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Molecular Marker Based Validation of Genetic Relatedness Among Maize (*Zea mays* L.) Inbred Lines and Its Influence on Heterosis

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ABSTRACT. *Random Amplified Polymorphic DNA (RAPD) analysis was employed among 11 maize inbred lines obtained from the germplasm of the Tamil Nadu Agricultural University. The 21 primers used in the study generated 179 markers of which 139 were polymorphic and the percentage polymorphism was 77.7%. Statistical analysis was carried out using NT Sys software and a dendrogram was generated using Jaccard's similarity coefficient values. The values of similarity coefficient ranged from 0.43 to 0.74 and the average similarity value was 0.60. Cluster analysis revealed that the genotypes were clustered at about 45% similarity into five groups but there was no correspondence between genetic relatedness and geographical origin of the accessions. Among the hybrids synthesized by crossing these inbreds in diallel fashion, 23 were observed to have a high degree of significance for yield contributing traits. Many of the hybrids were between parents with moderate similarity, which indicated that there was no relationship between genetic diversity and heterosis beyond a certain limit.*

INTRODUCTION

Maize (*Zea mays* L.) ranks third among cereal crops, next only to wheat and rice, with regard to its production at global level. World maize production during the year 2002 was around 602.5 million tones, as against the Indian production of 10.5 million tones. Average world productivity during the year 2002 was 4.3 tones per hectare. This is far greater than the average Indian productivity, which stood at 1.7 tones per hectare for the same period.

Maize has been used for multifaceted purposes by humans. It is used as food, feed and fibre. It also has several industrial applications such as manufacture of starch, gluten, germ oil, corn syrup, sugar, corn meal and corn flour. Seventeen per cent of maize produced in the world is used as food for humans and 66 per cent is used as feed for animals. In the Indian context, being an important cereal, over 85 per cent of its production in the country is consumed directly as food in various forms; the chapattis (Flat breads) are the commonest preparation, whereas roasted ears, popcorn and porridge are other important forms. The use of maize in animal feed, particularly for poultry and in the starch industry is increasing. Green maize plants also furnish a very succulent fodder during the spring and the monsoon, particularly in northern India (www.indiaagronet.com/indiaagronet/cropinfo/maize.htm#intro). This vividly indicates the importance of maize in India and the role it plays in meeting the ever-increasing demand for food. This also indicates the need to develop new, high yielding varieties and hybrids of maize.

Breeding methodologies of maize include population improvement, intervarietal crossing, development of synthetics, composites and hybrids. The plant breeder's choice of source germplasm determines the potential of improvement for traits under selection in a breeding programme. The success of any breeding method depends on the availability of genetic diversity in the base population. Utilisation of diverse parents in hybridisation programmes has been observed to yield better hybrids. A study was formulated with the objective of understanding the extent of diversity at molecular level among some maize inbreds and the influence it exerts on the hybrid performance.

MATERIALS AND METHODS

Plant material and DNA extraction

Five grams of fresh leaves from young seedlings of 11 inbreds *viz.,* UMI 946, UMI 852, UMI 752, UMI 679, UMI 615, UMI 577, UMI 556, UMI 532, UMI 497, UMI 470 and UMI 438 were used for DNA extraction (Table1). DNA was extracted by the method suggested by McCouch *et al.* (1998). The concentration of genomic DNA in the sample was determined with a flourimeter and DNA samples were diluted in TE buffer to a working concentration of 10 ng/µl. The diluted samples were stored in a freezer until amplification by Polymerase Chain Reaction (PCR). The stock genomic DNA was stored at -20° C till further use.

Table 1. List of accessions and their parentage.

Accession No.	Parentage	Origin
UMI 946	Hyd 92 R / 1040	Hyderabad, India
UMI 852	RICA 8926 Mex x 2474	Delhi, India
UMI 752	EH – 4003	Coimbatore, India
UMI 679	UMI – 65 x UMI –150	Coimbatore, India
UMI 615	(Sakthi x CM – 202) x C. Rattan x CM .111	Coimbatore, India
UMI 577	M – 13	Coimbatore, India
UMI 556	UMI – 140 x UMI –126	Coimbatore, India
UMI 532	UMI –79	Coimbatore, India
UMI 497	Not known	Bihar, India
UMI 470	K1	Coimbatore, India
UMI 438	EH – 450879	Delhi, India

DNA amplification and gel electrophoresis

The protocol for PCR amplification was standardized by using varying concentrations of template DNA, *Taq* DNA polymerase and Magnesium salt. A total of 21 primers, which were chosen based on their ability to produce high polymorphism in a separate study (*viz.*,OPAK – 02, 04, 05, 07, 08, 09, 16, 17, 19, 20; OPAM – 03, 04, 05, 07, 10, 11, 13, 16; OPAB – 01, 03 and 04 from Operon technologies Inc., CA, USA), were used to screen for genetic diversity among the 11 inbreds.

Amplification reaction mixtures were made up to 20 µl with 10 mM Tris HCl (pH-9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001 per cent gelatin, dATP, dCTP, dTTP and dGTP – 0.1 mM each , 1.0 pg of primer, 20 –30 ng of genomic DNA and 0.5 unit of *Taq* DNA polymerase (Bangalore Genei PVT, Ltd., Bangalore, India). Amplification was performed in 200 µl thin walled Tarsons PCR tubes in a PCR –300 (Perkin Elmer) thermal cycler programmed for 40 cycles each of 1 minute at 92°C, 1 minute at 34°C and 2 min at 72° C preceded and succeeded by 2 minutes at 92°C and 10 minutes at 72°C, respectively. PCR amplification products (20 µl) were subjected to electrophoresis in 1.4 per cent agarose gel in 1X TBE buffer at 120V for 3.5 hours using Hoefer super submarine electrophoresis unit (Pharmacia biotech). A 1kb DNA ladder (GibcoBRL) was used as a molecular standard. The electronic image of Ethidium bromide stained gel was captured using Kodak digital science dc-120 zoom digital camera (Eastman Kodak Co, Rochester, NY) and the gel was documented using Electrophoresis documentation and analysis system (EDAS-120) 1D Image analysis software (Scientific Imaging Systems, Eastman Kodak Company, N.Y.).

Scoring and analysis of RAPD bands

Clear and unambiguous bands were scored for their presence or absence, with the score 1 indicating their presence and 0 indicating their absence. Jaccard's similarity coefficient values were calculated between the inbreds and a similarity coefficient matrix was constructed. Based on the similarity coeffecient values, the inbreds were classified as less similar (less than 0.53), moderately similar (0.53 - 0.63) and highly similar (0.64 and above) (Table 2).

A dendrogram was generated by Sequential Hierarchial Agglomerative Nonoverlapping (SHAN) method with UPGMA distances based on Jaccard's similarity coefficient values. The entire analysis was performed using NTSYS pc version 2.02 (Rohlf, 1998) software.

Synthesis of hybrids

To study the influence of diversity on heterosis, the eleven inbred lines were raised in a crossing block and crossing was undertaken in all possible combinations among them excluding reciprocals. The 55 hybrids thus generated were raised along with their parents and a check hybrid (CoH 3) in a randomized block design with three replications. Observations on six morphometric traits (*viz,* plant height, leaf length, ear length, ear diameter, number of grains per row and single plant yield) were recorded in five random plants per replication in each entry. Standard heterosis was calculated for the six characters and all the hybrids were scored for their heterotic expression of the six characters. The hybrids were scored according to the degree of significance of standard heterosis for yield contributing traits, as per the method suggested by Manimaran and Raveendran (2001). A scores of +1, 0 and -1 were allotted for positive significant heterosis, non-significant heterosis and negative significant heterosis, respectively. Total score for each hybrid combination was arrived at by adding up the scores for all the characters.

RESULTS AND DISCUSSION

A total of 179 amplified products were generated with an average of 8.5 products per primer. Among them 139 bands were polymorphic and the percentage polymorphism was 77.7 percent. The number of bands generated by the primers ranged from 4 –13 (Plate 1a and Plate 1b; M- Marker, 1- UMI 438, 2 -UMI 470, 3 - UMI 497,4 - UMI 532, 5 - UMI 556, 6 - UMI 577, 7 - UMI 615, 8 - UMI 679, 9 - UMI 752, 10 - UMI 852, 11 - UMI 946).

Table 2. Similarity coefficient among the inbreds.

	UMI 946	UMI 852	UMI 752	UMI 679	UMI 615	UMI 577	UMI 556	UMI 532	UMI 497	UMI 470	UMI 438
UMI 946	1.00										
UMI 852	0.57	1.00									
UMI 752	0.58	0.61	1.00								
UMI 679	0.64	0.60	0.66	1.00							
UMI 615	0.59	0.59	0.69	0.74	1.00						
UMI 577	0.64	0.58	0.58	0.66	0.64	1.00					
UMI 556	0.61	0.43	0.58	0.53	0.60	0.60	1.00				
UMI 532	0.57	0.53	0.59	0.64	0.69	0.60	0.53	1.00			
UMI 497	0.62	0.54	0.57	0.65	0.69	0.62	0.59	0.65	1.00		
UMI 470	0.58	0.55	0.59	0.65	0.64	0.61	0.50	0.65	0.70	1.00	
UMI 438	0.60	0.45	0.53	0.56	0.56	0.60	0.60	0.52	0.66	0.59	1.00

Jaccard's pair wise similarity coefficient values for the 11 inbreds were calculated and are presented in Table 2. The genetic similarity coefficient values ranged from 0.43 (between UMI 556 and UMI 852) to 0.74 (between UMI 615 and UMI 679). The average similarity coefficient among the inbreds was found to be 0.60. Comparison of the genetic similarity coefficient values between inbreds indicated UMI 556 and UMI 852 to be the most dissimilar pair.

Cluster analysis revealed that the inbreds were clustered into five broad groups at about 45% genetic similarity (Fig. 1). The number of entries per cluster ranged from 1 to 3. The accession UMI 852 was observed to form a solitary cluster, which may be because of the pedigree of the genotype. In cluster 4, all the inbreds had originated from the same breeding station. However, in the clusters 2, 3 and 5, the genotypes had originated in different breeding stations. According to the above observations it is revealed that the clustering pattern does not reveal strong correspondence between genetic relatedness and the geographical origin. This corroborates with the finding of Moeller and Schaal (1999), who evaluated 15 American maize accessions using RAPD markers.

Based on the similarity coefficient and cluster analysis, it was observed that the 11 inbreds were of narrow genetic base and it emphasizes the need to exploit large germplasm collections having diverse morpho-agronomic traits in cultivar improvement. Shu *et al.* (1996) studied the genetic diversity between seven maize inbred lines using RAPD markers. They observed the similarity coefficient values in the range of 0.53 to 0.75, which indicated a high degree of similarity.

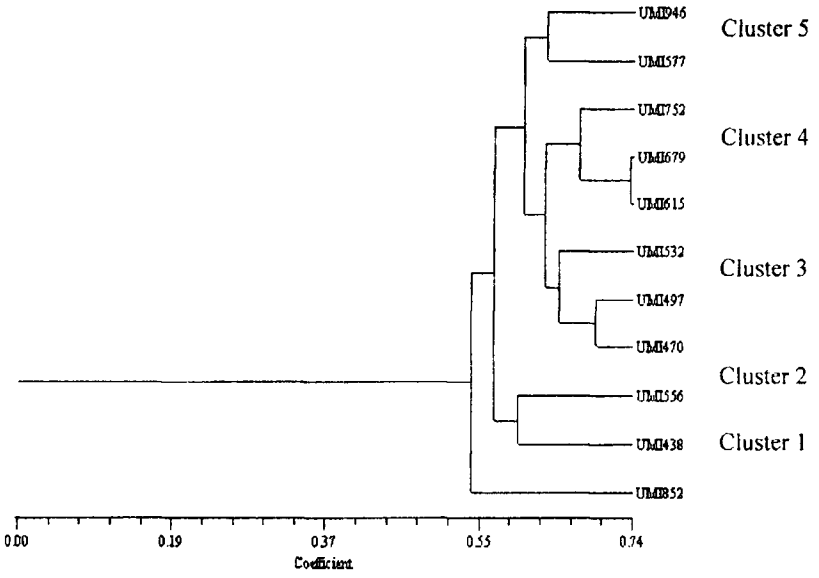

Fig. 1. RAPD phenogram of the 11 parental inbreds.

Among the hybrids produced, 34, 27, 25, 24,37 and 40 hybrids recorded significantly positive heterosis for plant hight, leaf length, ear length, ear diameter, no. of grains per new and grains yields per plant, respectively. Based on the scores it was observed that out of the 55 hybrids, 23 recorded a score of +1 and above (Table 3). The Results pertaining to these hybrids were tested for significance and given in Table 4.

The 23 hybrids thus selected were classified on the basis of similarity coefficient values recorded by their respective parents. It was observed that a majority of the hybrids were from cross combinations involving medium similarity parents. The parents of the hybrids UMI 946 x UMI 852, UMI 852 x UMI 556, UMI 852 x UMI 438, UMI 679 x UMI 556, UMI 556 x UMI 470 and UMI 532 x UMI 438 were observed to have similarity value lesser than or equal to 0.53, while the hybrid combinations UMI 752 x UMI 615, UMI 615 x UMI 497 and UMI 497 x UMI 438 involved parents with high similarity values as detected by molecular analysis. This clearly indicated that there is no relationship between genetic diversity and expression of heterosis beyond a certain limits. This is in accordance with Manimaran and Raveendran (2001) who based on their research on cotton concluded that the maximum heterosis for both yield and quality characters occurs at an optimal or intermediate level of genetic diversity.

This result also corroborates the findings of Shu *et al.* (1996) who, based on their study on seven maize inbred lines using RAPD markers, opined that there was no significant correlation between genetic diversity based on RAPD and hybrid performance. However, Verbitskaya *et al.* (1999) observed a positive correlation between genetic distance based on molecular marker polymorphism and productivity of hybrids.

Table 3. Scores recorded by hybrids selected based on standard heterosis.

Hybrids	Characters						Total score
	Plant height	Leaf length	Ear length	Ear diameter	No. grains / row	Yield	
UMI 946 x UMI 852	0	-1	0	1	1	1	2
UMI 946 x UMI 752	0	-1	0	1	1	1	2
UMI 946 x UMI 532	0	0	0	1	1	-1	1
UMI 852 x UMI 752	-1	0	1	1	1	1	3
UMI 852 x UMI 615	-1	0	1	1	1	1	3
UMI 852 x UMI 577	0	0	0	1	1	1	3
UMI 852 x UMI 556	0	0	0	1	1	1	3
UMI 852 x UMI 470	-1	-1	0	1	1	1	1
UMI 852 x UMI 438	-1	-1	0	1	1	1	1
UMI 752 x UMI 615	0	0	0	1	1	-1	2
UMI 752 x UMI 577	-1	-1	0	1	1	1	1
UMI 752 x UMI 556	0	1	0	1	1	-1	2
UMI 752 x UMI 532	-1	1	0	1	1	-1	1
UMI 752 x UMI 497	-1	1	0	1	1	1	3
UMI 752 x UMI 470	-1	1	0	1	1	-1	1
UMI 679 x UMI 556	0	-1	0	1	1	1	2
UMI 615 x UMI 497	0	0	0	1	1	-1	1
UMI 577 x UMI 470	0	0	0	1	1	1	3
UMI 556 x UMI 497	0	1	0	1	1	-1	2
UMI 556 x UMI 470	0	-1	0	1	1	1	2
UMI 532 x UMI 438	1	-1	0	1	1	-1	1
UMI 497 x UMI 438	-1	-1	0	1	1	1	1
UMI 470 x UMI 438	-1	-1	0	1	1	1	1

Table 4. Standard heterosis of selected hybrids.

Hybrids	Characters					
	Plant Height	Leaf length	Ear length	Ear diameter	No. grains / row	Yield
UMI 946 x UMI 852	-16.8	- 8.6**	6.8	62.2**	157.1**	2.6**
UMI 946 x UMI 752	-17.0	- 5.2**	5.7	53.1**	165.4**	8.2**
UMI 946 x UMI 532	- 9.8	3.8	-2.9	37.6**	138.7**	- 7.8**
UMI 852 x UMI 752	-22.9*	- 4.1	7.9*	88.3**	371.1**	5.2**
UMI 852 x UMI 615	-19.8*	1.8	9.9*	98.3**	321.5**	7.7**
UMI 852 x UMI 577	-10.2	1.2	4.4	146.4*	338.1**	5.4**
UMI 852 x UMI 556	-14.2	- 0.4	4.6	118.3**	310.7**	4.7**
UMI 852 x UMI 470	-34.6**	-13.2**	-6.1	68.7**	236.4**	1.1**
UMI 852 x UMI 438	-30.9**	-12.0**	-7.1	69.0**	471.4**	7.7**
UMI 752 x UMI 615	-11.2	- 0.2	1.2	99.6**	286.5**	- 8.5**
UMI 752 x UMI 577	-30.1**	- 8.2**	-3.5	101.4*	260.4**	4.2**
UMI 752 x UMI 556	-13.2	64.8**	1.1	125.0*	281.2**	-11.4**
UMI 752 x UMI 532	-21.4*	14.5**	0.2	97.6**	290.6**	- 9.7**
UMI 752 x UMI 497	-18.2*	8.8**	-7.3	92.6**	386.1**	9.0**
UMI 752 x UMI 470	-18.8*	5.7**	0.9	102.2*	213.7**	- 0.5*
UMI 679 x UMI 556	-14.2	- 4.2*	-6.6	80.3**	164.8**	0.7*
UMI 615 x UMI 497	-13.0	- 0.2	-7.0	84.7**	214.7**	-16.7**
UMI 577 x UMI 470	-12.1	0.6	6.8	99.6**	180.8**	1.7**
UMI 556 x UMI 497	-12.8	11.1**	-5.7	92.9**	203.7**	- 3.4**
UMI 556 x UMI 470	- 0.2	- 7.5**	1.3	98.2**	202.7**	3.7**
UMI 532 x UMI 438	-21.3**	- 7.0*	1.6	39.0**	252.6**	- 4.6**
UMI 497 x UMI 438	-30.5**	- 7.6**	-6.1	60.9**	378.2**	3.7**
UMI 470 x UMI 438	-26.2**	-10.7**	-3.9	60.7**	237.3**	1.9**

** Significant at 0.01 level; * Significant at 0.05 level

CONCLUSIONS

Based on this study it can be concluded the clustering pattern of maize inbred lines does not reveal strong correspondence between genetic relatedness and the geographical origin. There is no relationship between genetic diversity among the parents of maize, and the level of heterosis among the hybrids beyond a certain limit.

REFERENCES

Manimaran, R. and Raveendran, T.S. (2001). Relationship between genetic diversity and heterosis in cotton. Crop Res. 22 (1) : 72-77.

McCouch, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y., Khush, G.S., Coffman, W.R. and Tanksley, S.D. 1998. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76: 815-829.

Moeller, D.A. and Schaal, B.A. (1999). Genetic relationships among native American maize accessions of the great plains assessed by RAPD's. Theor. Appl. Genet. 99: 1061-1067.

Rohlf F.J. (1998). Numerical Taxonomy and Multivariate Analysis System, Version 2.02 i., 100. North Country Road, Setanket, New York.

Shu, C., Kang, H.K. and Cheng, C. (1996). Prediction of heterosis with genetic diversity among maize inbred lines revealed by RAPD. J. Agrl. Res. China 45: 147-155.

Verbitskaya, T.G., Sivolap, Y.M., Guzhva, D.V.S. and Sokolov, V.M. (1999). Analysis of the relations between the genetic divergence of the DNA of corn lines and hybrid productivity. Rus. Agric. Sci. 5: 28-33.

www.indiaagronet.com/indiaagronet/cropinfo/maize.htm#intro