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RESEARCH ARTICLE

Heterotic grouping methods reveal differential breeding efficiencies and season-specific categorization of inbreds in tropical maize (*Zea mays* L.)

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Abstract

The selection of diverse parents is a prerequisite for the exploitation of grain yield heterosis and is a real challenge to maize breeders**.** Heterotic grouping assists the breeder in selecting such diverse parents, which reduces the number of crosses to be made by avoiding intra-group crosses. An investigation was carried out to evaluate 54 inbreds along with two testers (LM 13 and LM14) and 108 hybrids derived using two testers with hybrid checks during the rainy and post-rainy seasons of 2019. Heterotic grouping based on three biometrical methods, *viz*., Specific Combining Ability combined with line Pedigree and hybrid Yield information (SCA-PY), Heterotic groups Specific and General Combining Ability (HSGCA), and Heterotic grouping by GCA of Multiple Traits (HGCAMT (HGCAMT) was inconsistent between the seasons and showed similar but not identical trends within a season. The HSGCA method could classify all the inbreds studied in both seasons. The SCA-PY and HGCAMT methods could classify 49 and 31 inbreds, respectively in the rainy season and 43 and 40 inbreds, respectively, in the post-rainy season. Only 10 inbreds were grouped identically in both seasons by all three methods and 15 inbreds were assigned to different groups between the seasons by all three methods. Besides, 44 lines (81.5%) were inconsistently grouped by at least one of the methods between the seasons, implying the season-specific response by the hybrids. The SCA-PY method was 2.2 and 6.2% more efficient than HSGCA and 10 and 18.1% more efficient than HGCAMT during rainy and post-rainy seasons, respectively. Thus, the results of the study revealed differential breeding efficiencies of grouping methods and suggested to have season-specific heterotic grouping in maize.

Keywords: Maize, heterotic group, HSGCA, SCA-PY, HGCAMT and combining ability

Introduction

Maize is the third most important cereal crop in the world and possesses high adaptability, versatility, and multiple uses. Hybrid maize is predominant in world agriculture and hence, the inbred lines are the key players in maize breeding. However, the immense diversity and the greater number of inbred lines available with the breeders pose challenges in identifying the parents that are the best specific combiners, which can be done through a series of crosses with selected inbred lines. This crossing scheme, if not planned strategically, would consume a lot of labour and resources. Thus, the approaches reducing the number of cross combinations while breeding hybrids would economize maize breeding. It is known that the diverse parental lines would produce heterotic hybrids. Consequently, the seed and pollen parents should be derived from genetically unrelated germplasm pools for optimum exploitation of heterosis in hybrid breeding (Melchinger and Gumber 1998). Among the large panel of inbred lines available with the breeders, some might be genetically similar while, others

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might be diverse, which cannot be assessed only by the phenotype. Therefore, methods to distinguish genetically similar and diverse inbreds have been devised. By these methods, genetically similar inbreds are assigned to one group, while those which are diverse from the first group, but are similar among themselves are assigned to another, and so on. Such groups are known as heterotic groups, and they are a prerequisite for increasing maize breeding efficiency (Fan et al. 2018), as these groups enable the development of highly heterotic hybrids with fewer crosses.

A heterotic group has been defined as a collection of germplasm that tends to exhibit a higher degree of heterosis when crossed with the germplasm from an external group than when crossed with the members of its own group (Lee 1995). The members of distinct heterotic groups are expected to differ in allelic frequencies and, hence are genetically diverse (Viana 2000). Since the magnitude of heterosis is the function of dominance and allelic frequency difference between the parents involved, it corroborates that the inter-group F_1 hybrids would manifest high heterosis. Thus, the formation of the heterotic group enables the exploitation of heterosis in an efficient manner through the identification of complementary lines. Therefore, constituting heterotic groups from the available inbreds is one of the foundation pillars of the hybrid breeding program of maize (Russell 1991; Cheres et al. 2000).

Heterotic grouping can be constituted by different methods such as pedigree analysis method (Reid et al. 2011; Hallauer et al. 1988; Fu et al. 2014), molecular method (Huang and Li 2002), and biometrical methods (Melani and Carena 2005; Wu et al. 2007). However, different heterotic grouping methods may assign the same inbred to different heterotic groups. Hence, the grouping method used by researchers has a great influence on assigning an inbred into a maize heterotic group. It is to be noted also that two or more inbreds (recycled inbreds) derived from a heterotic single cross may belong to different heterotic groups (Fu et al. 2014).

The superiority of a hybrid produced from a line depends upon two factors *viz*., the performance of the line itself and the behavior of the line in a hybrid combination. The behavior of a line in hybrid combinations is assessed through the estimation of general combining ability (*gca*) and specific combining ability (*sca*) effects. Therefore, these *gca* and *sca* information are mainly used in heterotic grouping by quantitative genetic/ biometrical methods (Fan et al. 2009a; Fan et al.2005b; Akinwale et al. 2014). Thus, among the different methods of heterotic grouping, the biometrical method is expected to be better as it assesses the actual performance of the line in hybrid combination resulting from the gene interactions of parental gene combinations.

Different biometrical methods are being used for heterotic grouping. The SCA-PY (Specific Combining Ability combined with line Pedigree and hybrid Yield information) method uses *sca* effects with pedigree and hybrid-yield information to assign inbreds to different heterotic groups. However, this method assigned the same inbreds into different heterotic groups in different studies/under environments because *sca* effects are greatly influenced by inbred × inbred and hybrid × environment interaction (Yadav et al. 2002; Oloyede and Oyekale 2015; Annor et al. 2019). Later, a novel HSGCA (Heterotic Groups Specific and General Combining Ability) method was proposed by Fan et al. (2008) based on both *gca* of lines and the *sca* of the combination. The above two methods (SCA-PY and HSGCA) consider SCA and/or GCA of grain yield alone as the criterion. Grain yield is a complex trait governed by polygenes with low heritability, the use of component traits with high heritability and strong correlation with grain yield might enhance the grouping efficiency (Badu-Apraku et al. 2013). Thus, a third method, Heterotic grouping by GCA of multiple traits (HGCAMT) was proposed by Badu-Apraku et al. (2013), by postulating that HGCAMT is expected to provide a more predictable heterotic grouping of the inbreds. Because *gca* measures additive gene effects for each trait. However, subsequent reports revealed that the efficiencies of these heterotic grouping methods were not consistent as expected and SCA-PY was reported to be more efficient in later studies. Fan et al. (2008) reported that the HSGCA method increased maize hybrid breeding efficiency over SSR marker and SCA-PY methods, while Badu-Apraku et al. (2013) reported higher efficiency of the HSGCA method. Subsequently, Laouali (2014) reported higher efficiency of the SCA method over the HSGCA method and concluded that the HSGCA method offered no additional advantage, while Oyetunde et al. (2020) reported marginal higher efficiency of SCA-PY over the HSGCA method and concluded that HSGCA and SCA-PY methods were the most efficient for heterotic grouping. Hence, more elaborate studies are required on methods of heterotic grouping and there is a need to assess the efficiency of different grouping methods across maize growing seasons, especially for multi-season maize growing regions, including India.

In India, maize is grown in three distinct seasons, *viz*., rainy, post-rainy, and spring seasons (Kumar et al. 2017), with the highest area in the rainy followed by post rainy season (Nirupma et al. 2012; FICCI 2018). With the increase in area under other seasons and increasing demand for maize grains, there is a need to accelerate and have dedicated hybrid maize breeding programs for different seasons. The choice of diverse parental lines from the germplasm collection requires knowledge of the applicability of heterotic groups formed for a season to other seasons. However, there are no reports on season-specific and comparative studies of combining ability and heterotic grouping between seasons. Thus, understanding the genetic relationships and heterotic groups in maize under different conditions is very important (Choukan et al. 2006; Zhang et al. 2018), including under different growing seasons. Besides, a maize breeder is developing and improving the inbreds continuously. Assigning newly developed inbreds into known heterotic groups or to a new group would increase efficiency while maintaining diversity (Richard et al. 2016). Hence, heterotic grouping is also one of the continuous processes in hybrid breeding. Therefore, the present study aimed to classify 54 potential inbred lines into heterotic groups to evaluate the efficiencies of different methods of heterotic grouping and the consistency of grouping between seasons.

Material and methods

Development and selection of lines

The base material for experimentation comprised newly developed 54 pre-evaluated tropical inbred lines of maize, which have the productivity of more than 2.5 tha^{-1} and tolerance to turcicum leaf blight and maydis leaf blight (Supplementary Table S1), of which 32 lines were developed through line breeding (8 lines) and recurrent selection (24 lines), while remaining 22 lines were derived from germplasm introductions from diverse sources such as International Maize and Wheat Improvement Centre (CIMMYT), Indian Institute of Maize Research (IIMR) and University of Agricultural Sciences, Raichur (UASR), Karnataka. Two testers, LM-13 and LM-14, belonging to two known opposite heterotic groups *viz.,* Makki safed (Dent) and Tuxpeno (Flint), respectively, were selected (Supplementary Table S1) for the grouping of the lines. The cross between these two testers (LM 13 ×LM 14), named PMH 1, representing the dent × flint heterotic pattern, is highly heterotic due to diverse seed and pollen parents and was a national check used in All India Coordinated Maize Improvement Project trials.

Generation of Line × tester crosses

Each of the 54 inbreds (designated as lines) was planted in four rows, each of two-meter length. Two testers were planted in 20 rows each to supply enough pollen to all 54 lines. Staggered sowing (2 times) of testers, separated by 10 days, was done to ensure pollen availability of testers for a longer period of time. The L × T crosses were developed by crossing one row of each line to each of the tester. The remaining two rows were selfed to produce a sufficient quantity of inbred seeds for replicated evaluation in the next season. This 54 × 2 (L× T) mating generated a total of 108 single cross hybrids.

Experimental design, site and evaluation

Experimental single cross hybrids (108) were evaluated along with 54 inbreds, two testers and five checks (PMH-1, a hybrid between the testers, and four national checks, NK-6240, CMH-287, DHM-121 and Bio-9544) in a Square Lattice Design (13 × 13 =169 genotypes per replication) with two replications at Indian Agricultural Research Institute's Regional Research Centre, Dharwad (IARI-RRCD). The experimental site is located at 15° 26′ N latitude and longitude of 70° 26′ E at an altitude of 678 m above mean sea level. It receives an average rainfall of 800 mm and medium-deep black soil with a pH of 7.2. In each replication, each genotype was sown in 2 rows of 4m length with a spacing of 60 × 20 cm on 10th June 2019 (rainy) and on 30th October 2019 (post-rainy). The weather data for the cropping period has been presented in Supplementary Table S2.

Phenotyping and data collection

The data on yield and yield components were recorded on randomly selected five competitive plants in each genotype (hybrids and inbred parents) using standard methods during the post-rainy season 2019 and rainy season 2019. The data were recorded on days to 50% tasselling (anthesis; DFT), days to 50% silking (DFS), plant height (cm), ear height (cm), cob length (cm), cob girth (mm), number of kernel rows per cob, number of kernels per row, 100 seed weight (g), shelling percentage and grain yield per plot (kg). DFT and DFS were recorded on a plot basis. The grain yield was recorded on an entire plot basis (avoiding the border plants to reduce error in yield estimate) and individual plant yield was estimated by dividing the plot yield by the plant stand at harvest. The grain moisture content was recorded and the grain yield per hectare was computed using the following formula.

$$\text{Grain yield(tha}^{-1}) = \frac{\text{Fresh cob yield (kg)} \times \text{MF} \times \text{SF} \times 1000}{\text{plot area (m}^2) \times 85}$$

Where, MF = Moisture factor = (1-moisture content in decimals) and

$$\text{SF = Shelling factor (in decimals)} = \frac{\text{Grain weight}}{\text{Cob weight (grain weight + pith weight)}}$$

Statistical model and analyses

The model used for analysis of square lattice design is as follows.

$$Y_{ij(l)} = \mu + R_i + B_{i(l)} + G_j + E_{ij(l)}$$

Where μ, $R_i, B_{i(l)}$, and G_j represent the effect of the mean, the replicate, the incomplete block, and the genotype, respectively. $E_{ij(l)}$ is the intra-block residual, assumed to be normally and independently distributed with mean 0 and variance $\sigma^2 e$.

Considering each season as a test environment, the analysis of variance (ANOVA) was carried out to generate genotype means adjusted for block effects according to the square lattice design (Cochran and Cox 1960) using SAS software (Version 9.4). The structure of ANOVA for square lattice design was followed according to Yates (1936).

The line × tester analysis and subsequent combining ability analyses were carried out following the procedure developed by Kempthorne (1957). The trait means obtained from the analysis was used for the line × tester analysis, as described by Singh and Chaudhary (1985). General and specific combining ability effects and variances were computed for grain yield and other traits using R Studio (Version 4.2.1) statistical package.

Combining ability

The general linear model for line × tester mating design used for each season is as follows.

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk},$$

Where Y_{ijk}= observed value of the hybrid between the i^{th} line and the j^{th} tester; μ = population mean;g_i = general combining ability (*gca*) of the i^{th} line; g_j = general combining ability (*gca*) effect of the j^{th} tester; s_{ij}= specific combining ability (*sca*) of the ij^{th} testcross; and e_{ijk} = residual effect.

For each trait, hybrid mean squares were partitioned into line, tester, and line × tester components. The *gca* effect of each of the two testers was obtained based on its performance in a hybrid combination with all 54 lines (females). Similarly, the *gca* effect of each line was determined based on the performance in combination with the two testers.

Heterotic grouping methods

SCA-PY method (Specific Combining Ability combined line Pedigree and hybrid Yield information)

In this method, a criterion given by Menkir et al. (2004) was used with some modifications. For each line, the *sca* effect and mean hybrid yield of the line with the two testers, *viz*., LM-13 (tester A) and LM-14 (tester B) were used to assign that line into a particular heterotic group. Besides, a test-cross hybrid yield of each line was compared with that of PMH 1 (the hybrid between two testers). Lines that showed a positive *sca* effect with tester A and showed >10% yield superiority over PMH 1, but had negative *sca* effects with tester B were placed in B group. On the contrary, lines exhibiting positive *sca* with tester B and showed >10% yield superiority over PMH 1, but had negative *sca* with tester A were assigned to A group. Lines having test cross yields (with both testers) greater than 10 % of the yield of PMH1 were placed in AB group.

Heterotic group's specific and general combining ability (HSGCA) method

This method considers both *gca* effects of parents and *sca* effects of hybrids to calculate HSGCA for each test cross-hybrid.

SCA = Cross mean (X_{ij}) – Line mean ($X_{i.}$) – Tester mean ($X_{.j}$) + Overall mean ($X_{..}$).

GCA = Line mean ($X_{i.}$) – Overall mean ($X_{..}$).

HSGCA = Cross mean X_{ij} – Tester mean ($X_{.j}$) = GCA + SCA.

Where X_{ij} = mean yield of the cross between i^{th} line and j^{th} tester, Xi. = mean yield of the i^{th} line$X_{.j}$ = mean yield of j^{th} tester

In HSGCA method, the heterotic grouping was done as per the procedure detailed by Fan et al. (2009). Lines showing negative HSGCA effect with tester A, but positive HSGCA with tester B were placed into A group and the lines showing negative HSGCA effect with tester B but positive HSGCA with tester A were placed in B group). Lines with positive HSGCA effects with both testers (tester A and tester B) were placed in AB group.

HGCAMT method (Heterotic Grouping by Combining Abilities of Multiple Traits)

The statistical model used by the HGCAMT method to assign the inbreds into the heterotic groups is as follows:

$$Y = \sum_{i=1}^{n} \frac{(Yi - \overline{Y\imath}}{S + e_{ij}}$$

Where,

Y is HGCAMT, which is the genetic value measuring relationship among genotypes based on the *gca*of multiple traits i to n;

Yi = individual *gca* effect of genotypes for trait i.

$\bar{Y}i$ = mean of *gca* effects across genotypes for trait i.

S = standard deviation of the *gca* effects of trait i.

e_{ij}= residual of the model associated with the combination ij.

Heterotic grouping by the HGCAMT was done by standardizing the *gca* effects (mean of zero and standard deviation of 1) of traits that had significant mean squares across test seasons to minimize the effects of different scales of the traits (Badu-Apraku, 2013). The standardized *gca* effects were subsequently subjected to Ward's method, and squared Euclidean distance was used for cluster analysis using SPSS Software version 16.0.

Breeding efficiency of heterotic grouping methods

To estimate the breeding efficiency, all the hybrids were first grouped into high and low-yield groups based on the per-plant grain yield of the hybrid between the two testers (PMH-1). Further, these groups were sub-classified as inter-group and intra-group crosses (both in high and low-yield groups according to the procedure of Annor et al. (2019) with some modifications. Only two group classifications (high and low yield group) instead of three (high, moderate, and low)were followed.

For computing the breeding efficiency of heterotic grouping, the number of crosses exceeding the grain yield ofPMH 1 at least by10% in inter-heterotic group crosses and intra-heterotic group crosses were recorded for both the tester combinations. The 10% superiority criterion was set to make sure that the hybrids identified as high-yielding were superior and not just numerically better. Such criteria are applied in practical plant breeding to promote hybrids to the next stage of testing. Also, the number of low-yielding crosses among intergroup and intragroup crosses was counted. Later, the breeding efficiency (BE) of heterotic grouping was computed using the following formula of Annor et al. (2019).

$$\text{Breeding efficiency (BE \%)} = \frac{\left[\frac{\text{NHYC inter HG}}{\text{TNC inter HG}} \times 100\right] + \left[\frac{\text{NLYC intra HG}}{\text{TNC intra HG}} \times 100\right]}{2}$$

Where,

NHYC interHG = Number of high yielding between group crosses,

TNCinterHG = Total number of between heterotic group crosses,

NLYC intra HG = number of low yielding within group crosses,

TNCintra HG = Total number of within heterotic group hybrids.

Results and Discussion

Lines and hybrids displayed significant variation and combining ability

Analysis of the variance of lattice design revealed that the mean sum of squares of different traits due to genotype was highly significant during both rainy and post-rainy seasons (Supplementary Table S3), indicating considerable variability among the genotypes studied. The pooled analysis was not done as the 'F' test (test for homogeneity of variances) was significant. This also implied that the genotypes showed differential responses to rainy and post-rainy seasons. This differential response might be due to the different weather conditions prevailing during the seasons (Supplementary Table 2). The ANOVA (not shown) revealed a highly significant variance due to genotypes, crosses, parents, lines, and parents *vs* crosses for days to 50% tasselling (anthesis), days to 50% silking, plant height, ear height, cob length, cob girth, number of kernel rows per cob, number of kernels per row, 100 seed weight, shelling percentage, and grain yield during both rainy and post rainy seasons. This indicated that crosses, parents, and parents *vs* crosses possessed significant variability for all the traits. In addition, partitioning of variance due to genotype suggested that the high variation observed among genotypes was due to contribution from parents as well as crosses studied justifying the classification of these 54 inbreds into heterotic groups. Hence, the information obtained on general and specific combining ability was utilized to categorize inbreds into different heterotic groups.

Heterotic grouping of new inbred lines of maize for rainy and post-rainy seasons

Maize is one of the very few crops where breeders have established well-defined heterotic groups such as Stiff Stalk and Non-Stiff Stalk in the North-American Corn Belt (Fu et al. 2014), Reid, Non-Reid, and Suwan 1 in southwest China (Fan et al. 2014), and Dent and Flint for the CIMMYT maize germplasm (Reifet al. 2003). In India, the dent ×flint heterotic pattern, being highly heterotic, is frequently exploited in heterosis breeding (Dhillon et al. 1993). However, new inbreds developed cannot be assigned directly to these heterotic groups. Many times, new inbred lines would be developed from bi-parental populations, commercial single crosses and double-crosses, etc., and inbreds developed from these may belong to a different group altogether (Fan et al. 2014; Lariepe et al. 2017). Hence, it is important to assign the newly developed lines to a known group or a different heterotic group based on systematic studies. In India, maize area is highest in the rainy season and is increasing rapidly in post rainy season and cultivation of hybrids predominates both seasons. These seasons witness different weather conditions and separate breeding programs catering to these seasons would be necessary. Therefore, heterotic grouping was done separately for rainy and post-rainy seasons to identify potential inbreds in the current study using three different methods.

SCA-PY method

In the rainy season, the SCA-PY method could classify a total of 49 inbreds into distinct heterotic groups (Table 1). Individuals classified under LM-13 were called group A and those who are classified under LM-14 were called group B. The inbred lines that don't fall under either of these groups were categorized either as AB group or unclassified depending upon the test cross performance. Accordingly, 19 inbreds were assigned to group A, 9 to group B, and 21 to the AB heterotic groups (Table 2).In the post-rainy season, 17, 13, and 13 inbreds were assigned to group A, group B and AB heterotic group, respectively and thus, assigned 43 of 54 inbreds to different heterotic groups (Table 2). The remaining inbreds, 5 in the rainy and 11 in post rainy season, were unclassified. This implied that unclassified inbreds belonged to a different heterotic group than those of the two testers used in the study (Menkir et al. 2004). Further, it can be noted that there was inconsistency between the grouping of inbreds between rainy and post-rainy seasons. The grouping of 29 inbreds was non-identical between the seasons. The inbred line, CDM-112 was placed in the A group during the rainy season, while the same line was placed in the B group during post rainy season because of its differential *sca* effect with the testers over seasons. This result implied that there is a differential response of hybrids to different seasons, which might be due to the different weather conditions in rainy and post-rainy seasons

Table 1. Heterotic grouping by SCA-PY method during rainy season and post rainy season 2019

Lines	Rainy season 2019				Heterotic Group	Post rainy season 2019				Heterotic Group
	Hybrid with T1		Hybrid with T2			Hybrid with T1		Hybrid with T2		
	Per se (tha^{-1})	*SCA*	*Per se (tha^{-1})*	*SCA*		*Per se (tha^{-1})*	*SCA*	*Per se (tha^{-1})*	*SCA*	
PDM-62	9.29	0.26	9.63	-0.26	UG	9.33	0.22	9.47	-0.22	AB
PDM-30-1	10.19	0.34	10.36	-0.34	AB	9.25	0.08	9.67	-0.08	AB
PDM-6507	6.22	-2.3**	11.77	2.3**	A	6.24	-1.76*	10.33	1.76*	A
PDM-6506	7.69	-1.58	11.69	1.58	A	6.11	-2.00*	10.69	2.00*	A
PDM-6552	12.79	0.82	12.01	-0.82	AB	10.69	0.1	11.07	-0.1	AB
PDM-89-2	12.92	1.51	10.75	-1.51	AB	11.02	-0.58	12.76	0.58	AB
PDM-200	10.45	1.04	9.22	-1.04	B	9.95	0.7	9.14	-0.7	B
PDM-210-1	8.53	-0.51	10.39	0.51	A	8.17	0.08	8.58	-0.08	UG
PDM-4241	12.61	0.26	12.94	-0.26	AB	8.21	1.02	6.76	-1.02	B
CDM-310	12.47	0.45	12.42	-0.45	AB	8.3	-0.77	10.42	0.77	A
CDM-311	9.84	0.29	10.11	-0.29	AB	10.5	-0.07	11.22	0.07	AB
CDM-328	11.77	0.99	10.64	-0.99	AB	10.21	0.22	10.36	-0.22	AB
CDM-312	9.88	-0.34	11.41	0.34	AB	10.31	0.08	10.73	-0.08	AB
CDM-330	11.22	0.58	10.92	-0.58	AB	9.12	0.83	8.05	-0.83	B
CDM-306	13.36	1.62*	10.97	-1.62*	AB	9.68	0.82	8.62	-0.82	B
CDM-332	9.25	0.26	9.58	-0.26	UG	8.55	0.97	7.2	-0.97	B
CDM-333	8.17	-0.58	10.17	0.58	A	7.2	-0.04	7.85	0.04	UG
CDM-334	10.92	0.44	10.9	-0.44	AB	8.48	0.37	8.32	-0.37	UG
DDM-312	9.41	0.04	10.18	-0.04	A	6.85	0.11	7.21	-0.11	UG
DIM-301	9.26	1.25	7.62	-1.25	B	7.49	1.04	6	-1.04	B
DIM-309	11.23	2.16**	7.77	-2.16**	B	7.19	-2.4**	12.67	2.4**	A
BGD-48(Y)	9.36	2.13**	5.94	-2.13**	B	8.4	1.13	6.73	-1.13	B
PDM259	7.48	-1.43	11.19	1.43	A	6.58	-1.23	9.61	1.23	A
CDM-309	7.43	-0.88	10.04	0.88	A	8.15	-0.37	9.47	0.37	A
DIM-303	11.19	1.41	9.22	-1.41	B	8.42	-0.43	9.86	0.43	A
DIM-334	8.37	-2.2**	13.71	2.2**	A	11.73	-0.21	12.72	0.21	AB
DIM-321	10.09	0.11	10.71	-0.11	AB	9.45	1.37	7.29	-1.37	B
BGD-106-1	9.76	0.46	9.7	-0.46	AB	9.37	1.868*	6.22	-1.868*	B
PDM-256-4	10.31	-0.42	11.99	0.42	AB	6.91	-0.285	8.06	0.285	A
PDM-4611	10.76	0.5	10.61	-0.5	AB	8.88	0.285	8.89	-0.285	UG
PDM-4591	8.97	-0.3	10.41	0.3	A	8.33	0.293	8.33	-0.293	UG
PDM-4131R-2	9.77	-0.41	11.44	0.41	AB	7.58	-0.872	9.9	0.872	A
PDM-4251R	8	0.22	8.42	-0.22	UG	11.01	2.38*	6.82	-2.38*	B
BLSB-5	7.67	0.12	8.28	-0.12	UG	7.9	0.05	8.38	-0.05	UG

Contd….

PDM-36389-1	10.2	-0.77	12.59	0.77	AB	7.77	0.143	8.06	-0.143	UG
BML-7	14.48	2.85**	9.62	-2.85**	B	11.11	0.91	9.87	-0.91	AB
BML-6	11.26	1.95*	8.22	-1.95*	B	7.34	0.333	7.26	-0.333	UG
PDM-91-2	7.43	0.1	8.07	-0.1	UG	8.71	-0.56	10.41	0.56	A
PDM-253-2	5.9	-1.86*	10.46	1.86*	A	6.71	-0.78	8.85	0.78	A
PDM-6505	6.29	-1.62*	10.39	1.62*	A	5.86	-1.152	8.74	1.152	A
CDM-112	7.89	-0.67	10.07	0.67	A	7.55	0.79	6.55	-0.79	B
CDM-113	9.92	0.5	9.78	-0.5	AB	13.45	2.78**	8.46	-2.78**	B
CDM-210	11.31	0.89	10.38	-0.89	AB	10.19	0.463	9.85	-0.463	AB
CDM-207	8.51	-0.75	10.85	0.75	A	6.46	-1.82*	10.68	1.82*	A
CDM-119	7.72	-2.04*	12.65	2.04*	A	7.21	-1.995*	11.78	1.995*	A
DDM-207	7.02	-2.53**	12.92	2.53**	A	6.4	0.165	6.65	-0.165	UG
DIM-204	10.28	0.8	9.54	-0.8	B	6.81	0.008	7.38	-0.008	UG
DIM-111	5.87	-1.51	9.73	1.51	A	6.51	-1.722*	10.53	1.722*	A
PDM-258-1	11.95	0.41	11.98	-0.41	AB	9.03	0.295	9.02	-0.295	AB
PDM-6571	7.97	-0.75	10.32	0.75	A	6.68	-1.69*	10.64	1.69*	A
PDM-194-2	8.58	0.38	8.66	-0.38	UG	10.66	0.24	10.76	-0.24	AB
PDM-4131R-1	9.81	-1.51	13.68	1.51	AB	10.02	-0.582	11.76	0.582	AB
HKI-163-1	7.59	-1.38	11.21	1.38	A	7.95	-0.28	9.09	0.28	A
PDM-138	9.59	1.29	7.86	-1.29	B	9.36	1.49	6.96	-1.49	B
PMH 1 (LM-13 × LM-14)	9.23					8.76				

UG-Unknown group

Table 2. Heterotic grouping of 54 inbred lines by three methods in rainy season and post rainy season-2019

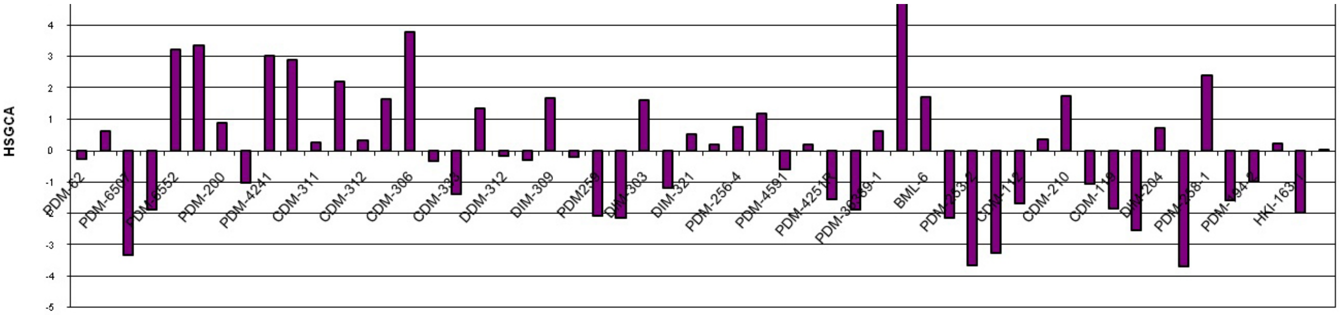
Rainy season 2019.				
Grouping method	HG	Inbred line	Sub total	Total
SCA-PY	A	PDM-62, PDM-6507, PDM-6506, PDM-210-1, CDM-333, DDM-312, PDM259, CDM-309, DIM-334, PDM-4591, PDM-253-2, PDM-6505, CDM-112, CDM-207, CDM-119, DDM-207, DIM-111, PDM-6571, HKI-163-1	19	
	B	PDM-200, DIM-301, DIM-309, BGD-48(Y), DIM-303, BML-7, BML-6, DIM-204, PDM-138	9	49
	AB	PDM-30-1, PDM-6552, PDM-89-2, PDM-4241, CDM-310, CDM-311, CDM-328, CDM-312, CDM-330, CDM-306, CDM-334, DIM-321, BGD-106-1, PDM-256-4, PDM-4611, PDM-4131R2, PDM-36389, CDM-113, CDM-210, PDM-258-1, PDM-4131R1	21	
HSGCA	A	PDM-6507, PDM-6506, PDM-210-1, CDM-333, PDM259, CDM-309, DIM-334, PDM-4591, PDM-253-2, PDM-6505, CDM-112, CDM-207, CDM-119, DDM-207, DIM-111, PDM-6571, HKI-163-1	17	
	B	PDM-62, PDM-30-1, PDM-200, CDM-311, CDM-332, DDM-312, DIM-301, DIM-309, BGD-48(Y), DIM-303, BGD-106-1, PDM-4251R, BLSB-5, BML-7, BML-6, PDM-91-2, CDM-113, CDM-210, DIM-204, PDM-194-2, PDM-138	21	54
	AB	PDM-6552, PDM-89-2, PDM-4241, CDM-310, CDM-328, CDM-312, CDM-330, CDM-306, CDM-334, DIM-321, PDM-256-4, PDM-4611, PDM-4131R2, PDM-36389, PDM-258-1, PDM-4131R1	16	
HGCAMT	A	PDM-6507, PDM-6505, DDM-207, PDM-6571, HKI-163-1, PDM-194-2, PDM-4251R, PDM-6506, CDM-333, PDM-259	10	
	B	DIM-301, CDM-309, BGD-48(Y), PDM-138, PDM-91-2, CDM-112, CDM-113, CDM-207, PDM-253-2, DIM-111	10	31
	AB	PDM-6552, PDM-4241, CDM-310, DIM-334, CDM-306, PDM-258-1, PDM-36389, BML-7, PDM-4131R2, PDM-4131R1, PDM-256-4	11	

Post rainy season-2019				
SCA-PY	A	PDM-6507, PDM-6506, CDM-310, DIM-309, PDM-259, CDM-309, DIM-303, PDM-4131R-2, PDM-91-2, PDM-253-2, PDM-6505, CDM-207, CDM-119, DIM-111, PDM-6571, HKI-163-1, PDM-256-4	17	
	B	PDM-200, PDM-4241, CDM-330, CDM-306, DIM-301, BGD-48(Y), DIM-321, BGD-106-1, PDM-4251R, CDM-113, PDM-138, CDM-112, CDM-332	13	43
	AB	PDM-62, PDM-30-1, PDM-6552, PDM-89-2, CDM-311, CDM-328, CDM-312, DIM-334, BML-7, CDM-210, PDM-258-1, PDM-194-2, PDM-4131R-1	13	
HSGCA	A	PDM-6507, PDM-6506, CDM-310, CDM-333, DIM-309, PDM259, CDM-309, DIM-303, PDM-256-4, PDM-4131R-2, BLSB-5, PDM-253-2, PDM-6505, CDM-207, CDM-119, DIM-111, PDM-6571, HKI-163-1	18	
	B	PDM-210-1, PDM-4241, CDM-330, CDM-306, CDM-332, CDM-334, DDM-312, DIM-301, BGD-48(Y), DIM-321, BGD-106-1, PDM-4611, PDM-4591, PDM-4251R, PDM-36389-1, BML-6, CDM-112, CDM-113, DDM-207, DIM-204, PDM-258-1, PDM-138	22	54
	AB	PDM-62, PDM-30-1, PDM-6552, PDM-89-2, PDM-200, CDM-311, CDM-328, CDM-312, DIM-334, BML-7, PDM-91-2, CDM-210, PDM-194-2, PDM-4131R-1	14	
HGCAMT	A	PDM-6507, PDM-6506, CDM-330, DIM-321, PDM-259, PDM-210-1, PDM-4591, BLSB-5, BGD-106-1, PDM-253-2, CDM-207, DIM-111, PDM-6571, HKI-163-1, CDM-334, CDM-332, PDM-36389-1	17	
	B	DIM-301, BGD-48(Y), PDM-256-4, CDM-333, DDM-312, BML-6, CDM-112, DDM-207, DIM-204, PDM-138, PDM-6505	11	40
	AB	DIM-309, PDM-6552, PDM-89-2, CDM-311, CDM-328, CDM-312, DIM-334, BML-7, CDM-210, PDM-194-2, PDM-4131R-1, CDM-113	12	

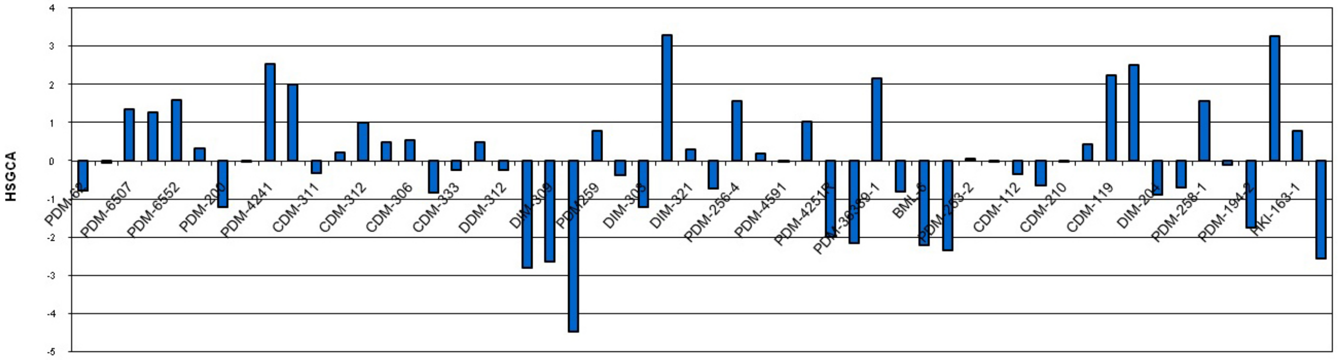
HG-Heterotic group

Heterotic group's specific and general combining ability (HSGCA) method

The HSGCA effects include both *sca* and *gca* effects and hence the HSGCA method is expected to be biometrically superior to other methods (Fan et al. 2009). The computed HSGCA effects for grain yield of the 54 maize inbred lines and the two testers are presented in Figs. 1 and 2, respectively. The HSGCA method assigned 17 inbreds to the A group, 21 to B group, and 16 to AB group (Table 2) during the rainy season. During the post-rainy season, 18 inbreds were

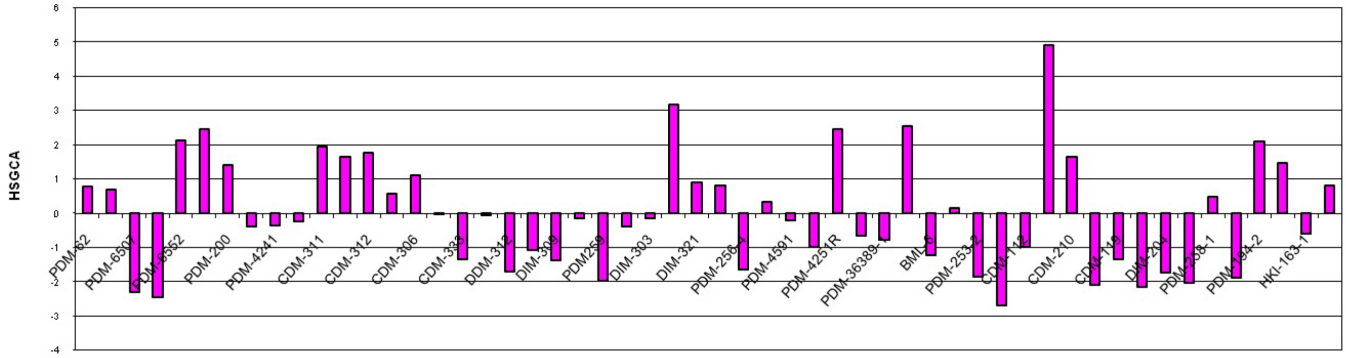


HSGCA with tester 1 (LM-13)

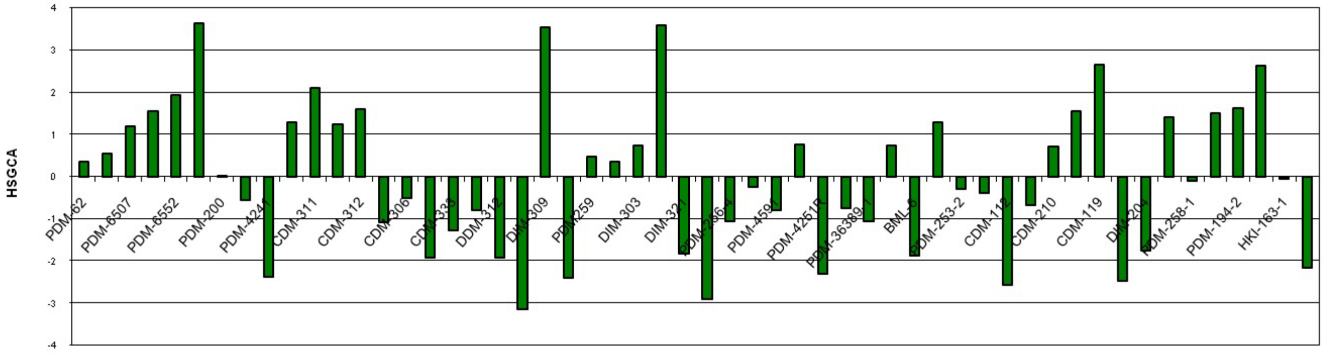


HSGCA with tester 1 (LM-14)

Fig. 1. HSGCA method of heterotic grouping during rainy season 2019



HSGCA with tester 1



HSGCA with tester 1

Fig. 2. HSGCA method of heterotic grouping during post rainy season 2019

Table 3. Similarity among grouping methods between rainy and post rainy seasons (2019)

Group	Inbred lines assigned to same heterotic group in both the seasons by all three methods	
	Inbred	Number
A	PDM-6507, PDM-6506, PDM259, PDM-6571, HKI-163-1	5
B	DIM-301, BGD-48(Y), PDM-138	3
AB	PDM-6552, PDM-4131R-1	2

categorized into the A group (LM-13), 22 into the B group (LM-14), and 14 into the AB heterotic group (Table 2). It can be noted that the HSGCA method classified all the inbreds studied during both rainy and post-rainy seasons. Here also, 29 inbreds were grouped differentially between rainy and post-rainy seasons. For instance, the inbred lines CDM-112 and PDM-4591 were differentially grouped over seasons by this method. This might be due to the G × E interaction of these inbreds with different environmental conditions existing between the seasons.

Heterotic grouping based on GCA of multiple traits (HGCAMT)

Inbred lines grouped under different heterotic groups by different methods during rainy and post-rainy seasons are presented in Table 2. Classification by the three methods showed similar but not identical trends within each season. For example, even though 39 inbred lines were similarly grouped by SCA-PY and HSGCA methods, two inbred lines, PDM-62 and DDM-312 were placed in A group by SCA-PY method but these lines were placed in B group by HSGCA method. Besides, Inbreds CDM-112, DIM-111, CDM-309 and PDM-253-2 were placed into the A group by HSGCA method, while HGCAMT placed these inbreds into B group during the rainy season. Such differential grouping could be due to the differential performance of these inbreds and associated hybrids during rainy and post-rainy seasons due to G × E interactions. In post rainy season, inbreds, PDM-6505 and CDM-333 were differently grouped into A group and B group by HSGCA and HGCAMT methods, respectively. This differential grouping by different methods might be due to the differences in the procedures followed in grouping methods. This is expected because the HGCAMT method considers multiple traits and some genotypes are expected to show G × E interaction between seasons.

Similarity among grouping methods

Similarity among grouping methods within rainy and post-rainy seasons are presented in Table 3. During the rainy season, eight inbreds were placed into group A by all three methods, while additionally, nine inbreds were placed into the same group by SCA-PY and HSGCA methods *i.e.*, a total

Table 4. Breeding efficiency of different grouping methods duringrainy and post rainy season 2019

Season	Yield group (g)	Cross type	Number of crosses			Breeding efficiency of		
			HSGCA	SCA-PY	HGCAMT			
Rainy 2019	> 145	Inter group	50	53	25	Method	Rainy	Post rainy
		Intra group	3	0	3	HSGCA	78.9%	80.6%
	<145	Inter group	20	17	16	SCA-PY	81.1%	86.8%
		Intra group	35	28	18	HGCAMT	71.1%	68.7%
Post rainy 2019	> 127	Inter group	48	48	28	Superiority of SCA-PY method		
		Intra group	2	1	4	over the method	Rainy	Post rainy
	<127	Inter group	20	8	24	HSGCA	2.2%	6.2%
		Intra group	38	29	24	HGCAMT	10.0%	18.1%

For estimating breeding efficiency number of high yielding crosses (> 145g in rainy and > 127 g per plant in post rainy) and the number of low yielding crosses werecounted and were classified as inter group and intra group crosses. The breeding efficiency was estimated using the formula presented in material and methods section

of 17 lines were commonly categorized into A group by these two methods. During the post-rainy season, eight inbreds were placed in the same heterotic group (A group) by all three methods, while an additional eight inbreds were placed into the same group by two (SCA-PY and HSGCA) of the three methods. Thus, a total of 16 lines were commonly categorized into A group by these two methods in post rainy season (Supplimentary Figs. 1 and 2).

During the rainy season, three inbreds were placed into group B by all three methods. While six inbreds were similarly placed into the same group by SCA-PY and HSGCA methods *i.e.*, a total of nine inbreds were commonly categorized into the B group by these two methods. During post rainy season, four inbreds were placed in the same heterotic group B by all three methods. While eight inbreds were placed into the same group by two (SCA-PY and HSGCA) of the three methods *i.e.*, a total of 12 lines were commonly categorized into B group by these two methods.

During the rainy season, nine lines were placed into the group AB by the three methods, while seven lines were similarly placed into the same group by SCA-PY and HSGCA methods, *i.e.*, a total of 13 lines were commonly categorized into the AB group by these two methods. During post rainy season, 10 inbreds were placed in the same heterotic group AB by all three methods. While one more inbred, PDM-258-1 placed into the same group by SCA-PY and HSGCA methods. CDM-113 is commonly categorized into AB group by HSGCA and HGCAMT method. This indicated the differential grouping of some inbreds by different methods, which suggested there is a scope for improving these methods (Laouli 2014). However, reports by Badu et al. (2015) showed close correspondence with the classification by the HGCAMT and HSGCA methods under striga infestation, low N and optimum conditions while studying QPM inbreds. In the present study of tropical germplasm, the SCA-PY and the HSGCA methods in the rainy season and post-rainy season showed close correspondence as 39 inbreds were grouped similarly, indicating that all these methods were effective in classifying the inbreds into heterotic groups, while other inbreds were grouped differentially.

Heterotic grouping methods revealed season-specific grouping of most inbreds between rainy and post-rainy seasons

In India, in addition to traditional rainy season cultivation, maize area is fast expanding in post rainy season and is catching up in the spring season, especially in certain regions of northern India (Kumar et al. 2013; Kumar et al. 2017). The maize inbreds and their combinations are expected to respond differentially to distinct seasons due to different growing conditions. Therefore, such responses of inbreds and their combinations prompt us to consider separate heterotic grouping of inbreds for different seasons to increase hybrid breeding efficiency. In the current study, we have attempted to group the selected 54 lines separately for rainy and post-rainy seasons. In fact, between rainy and post-rainy seasons, similar grouping was observed for only 10 inbred lines across three grouping methods (Table 3).This might be due to the similar response of these inbreds to rainy season and post-rainy season environments due to the lower G × E component. Fifteen out of 54 lines were assigned to an altogether different group or not assigned to any group, in few cases, in rainy and post-rainy seasons by three grouping methods used. Furthermore, the remaining 29 lines were differently grouped in any one or two of the three grouping methods. This might be due to the different growing conditionsto temperature, relative humidity, and rainfall under rainy and post-rainy seasons. Kang (1996) and Akinwale et al. (2014) reported that the environment plays a predominant role in the phenotypic expression of agronomic traits and, hence, the differential response of genotypes due to G × E interaction under different environmental conditions. Laouali (2014) found differential grouping of inbreds under different environmental conditions such as drought and optimum conditions in tropical maize. In our study, the deferential grouping of inbreds for rainy and post-rainy seasons was observed. It implied some genotypes might be better adapted to a particular season than others. This suggested the need for separate heterotic grouping exercises for different seasons to aid in the selection of complementary parental lines and to increase hybrid breeding efficiency in maize.

Breeding efficiency of heterotic grouping methods

The primary purpose of heterotic grouping is to constitute genetically diverse groups and to exploit the high heterosis expected from the intergroup crosses. The clue for this is derived from the heterosis equation $H_{F1}= \Sigma dy^2$, which suggests that heterosis is the function of dominance effect (*d)* and allelic frequency difference (*y:*a measure of diverse nature) between the parents (Falconer and Mackay 1996). Accordingly, several heterotic grouping methods have been proposed and used, but none of them seems to be perfect due to the possibility of unlimited genetic combinations between any two inbred lines (Akinwale et al. 2014). This might also be due to the polygenic nature of yield, G × E, and the existence of considerable residual heterozygosity (McMullen et al. 2009) leading to a change in the quantum of dominance effects. Hence, it is quite possible to obtain a heterotic hybrid even from intra-heterotic group crosses (Fan et al. 2009; Oyetunde et al. 2020).

The assignment of inbreds into different heterotic groups depends on the method followed as each method uses a different estimate and/or follows a different procedure for grouping. The estimates used for grouping would be influenced by lines, testers, environments, and their interactions. Thus, no heterotic grouping method is perfect. Consequently, some intergroup crosses might be low yielding while, a few of the intra-group crosses could be highly heterotic. Hence, the best or most efficient method is one that produces a maximum number of intergroup heterotic crosses coupled with a minimum number of intra-group heterotic crosses (Annor et al. 2019). Thus, the breeding efficiency of the heterotic grouping method can be defined as the percentage of superior high-yielding intergroup hybrids out of the total number of crosses (Fan et al. 2009). Besides, the number of low-yielding intragroup crosses is also to be considered (Annor et al. 2019).

In the present study considering 108 crosses, the hybrids with >10% superiority of yield over PMH 1 (the hybrid between two testers) were assigned to a high grain yield group/category and the rest of the hybrids were assigned to a low yield group/category. Subsequently, these were categorized as inter-heterotic group and intra-heterotic group crosses in both seasons. The estimation of breeding efficiency was done separately for rainy and post-rainy seasons. In the rainy season, the hybrids that had a mean grain yield >145 g per plant (>10% of PMH 1) were put in the high yield group, while those with <145 g per plant were assigned to the low grain yield group. Similarly, in post rainy season, crosses with a mean grain yield >127 g per plant (>10% of PMH 1) were assigned to the high grain yield group and the remaining hybrids were assigned to the low grain yield group. Subsequently, in both high yield group and low-yield groups, crosses were categorized into intergroup and intra-group by referring to the heterotic groups assigned to the parents by respective grouping methods for each season separately. Further, from rainy season evaluation using the HSGCA method identified 50, SCA-PY 53 and HGCAMT 25 high-yielding (>145 g per plant) inter-heterotic group crosses (Table 4). However, low-yielding hybrids were also found among the inter-group crosses. The number of low-yielding inter-group crosses was 20, 17 and 16 for HSGCA, SCA-PY and HGCAMT methods, respectively.

In post-rainy season 48, 48 and 28 high-yielding intergroup hybrids were identified by HSGCA, SCA-PY, and HGCAMT methods, while for low-yield intergroup hybrids, these numbers were 20, 8, and 24, respectively. However, all three methods identified a few high-yielding intragroup crosses and several low-yielding intergroup crosses in both seasons. Similar results were also reported in previous studies and the high yielding intra group crosses and low-yielding intergroup crosses are not expected or expected in low frequency if the grouping method is highly efficient (Fan et al. 2009; Akinwale et al. 2014; Oyetunde et al. 2020). Hence, the breeding efficiency of different grouping methods was estimated to determine the best method available at present.

In the rainy season, the estimated breeding efficiencies of HSGCA, SCA-PY and HGCAMT were 78.9, 81.1 and 71.1%, respectively (Table 4). In post rainy season, efficiencies got improved for HSGCA and SCA-PY (80.6 and 86.8%, respectively), while it were reduced for HGCAMT. Furthermore, the SCA-PY method was 2.2% more efficient than HSGCA and 10 % more efficient than the HGCAMT method during the rainy season. In post rainy season, the efficiency of the SCA-PY method was higher at 6.2 and 18.1% over HSGCA and HGCAMT methods, respectively in classifying inbred lines into heterotic groups. This implied that the SCA-PY method was superior in both seasons and the HSGCA method was the next best method. Laouali (2014) reported that the SCA method was more efficient over the HSGCA method, which offered no additional advantage. Oyetunde et al. (2020) reported marginal higher efficiency of SCA-PY over the HSGCA method.

On the contrary, several researchers (Fan et al. 2009; Badu-Apraku et al. 2013; Akinwale et al. 2014; Kumar et al. 2022) earlier reported that the HSGCA method was more efficient. Such contrasting results are expected as the inbreds used and environmental conditions for the evaluations were different. The efficiency of HGCAMT might be lower when just a few traits with significant and positive *gca* effects are available for grouping and higher efficiency of the HGCAMT is expected with a greater number of positive *gca* traits (Badu-Aprakuet al. 2013). The results of the present study reiterated that none of the heterotic grouping methods are perfect as the three methods showed inconsistency in grouping and suggested to keep refining the existing and devising new methods to have a better heterotic grouping and to increase hybrid breeding efficiency in maize.

The heterotic groups are connected with gene interactions but there is a lack of approaches to distinguish the gene interactions for analysis of genetic diversity responsible for high heterosis, and hence, diversity analysis itself is not enough for heterotic grouping. Therefore, different methods of heterotic grouping have been devised and used. In the current study, the SCA-PY method had higher breeding efficiency in both rainy and post-rainy seasons. The hybrid performance includes *gca* of parents and *sca* of combination. HSGCA method is expectedly the best as it exploits both *gca* and *sca* in classifying inbreds into heterotic groups. However, in our study, HSGCA was the next best method to the SCA-PY method. Hence, at present, either SCA-PY or HSGCA can be used for heterotic grouping. These methods may further be refined by considering additional parameters such as the critical difference between hybrid means, involving more than two testers, *etc*. In addition, the study suggested having season-specific heterotic grouping to get higher efficiency in hybrid maize breeding programs aiming for different seasons, such as rainy and post-rainy. The present heterotic grouping suggested to attempt inter group crosses such as CDM119 × PDM4241, CDM207 × PDM4251R, PDM6571 × DIM301, and PDM6507 × CDM113 and subject them to multi-location trials to evaluate their superiority, stability, and suitability for commercialization. The possible heterotic pattern is A × B, which should be further tested and utilized for developing high-yielding heterotic hybrids. Besides, within a group inbred improvement programme may be planned through recurrent selection procedures.

Supplimentary material

Supplimentary Tables S1 to S3 and Supplimentary Figs. 1 and 2 are provided which can be accessed at www.isgpb.org.

Authors' contribution

Conceptualization of research (JSB); Designing of the experiments (JSB, GM, SN, GKN); Contribution of experimental materials (JSB); Execution of field/lab experiments and data collection (CSKK); Analysis of data and interpretation (GM, SN, GKN); Preparation of the manuscript (CSKK, JSB, GM).

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Supplementary Table S1. Details of the lines and testers used in the study

Source	Inbred line (newly developed)	Inbred development
ICAR IARI RRC Dharwad (8)	(1) BLSB-5,(2) DIM-111,(3) DIM-204,(4) DIM-301, (5) DIM-303,(6) DIM-309,(7) DIM-321,(8) DIM-334,	Line breeding with heterotic hybrids
ICAR IARI RRC Dharwad (24)	(9) PDM-138,(10) PDM-194-2, (11) PDM-200, (12) PDM-210-1,(13) PDM-253-2, (14) PDM-256-4, (15) PDM-258-1,(16) PDM259,(17) PDM-30-1, (18) PDM-36389, (19) PDM-4131R1, (20) PDM-4131R2, (21) PDM-4241,(22) PDM-4251R,(23) PDM-4591, (24) PDM-4611, (25) PDM-62,(26) PDM-6505, (27) PDM-6506,(28) PDM-6507,(29) PDM-6552, (30) PDM-6571,(31) PDM-89-2,(32) PDM-91-2,	Recurrent selection and line breeding
UAS Raichur (2)	(33) BGD-106-1,(34) BGD-48 (Y),	Repeated selfing of introduced germplasm
ICAR-IIMR (5)	(35) BML-6,(36) BML-7,(37) DDM-207, (38) DDM-312,(39) HKI-163-1,	
CIMMYT (15)	(40) CDM-112,(41) CDM-113,(42) CDM-119, (43) CDM-207,(44) CDM-210,(45) CDM-306, (46), CDM-309,(47) CDM-310,(48) CDM-311, (49) CDM-312,(50) CDM-328,(51) CDM-330, (52) CDM-332,(53) CDM-333,(54) CDM-334,	
	Tester	
MS heterotic pool	LM-13 (Group A)	Line breeding
Tuxpeno pool	LM-14 (Group B)	Line breeding

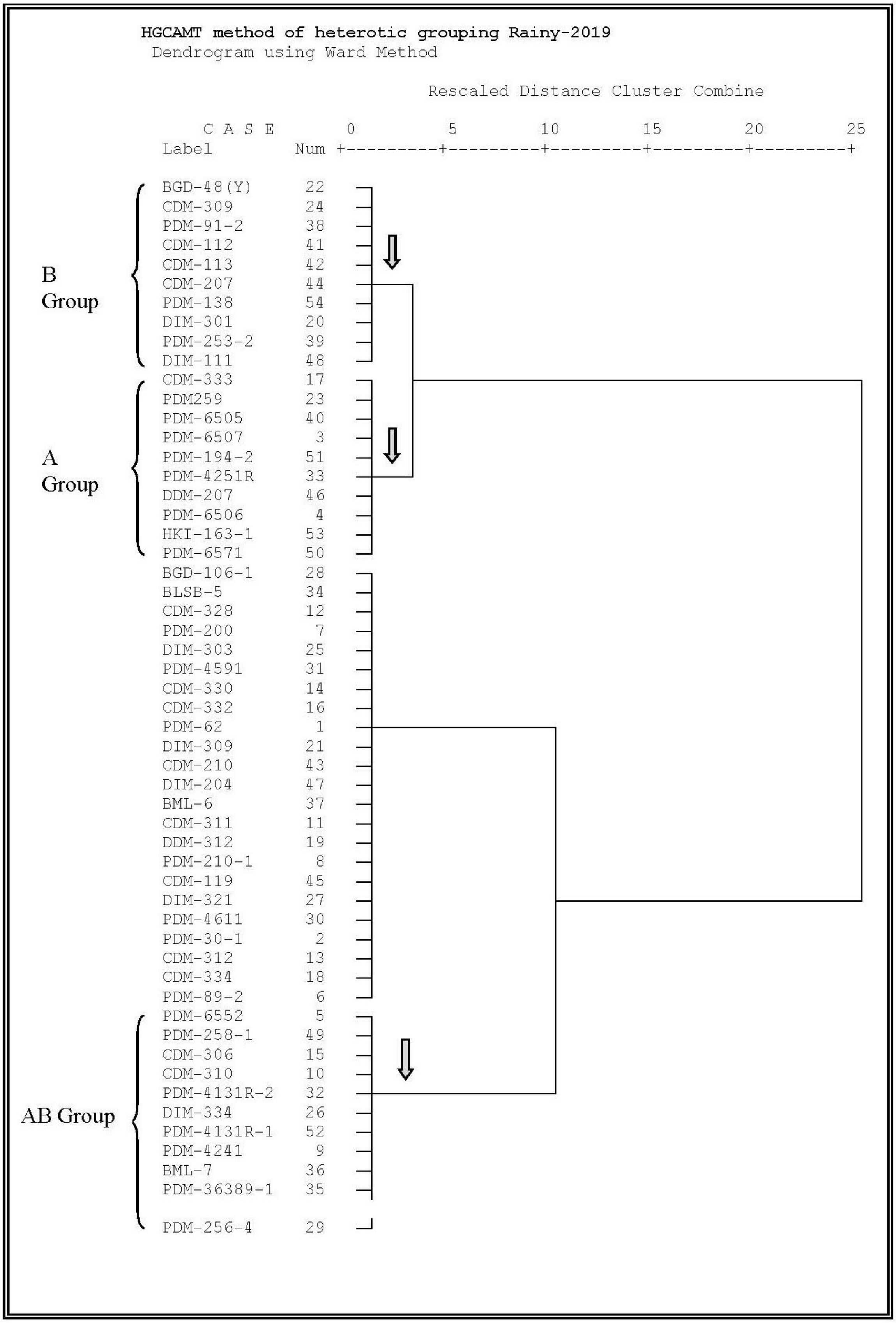
Supplementary Table S2. The meteorological data during the rainy andpost-rainy seasons of 2019 at ICAR-IARI RRC, Dharwad, Karnataka, India

Rainy season-2019 cropping period					
Months	Temperature		RH%	Rainfall (mm)	No. of rainy days
	Maximum°C	Minimum°C			
June 2019	31.5	21.5	76	104.7	6
July 2019	27.1	20.3	87	230.8	17
August 2019	26.4	20.4	88	451.2	17
September 2019	27.3	20.9	87	106.8	10
October 2019	28.8	20.3	86	323.2	12
Total				1216.7	62
Post rainy season cropping period 2019-20					
Months	Temperature		RH%	Rainfall (mm)	No. of rainy days
	Maximum°C	Minimum°C			
November 2019	27.7	18.0	72	21.0	2
December 2019	28.6	16.5	69	7.8	1
January 2020	29.8	15.5	60	0.0	0
February 2020	31.8	16.8	49	0.0	0
March 2020	34.0	19.4	49	13.6	2
April 2020	35.8	21.2	58	34.6	4
Total				1216.7	62

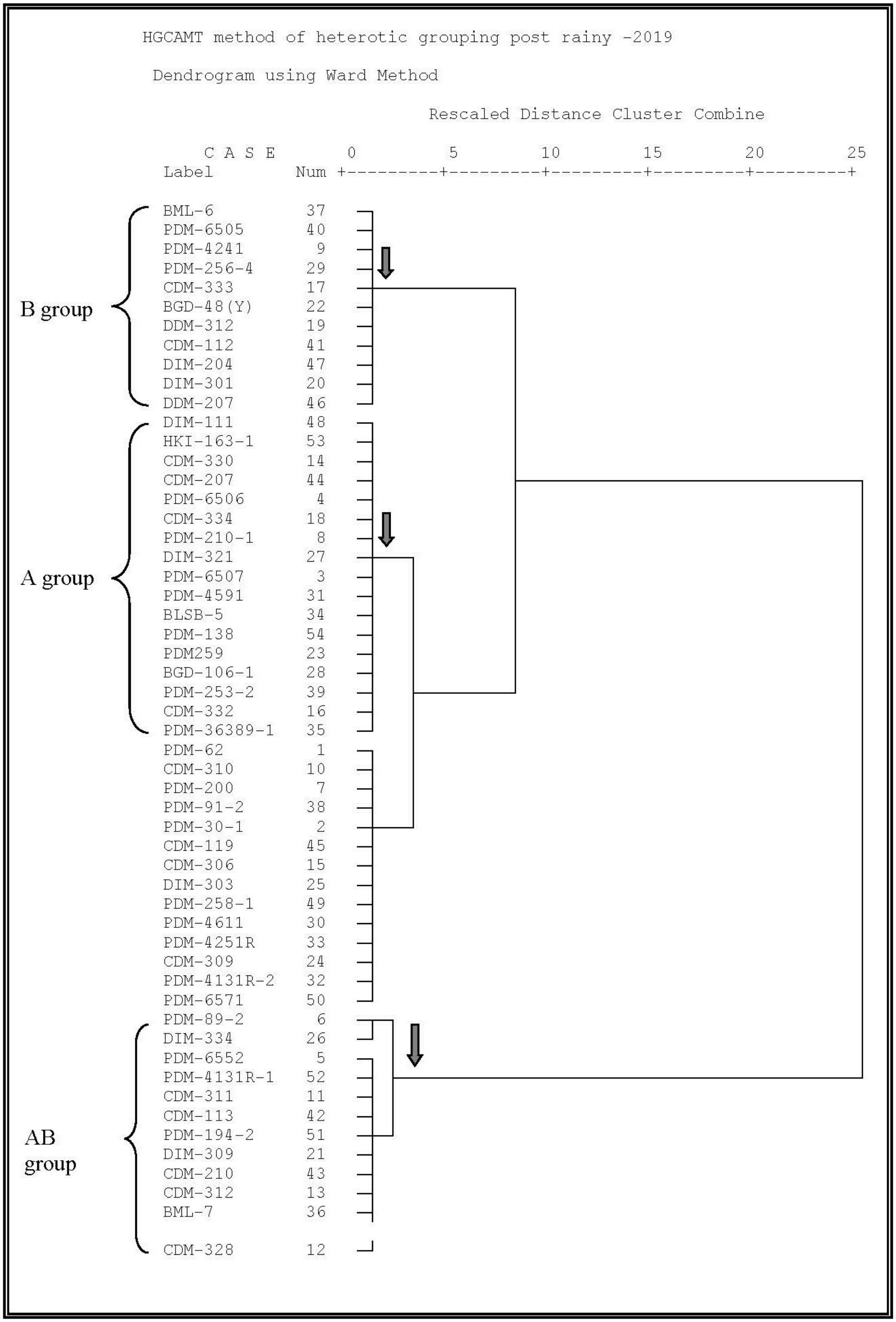
Supplementary Table S3. Lattice design analysis of variance for various traits during rainy and post rainy season-2019

Source of variation	d.f.	Mean sum of squares										
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Rainy Season 2019												
Replications	1	8.92	9.50	242.11	325.28	1.31	0.15	0.38	19.94	31.39	11.41	5.39
Blocks	24	2.97	2.93	228.57*	97.86	1.48	0.03	0.50	13.18	13.05	3.54	2.40
Genotypes	168	19.53**	23.56**	3421.92**	1471.21**	13.39**	0.62**	3.20**	93.61**	61.78**	31.10**	21.65**
Error (Intrablock)	144	2.67	2.95	90.88	62.46	1.09	0.03	0.39	8.66	16.07	5.83	1.71
LSD at 1%	1	4.26	4.47	25.93	20.59	2.72	0.46	1.62	7.67	10.44	6.29	3.40
LSD at 5%	5	3.23	3.39	19.64	15.60	2.06	0.35	1.23	5.81	7.91	4.77	2.58
Post rainy season-2019												
Replications	1	18.0	14.5	525.97	333.07	0.12	0.43	0.89	22.62	70.17	6.67	1.83
Blocks	24	16.80	14.66	1790.83*	536.55*	11.40	0.34	0.90	48.15	42.92*	10.17	3.93
Genotypes	168	45.88**	50.82**	3343.52**	1712.88**	10.75**	0.387**	3.36**	74.80**	30.95**	43.12**	15.96**
Error (Intra-block)	144	7.47	6.07	145.81	86.30	1.27	0.03	0.75	8.70	8.18	7.23	0.84
LSD at 1%	1	7.41	6.70	33.53	25.66	3.13	0.49	2.26	8.14	7.89	7.01	2.52
LSD at 5%	5	5.61	5.07	25.38	19.43	2.37	0.37	1.71	6.16	5.97	5.31	1.91

X_1 = Days to 50 % tasseling
X_4 = Ear height (cm)
X_7 = Number of kernel rows per cob
X_{10} = Shelling %
X_2 = Days to 50 % silking
X_5 = Cob length (cm)
X_8 = Number of kernels per row
X_{11} = Grain yield (tha^{-1})
X_3 = Plant height (cm)
X_6 = Cob girth (cm)
X_9 = 100-seed weight (g)
df = degrees of freedom
*,**: Significant at 5% and 1% level of probability



Supplimentary Fig. 1. Dendrogram of HGCAMT values (Wards method) of cluster analysis in rainy season 2019



Supplimentary Fig. 2. Dendrogram of HGCAMT values (Ward's cluster analysis) during post rainy season 2019