) and the lowest one H2 (L8xL2). The differences in yields of different hybrids are possible in hybrids of various genetic backgrounds (ALI *et al*., 2007), which is confirmed by our results. Among lines, the highest yield has L4 (5.68 tha^{-1}) and the lowest one L2 (3.37 tha^{-1}).

The averaged yield of parental lines was 4.20 tha^{-1}. Generally, the yield of hybrids and parental lines was lower in location 2 than location 1 (data not shown). Midparent heterosis (MPH) from grain yield averaged 164.25%, ranged from 136.72% (L4xL6) to 218.07% (L8xL2). The highest best parent heterosis was in H2 (212.60%) and the lowest in H4 (100.70%), averaged 137.36%. The hybrid with the highest yield, combination of two good yielding inbred lines, has the lowest MPH and low BPH. The parents of H2 with the lowest yield but the highest both MPH and BPH are low yielded inbred.

All hybrids, as crosses between inbred lines from different heterotic group, has positive yield heteroses which is in agreement with another investigators, who commonly assumed that the combination of lines of different heterotic groups originates hybrids with higher chances of genetic expression of the target effects of hybridization (TROYER, 1999; TOLLENAAR *et al*., 2004).

Table 2. Yield, yield heterosis and GD for hybrids and their parental inbred lines

genotype	Yield (t/ha)	genotype	Yield (t/ha)	Midparent heterosis (%)	Best parent heterosis (%)	GD
Parental genotypes		F1 hybrids				
L1	4.03	L1xL7	11.10	183.88	175.43	0.59
L2	3.37	L8xL2	10.91	218.07	212.60	0.61
L3	5.25	L3xL7	11.05	144.46	110.47	0.55
L4	5.68	L4xL8	11.40	148.90	100.70	0.59
L5	3.47	L4xL6	12.12	136.72	114.79	0.61
L6	4.56	L3xL6	11.82	141.22	125.15	0.57
L7	3.79	L3xL8	10.98	151.25	109.14	0.55
L8	3.49	L6xL5	11.61	189.52	150.60	0.57

The 15 SSR primer pairs were used to reveal the genetic diversity among eight hybrids and their parental lines. From screening 20 SSRs primers, 3 primers failed to amplify products consistently, 2 primers were difficult to score accurately. Fifteen pairs of primers were chosen which could produce stable and repeatable bands (Tab. 1). The number of SSR loci used to screen the maize genotypes was considerably lower than those reported previously in maize (PINTO *et al*., 2003, WARBURTON *et al.,* 2002, LEGESSE *et al.,* 2007) but according to SOUZA *et al*., (2008), 16 microsatellite loci were enough to analyze the genotypes with accuracy. A total of 121 alleles were scored from which 110 (89.4%) was polymorphic. The average number of alleles per primer was 8.07, ranging from 2 (UMC1827) to 16 (bngl 1633). The mean allele per markers in this study was little higher than those detected by previous SSR studies (SUN *et al*. 2001). XIA *et al* (2004) found an average of 7.4 allelles per markers and RANATUGA *et al* (2009) notice 6.0 alleles per primers using 22 SSR primers for analysis of maize inbreds.

The lowest genetic distance (0.12) was between two sister lines L7 and L8 and the highest one between L2 and L3 (Tab3.). L2 is from independent source and L3 belonging to BSSS germplasm. Also, inbred line L2 was genetically distinct from sister lines L7 and L8, belonging to Lancaster germplasm. Genetic distance was lower between inbred belonging to Lancaster heterotic group compared to inbreds belonging to BSSS heterotic group. The genetic distance between inbred lines is in agreement with data on the origin of the inbreds, and also with the grain yield heterosis of their crosses. Hybrid H4, with the highest yield, is cross between L4 and L6, lines with the highest genetic distance, which is in accordance with SAMPHANTARAK (2003), who had reported that the two main factors affected high grain yield of hybrids were high GD and adaptability of both parental lines.

The genetic distance between parental inbred lines of analyzed hybrids was in range 0.55-0.61. The lowest genetic distance was estimated between hybrids H8 and H6 which have one common parent and the highest one between hybrids H6 and H4 (Tab 4.). In hybrids H1, H3, H7 and H8 genetic distance between hybrid and both parent lines was almost similar. Hybrids H5 and H6 were more similar to female parent as well as hybrids H2 and H4 to male parent.

Table 3 Genetic distance between inbred lineas

	L1	L2	L3	L4	L5	L6	L7
L2	0.47						
L3	0.53	0.62					
L4	0.56	0.47	0.57				
L5	0.43	0.49	0.58	0.64			
L6	0.56	0.71	0.57	0.61	0.57		
L7	0.59	0.61	0.55	0.59	0.52	0.41	
L8	0.59	0.61	0.55	0.59	0.57	0.41	0.12

Table 4. Genetic distance between hybrids

	H1	H2	H3	H4	H5	H6	H7
H2	0.46						
H3	0.48	0.59					
H4	0.46	0.50	0.46				
H5	0.53	0.42	0.61	0.40			
H6	0.54	0.43	0.42	0.62	0.46		
H7	0.48	0.57	0.39	0.44	0.56	0.40	
H8	0.56	0.49	0.51	0.59	0.53	0.37	0.47

The cluster analysis based on genetic distance computed from SSR data classifies each of 16 genotypes into one of two principal clusters, designated GI, GII. Results are presented in Figure 1. The cluster I consist of two subclusters A and B. Subcluster A encompasses 6 genotypes, hybrids H1 and H2 form one subgroup and

hybrids H4 and H5 another subgroup. Hybrids H4 and H5 have L4 as a common parent. Two inbred lines, L2 and L4 are linked with those hybrids. Subcluster B contains four hybrids H3, H7, H6, H8 with one common inbred line in background. Clustering of hybrids based on SSR markers showed good agreement with their pedigree data, hybrids with similar parental components were joined together in similar group. Cluster GII includes two subclusters C and D. Three inbred lines from BSSS heterotic group, L1, L5 and L3, formed subclaster C. Claster D formed two sister lines and L6 loosely linked to them All inbred lines from subclaster D belonging to Lancester heterotic group. The results show that lines the most closely related by pedigree are those that are also closely related on the basis of SSR marker information.

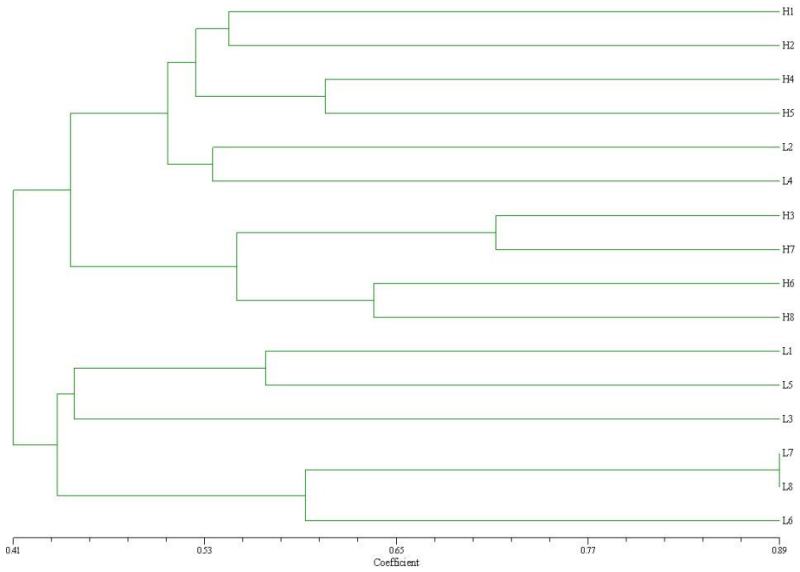

Fig. 1. Association among hybrids and parental inbred lines revealed by cluster analysis of SSR distance data

The correlation between the GD and grain yield, grain yield heterosis was established by Spearman's rank correlation coefficient. The GD between parental inbred lines was positively correlated with the hybrid grain yield (r=0.22), as well as with MPH and BPH with value of 0.12 and 0.45, respectively. Our result was similar to that of BETRAN *et al.* (2003) who showed the GD was positively correlated with the grain yield, MPH, and best parent heterosis of F1 hybrids. PAJIC *et al* (2010) obtained positive and significant correlation between GD and grain yield and positive correlation with grain yield heterosis in set of popcorn inbred lines.

The SSR markers represent a powerful tool in the assessment of the genetic diversity between inbred lines. Using them, field trials for the identification of promising heterotic patterns can be planned more efficiently based on the prior obtained information by markers, and that would make a great contribution to the efficiency of maize breeding.

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KORELACIJA PRINOSA I HETEROZISA HIBRIDA KUKURUZA I NJIHOVIH RODITELJSKIH LINIJA SA GENETIČKOM DISTANCOM NA OSNOVU SSR MARKERA

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Prinos, heterozis za prinos zrna i genetička distanca izračunata na osnovu SSR markera, su ispitivani za osam hibrida kukuruza i njihove roditeljske komponente. Prosečan prinos zrna F1 hibrida je bio 11.37 tha^{-1}. Hibrid dobijen ukrštanjem linija L4xL6 je imao najveći prinos od 12.12 tha^{-1}. Prosečna vrednost heterozisa u odnosu na prosečnog roditelja za prinos zrna za hibride je bila 164.25%, i varirala je od 136.72% (L4xL6) do 218.07% (L8xL2), i za heterozis u odnosu na boljeg roditelja od 100.70% (H4) do 212.60% (H2), prosečno 137.36%. Prosečna genetička distanca između roditeljskih linija ispitanih hibrida je bila 0.58 sa opsegom od 0.55 do 0.61. Korelacija između GD i prinosa hibrida je bila pozitivna (r=0.22), kao i sa heterozisom u odnosu na prosečnog roditelja (r=0.12) odnosno heterozisom u odnosu na boljeg roditelja (r=0.45).

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