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ASSOCIATION OF GENETIC DIVERGENCE WITH HETEROSIS, COMBINING ABILITY AND MEAN VALUE FOR QUANTITATIVE TRAITS IN POPCORN (*ZEA MAYS* VAR. *EVERTA*)

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ABSTRACT
The study was aimed to analyze the genetic diversity among popcorn inbred lines using Random Amplified Polymorphic DNA profiling and to associate it with heterosis, specific combining ability and mean values of hybrids for quantitative traits viz., days to 50% tasseling, days to 50% silking, days to maturity, plant height, ear height, seed index, grain yield per plant, dry fodder yield per plant and popping expansion obtained in diallel study. The highest heterosis was observed for grain yield (99.16%) followed by dry fodder yield (96.57%). Fifteen polymorphic RAPD primers were used for polymerase chain reaction amplification. In dendrogram, most of the genotypes were clustered in a single cluster except I-07-35-7-3 which grouped in another cluster. The estimate of genetic diversity varies from 0.49 to 0.86. Correlation coefficients (r) ranged from -0.472 to 0.295. These correlations were extremely low or non-significant for all the quantitative traits. The results depicted that RAPD technique was efficient in detecting polymorphism in popcorn populations. Genetic diversity showed no significant association with all parameters of hybrid performance for days to maturity, plant height and seed index.

INTRODUCTION

Maize is one of the top three cereal crops grown and consumed throughout the world. There are different types of maize grown for commercial purpose viz., sweet corn, popcorn and baby corn. Among which, popcorn (*Zea mays* var. *everta*) is a type of maize that expands from the kernel and pops up when heated. It is popular as a fibre rich and nutritious snack in all the parts of the world. In India, present growing cultivars are composite varieties viz., Amber popcorn, VL popcorn and Jawahar popcorn having lower yield with less popping quality. The aim of any breeder for popcorn improvement is to develop hybrid with both high grain yield and popping expansion. However, these two traits showed negative correlation for their inheritance (Viana and Matta, 2003; Pajic *et al.,* 2008; Rangel *et al.,* 2008).

Heterosis is an important tool for enhancing hybrid vigour for growth and yield traits due to its success in different types of maize (Bruel *et al.,* 2006; Miranda *et al.,* 2008). The diallel analysis provides information on the type of gene action and general combining ability and specific combining ability (SCA) of genotypes (Silva *et al.,* 2010; Moterle *et al.,* 2011). Selection of parents is most important and critical criteria for developing hybrids. The recent trend in breeding programmes is use of molecular fingerprinting of genotypes which has an advantage of speed and accuracy compared to previous methods of forming heterotic groups (Miranda *et al.,* 2008). The use of molecular markers viz., ISSR, RAPD and SSR prevent the environment interferences for assessing genetic divergence (Leal *et al.,* 2010). Among different molecular markers, Random Amplified Polymorphic DNA (RAPD) is used due to its capacity to detect a high-level of polymorphism in plants based on the exploration of wide genome regions (Williams *et al.,* 1990). In popcorn, this low-cost technique allows simple and fast polymorphism detection. Some studies have been conducted for a better understanding of genetic diversity in popcorn using RAPD (Vilela *et al.,* 2008; Leal *et al.,* 2010). It is widely believed that the level of genetic distances between two inbred lines has an influence on the performance of resulting hybrids (Pajic *et al.,* 2010). Studies of molecular diversity in relation to hybrid performance have been undertaken in several crops. The performance of hybrid can be reflected by three parameters viz., heterosis, specific combining ability and mean value (MV) of hybrids in the field experiments. Therefore, there is a need to study all these parameters with genetic divergence for better understanding the relationships.

Hence, the present study was conducted with the aim to identify superior hybrids with high yield and desirable popping expansion with the following objectives 1) to reflect the RAPD profiling of ten popcorn inbred lines 2) to assess the heterosis, combining ability and mean performance of the hybrids developed from diallel mating design 3) to determine the association between distance matrices of molecular markers

and hybrid performance for quantitative traits in popcorn. Development of such hybrids will not only enhance the market potential, but also bring huge returns to farming and trading communities.

MATERIALS AND METHODS

Genotypes

Ten inbred parental lines viz., I-07-44-3-2 (P_1), I-07-44-4-3 (P_2), I-07-44-7-1 (P_3), I-07-43-7-3 (P_4), I-07-42-1-2 (P_5), I-07-42-6-3 (P_6), I-07-35-7-3 (P_7), I-07-63-1-2 (P_8), I-07-63-18-3 (P_9) and I-07-63-36-1 (P_{10}) were crossed in a diallel fashion to produce 45 single cross hybrids during the wet season of 2011 (Griffing, 1956).

Molecular descriptor

Total DNA was extracted from the leaves by Cetyl trimethyl ammonium bromide (CTAB) method (Zidani *et al.*, 2005). Spectrophotometry was performed to determine DNA concentration by using Nanodrop N.D.1000 at absorbance ratio 260/280 nm. The DNA samples were amplified in a thermal cycler (Apollo, USA) with an initial denaturing step of 5 min at 96°C followed by 45 cycles of 1 min at 94°C, 1 min 38°C and 2 min at 72°C and a final extension step of 7 min at 72°C. Eighty primers were initially screened for presence of sharp and distinct amplified products in all the genotypes. Among them 15 primers with good results were obtained have been used for final analysis (Table 2).

Field experiment

The hybrids and parents were raised in Randomized Complete Block Design with three replications during winter 2011-12 at Agronomy Farm, Anand Agricultural University, Anand (22°-35' North latitude and 72°-55' East longitude, elevation 45.1 m a.s.l., yearly rainfall 877.6 mm). The plot size was two rows of 4.0 m with distance of 0.60 m between rows and 0.20 m between plants within a row. Irrigation, plant protection measures, fertilizer and other cultural practices were followed to raise a normal maize crop.

Quantitative traits evaluated

Different quantitative traits viz., days to 50% tasseling, days to 50% silking, days to maturity, plant height, ear height, seed index, grain yield per plant, dry fodder yield per plant and popping expansion were studied. Popping expansion was measured in sample of 30.0 g of seeds that were popped in an automatic Popcorn Maker developed by Skyline according to Vieira *et al.* (2011). Seed index was measured by taking 100-seeds weight.

Statistical analysis

For molecular data analysis, amplification profiles of genotypes were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). The data matrix was read by NTSYS-pc version 2.02 developed by Rohlf (1994) and analysed using Jaccard's similarity coefficient (Jaccard, 1908). The resulted similarity matrix was used to produce dendrogram of genotypes. Genetic distance was estimated according to Spooner *et al.* (1996) as Genetic Diversity = 1-J (J = similarity coefficient).

The sums of squares of treatments were partitioned into general combining ability and specific combining ability using method 2, on model 1 of Griffing (1956), followed by heterosis evaluation by the method of Turner (1953) for relative heterosis (RH) and Fonseca and Patterson (1968) for heterobeltiosis (HB). Finally, association of genetic diversity with heterosis, SCA and mean value was evaluated by Pearson's correlations. Statistical packages SPSS 15.0 was used for correlation analysis. The test of significance was verified by t test.

RESULTS AND DISCUSSION

Yield, its attributing traits and popping expansion

Analysis of variance indicated significant differences among the genotypes for all the traits evaluated. It indicated that experimental genotypes had sufficient genetic variability for all the characters evaluated. ANOVA of diallel analysis showed highly significant estimates ($p < 0.01$) for both GCA and SCA for all biometrical traits. The quadratic components associated with the specific combining ability effect were greater than those associated with the general combining ability for all traits under the study, which revealed the greater importance of the dominance effects for their inheritance.

The range of heterosis, specific combining ability and mean performance of hybrids observed for all the traits are presented in Table 1. The highest effect of relative heterosis was observed for grain yield (99.16%), while for heterobeltiosis highest heterotic value was 90.91% (dry fodder yield). For SCA effects, 13, 13, 9, 9, 12, 21, 17, 22 and 17 hybrids out of 45 hybrids recorded significant effects in desired direction for days to 50% tasseling, days to 50% silking, days to maturity, plant height, ear height, seed index, grain yield per plant, dry fodder yield per plant and popping expansion, respectively.

Genetic diversity of popcorn genotypes

The RAPD profile products generated can be classified into two types as monomorphic and polymorphic electromorphs. These differences can be used to examine and establish systematic relationships. In present study, best 15 polymorphic primers were selected which amplified total of 753 fragments with 259 loci, among that 253 loci were found polymorphic, showed 97.68% polymorphism and fragment sizes varying from 117 to 4874 bp. The Polymorphism information content (PIC) value of primers ranged from 0.82 to 0.94 (Table 2). The RAPD profile for 22ES10G33 primer is presented in Fig. 1.

The RAPD marker 22ES10G33 produced maximum number of 68 scorable bands and OPC 11 produced minimum number of 29 bands. With regard to the cluster analysis of genotypes P_7 and P_9 (P_7:P_9) were the most distant (0.86). The highest genetic distance revealed their distinctiveness between them. While, P_5:P_6 and P_6:P_8 were the less distant (0.49). Since it was not possible to obtain the pedigree of most of these cultivars, it was presumed that these genotypes have common parentage. The genetic distances were calculated for all 45 combinations involving 10 genotypes presented in Table 3.

Based on the RAPD data, clustering pattern of dendrogram

Table 1: Range of heterosis, specific combining ability and mean performance of hybrids

Traits	Range			
	Relative heterosis(%)	Heterobeltiosis(%)	Specific combining ability	Mean value
DFT	-14.22** to 2.24*	-11.44** to 5.24**	-5.14** to 3.66**	59 to 71.7 days
DFS	-15.84** to 1.90	-13.49** to 4.90**	-6.20** to 3.36**	62 to 73.7 days
DM	-8.68** to 2.26	-8.43** to 3.82	-5.24** to 3.84**	108.7 to 122.3 days
PH	-13.62** to 39.07**	-10.80** to 57.71**	-23.36** to 20.64**	148.4 to 209.1 cm
EH	-19.39** to 50.33**	-18.19** to 62.85**	-16.58** to 21.55**	76.4 to 128.0 cm
SI	-18.93** to 87.14**	-21.79** to 66.61**	-3.36** to 9.13**	11.4 to 27.2 g
GY	-17.63** to 99.16**	-26.00** to 80.72**	-32.59** to 48.48**	68.7 to 177.9 g
DFY	-26.91** to 96.57**	-33.96** to 90.91**	-65.83** to 45.81**	93.3 to 210 g
PE	-64.96** to 67.31**	-72.99** to 49.96**	-8.39** to 8.34**	4.3 to 28.2 cc/g

*,** indicate level of significance at $p < 0.05$ and $p < 0.01$, respectively. DFT = Days to 50% tasseling, DFS = Days to 50% silking, DM = Days to maturity, PH = Plant height, EH = Ear height, SI = Seed index, GY = Grain Yield per plant, DFY = Dry fodder yield per plant, PE = Popping expansion

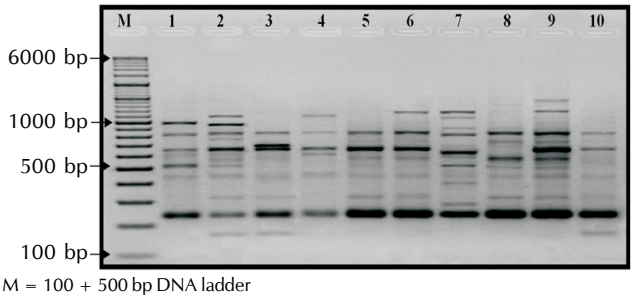
Table 2: Selected RAPD primers in terms of their sequence, number of detected loci, number of polymorphic loci and size of the amplified fragments

S. No.	Primer	Sequence(5′ → 3′)	Number of detected Loci	Number of polymorphic Loci	PIC value	Fragment size (bp)
1	OPA 02	TGCCGAGCTG	14	13	0.89	209-2911
2	OPA 03	AGTCAGCCAC	12	11	0.87	388-3239
3	OPA 20	GTTGCGATCC	21	21	0.91	224-3271
4	OPC 11	AAAGCTGCGG	11	11	0.82	125-2860
5	OPE 01	CCCAAGGTCC	20	20	0.91	240-3546
6	OPH 20	GGGAGACATC	20	20	0.92	123-4874
7	OPK 07	AGCGAGCAAG	18	18	0.90	127-2970
8	OPL 11	ACGATGAGCC	18	18	0.91	217-3410
9	OPM 02	ACAACGCCTC	21	21	0.93	119-2795
10	OPU 17	ACCTGGGGAG	11	09	0.86	128-3276
11	OPW 01	CTCAGTGTCC	19	19	0.91	130-3576
12	OPW 05	GGCGGATAAG	22	22	0.94	117-3572
13	13ES10C24	GGCTCGTACC	14	13	0.88	146-2257
14	16ES10C27	CGCCACGTTC	17	17	0.92	261-3014
15	22ES10G33	AGGCCCGATG	21	20	0.92	159-2499
		Total / Range	259	253	0.82-0.94	117-4874

Table 3: Diversity matrix for 10 popcorn genotypes based on RAPD analysis

	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
P_1	0									
P_2	0.64	0								
P_3	0.67	0.64	0							
P_4	0.79	0.81	0.74	0						
P_5	0.71	0.59	0.63	0.74	0					
P_6	0.71	0.66	0.68	0.75	0.49	0				
P_7	0.77	0.79	0.77	0.80	0.79	0.81	0			
P_8	0.75	0.59	0.70	0.82	0.57	0.49	0.79	0		
P_9	0.81	0.69	0.74	0.83	0.53	0.57	0.86	0.53	0	
P_{10}	0.77	0.74	0.75	0.83	0.64	0.68	0.83	0.71	0.73	0

P_1 = I-07-44-3-2, P_2 = I-07-44-4-3, P_3 = I-07-44-7-1, P_4 = I-07-43-7-3, P_5 = I-07-42-1-2; P_6 = I-07-42-6-3, P_7 = I-07-35-7-3, P_8 = I-07-63-1-2, P_9 = I-07-63-18-3, P_{10} = I-07-63-36-1



M = 100 + 500 bp DNA ladder
1. I-07-44-3-2, 2. I-07-44-4-3, 3. I-07-44-7-1, 4. I-07-43-7-3, 5. I-07-42-1-2
6. I-07-42-6-3, 7. I-07-35-7-3, 8. I-07-63-1-2, 9. I-07-63-18-3, 10. I-07-63-36-1

Figure 1: RAPD profiling with 22ES10G33 primer

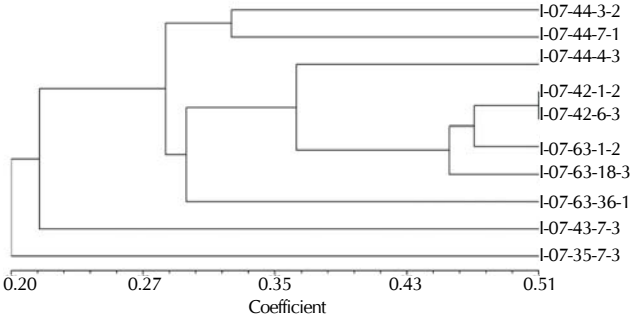


Figure 2: Dendrogram showing clustering of 10 popcorn genotypes using UPGMA based on Jaccard's coefficient obtained from RAPD profiling

Table 4: Pearson's correlation between genetic diversity (GD) with relative heterosis (RH), heterobeltiosis (HB), specific combining ability (SCA) and mean (MV) in hybrids

Characters	GD vs. RH	GD vs. HB	GD vs. SCA	GD vs. MV
Days to 50% tasseling	-0.086	0.079	-0.116	-0.412**
Days to 50% silking	-0.146	-0.039	-0.136	-0.371*
Days to maturity	-0.049	0.012	-0.040	-0.172
Plant height	0.014	0.270	0.016	0.286
Ear height	0.023	0.259	0.012	0.295*
Seed index	0.142	0.140	0.019	0.193
Grain yield	0.174	0.148	0.056	0.294*
Dry fodder yield	-0.370*	-0.408**	-0.170	-0.148
Popping expansion	-0.472**	-0.430**	-0.198	-0.416**

*,** indicate level of significance at 5% and 1%, respectively

was generated using the pooled data (Fig. 2). The dendrogram is having two main clusters. All the genotypes were clustered together in a common cluster except I-07-35-7-3 (P_7), which favours its allocation in another cluster. And, this clustering pattern supports and proves the narrow genetic base of popcorn genotypes. The genotypes which are clustered together supposed to have the same ancestors in their parentage.

Association of RAPD genetic divergence with quantitative traits

Evaluating the data obtained in the field experiments, the cross that resulted in highest relative heterosis (99.16 %), SCA (48.48) and mean value (177.9 g) for grain yield was $P_4 \times P_7$, which disagrees with the hypothesis that greater genetic distance is highly associated with the greater heterosis, SCA and mean value expressed by the hybrid. Similarly, for popping expansion, the results of the diallel analysis did not coincide with that of genetic dissimilarity matrix, where $P_8 \times P_{10}$ was the most outstanding cross with highest relative heterosis (67.31 %), SCA (8.34) and expansion 28.2 cc/g. These results were supported by Munhoz *et al.* (2009) and Souza *et al.* (2012) in popcorn for grain yield and popping expansion. Similar type of trend was observed for all other traits viz., days to 50% tasseling, days to 50% silking, days to maturity, plant height, ear height, seed index and dry fodder yield. The hybrids which perform better than other hybrids for these entire traits are developed from the parents which are less diverse to each other. To have a clear-cut conclusion we need to compare laboratory data and field experiment results statistically. For that genetic divergence estimated using RAPD fingerprinting and parameters of hybrid performance for all the traits were correlated (Table 4).

Correlation coefficients (r) of genetic diversity with heterosis, SCA and mean ranged from -0.472 (popping expansion for relative heterosis) to 0.295 (ear height for mean). These correlations were extremely low and significant or non-significant (at 5% and 1% probability), making the use of RAPD markers unfeasible for conclusions such as the prediction of hybrid performance for establishment of heterotic groups based on genetic divergence. The results of association between distance matrix and heterosis and SCA were non-significant for days to 50% tasseling, days to 50% silking, days to maturity, plant height, ear height, seed index and grain yield. These results were in accordance with Rinaldi *et al.* (2007) for flowering traits viz., days to 50% tasseling and days to 50% silking. However, Pajic *et al.* (2010) obtained positive and significant relationships of genetic diversity with heterosis and SCA for grain yield which differ from results obtained in present study. The results of correlation of genetic diversity with mean showed positive and significant correlation for grain yield. But, these results were contradictory to Souza *et al.* (2012), who obtained non-significant correlations. Negative and significant association was observed with heterosis for dry fodder yield, but it correlated non-significantly for SCA and mean. Popping expansion showed negative and significant correlation for heterosis and MV that is contradictory with the results of Pajic *et al.* (2010) who portrayed positive and significantly correlation of genetic diversity with heterosis and SCA and Rinaldi *et al.* (2007) and Munhoz *et al.* (2009) who obtained non-significant association between diversity matrix with heterosis and SCA for popping expansion.

The applications of molecular marker in relation to hybrid performance have been studied in different types of maize. Some of the authors reported significant correlations (Betran *et al.,* 2003; Amorim *et al.,* 2006 ; Srdic *et al.,* 2007) while, the others stated non-significant correlation between genetic diversity and heterosis and SCA (Rinaldi *et al.,* 2007; Legesse *et al.,* 2008; Dhliwayo *et al.,* 2009; Balestre *et al.,* 2010; Devi & Singh, 2011). The absence of significant associations between genetic diversity and heterosis, SCA and mean performance may be explained by the fact that amplified polymorphic fragments were from any part of the genome, including areas without selection pressure. These sequences may not code traits of economic importance (Joyce *et al.,* 1999). Also, the genotypes used for study, G x E interaction and RAPD primers used for the study were different from the previous workers. The fact is that this problem is not completely solved and will need further investigations.

CONCLUSION

Based on the present results the authors report that the RAPD technique was efficient in detecting polymorphism and determining genetic divergence among popcorn genotypes. Nevertheless, genetic distance did not correlate significantly with the heterosis, specific combining ability and mean value of hybrids for biometric traits.

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