Genetic distance based on SSR markers and testcross performance of maize inbred lines

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Abstract

The identification of parental inbred lines to develop superior hybrids is a rather costly and time-consuming step in maize breeding. In some cases, pedigree information has been used to select diverse parental lines. In the case of Iranian maize inbred lines, this information is not fully available. In this study we investigated the genetic distance (GD) based on Simple sequence Repeats (SSR) markers between pairs of five maize testers and 28 inbred lines and assessed the relationship between GD and F_1 hybrid performance, specific combining ability (SCA) and midparent heterosis (MPH). One hundred and forty testcrosses were evaluated for grain yield in 2003, 2004 and 2005 at two locations, Karaj and Gorgan (only 2004), Iran. Significant positive but low correlations were found between GD and F_1 performance, SCA and MPH (0.27**, 0.39** and 0.28**, respectively). Testers affected the magnitude of correlations, with relatively high values revealed in the Mo17 crosses (0.54**, 0.61** and 0.61** for F_1, SCA and MPH, respectively) and lowest values in the B73 crosses. Although GD between parents correlated significantly with hybrid performance, the estimates of GD did not consistently identify the best crosses.

Keywords: Genetic Distance-Maize (*Zea mays* L.); Midparent Heterosis; Simple Sequence Repeats (SSRs); Specific Combining Ability (SCA); Yield prediction.

INTRODUCTION

In hybrid maize breeding programs, efficiency of procedures to identify inbreds used to develop outstanding single crosses strongly affects the success of the program (Hallauer and Lopez-Perez, 1979). The best hybrid combinations can be identified using informa-tion from diallel or topcrosses to testers (Gonzalez *et al*., 1997; Terron *et al*., 1997), pedigree information, morphological traits (which may be greatly influenced by the environment), and molecular markers that detect variation at the DNA sequence level (Smith and Smith, 1992).

With large number of inbreds, diallel crosses are not possible in practice due to a prohibitive number of crosses. Hallauer *et al*. (1988) suggested relative performance of inbred lines in testcrosses with divergent testers to determine the heterotic patterns among lines, as has been carried out at the International Maize and Wheat Improvement Center (CIMMYT) maize breeding program (Vasal *et al*., 1992a, 1992b). Grouping of Iranian inbred maize lines into heterotic groups is not yet as well defined as in other temperate maize programs. Information on pedigree would aid in grouping, but is often lacking for Iranian breeding materials. DNA markers could help to establish initial heterotic groups based on GD, allowing breeders to avoid crosses among related inbreds. Choukan *et al*. (2006) and Choukan and Warburton, (2005) used this approach in grouping 56 late to medium and 36 early maturing Iranian maize inbred lines using 46 and 36 SSR markers, respectively.

Several previous studies have demonstrated the possibility of using molecular markers to assign maize germplasm to heterotic groups or form new groups (Lee *et al*., 1989; Livini *et al*., 1992; Dubreuil *et al*., 1996). Nevertheless, the correlation between GD based on molecular markers and hybrid performance has not been consistent. Low to high correlations between molecular divergence and hybrid performance have been reported (Dhillon *et al*., 1993; Ajmon-Marsan *et al*., 1998; Smith *et al*., 1990), indicating that

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GD may or may not be useful to predict hybrid performance. The objective of this study was to evaluate the correlation between the SSR markers based on GD and hybrid performance, MPH and SCA in crosses between Iranian maize inbred lines, in order to determine the possible use of marker based GD in Iranian maize improvement programs.

MATERIALS AND METHODS

Plant materials and field experiments: A total of 28 maize inbred lines currently used in Iranian maize breeding programs and five tester lines were used for this study (Table 1). Each of the 28 inbred lines was crossed with five tester lines (Mo17, B73, K74/1, K1264/1 and KL17/2-5) to produce a total of 140 hybrid combinations. These testers have been used in hybrid production in Iran, and are the parents of commercial single cross hybrids, except KL17/2-5. The 140 line x tester crosses and four promising hybrids were evaluated at two locations for grain yield: Karaj in 2003, 2004 and 2005 (35º49′ N, 51º00′ E), and Gorgan in 2004 (35º50′ N, 54º22′ E). Plot data were used to adjust the grain moisture to 14%, calculating the grain yield based on t/ha.

SSR analysis: DNA extraction and SSR assays were previously described in detail by Choukan *et al*. (2006). Briefly, inbred lines were characterized with forty-six SSR primers, which were chosen based on repeat unit and bin location to provide uniform coverage of the entire maize genome from the MaizeGDB database (http://www.maizegdb.org/ssr.pho). The SSRs were multiplexed for maximum efficiency. DNA fragments were separated using acrylamide gels run on an ABI377 automatic DNA sequencer. Fragment sizes were calculated with GeneScan3.1 (Perkin Elmer/Applied Biosystems) using the Local Southern sizing method. The fragments were assigned to alleles using the category function of Genotype 2.1 (Perkin Elmer/Applied Biosystems), and exported as an excel file recording allele identity for each individual.

Statistical analysis: Line x tester analysis was carried out using the adjusted means of grain yield (t/ha) based on the method described by Kempthorne (1957). Specific combining ability SCA effects for grain yield were calculated according to the line x tester model. The MPH of each cross was calculated as the difference between F_1 means and the respective midparent

Table 1. List and pedigree or origin (when known) of maize testers and inbred lines involved in this study.

Inbred lines	Pedigree sources/origin
Lancaster Sure Crop (LSC) related lines	
Mo17(Tester)	CL.187-2 x C103
K18	Derived from Mo17 changes
K19	Derived from Mo17 changes
K19/1	Derived from K19 changes
Reid Yellow Dent (RYD) related lines	
B73(Tester)	BSSS C5 (Iowa Stiff Stalk Synthetic)
Lines derived from unknown source XL17/2	
KL17/2-2	Derived from XL17 changes
KL17/2-3	Derived from XL17 changes
KL17/2-5(Tester)	Derived from XL17 changes
Lines derived from unknown local source	
K1264/1(Tester)	Unknown
K1263/8	Unknown
Lines with unknown pedigree	
K3304/1-2	Unknown
K74/1(Tester)	Unknown
K1259	Unknown
K1259/4	Unknown
K760/7	Unknown
K2818	Unknown
K2836/2	Unknown
Lines extracted from Late Synthetic	
K3651/1	SYN-Late
K3653/2	SYN-Late
K3653/5	SYN-Late
K3640/3	SYN-Late
K3640/5	SYN-Late
K3640/6	SYN-Late
K3640/8	SYN-Late
K3615/1	SYN-Late
K3615/2	SYN-Late
Lines extracted from CIMMYT materials	
K3545/7	Telaltizapan-8946
K3493/1	Unknown from EVT 16A
K3530/3	Pool 42 (Based on germplasm from Mexico, Peru, Bolivia, Pakistan, Hungary, the USA and Yemen)
K3547/5	Srinagar 8848
K166/629B	(P33/ Mo17) F_2 population received from CIMMYT P33, a Subtropical intermediate-maturity population, contains predominantly Argentinean (Cateto) flints
Other Lines	
K3218	EA-2920 x EA-3148(Spain)
K3047/2	BR 105 III(Brazil)

mean across all environments. GDs between all possible pairs of testers and lines, based on the SSR data,

were estimated from the Rogers' distance (Rogers 1972):

$$RDij = \frac{1}{m}\sum_{j}^{m}\sqrt{\frac{1}{2}\sum_{i}^{a_j}(p_{ij}-q_{ij})^2}$$

where RDij= Rogers' distance between individuals, p_{ij} and q_{ij} are the frequency of ith allele at the jth locus in parents x and y, respectively, while aj is the number of alleles at the jth locus, and m is the number of the loci examined. Correlation coefficients (r) were calculated

between GD's and F_1 performance and SCA of grain yield for the 140 line x tester testcrosses, as well as within each of five tester lines.

RESULTS

Hybrid performance: Grain yields for 140 line x tester combinations are presented in Table 2. Mean grain yield of testcrosses averaged over environments

Table 2. Genetic distance (GD) calculated from SSR data and F1 grain yield (Yi) for each line x tester crosses.

Lines	Tester KL17/2-5		Tester K74/1		Tester K1264/1		Tester B73		Tester Mo17	
	GD	Y	GD	Y	GD	Y	GD	Y	GD	Y
K18	0.686	6.65	0.676	10.62	0.595	6.21	0.447	7.66	0.400	5.47
K19	0.600	7.78	0.619	10.13	0.571	7.27	0.721	7.70	0.359	5.61
K19/1	0.622	7.83	0.568	9.63	0.607	8.06	0.602	7.43	0.458	5.46
K3218	0.609	5.95	0.637	5.59	0.533	5.91	0.714	6.67	0.555	9.37
KL17/2-3	0.231	3.94	0.610	4.63	0.561	6.22	0.714	7.77	0.658	9.92
KL17/2-2	0.470	4.03	0.672	5.72	0.522	5.91	0.655	7.39	0.591	9.95
K1263/8	0.641	6.52	0.634	6.98	0.317	5.42	0.524	7.14	0.553	8.74
K3304/1-2	0.628	6.88	0.646	6.27	0.509	6.15	0.616	7.77	0.645	9.89
K3530/3	0.525	6.98	0.476	5.87	0.667	6.26	0.674	9.81	0.590	10.08
K3545/7	0.625	6.56	0.463	4.56	0.659	6.28	0.714	8.10	0.632	9.25
K3547/5	0.700	7.31	0.571	7.21	0.500	5.86	0.628	8.20	0.667	9.99
K3493/1	0.700	6.84	0.548	6.26	0.595	7.32	0.326	8.00	0.692	8.59
K1259	0.705	5.37	0.606	6.54	0.476	6.26	0.583	7.52	0.645	9.18
K1259/4	0.688	6.32	0.582	6.57	0.549	5.90	0.607	7.55	0.697	8.88
K2818	0.736	6.74	0.740	5.48	0.715	6.03	0.497	6.15	0.736	8.52
K760/7	0.554	7.10	0.586	7.20	0.423	6.03	0.663	9.84	0.597	7.01
K166B	0.705	7.39	0.476	7.09	0.646	6.61	0.440	8.30	0.526	7.09
K3615/1	0.539	6.24	0.613	6.44	0.538	6.33	0.549	9.70	0.510	7.48
K3615/2	0.612	6.79	0.490	7.09	0.597	7.09	0.542	9.04	0.520	11.20
K3651/1	0.600	5.55	0.643	5.76	0.500	4.91	0.605	7.13	0.641	9.97
K3653/2	0.591	7.63	0.526	6.52	0.509	5.49	0.663	8.68	0.446	9.68
K3640/3	0.654	5.77	0.447	6.44	0.588	6.94	0.704	10.42	0.689	9.80
K3640/5	0.500	6.18	0.429	5.32	0.619	5.64	0.651	8.52	0.538	9.95
K3640/6	0.500	7.68	0.381	4.53	0.595	6.76	0.581	9.12	0.538	8.27
K3640/8	0.590	6.23	0.512	5.03	0.610	5.41	0.595	8.81	0.474	7.94
K3047/2	0.641	5.83	0.585	6.60	0.512	7.52	0.548	9.63	0.500	8.93
K3653/4	0.577	7.61	0.422	5.59	0.588	6.36	0.598	9.41	0.500	8.62
K2836/2	0.618	7.32	0.522	7.66	0.513	5.99	0.485	10.68	0.510	9.02
SE(GD)	0.019		0.017		0.015		0.018		0.018	

Standard error GD for entire set of 140 testcrosses = 0.008   Standard error Grain yield = 0.584

varied from 3.940 t/ha to 11.200 t/ha. The highest grain yield was seen in the cross K3615/2 x Mo17. Six of the ten highest yielding hybrids correspond to crosses with Mo17, two with tester B73, and two with K74/1 (including one commercial hybrid). Among the ten lowest yielding hybrids, three correspond to KL17/2-5, five to K74/1 and two to K1264/1 crosses. Most of the inbred lines which presented the highest grain yield had Mo17 or B73 tester lines as a parent. This was probably due to previous selection for combining ability of inbred lines in the earlier stages of line development using Mo17 as a tester line, and possibly the higher yield *per se* of B73. Estimates of positive SCA effects (Table 3) were greatest for Mo17 sister lines [lancuster suree crop (LSC) related lines], K18, K19 and K19/1 with the K74/1 tester line. Crosses among Late Synthetic related inbred lines had in several instances a relatively high yield and significantly positive SCA estimates.

Genetic distances among inbred lines and testers: The average GD coefficient based on the polymorphic SSRs among inbred lines with testers was 0.576, ranging from 0.231 to 0.740 with significant ($p < 0.01$) differences between GD estimates (Table 2). The highest GD (0.740) was detected between K2818 and K74/1, which are of unknown origin/pedigree. The lowest GD (0.231) was observed between the sister lines KL17/2-5 and KL17/2-3. In general, lower distances were observed between sisters lines (KL17/2-5 with Kl17/2-3 and KL17/2-2, Mo17 with K19, K19/1 and K18). However, two notable exceptions to this observation were seen in crosses between Mo17, an LSC derived inbred, and the two LSC sister lines K18 and K19 (which showed GD estimates of 0.447 and 0.721). The latter, in particular, was unexpectedly high, and may indicate a mis-reported pedigree. K2818 was the most distant line from all the testers, except B73. The GDs between inbred lines and testers spanned a great range,

Table 3. Specific combining ability (SCA) and midparent heterosis (MPH) for each line x tester crosses.

Lines	Tester KL17/2-5		Tester K74/1		Tester K1264/1		Tester B73		Tester Mo17	
	SCA	MPH	SCA	MPH	SCA	MPH	SCA	MPH	SCA	MPH
K18	0.04	126.1	4.11	199.6	-0.08	65.6	-0.81	151.7	-3.26	68.1
K19	0.79	182.6	3.25	201.9	0.60	104.0	-1.15	169.6	-3.5	82.9
K19/1	0.86	147.8	2.76	156.1	1.41	103.2	-1.40	127.9	-3.63	57.2
K3218	-0.04	72.7	-0.29	38.1	0.24	38.9	-1.17	88.0	1.26	149.1
KL17/2-3	-1.84	40.6	-1.05	35.8	0.75	72.1	0.12	167.1	2.02	218.1
KL17/2-2	-1.86	39.8	-0.07	64.1	0.34	60.1	-0.36	147.5	1.95	211.4
K1263/8	0.27	109.4	0.84	87.9	-0.51	38.2	-0.97	121.8	0.37	154.9
K3304/1-2	0.20	131.9	-0.31	75.7	-0.21	63.0	-0.77	153.4	1.09	201.5
K3530/3	-0.11	74.7	-1.12	27.7	-0.51	30.2	0.86	139.5	0.88	134.1
K3545/7	0.32	144.4	-1.58	38.7	0.36	79.8	0.00	190.7	0.89	208.5
K3547/5	0.31	151.2	0.31	105.3	-0.83	57.5	-0.66	172.0	0.87	209.7
K3493/1	0.14	130.2	-0.32	75.4	0.95	93.7	-0.55	160.3	-0.22	161.7
K1259	-0.89	121.7	0.38	116.2	0.32	93.8	-0.60	197.8	0.8	235.4
K1259/4	-0.01	170.3	0.34	123.3	-0.11	87.5	-0.64	209.3	0.43	234.7
K2818	0.87	142.9	-0.29	62.2	0.48	68.3	-1.58	113.6	0.53	175.8
K760/7	0.38	167.6	0.58	121.2	-0.37	74.2	1.25	256.9	-1.84	136.1
K166B	0.81	190.6	0.61	125.4	0.34	97.1	-0.14	213.6	-1.62	148.0
K3615/1	-0.29	144.2	0.01	103.7	0.12	88.0	1.32	264.8	-1.16	160.7
K3615/2	-0.74	110.9	-0.33	85.6	-0.12	75.9	-0.35	172.0	1.55	216.9
K3651/1	-0.41	102.4	-0.09	72.4	-0.73	38.2	-0.68	150.9	1.90	226.5
K3653/2	0.74	122.9	-0.26	62.1	-1.09	29.6	-0.06	146.3	0.67	158.9
K3640/3	-1.39	121.7	-0.61	101.1	0.09	103.3	1.40	285.2	0.51	235.9
K3640/5	-0.23	108.7	-0.98	49.4	-0.45	49.6	0.25	178.0	1.42	203.8
K3640/6	1.11	177.6	-1.92	34.6	0.52	89.1	0.70	217.9	-0.41	168.6
K3640/8	0.26	133.8	-0.83	54.2	-0.25	55.7	0.97	218.3	-0.15	166.8
K3047/2	-1.16	142.6	-0.28	119.7	0.85	134.1	0.78	284.1	-0.18	228.7
K3653/4	0.80	171.7	-1.11	64.4	-0.13	76.4	0.75	224.4	-0.31	176.9
K2836/2	-0.10	196.4	0.34	149.3	-1.11	82.6	1.40	314.9	-0.53	223.7

SE(SCA)= 0.630   SE(MPH)= 0.95

Table 4. Correlations of SSR based GD with mean grain yield, MPH and SCA of five tester lines combination with 28 inbred lines.

	Grain yield	Midparent Heterosis	Specific combining abilty
Entire set of crosses	0.27**	0.28**	0.39**
Mo17 tester crosses	0.54**	0.61**	0.61**
B73 tester crosses	0.03[ns]	0.09[ns]	0.10[ns]
K1264/1 tester crosses	0.32[ns]	0.16[ns]	0.35[ns]
K74/1 tester crosses	0.30[ns]	0.29[ns]	0.49**
KL17/2-5 tester crosses	0.45*	0.51**	0.41*

indicating the presence of a broad genetic base when comparing inbred lines to testers.

Relationship of genetic distance with hybrid performance: The correlation coefficients between SSR based GD and grain yield, SCA and MPH for the entire set of 140 line x tester combinations were moderate but significant (r= 0.27**, 0.39** and 0.28, respectively), showing a positive association between GD and grain yield and MPH (Table 4). Correlation between GD and grain yield, SCA and MPH across testers varied from 0.03 to 0.54, 0.10 to 0.61 and 0.09 to 0.61, respectively. Grain yield, SCA and MPH correlated significantly with GD in Mo17 and KL17/2-5 testcrosses to 28 inbred lines, as well as K74/1 testcrosses only for SCA. In general, correlations between GD and yield or heterosis were low in B73 crosses and highest in Mo17 crosses. Although GD between parents was significantly correlated with hybrid performance, it did not consistently identify the best crosses. This is similar to results already published (Godshalk *et al*., 1990; Melchinger *et al*., 1990; Ajmon-Marsan *et al*., 1998).

DISCUSSION

The difference in predictive ability of markers in different genetic backgrounds has been reported earlier (Melchinger, 1993). Bernardo (1992) stated that molecular marker heterozygosity would be most valuable for predicting hybrid performance in crop species under conditions such as strong dominance effects, and high trait heritability. Charcosset *et al*. (1991) anticipated the need for linkage disequilibrium between marker loci and quantitative trait loci (QTL) for grain yield in order to associate marker heterozygosity with heterosis. In other studies, correlations of GD based on marker heterozygosity with performance and heterosis differed from one tester to another and depended on the genetic background of the germplasm

(Lee *et al*., 1989; Boppemmaier *et al*., 1993; Zhang *et al*., 1996).

In the current study, although the magnitude of the correlation is very low to be predictive, a significant conclusion can be made favoring the use of SSR based GD estimates in some Iranian testers. Because many productive hybrids have been made in Iran with Mo17, it will continue to be a major tester in hybrid maize programs. It would be beneficial to make all hybrids with Mo17 (and possibly KL17/2-5) only after fingerprinting to determine highest the GD, allowing a significant reduction in number of crosses tested, (and thus a reduction in costs and labor). The GDs based on SSR data for crosses to B73 and other testers with low correlations may also predict poor hybrid performance, which can then these can be avoided, thus reducing the number of crosses required under field evaluation.

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