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Heterotic grouping of late-maturing maize inbred lines based on combining ability and molecular marker studies

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Abstract

Aim : To examine concurrence between yield based and SSR marker based heterotic grouping of late-maturing maize inbred lines.

Methodology : A total of 45 F₁ crosses derived from 10x10 diallel mating scheme were evaluated along with their parents and hybrid checks across two locations (New Delhi and Dharwad). To group the inbred lines at molecular level, 35 polymorphic SSR primers were used for PCR amplification of repeat sequences from the genomic DNA of each inbred isolated by CTAB method. Cluster analysis was carried out by using NTSYS-pc-2.02.

Results : Analysis of variance revealed significant ($P < 0.01$) variation among the parents and their hybrids across two locations for all the traits. The yield SCA classified 10 late-maturing maize inbred lines into three heterotic groups. Similarly, molecular genetic diversity analysis also categorized the inbred lines into three major clusters. However, correlations between SCA effects and genetic distance were low ($r = 0.161$) for grain yield.

Interpretation : Heterosis prediction with the help of molecular markers alone was not found effective. Further, field-testing can be complemented with molecular markers for the elimination of inferior crosses.

Key words: Combining ability, Dendrogram, Heterotic groups, Maize, Simple sequence repeats

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Introduction

Genetic diversity is a prerequisite for the success of any crop improvement programme. In maize, introduction of exotic germplasm lines were found promising in enhancing the genetic variation and also to improve the productivity of local maize varieties. However, for the efficient use of introduced germplasm, preliminary information on genetic diversity and heterotic pattern between exotic and local maize germplasm is essential (Fan *et al.*, 2008). In heterosis breeding and population improvement programmes, breeders have to evaluate thousands of crosses in replicated field trials to eliminate inferior crosses. The success of such breeding effort lies in the efficiency of procedure employed to identify parental lines, which can be crossed to develop outstanding single cross hybrid. Heterotic grouping of inbred lines is one such strategy, where probability of obtaining superior heterotic hybrids increases when inbred lines belonging to different heterotic groups are crossed (Reif *et al.*, 2003; Singode *et al.*, 2017).

Heterotic group is defined as “a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups”. There are several methods such as quantitative genetics analysis, pedigree analysis and molecular marker analysis for classifying the genotypes into different heterotic groups. Combining ability analysis using diallel mating design developed by Griffing (1956) is one of the widely used biometrical techniques. This method has been employed by several researchers to define heterotic patterns in maize (Laude and Salazar, 2008; Bidhendi *et al.*, 2011; Laude and Carena, 2015; Amegbor *et al.*, 2017) and other crops (Torres and Geraldini, 2007; Farshadfar *et al.*, 2013) and animals (Seidavi, 2010; Balaguer, 2014; Roy *et al.*, 2018).

Combining ability studies alone may not be efficient to classify inbred lines into distinct heterotic groups. It can be better complimented with the use of molecular markers that allow direct comparison of genetic similarity between inbred lines at DNA level (Reif *et al.*, 2003). Among various molecular tools, simple sequence repeats (SSRs) were widely used in maize for heterotic grouping of inbred lines (Pabandon *et al.*, 2009; Akinwale *et al.*, 2014; Rajendran *et al.*, 2014). In the present study, an effort was made to understand the heterotic pattern of available superior late-maturing inbred lines and to test the concurrence between yield based and SSR marker based classification of the inbred lines.

Materials and Methods

Plant material and field evaluation: The material selected for the present study consisted of ten late-maturing maize inbred lines (Table 1) maintained in the Maize Genetics Unit, Indian Agricultural Research Institute, New Delhi. Hybridization in half diallel fashion was done among the inbred lines according to Griffing's diallel method II (Griffing, 1956) during *rabi* season at

Winter Nursery Centre, Indian Institute of Maize Research, Hyderabad. Forty five F1 hybrids, parents and two checks (PMH-1, PMH-3) were evaluated under randomized complete blocks design with three replications in kharif season at two test locations viz., New Delhi and Dharawada.

Each genotype was grown in a single-row plot of 5 m long, spaced 75 cm apart. Further, plant to plant spacing of 25 cm within each row was maintained. Proper package of practices was followed to raise the good crop at both the test locations. Data on 11 morphological traits were recorded on five randomly selected plants in each row. The grain yield data was subjected to *F* test in order to test the homogeneity of variance (Gomez and Gomez, 1984). Analyses of variance and combining ability analysis was performed using Windost software package (WINDOSTAT ver. 9.1) following method II of Griffing's fixed effect model. The inbred lines were classified into different heterotic groups based on specific combining ability (SCA) effects of grain yield per plant. The SCA data was used as dissimilarity coefficients for cluster analysis in unweighted pair group method with arithmetic averages (UPGMA) method.

Analyses of molecular diversity: Leaf sample from 21-day-old plants were collected and DNA was isolated using modified CTAB procedure (Saghai-Marouf *et al.*, 1984). Primers with banding pattern difficult to score and monomorphic were excluded. Final set of 35 SSR primers (Table 2) were selected out of 50 SSR primers for further analysis. SSR alleles were scored sequentially from the largest to the smallest size band based on their position relative to the ladder. Genetic similarity (GS) matrix was generated using Jaccard's coefficient. Similarity matrix was analysed using NTSYS-pc-2.02 to draw cluster diagram.

Results and Discussion

Analysis of variance revealed highly significant ($P < 0.01$) differences among the parents and hybrids for grain yield across environments (Table 3), which indicate the presence of ample variation in the genotypes studied and it also infers that inbreds can be utilized for the development of location specific varieties. Interaction of environment $\times$ genotype was highly significant ($P < 0.01$) indicating that yield of genotypes were different in different environments. GCA and SCA variances were highly significant for most of the traits indicating the importance of both additive and non-additive gene actions in the inheritance of these traits (Choukan and Mosavat, 2006; Zare *et al.*, 2010; Badu-Apraku *et al.*, 2015; Amegbor *et al.*, 2017).

Highly significant differences were observed for GCA interaction variance in the traits like ear length, ear girth, plant height and hundred seed weight. Similarly, for SCA interaction variance almost all the traits exhibited highly significant differences. Significance of interaction variance for majority of traits indicate that variations in the combining abilities of inbred lines and yield performance of single-crosses evaluated across environment was not consistent. Hence, this suggested the need

Table 1: Description of 10 maize inbred lines and two checks used in the study

Abbreviation	Inbred line	Preliminary group	Days to 50% silk	Other characteristics
P1	IPA26368	1	63	Yellow-orange, flint
P2	IPA26011	2	59	Orange, flint
P3	IPA26023	3	61	Orange, flint
P4	IPA26038	2	61	Yellow-orange, flint
P5	IPA26051	2	56	Orange, flint
P6	IPA26055	2	57	Yellow-orange, flint
P7	IPA26063	2	58	Orange, flint
P8	IPA26076	2	56	Yellow, flint
P9	IPA26077	2	59	Yellow, flint
P10	IPA26085	3	59	Yellow-orange, flint
Check 1	PMH 1	-	60	Yellow-orange, flint
Check 2	PMH 3	-	60	Orange, flint

to identify and select specific inbred lines and single-crosses for specific environments (Akinwale *et al.*, 2014). The inbred lines with significant ($P < 0.05$) positive GCA for grain yield were P_5 (0.42) and P_6 (0.19) (Table 4). These two inbred lines were identified as best general combiners for majority of traits. Since GCA effect is controlled by additive genes inclusion of inbreds with high GCA effect in breeding program can accelerate yield improvement process (Dar *et al.*, 2016).

A total of seventeen crosses revealed highly significant positive SCA effect for grain yield across two locations, and top three crosses with highest SCA value were $P_9 \times P_{10}$ (1.95), $P_4 \times P_5$ (1.44) and $P_3 \times P_6$ (1.43). Since these hybrids produced high grain yield across two test environments, they can be readily evaluated

under coordinated yield trials as single cross hybrids. A significant positive SCA for grain yield of some of the hybrids were a product of certain inbred lines observed with low and negative GCA estimates. Such positive performance of high $\times$ low combination may be due to the interaction between dominant favourable alleles from good general combiners and unfavourable recessive alleles from poor combiners (Owusu *et al.*, 2017). Three heterotic groups were formed based on cluster analysis using UPGMA on SCA effects of yield data (Fig. 1a). Group A consisted of lines P1, P6, P7, P8 and P9. Inbreds P5 and P10 fall into group B. Group C contained P2, P3 and P4. According to the assumption, SCA of two lines from different heterotic groups are greater than those from the same group (Amegbor *et al.*, 2017). The heterotic group with high performing inbred lines is best suited for the

Table 2: Genomic location, SSR locus, repeat type and number of alleles produced by each of the 35 SSR locus

Marker	Bin no.	Repeat type	No. of alleles	Marker	Bin no.	Repeat type	No. of alleles
umc2226	1.02	(TGG)6	4	umc2319	6.05	(GAGGAG)5	2
bnlg439	1.03	Unspecified	3	umc2165	6.07	(TTC)12	2
umc1452	1.04	(GCC)4	3	umc2324	6.08	(CAC)4	2
umc2083	1.06	(CGG)7	2	umc2364	7.01	(GGA)7	2
umc1446	1.08	(TAA)7	2	umc1831	7.02	(AG)8	2
umc1704	2.09	Unspecified	2	umc2330	7.03	(GAGA)5	2
umc2220	2.07	Unspecified	2	umc2332	7.04	(CTC)5	3
umc2262	3.04	(CATCT)5	3	umc2334	7.06	(GGA)4	2
umc1102	3.05	GGAT	3	phi125	8.03	AG	2
umc1690	3.07	(GCA)4	2	bnlg240	8.06	Unspecified	2
umc1757	4.01	(TCC)7	2	phi028	9.01	GAA	3
phi021	4.03	AG	3	umc1714	9.07/08	(GGC)4	2
umc2061	4.05	(CTG)8	2	umc2130	9.02	Unspecified	2
umc1869	4.06	(GGT)6	2	umc1318	10.02	(GTC)5	2
umc2373	5.04	(GCT)4	3	umc2017	10.03	(CAA)4	2
umc1153	5.09	(TCA)4	2	umc2043	10.05	(TCC)4	2
umc1723	6.02	(CTT)6	2	umc2021	10.07	(TGG)4	3
bnlg1014	6.04	Unspecified	2				

Table 3: Mean squares from the combined ANOVA of grain yield and other traits of late-maturing inbred lines evaluated across research environments

Source	DF	Mean square										
		DFA	DFS	ASI	PH	EH	EL	EG	NGPR	NRW	HSW	GYPTT
Env. (E)	1	2965.46**	2644.01**	9.70**	61621.14**	25432.44**	98.72**	2.61**	56.35**	0.06	2.94	6.79**
Genotypes (G)	54	8.83**	9.49**	0.81**	822.68**	870.88**	11.89**	1.06**	88.47**	7.14**	30.95**	13.10**
G*E	54	16.78**	14.09**	1.10**	696.06**	481.53**	4.20**	0.28**	22.62**	1.39**	17.40**	1.46**
GCA	9	1.87	3.04**	0.42**	249.95**	77.07**	4.36**	0.24**	19.74**	2.50**	12.09**	0.91**
SCA	45	3.16*	3.19**	0.24*	279.08**	332.94**	3.89**	0.38**	31.44**	2.36**	9.97**	5.06**
GCA*E	9	1.93	2.01	0.22	106.72**	58.65*	1.81**	0.13**	3.89*	0.55*	5.05**	0.42*
SCA*E	45	6.33**	5.24**	0.40**	257.08**	180.88**	1.32**	0.09**	8.27**	0.45**	5.95**	0.50**
Error	216	1.33	1.21	0.15	35.26	29.74	0.56	0.02	1.63	0.27	1.23	0.21

DF: degree of freedom, DFA: days to 50% anthesis, DFS: days to 50% silking, ASI: anthesis-silking interval, PH: plant height (cm), EH: ear height (cm), EL: ear length (cm), EG: ear girth (cm), NGPR: no. of grains per row, NRW: no. of grain rows per ear, HSW: 100 seed weight (gm), GYPPT: seed yield per plot (kg plot⁻¹). *, ** Significant levels at 0.05, and 0.01 probability levels

Table 4: Estimates of GCA and SCA effects among 10 late-maturing maize inbred lines for grain yield (kg plot⁻¹)

Inbreds	GCA	SCA								
		P ₁	P ₂	P ₃	P ₄	P ₅	P ₆	P ₇	P ₈	P ₉
P1	-0.25**									
P2	-0.1	1.24**								
P3	-0.09	0.65*	-0.09							
P4	0.06	0.74*	0.69*	-0.42						
P5	0.42**	0.41	0.95**	1.23**	1.44**					
P6	0.19*	-0.84**	1.24**	1.43**	0.49	0.92**				
P7	-0.17	0.54	0.46	0.97**	0.46	1.33**	0.39			
P8	-0.09	0.84**	0.80**	0.71*	1.09**	0.61*	0.51	0.26		
P9	0.07	0.74*	1.20**	1.21**	0.59	0.22	0.28	-0.65*	0.25	
P10	-0.05	1.04**	-0.03	0.61*	0.87**	-0.44	0.67*	0.60*	0.28	1.95**

*, ** Significant levels at 0.05, and 0.01 probability levels

Fig. 1: Association among 10 late-maturing maize inbred lines revealed by UPGMA cluster analysis generated by (a) SCA effect and (b) 35 SSR markers.

development of synthetic and composite varieties. Further, manifestation of high degree of heterosis in crosses between heterotic groups denotes genetic diversity between groups (Badu-Apraku *et al.*, 2015). Probability of obtaining superior hybrids increases when parental lines are selected from diverse groups.

35 SSR primers produced 83 alleles among the ten late-maturing inbred lines. The average number of alleles amplified per locus was 2.3 ranging from two to four (Table 2). In a similar study, Fan *et al.* (2004) reported a range of 2–5 alleles per locus and a mean of 2.84 while assessing the genetic diversity of 10 QPM inbred lines with 37 SSR primers. The lowest genetic similarity of 0.308 was observed between P_1 vs P_5 whereas the highest was found between P_1 vs P_2 with 0.625. Based on the UPGMA cluster analysis, 10 late-maturing inbred lines were grouped into three major clusters (Fig. 1b). Cluster I harbored lines P_1 , P_2 , P_3 and P_8 . Inbreds P_4 , P_7 , P_9 and P_{10} fall in cluster II, while Cluster III contained the lines P_5 and P_6 . Among the molecular markers, use of SSR markers for heterotic grouping in maize has proven valuable (Akinwale *et al.*, 2014; Rajendran *et al.*, 2014; Sharma *et al.*, 2017).

The correlation coefficient between genetic distance estimates of ten late-maturing inbred lines and their corresponding grain yield SCA effect ($r=0.161$) was positive and insignificant. It depicts existence of poor correlation between heterosis and genetic distance. There may be several factors responsible for poor correlation like random distribution of marker, use of few markers for the study, unequal genome coverage and lack of linkage between genes controlling yield related traits (Kwon *et al.*, 2002; Akinwale *et al.*, 2014; Rajendran *et al.*, 2014). From the above results, it can be concluded that with the help of molecular markers alone it is not possible to accurately predict the hybrid performance.

The present study revealed highly significant variations among the study material for all the yield-contributing traits. Based on combining ability results, the parents P_5 (IPA26051) and P_6 (IPA26055) were identified as donors for grain yield improvement in hybrid breeding. Based on the grain yield SCA, the cross combination $P_9 \times P_{10}$ (IPA26077 x IPA26085), $P_4 \times P_5$ (IPA26038 x IPA26051) and $P_3 \times P_6$ (IPA26023 x IPA26055) have been identified as promising F_1 hybrids for grain yield, hence these hybrids have been recommended for heterosis breeding. Our findings further confirmed that heterosis prediction with the help of set of molecular markers alone was not found effective.

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