

# Genetic distance based on simple sequence repeats and its relationship to specific combining ability and heterosis in maize (*Zea mays* L.)

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## Abstract

Genetic relationship among ten maize inbred lines derived from four different source populations was assessed using forty microsatellite markers. Correlations were estimated between Simple Sequence Repeat (SSR) markers based genetic distance with  $F_1$  hybrid performance, midparent heterosis and combining ability for grain yield, days to silking and plant height. The genetic distances based on SSRs were almost congruent with pedigree information and depicted positive linear relationship with  $F_1$  hybrid performance, heterosis value and specific combining ability for grain yield. From the present results it is concluded that the Jaccard similarity coefficient based on SSR data might be used for determining genetic relationship among inbred lines. Although positive relation was revealed between genetic distance and midparent heterosis in this analysis but due to its low magnitude it couldn't be used to precisely predict the  $F_1$  hybrid yield performance.

**Key words:** Maize, genetic distance, SSRs, heterosis, combining ability, *Zea mays*

## Introduction

The heterosis has been extensively exploited in the improvement of many crops leading to significant increase in yield. Identification of inbred lines that form superior hybrids is the most costly and time-consuming task in hybrid maize development, which is based on a *priori* and a *posteriori* choice. The former consists of selection methods based on *per se* performance, such as midparental value, divergence according to coefficient of parentage, multivariate analysis and parental distances. *Per se* performance of maize inbred lines does not predict the performance of maize hybrid for grain yield [1]. In the *posteriori* choice, parents are evaluated on the basis of  $F_1$ ,  $F_2$  and advanced

generations. Specific combining ability and heterosis are analyzed to identify better hybrid combinations, but it becomes a cumbersome task to analyze large number of genotypes and their cross products.

To increase the breeding efficiency, parental genetic distance based on morphological and molecular markers has been used as a potential tool to predict hybrid vigour of single cross. The relationship between genetic distance and heterosis was reported even before the development of molecular markers [2]. In maize molecular markers have been used to analyze the genetic relationships among inbred lines and to examine the relationship between estimated genetic distance and single cross grain yields [3, 4, 5]. Some researchers have indicated that the genetic distance from RFLP cannot be used for predicting the yield performance and heterosis for  $F_1$  hybrids. However, Smith *et al.*, [6] and Bernardo [7] believed that the degree of similarity calculated from RFLP data could allow maize breeders to predict the combinations of lines that would result in high yielding single cross hybrids. Lanza *et al.* [8] and Liu *et al.* [9] reported that RAPD could be used as a tool for determining the extent of genetic diversity among maize inbred lines, for allocating genotypes into distinct heterotic groups. However, Shieh and Thseng [10] reported non-correlation between RAPD-based genetic distance and maize single cross hybrid grain yield.

Microsatellite (SSR) markers are an important class of molecular markers due to their abundance, codominant nature and high level of variability. However, their importance in predicting heterosis is not unambiguous ranging from the reports implying high utility [11] to those indicating limited use [12]. Attempts

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have been made to use maize genotypes belonging to different breeding groups in India for the analysis of genetic distance and relationship [13]. In this context present investigation was carried out using maize inbred lines belonging to particular breeding group with the following objectives, (i) to estimate the heterosis and SCA for grain yield, days to 50% silking and plant height, (ii) to determine the genetic relationship by means of SSR markers, (iii) to calculate genetic distance based on SSR markers and (iv) to estimate correlation between genetic distance (GD) with  $F_1$  hybrid performance ( $F_1$ ), percent midparent heterosis (%MPH) and specific combining ability (SCA).

## Materials and methods

### Materials

Ten maize inbred lines used in the present study were derived from four different source populations (Table

1). These inbred lines were crossed in a 10 x 10 half diallel mating design at maize winter nursery, Hyderabad during *Rabi* season (2000-01), to obtain 45  $F_1$  hybrids. The experimental material consisted of ten parental lines, 45  $F_1$ 's and five checks (Pro311, Him 129, PEHM2, PEHM3 and Parbhat). All the sixty entries were evaluated for grain yield, days to silking, and plant height as per the standard procedure in field trials at two locations (Delhi and Karnal) during *Kharif* season (2001) in randomized complete block design with 3 replications. Each experimental unit consisted of two rows of 5 m length with 75 x 25 cm spacing.

### SSR analysis

DNA was extracted using the CTAB procedure [14] with minor modifications from leaf samples of 20-day old seedlings. Forty SSR primers were used for the PCR amplification (Table 2) and the amplified products were resolved on 3.5% SFR (Super Fine Resolution;

**Table 1.** Pedigree and source population of maize inbred lines used in the present study

Inbred code no.	Pedigree	Source population	Inbred code no.	Pedigree	Source population
DMB101	IPA3-6-10-3-I-I-I-2-I-#	A-64	DMB106	IPA21—f-I-#	AD-609
DMB102	IPA3-f-I-#	A-64	DMB107	IPA 34 -5-f-#	MDR-1
DMB103	IPA3-f-2-#	A-64	DMB108	IPA 34-62-f-#	MDR-1
DMB104	IPA I-f-16-2-#-#	A-64	DMB109	TCA 22-f-#-#	A-64
DMB105	IPA21-f-#-#	AD-609	DMB110	SC 7-2-f-#	MDR-1XA-64

**Table 2.** Microsatellite markers used in the present study

SSR locus type	Repeat location	Bin les #	Alle-range	Size value	PIC locus	SSR type	Repeat location	Bin	Alleles # range	Size value	PIC
<i>bnlg147</i>	GT	1.02	2	100-140	0.38	<i>Phi075</i>	CT	6.00	3	100-150	0.66
<i>bnlg615</i>	CT	1.07	3	75-150	0.61	<i>umc1006</i>	GCCAGA	6.02	1	125	0.00
<i>bnlg400</i>	AG	1.09	1	100	0.00	<i>umc1014</i>	GA	6.04	3	100-150	0.56
<i>umc1331</i>	GGT	1.11-1.12	3	140-200	0.55	<i>phi089</i>	ATGC	6.08	2	80-100	0.48
<i>bnlg371</i>	CA	2.05	4	60-90	0.67	<i>bnlg572</i>	AG	7.03	3	100-150	0.58
<i>mage05</i>	AG	2.05	3	80-120	0.40	<i>bnlg339</i>	CT	7.03	4	100-150	0.66
<i>phi029</i>	CCCTCT	3.04	1	100	0.00	<i>phi045</i>	AAC	7.06	3	150-175	0.50
<i>bnlg1452</i>	AG	3.04	4	60-140	0.70	<i>phi119</i>	AG	8.02	3	150-200	0.65
<i>bnlg1796</i>	AG	3.06	4	70-100	0.70	<i>phi014</i>	GGC	8.04	1	175	0.00
<i>phi079</i>	AGATG	4.05	2	180-200	0.49	<i>phi121</i>	CCG	8.04	2	50-80	0.45
<i>dupssr34</i>	TTG	4.07	4	100-200	0.70	<i>bnlg162</i>	CT	8.05	4	100-160	0.53
<i>phi093</i>	AGCT	4.08	2	200-250	0.50	<i>phi033</i>	AAG	9.01	2	150-200	0.38
<i>Bnlg143</i>	CA	5.01	4	100-200	0.68	<i>bnlg127</i>	CT	9.04	3	200-300	0.59
<i>Bnlg105</i>	AG	5.02	3	80-120	0.56	<i>phi108411</i>	AGCT	9.05-9.08	2	70-100	0.41
<i>Umc1225</i>	AG	5.08	4	100-125	0.68	<i>phi062</i>	ACG	10.04	1	150	0.00
<i>bnlg389</i>	CT	5.09	3	110-160	0.65	<i>bnlg2336</i>	AG	10.04	3	100-150	0.57

Amresco) agarose gel. The polymorphic bands were used for the construction of the binary value matrix, representing the absence and presence of bands by 0 and 1, respectively. Polymorphism information content (PIC) was estimated for each SSR markers as per the procedures outlined by Senior *et al.*, [16]. Genetic similarity estimates between each pair of inbred lines were obtained using Jaccard's similarity coefficients (GS) =  $a/(a+b+c)$ , where a = number of bands present in both parents, b = number of bands present in the first parent and c=number of bands present in the second parent. Genetic distance between pairs of inbred lines were estimated as  $GD = 1-GS$ . The distance matrix was analyzed and association among the inbreds was revealed with cluster analysis [15] and principal coordinate analysis (PCoA) based on Jaccard distances and UPGMA method using NTSYS-pc version 2.02.

### Statistical analysis

Analyses of variance were computed for individual environment following the procedures for CRBD given by Cochran and Cox [17]. A combined ANOVA was carried out for data pooled across the environments after testing for homogeneity of error variance. Combining ability analysis was done according to Griffing [18] method 2 and model I using statistical software package SPAR 1. Pearson correlation coefficients ( $r$ ) between genetic distance (GD) and single cross hybrid performance ( $F_1$ ), midparent value (MP), percent midparent heterosis (MPH) and specific combining ability (*sca*) for all three traits were calculated from means per environment and across environments using statistical software, SPSS 10.0. Linear regression of hybrid performance, heterosis and specific combining ability of grain yield on genetic distance was performed.

### Results and discussion

Analysis of variance showed significant differences among the inbred lines and their cross combinations for all three traits (Table 3). Combined ANOVA revealed that mean sum of squares due to genotype and genotype x environment interaction was highly significant [19]. *gca* and *sca* variance across environments was highly significant for all the traits (Table 3). The *sca* effect for grain yield was maximum in DMB 101 x DMB109 (29.14) followed by DMB103 x DMB104 (23.09) as depicted in Table 4 while for earliness it was highest in DMB101 x DMB110 (-3.85) followed by DMB103 x DMB 105 (-3.15). Maximum percent midparent heterosis (MPH) for grain yield was observed in cross DMB 101 x DMB 109 with 105 %

**Table 3.** Components of variance ( $cr^2$ ) for three agronomic traits determined from the combined analysis of variance of 10 inbred lines and 45 hybrids grown at two locations

Source	df	Grain yield (gm)	Days to silking (d)	Plant height (cm)
Treatment (T)	54	1815.85**	32.85**	1566.62**
Location (L)	1	47424.01**	22.4**	124548.24**
TxL	54	538.59*	8.35**	364.47**
Error	216	378.83	2.57	203.13
$s^2_{gca}$	9	512.87**	26.05**	768.29**
$s^2_{sca}$	45	623.76**	7.92**	472.99**
$s^2_{gca} \times L$	9	131.80	3.49**	110.48
$s^2_{sca} \times L$	45	189.07*	2.64**	123.69**
Error	216	126.27	0.85	67.71

gain, which was also the top yielding hybrid with maximum *sca* followed by DMB 102 x DMB 109 (Table 4). The percent midparent heterosis for days to silking was most negative in hybrid DMB101 x DMB110 (-12.44) followed by DMB102 x DMB109 (-11.64). Thus due to its contribution to early maturity DMB109 can also be recommended for developing early maturing hybrids. Most of the hybrids showed MPH for earliness. Maximum percent MPH for plant height was observed in the hybrid DMB102 x DMB110 (31.77) followed by DMB101 x DMB110 (31.03). Only two hybrids showed negative midparent heterosis for plant height, which indicated high vigour and growth among hybrids. The average degree of percent MPH for grain yield varied from 31.79 (Karnal) to 38.42 (Delhi). For days to silking average MPH was -2.91 at Karnal and -6.59 at Delhi, while it was -4.80 across the environments (Table 5). The average degree of percent MPH for plant height was more at Karnal in comparison to Delhi but the latter was favourable for earliness and yield attributes.

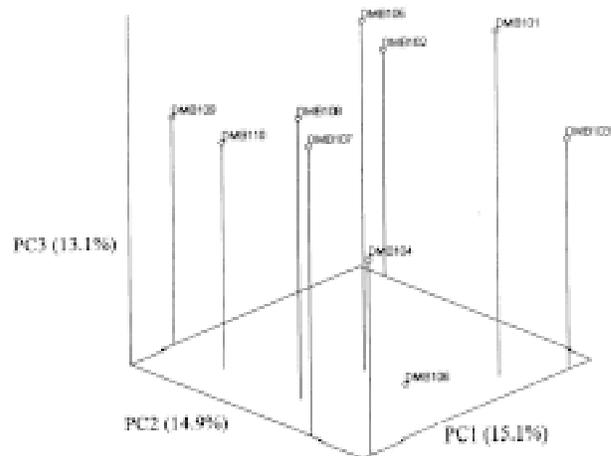
Out of forty SSR markers, 32 revealed clear and consistent amplification profiles. Gene or allelic diversity, measured by PIC at a locus ranged from 0.38 to 0.70 except monomorphic loci (Table 2). The loci showing the lowest PIC value could be associated with stronger selection pressure during the improvement of the source populations and inbred line development. The SSR based Jaccard similarity coefficient analysis showed genetic distances between pair of inbred lines ranging from 0.55 (DMB109 and DMB110 showing maximum similarity) to 0.81 (DMB109 and DMB103 exhibiting



inbred lines also originated from different source populations [15]. Genotypes (DMB 107 and DMB 108) belonging to MDR-1 source population were found to be relatively closer. Genotype DMB 110 derived from A-64 and MDR-1, appeared to be more closely related to DMB 107, DMB 108 and DMB 109, which shared these two source populations. Since PCoA and CA complement each other to a certain degree, Messmer *et al.* [20] recommended performing both types of analysis in order to extract maximum information from molecular data. Grouping of the ten genotypes on the basis of SSR profiling was found to be congruent with their pedigree and breeding history except in few cases, like DMB 105 and DMB 106 belonging to AD-609 population were far apart in the diagram (Fig. 1). Some inconsistencies in clustering patterns were also reported by Yu *et al.* [22] using SSR data. Further, pedigree and molecular marker data may not always be congruent in highly cross-pollinated crops like maize due to selection drift during the development of inbreds [21]. In general, cluster analysis as well as PCoA revealed high genetic divergence among the inbred lines even within the same cluster. The average distance among inbred lines was 0.70 suggesting large diversity among the genotypes.

The correlation among GD with  $F_1$ , MP, MPH and *sca* was estimated using Pearson product moment correlation coefficient for grain yield, days to silking and plant height and subsequently the GD was plotted against MPH, *sca* and mean value for grain yield (Fig.

2). Grain yield for hybrid was positively correlated with GD, MPH, and *sca* while negatively for MP across environments (Table 5). The correlation 'r' between  $F_1$  and *sca* is higher than  $F_1$  and MPH for all three traits. This was an indication that the *sca* among parental lines can predict hybrid performance better than the heterosis observed which is highly dependent on the performance of inbred lines and this was similar to the reports by Betran *et al.*, [5]. The correlation between midparent

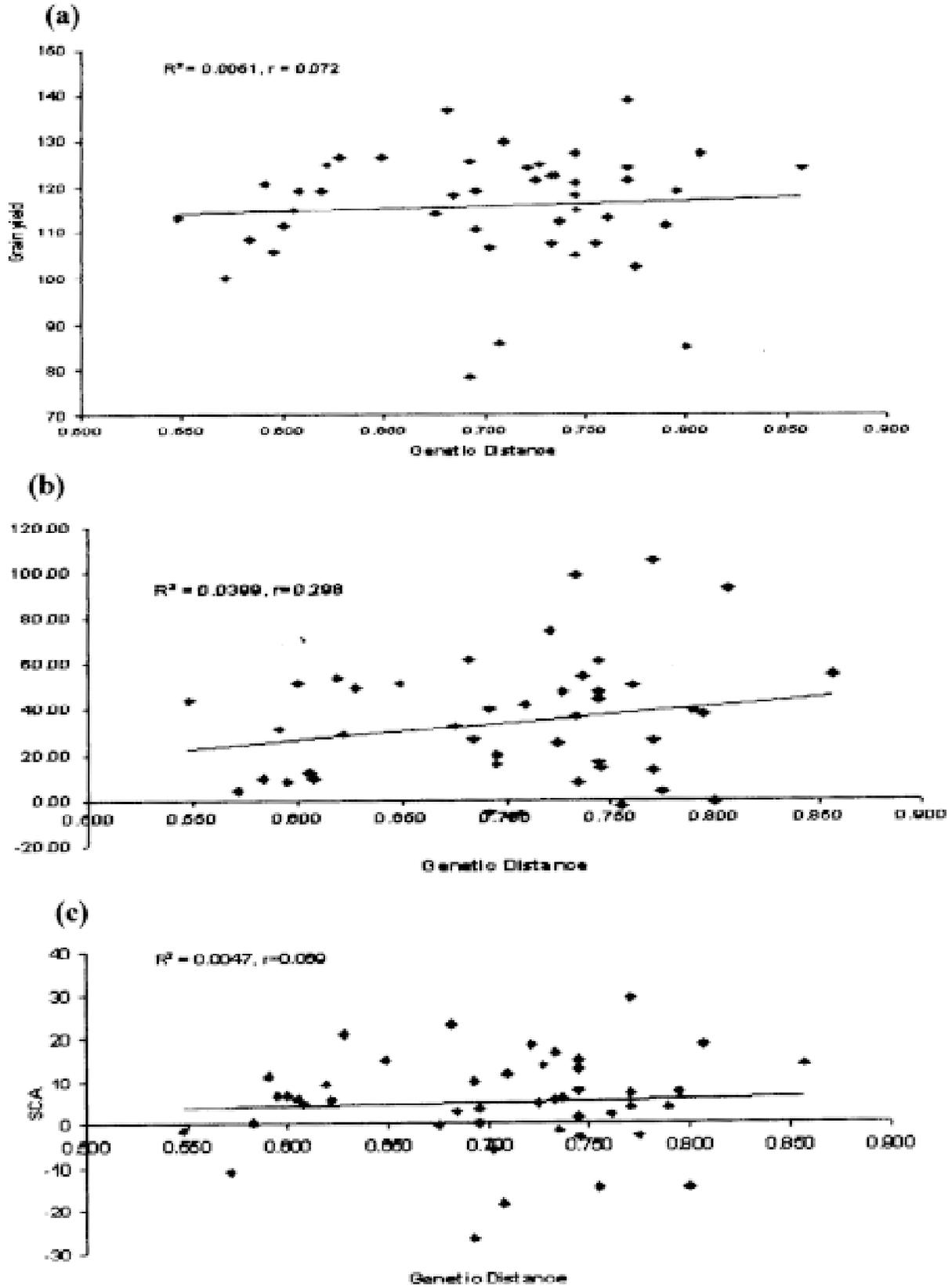


**Fig. 1. Principal coordinate analysis of ten maize inbred lines based on SSR markers; PC1, PC2 and PC3 are the first, second and third principal coordinates, respectively**

**Table 5.** Average midparent heterosis (MPH) and correlation among  $F_1$  hybrid performance, percent midparent heterosis, specific combining ability and genetic distance for all the hybrids among 10 maize inbred lines

Environment	%MPH (average)	Correlations							
		$r(F_1, SCA)$	$r(F_1, MPH)$	$r(F_1, MP)$	$r(SCA, MPH)$	$r(F_1, GD)$	$r(SCA, GD)$	$r(MPH, GD)$	$r(MP, GD)$
Grain yield (gm/plant)									
Delhi	38.42	0.92**	0.68**	-0.18	0.77**	0.22*	0.17	0.25*	-0.19
Karnal	31.79	0.85**	0.57**	-0.13	0.83**	0.16	0.09	0.33*	-0.11
Across	33.71	0.87**	0.67**	-0.17	0.82**	0.07	0.07	0.29*	-0.18
Days to silking (d)									
Delhi	-6.59	0.61**	0.44*	0.15	0.84**	-0.31*	-0.04	0.08	-0.28*
Karnal	-2.91	0.79**	0.76**	0.37*	0.91**	-0.11	0.12	-0.03	-0.12
Across	-4.80	0.70**	0.61**	0.31*	0.88**	-0.19	0.09	0.02	-0.22*
Plant height (cm)									
Delhi	12.55	0.83**	0.74**	0.09	0.86**	-0.25	-0.16	-0.25*	-0.08
Karnal	22.34	0.84**	0.61**	0.10	0.82**	-0.21*	-0.21*	-0.16	-0.02
Across	16.54	0.79**	0.64**	0.19	0.85**	-0.27*	-0.23*	-0.26*	0.05

\*, \*\*Correlation is significant at the 0.01 and 0.05 % levels respectively.



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Fig. 2. Regression of  $F_1$  on GD (a), midparent heterosis (MPH) on GD (b) and sca on GD (c) for grain yield; 'r' is the correlation coefficient

value and hybrid across environments were  $-0.17$ ,  $0.31$  and  $0.19$  for grain yield, days to silking and plant height, respectively. Specific combining ability was positively correlated with MPH with the value being  $0.82$ ,  $0.88$  and  $0.85$  for GY, DS and PH, respectively. The GD was positively correlated with  $F_1$ , *sca* and MPH in individual as well as across the environments (Table 5) for the grain yield. For days to silking, GD was correlated negatively with  $F_1$  and MP, while with *sca* and MPH it was associated in both directions. The GD was negatively correlated with  $F_1$ , *sca*, MPH and MP for plant height. The cross between more similar genotypes exemplified by DMB109 x DMB110 and DMB108 x DMB110 were least heterotic ( $43.91$  and  $4.35$  respectively) in comparison to hybrid between distant parents (DMB103 x DMB109,  $92.45\%$ ) for grain yield. The low correlation between GD and MPH for plant height and days to silking were mostly due to small MPH estimates (Table 3) for these traits. By comparison, the corresponding correlation for grain yield was high ( $r = 0.29$ ,  $p = 0.05$ ). This was consistent with the relatively large contribution of *sca* effect to the total sum of squares. From the distribution of SSR genetic distance and mean of grain weight (Fig. 2a), the linear regression produced smaller 'r'. It was small for *sca* also, but for MPH,  $r = 0.29$  was produced (Fig. 2b). Melchinger [4] pointed out that only intragroup crosses show a correlation between parental genetic distance and mid parent heterosis, but no intergroup crosses. This study mainly involved intragroup crosses and related lines as most of the inbred lines were derived from the same heterotic group based on marker data as well as pedigree. However, the low correlation between GD and percent MPH may be due to the broad genetic base of the source population, as revealed by average genetic distance ( $0.70$ ) or relatively less number of markers employed. Ajmone Marson *et al.* [23] also found correlation between GD and MPH as  $0.31$  using RFLP markers.

Thus simple sequence repeats markers can help to estimate the level of genetic diversity in breeding material, determine genetic relationship among inbred lines and are a good complementation to field trials for identifying groups of genetically similar germplasms. The results of present study demonstrated positive correlation between SSR based GD with percent MPH and *sca* for grain yield, but due to the fact that their coefficients of determinations for heterosis value ( $0.039$ ) and *sca* ( $0.004$ ) are small, the predictive value is limited. In essence, this study revealed that SSR analysis couldn't be used to precisely predict the yield

performance and heterosis value of  $F_1$  Hybrids. Yuan *et al.*, [24] also reported that correlation between hybrid performances; *sca* and GD were not sufficient to have a practical utility in prediction of hybrid performance. There are many conditions which negatively influence the prediction of genetic distance and heterosis, such as increased similarity in a gene pool due to strong selection pressure [25], lack of linkage between genes controlling the traits and the markers used [26], gene pool with a narrow genetic base [27], epistasis [2] and G x E interaction. Therefore, it can be concluded that GD estimates based on unselected set of DNA markers alone are not promising for predicting hybrid performance, there must be preselection of specific markers linked to quantitative trait loci correlated to heterosis.

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