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Heterotic analysis using AFLP markers reveals moderate correlations between specific combining ability and genetic distance in maize inbred lines

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Heterotic breeding strategies in maize (*Zea mays* L.) can be improved if high specific combining abilities in hybrid performance and a DNA marker-based genetic distance in the inbred lines is correlated, and hence heterosis can reliably be predicted. In this study, the genetic diversity across 9 elite maize inbred lines was evaluated using the amplified fragment length polymorphism (AFLP) marker. The genetic distance (GD) between each of all possible inbred pairs and the specific combining ability (SCA) and heterosis in the F1 hybrids were evaluated in a diallel set of crosses (Griffing II). Nineteen AFLP primers produced 1019 reproducible bands of which 691 (67.81%) were polymorphic. This gave an average of 53.6 bands per primer combination. A matrix of Genetic similarity (GS) according to UPGMA clustered the inbred lines into 4 groups with a GD ranging from 0.2442 to 0.4093. The results indicated that GD was moderately correlated with grain yield (0.4096), mid-parent heterosis (MH) (0.3624), better-parent heterosis (BH) (0.3309) and SCA (0.4725). Although the AFLP markers have high polymorphisms and can be used to detect the genetic divergences, place maize inbred lines in different heterotic pools and identify the most positive SCAs and heterosis, they are still limited in fully predicting hybrid performance.

Keywords: Maize, AFLP, genetic distance, heterosis, combining ability.

INTRODUCTION

It is widely believed that the level of genetic variation between two inbred lines has an influence on the general performance or heterosis in the resulting hybrids (Hinze and Lamkey, 2003). Hence molecular markers which reflect such genetic variation can hasten the selection of parental inbred lines. Previous methods have included

diallel crossing methods, multivariate analyses (Aydin et al., 2007). However, several studies have shown that a multifaceted approach which includes morphological, biochemical and intense molecular trait evaluation of candidate inbred lines can be more reliable in heterotic breeding (Rencher, 1995). Currently, several molecular markers like *Restriction Fragment Length Polymorphism (RFLP)*, *Amplified Fragment Length Polymorphism (AFLP)*, *Random Amplified Polymorphic DNA (RAPD)*, and *Simple Sequence Repeat (SSR)* have been employed in analyzing genetic diversity, QTL identification, and in forecasting heterosis in maize although the results on the later respect have been inconsistent (Smith et al., 1997; Ajmone-Marsan et al., 1998; Pejic et al., 1998; Melchinger, 1999; Phumichai et al., 2008; Dhliwayo et al., 2009). The inconsistency might have been due to the differences in approach when dealing with QTLs which do not normally follow the Mendelian pattern of inheritance. The objective of this study was to estimate relationships among the specific combining ability, heterosis and genetic distance based on

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Abbreviations: AFLP, Amplified fragment length polymorphism marker; GD, genetic distance; SCA, specific combining ability; GS, similarity; UPGMA, unweighted pair-group method with arithmetic mean; MH, mid-parent heterosis; BH, better-parent heterosis; RFLP, restriction fragment length polymorphism; DNA, deoxyribonucleic acid; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeat; PIC, polymorphism information content; CTAB, cetyltrimethyl ammonium bromide; QTLs, quantitative traits loci.

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Table 1. The identity and pedigree of the nine maize inbred lines used in this study.

Code	Genotypes	Pedigree	Source
L1	H8215	Huangzao 4 improved line	Jilin Agricultural University
L2	Q9563-1	Unknown hybrid	Jilin Agricultural University
L3	5079	Mo17 improved line	Jilin Agricultural University
L4	3298	Dan340×Zheng58	Jilin Agricultural University
L5	3318	Nongda3138	Jilin Agricultural University
L6	G9716	330 improved line	Jilin Agricultural University
L7	Q6847	4112×B73	Jilin Agricultural University
L8	D598-1	598 variant plant	Jilin Agricultural University
L9	E221	C8605×BSSS	Jilin Agricultural University

AFLP in 9 maize inbred lines for grain yield. The AFLP can be used to molecularly identify QTLs and assist in mapping genomic regions that contain specifying a particular quantitative trait like yield. One of the assumptions made in dealing with QTL inheritance has been that there is an equal contribution from all the genes involved in the eventuality of a trait. The ability of AFLP to detect more polymorphisms in the inbred genome might give more statistically reliable gene interactions and associations amongst parental lines. The diallel mating designs especially the Griffins' (1956) provide useful selection tools for obtaining information about QTLs concerning GCA and SCA during plant breeding programs (Hayman, 1954; Fry 2004; Zhang and Kang, 1997).

MATERIALS AND METHODS

Plant materials

In this diallel study, nine maize inbred lines from Jilin Agricultural University in Northeast China were selected and 36 crosses made following the Griffing mating II design (Griffing, 1956) during the 2006 summer season (Table 1).

Field testing

In 2007, the hybrids and inbred lines were planted in the same location during the spring season. The experimental design used was the randomized complete block with three replications. The plot sizes were in double 6-m rows with 0.65 m between rows and 0.30 m between plants within a row. The final population density was 51,282 plants ha⁻¹. They were hand harvested after which the grain yield (Kg ha⁻¹) was calculated as ear weight at harvest, assuming 80% shelling at 15% moisture adjustment.

DNA isolation and AFLP analyses

Genomic DNA was extracted from young leaves each of the 9 maize inbred lines using a modified CTAB procedure (Saghai-Maroo et al., 1984). Approximately 300 ng of DNA was used to do AFLP analysis for each accession. The EcoRI and MseI restriction enzymes were used in digesting the DNA. The AFLP protocol with slight modifications was followed as described by Vos et al. (1995). Nineteen primers combinations were used in the selective

amplification of the fragments. The PCR products were denatured and visualized using 5% polyacrylamide gel electrophoresis and silver staining.

Statistical analysis

Specific combining ability (SCA) effects for the hybrids were estimated using Griffing's Method II Diallel analysis (Griffing, 1956). The estimate of heterosis over the mid-parent and better-parent was calculated using the procedure by Matzingar et al. (1962). The difference of F₁ mean from the respective mid-parent (MH) and better-parent (BH) value was evaluated as used by Wvne et al. (1970). The amplified AFLP bands were scored using a 1/0 (presence/absence) code system. Variability for each locus was used to estimate the polymorphism information content (PIC) as used by Smith et al. (1997). Genetic similarity (GS) was calculated between each pair of lines on the basis of the simple matching coefficient (Sneath and Sokal, 1973). While the genetic distance was computed as GD = 1-GS (Nei and Li, 1979). A dendrogram were constructed using unweighted pair-group method with arithmetic mean (UPGMA) clustering using the computer software NTSYS-pc version 2.10e (Rolf, 1998). Finally Simple correlation (r) between GD and SCA, MH and BH were calculated.

RESULTS

The combining ability

The analysis of variance (ANOVA) for grain yield of the F₁ hybrids was carried out. It showed that the genotypic mean squares was highly significant (Table 2), indicating that a substantial variability existed in the parental inbred lines.

The estimates of general combining ability effects (Table 3) showed that inbred line L9 was the best general combiner. GCA and SCA effects were significant and positive in breeding material used for grain yield. The inbred lines L9, L6, L3 and L8 possessed higher GCA, and hence, they had the potential in being used to improve in maize breeding programs. The inbred lines L1, L5, L4, L7 and L2 were found to have a generally poor combining ability for grain yield. The revealed SCA effects (Table 3) for 34 out the total of 36 cross combinations were positive with L1×L9 making the best specific combination for grain

Table 2. Analysis of variance (F value) for grain yield.

Source	SS	DF	MS	F	Prob F
Rep.	212326.6	2	106163.2	0.464	0.6300
Genotype	32598000.0	44	37104500.0	162.326	0.0001
Error	20115230.0	88	228582.1		
Total	52925000.0	134			

SS, Sum of squares; MS, mean sum of squares; DF, degree of freedom; F, frequency; Prob., probability.

Table 3. Estimates of specific combining ability and general combining ability effects.

Line	L1	L2	L3	L4	L5	L6	L7	L8	L9
L2	1478.87								
L3	866.86	770.59							
L4	-271.77	2245.64	2598.58						
L5	1119.19	3182.75	605.79	1621.86					
L6	2500.24	945.85	2084.26	1102.05	165.66				
L7	-2877.75	829.40	1760.97	418.68	1925.28	2754.18			
L8	1043.31	1825.68	2322.21	1963.93	2381.38	1248.75	2487.00		
L9	3428.36	1614.14	2852.56	2245.56	887.81	579.10	2204.53	2905.08	
GCA	-909.89	-118.75	635.14	-601.44	-840.27	701.77	-248.45	475.78	906.12

Table 4. Mid-parent heterosis (MH) and better-parent heterosis (BH) for grain yield of single crosses among 9 maize inbred lines.

Cross	MH (%)	BH (%)	Cross	MH (%)	BH (%)	Cross	MH (%)	BH (%)
L1×L2	206.35	158.09	L2×L7	189.34	137.18	L4×L8	417.71	405.49
L1×L3	164.54	158.27	L2×L8	363.12	330.53	L4×L9	396.40	344.66
L1×L4	144.67	90.94	L2×L9	333.84	326.64	L5×L6	178.26	84.84
L1×L5	221.76	130.61	L3×L4	315.56	230.09	L5×L7	245.70	142.79
L1×L6	163.10	131.27	L3×L5	274.89	250.90	L5×L8	491.96	413.95
L1×L7	32.68	28.28	L3×L6	194.28	153.39	L5×L9	374.47	282.37
L1×L8	231.19	163.05	L3×L7	197.46	181.00	L6×L7	177.87	151.75
L1×L9	296.75	238.91	L3×L8	335.28	252.10	L6×L8	221.60	132.54
L2×L3	241.78	193.76	L3×L9	337.64	281.55	L6×L9	196.90	128.17
L2×L4	356.29	314.93	L4×L5	396.23	339.89	L7×L8	277.45	192.42
L2×L5	438.97	340.01	L4×L6	197.90	112.26	L7×L9	259.49	198.62
L2×L6	188.76	119.34	L4×L7	187.18	118.76	L8×L9	454.83	407.87

yield.

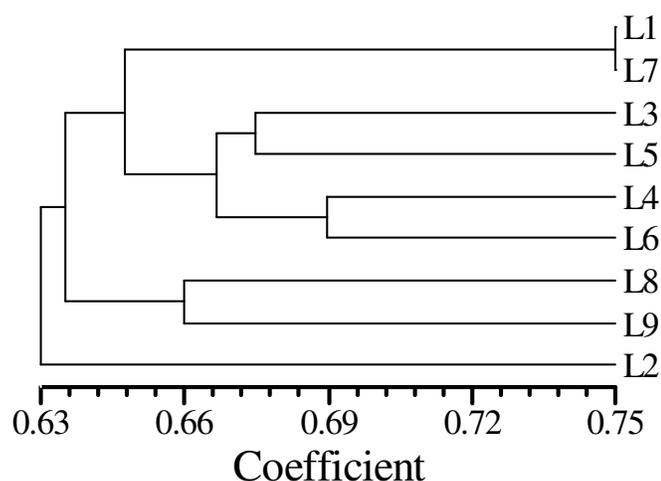
Performance of heterosis

The average degree of MH and BH ranged from 32.68 to 491.96% and 28.28 to 413.95%, respectively (Table 4). The results showed that MH correlated well to the BH with the highest cross being found in L5×L8, while the lowest was L1×L7. The BH of seven among 36 crosses (19.4%) was over 200%, and therefore it was hopeful to

select superior cross combinations from them. Compared with topcross and double hybrids, the single crosses commonly gave higher yields due to homozygous advantages of their parents and the interaction of the genes in favour of cumulative dominant alleles which were useful in the expression of heterosis. However, we could not judge utility of cross combinations on the basis of MH or BH, except for the performance of grain yield in maize hybrids which were significantly better than the control varieties.

Table 5. Genetic distances between the 9 maize inbred lines on AFLP analysis.

	L1	L2	L3	L4	L5	L6	L7	L8	L9
L1	0								
L2	0.3672	0							
L3	0.3697	0.3594	0						
L4	0.3416	0.3510	0.3317	0					
L5	0.3831	0.3691	0.3235	0.3124	0				
L6	0.3867	0.3759	0.3461	0.3077	0.3375	0			
L7	0.2442	0.3680	0.3705	0.3258	0.3362	0.3001	0		
L8	0.3944	0.4093	0.3993	0.3512	0.3859	0.3370	0.3317	0	
L9	0.3974	0.3611	0.3929	0.3578	0.3694	0.3396	0.3197	0.3390	0

**Figure 1.** Clustering analysis of the nine maize inbred lines into four groups as revealed by AFLP markers.**Table 6.** Coefficients between parameters of genetic distance (GD), grain yield, MH, BH and SCA.

Crosses	Grain yield	MH	BH	SCA
MH	0.5209**			
BH	0.5034**	0.9617**		
SCA	0.8666**	0.6361**	0.5927**	
GD	0.4096*	0.3624*	0.3309*	0.4725**

** Significant at 0.01 probability level.

* Significant at 0.05 probability level.

Genetic diversity

From the 19 AFLP primer combinations used for the PCR amplification, 1019 bands were obtained in which 691 (67.81%) were polymorphic. This gave an average of 53.6 bands for each primer combination. Genetic distances among the 9 lines ranged from the lowest value of 0.2442 which was between L1 and L7 to the highest 0.4093 between L2 and L8. The average was 0.3526 (Table 5).

The inbred lines were classified into four groups based on the matrix of GS according to UPGMA (Figure 1). Cluster A contained the inbred lines L1 and L7. Cluster B consisted of L3, L5, L4 and L6, including two subgroups (L3 and L5; L4 and L6). L8 and L9 were found in cluster C. Cluster D only centered on L2. The dendrogram constructed based on the AFLP data showed an excellent fit with the GD values.

Correlation analysis

A comparison of the correlation coefficients for the parameters decreased from 0.9617 to 0.3309, most of which were highly significant ($P < 0.01$) except for the GD with grain yield, MH and BH, which were moderately significant ($P < 0.05$). A few exceptions to these general trends were noted. For example, correlation coefficients of MH with BH (0.9617) and SCA with grain yield (0.8666). Thus, grain yield of hybrids could be reflected by SCA in this study, but grain yield, MH, BH and SCA could not exactly and wholly be detected by GD (Table 6).

DISCUSSION

The interest of predicting hybrid performance in maize has continued to increase over the decades although specific markers to fully detect heterosis are largely elusive. The prediction of hybrid performance through genetic distance has been very difficult (Melchinger, 1999). This has been attributed to the small role of the dominance gene action, low heritability of the grain yield trait and the few trait-relevant quantitative trait loci linked to particular molecular markers.

Recently, Dhliwayo et al. (2009) demonstrated that genetic distances were not significantly associated with hybrid performance, specific combining ability effects, or midparent heterosis for grain yield in maize. But Phumichai et al. (2008) found that GD correlated positively with specific combining ability (SCA) in predicting heterosis. Moreover studies by Balestre et al. (2008) concluded that

there is moderate correlation between GD and heterosis with $r = 0.40$ and a SCA with $r = 0.38$, which is similar to the results of our study in which the $r = 0.4096$ for GD and $r = 0.8666$ for SCA were obtained. This could be termed as moderate although the SCA was much higher. The variances of general and specific combining ability are related to the type of gene action involved. The variance for GCA includes an additive portion while that of SCA includes nonadditive portion of total variance arising largely from dominance and epistatic deviations (Rojas and Sprague, 1952). These views of combining ability indicated that in case of grain yield in maize, the parents which had the best GCA on their exploitation in cross combinations as one of the parents did not produce excellent hybrid combinations. Nevertheless, the parents with highest SCA produced good hybrid combinations. The results are in partial agreement with the findings of Singh et al. (1977), Castiglioni et al. (1999), Wu and Dai (2000) and Crossa et al. (1990). However, when maize breeders desire high yielding specific combinations, especially in heterosis utilization, SCA effects could help in the selection parental material for hybridization. Other genetic and epigenetic dimensions coupled with possibilities of habitat fragmentation and the resultant homogenization of cultivars can be explored in the selection of parental materials (Zhang et al., 2009).

Compared to previous studies, AFLP markers are still regarded as considerably efficient because of their capacity to reveal several bands in a single amplification (Pejic et al., 1998). Our results suggest that genetic distance information based on AFLP markers would be useful in excluding genetically similar lines such as more than one of L1 and L7, although they are limited in predicting the hybrid grain yield performance. The correlation co-efficients (r) of the parameters among SCA, MH, BH, grain yield and GD were significant, but the determination coefficient (r^2) was low except for that of MH with BH, SCA with grain yield. Hence the genetic distance based on AFLP marker data is not conclusive in predicting heterosis and high-yielding crosses; so there is need to develop more efficient markers probably multidimensional ones.

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