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Comparison of the effectiveness of heterotic grouping methods in classifying intermediate maturing maize (*Zea mays* L.) inbred lines under stressful and non-stressful environments

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The effectiveness of a hybrid breeding program depends on the heterotic patterns that can be used to utilize grain-yield heterosis. This study was carried out to (a) categorize inbred lines into heterotic groups using three different methods and (b) determine the most effective heterotic grouping method for categorizing set of inbred lines. A total of 96 hybrids generated from thirty-two set of inbred lines crossed to three elite testers (87036, 1368 and 9071) using the line × tester design were evaluated together with three checks under low N (30 kg ha^{-1}) and high N (90 kg ha^{-1} N) environments at three locations in Ghana. Classification of inbred lines were based on three different methods: Heterotic group's specific and general combining ability (HSGCA), specific combining ability (SCA), and general combining ability effects of multiple traits (HGCAMT). The SCA approach, which had the highest breeding efficiency across all test environments, was ultimately determined to be the most effective way for classification. The inbred in each heterotic group may be recombined to form populations which could be improved through recurrent selection. The various heterotic groups can be useful in designing hybridization strategies to create maize hybrids that are both high-yielding and tolerant to low levels of nitrogen in stressful environments.

Key words: Inbreds, nitrogen, heterotic, grouping, efficiencies, hybrids, maize.

INTRODUCTION

Nitrogen-efficient maize genotypes can play a role in addressing food security challenges in West and Central Africa (WCA) by reducing the cost of soil fertility amendments while maintaining acceptable yields and raising agricultural incomes (Ribeiro et al. 2020). Food and feed supplies would undoubtedly be greatly reduced

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if hybrids were not available to the producer (Stuber 1994). According to Singh (2005), most of the commercial hybrid varieties are F1's from two inbreds. An inbred is defined as an essentially homozygous line obtained through continuous inbreeding of cross-pollinated species (Singh 2005).

The development of hybrid maize depends on the breeding program's ability to quickly discover lines that combine well in hybrid combinations and fast identify suitable heterotic combinations to maximize the vigor of the hybrid (Kim and Ajala, 1996; Fan et al., 2009). A heterotic group is a collection of closely related genotypes that have been divided into genetically different groups and clusters. Inbred lines from different heterotic groups should, in theory, cross to produce hybrids that are more robust and productive (Lee, 1995).

The highest expression level of heterosis requires parents with different genetic backgrounds, so understanding the heterotic patterns of the germplasm is essential for hybrid development programs. Studies have shown that the environment in which inbred lines and populations are evaluated affects heterotic patterns, but superior combining pairs of inbreds can be found that produce good hybrids across environments (Menkir et al., 2003; Badu-Apraku et al., 2006). It is necessary to identify the heterotic groupings of inbred lines in low and high N conditions. This will allow the selection of lines that combine well under both stressed and non-stressed environments.

The classification of a maize line to a certain heterotic group can considerably be impacted by the techniques used by researchers. Inbred lines must always be grouped into heterotic groups in maize breeding programs, despite the fact that several researchers in SSA have done so based on various environmental factors (Agbaje et al., 2008; Badu-Apraku et al., 2015; Badu-Apraku et al., 2013; Menkir et al., 2003; Annor et al., 2020). The most common techniques are (i) relying solely on the specific combining ability (SCA) effects of grain yield, (ii) incorporating both the specific and general combining ability effects, and (iii) integrating numerous features with large GCA effects (HGCAMT).

However, heterotic groupings that depend on combining ability are significantly influenced by the environment, leading to irregularities in the grouping of breeding lines (Annor et al., 2020). In order to resolve these discrepancies, genetic distances based on molecular markers (GD) are frequently used in the division of maize germplasm into distinct groups (Balestre et al., 2008; Reif et al., 2003). The heterotic grouping of maize germplasms using marker-based genetic distances, however, has produced contradictory results. This is mostly caused by variances in the genotyping platforms' effectiveness. The results of maize breeders' efforts to identify the best heterotic grouping technique have been contradictory. Fan et al. (2009), for instance, proposed the heterotic groups' specific and general combining ability (HSGCA) method, which combines both SCA and GCA, as a more acceptable method for classifying inbred lines into heterotic groups.

In their studies, Fan et al. (2009) and Badu-Apraku et al. (2015) performed a meticulous comparison of the specific combining ability (SCA) of various lines using molecular markers. Additionally, they applied the heterotic group's specific and general combining ability (HSGCA) methods. Their investigation revealed that the HSGCA method emerged as the most efficient approach in terms of breeding efficiency. To assess breeding efficiency accurately, they considered two crucial factors: the average proportion of total inter-heterotic group hybrids arising from superior high-yielding inter-heterotic group hybrids, and the proportion of total low-yielding intra-heterotic group hybrids attributed to the low-yielding intra-heterotic group hybrids. On the contrary, in a study focused on early maturing quality protein maize (QPM) inbred lines, Badu-Apraku et al. (2015, 2016) discovered that heterotic grouping based on molecular markers proved to be the most effective approach. The contradictory outcomes reported in previous studies can be attributed to the variations in the genetic materials employed (Badu-Apraku et al., 2016). Badu-Apraku et al. (2015) demonstrated the superiority of the HSGCA method over the HGCAMT, SNP-GD, and SCA methods in assigning 17 out of 20 maize inbred lines into heterotic groups across multiple stress environments. Similarly, in a related study, Badu-Apraku et al. (2015) identified the SNPGD classification procedure as the most efficient method, outperforming other approaches such as HGCAMT, HSGCA, and SCA, in grouping 14 quality protein maize inbred lines under varying environmental conditions.

A primary limitation of heterotic grouping, whether through SCA or HSGCA, is its focuses predominantly on a single trait, typically grain yield. However, grain yield is a complex trait governed by multiple genes and exhibits low heritability, particularly under stressful environments. Specifically, severe drought stress diminishes the effectiveness of selecting for grain yield as it leads to reduced yield levels and decreased grain yield heritability estimates (Bolaños and Edmeades, 1993).

Consequently, selecting for improved yield in stressful environments becomes challenging. An alternative approach proposed by Badu-Apraku et al. (2013) suggests considering component traits that demonstrate a robust correlation with grain yield for indirect selection. This approach involves assessing multiple traits of inbred lines that exhibit significant general combining ability (GCA) effects across diverse environments. By incorporating a broader range of traits, especially in situations where lines and hybrids are being developed for resistance or tolerance to multiple stresses, this method allows for a more precise classification of inbred lines into heterotic groups.

The adoption of the HGCAMT (classification based on

general combining ability effects of multiple traits) is anticipated to enhance the accuracy and predictability of heterotic grouping for lines. This method takes into account the additive gene effects associated with each trait, enabling a comprehensive evaluation. Optimal selection of the method for grouping parental inbreds into heterotic groups offers significant advantages to breeders, including resource efficiency and the expedited attainment of breeding goals. Thus, the aims of this study were to:

(1) Categorize inbreds lines into heterotic groups using three different methods
(2) Compare the efficiencies of the three grouping methods.

MATERIALS AND METHODS

Genetic material and evaluation

The study, as described in Ribeiro et al. (2020) and illustrated in Table 1, involved the utilization of 32 inbred lines and three elite inbred testers sourced from the breeding program of IITA and CIMMYT. To generate a total of 96 hybrids, the inbred lines were subjected to crossing with the three testers, employing the line by tester mating design. The experimental trials were conducted across two seasons and three distinct locations, namely Fumesua (Latitude 6° 41′N and Longitude 1°28′W), Ejura (Latitude 70 40N and Longitude 10 39W), and Kwadaso (60 43N and Longitude 10 36W). In each environment, the hybrids, along with four reference checks, were evaluated in separate trials, with adjacent blocks assigned for both Low-N (30 kg N/ha) and high-N (90 kg N/ha) conditions. Prior to field preparation, soil samples were collected from a depth of 0-15 cm and analyzed for nitrogen (N), phosphorus (P), and potassium (K) levels using the Kjeldahl digestion and colorimetric method (Bremner and Mulvaney, 1982) at the Analytical Services Division of CSIR-Soil Research Institute in Kwadaso, Kumasi. The soil analysis revealed that the Kwadaso soil contained 0.09% N, 124 Cmolc/kg P, and 0.37 Cmolc/kg K, while the Ejura soil had 0.03% N, 17.41 Cmolc/kg P, and 0.04 Cmolc/kg K. The Fumesua soil exhibited 0.12% N, 27.89 Cmolc/kg P, and 0.28 Cmolc/kg K. For the experimental design, a 10 × 10 alpha lattice arrangement was employed with two replications. Single-row plots, measuring 5 m in length, were established with a spacing of 0.75 m between rows and 0.5 m between plants within each row. Initially, three seeds of the lines were planted in each hole and subsequently thinned to two plants per hill at two weeks after emergence, resulting in a population density of 53,333 plants per hectare. At two weeks after sowing, nitrogen fertilizer was applied to achieve a total available N of 30 kg/ha in the Low-N block. Additionally, single superphosphate and muriate of potash were applied at rates of 60 kg/ha. Emamectin benzoate, at a rate of 0.30 L/ha, was used as needed for insect pest management, particularly targeting fall armyworm. Post-emergence weed control was conducted through selective herbicide spraying using dicamba (1.0 L/ha) and manual weeding, as required.

Field phenotyping

The collected data included several parameters: days to 50% silking (DTS), which represents the number of days from planting to 50% emergence of silks; days to anthesis (DTA), which indicates the duration to 50% pollen shedding; and the anthesis-silking interval (ASI), calculated as the difference between days to 50% silking and 50% anthesis. Plant height (PHT) was measured as the length from the plant base to the first tassel branch's height, while ear height (EHT) represented the distance to the node carrying the upper ear. The assessment of lodging included two aspects: root lodging (RL), indicated by the percentage of plants leaning more than 30 degrees from the vertical, and stalk lodging (SL), measured as the proportion or percentage of plants with broken stalks below the ear or stalks bending more than 45 degrees from the upright position. Ear aspect (EASP) was rated on a scale of 1 to 9, where 1 indicated clean, uniform, large, and well-filled ears, while 9 represented ears with undesirable features. The visual appeal of plant architecture within a plot was evaluated using a scale of 1 to 5, where 1 indicated excellent overall phenotypic appeal and 5 indicated very poor overall phenotypic appeal (Plant aspect - PASP). To determine the ear number per plant (EPP), the total number of ears per plot was divided by the number of harvested plants. The chlorophyll concentration of the ear leaf was measured using a portable SPAD meter (CCM-200 plus-opti sciences) on five randomly selected plants per plot at approximately 2 weeks after anthesis (WAA). Disease severity, such as maize streak and blight, was assessed on a scale of 1 to 5, with 1 indicating the absence of disease and 5 representing severe infection. For trials conducted under nitrogen (N) stress, harvested ears from each plot were shelled to determine the percentage grain moisture. Grain yield in kg/ha was calculated by adjusting the shelled grain weight to 15% moisture content. An assumption of 80% shelling percentage was applied to all genotypes in the high N plot. The resulting grain yield, obtained from ear weight, was then converted to kg/ha and adjusted to 15% moisture.

Statistical analysis

A line x tester analysis of variance was used to determine the statistical significance of GCA-line, GCA-tester, SCA-hybrid and their interactions with the environments as described by Amegbor et al. 2017). The interaction between SCA and the environment was fundamental to the study's findings, demonstrating that different heterotic groupings of the lines may occur depending on the environment.

Heterotic grouping of inbreds under contrasting environments

Three approaches were employed to categorize lines into heterotic groups:

(1) The first method involved the classification of inbred lines into heterotic groups based on their specific combining ability (SCA) effects for grain yield and the average grain yield of test crosses, as proposed by Menkir et al. (2004). In this approach, lines exhibiting positive SCA with a specific tester and an average grain yield higher than the best check were assigned to the heterotic group opposite to that of the tester. Conversely, lines displaying negative SCA when crossed with a tester were classified into the same heterotic group as the tester.
(2) Another method for establishing heterotic groups relied on specific and general combining ability, as suggested by Fan et al. (2009). The computation of heterotic specific general combining ability (HSGCA) involved the formula:

HSGCA = Cross mean Xij - Tester mean (Xi) = GCA + SCA

where Xij represents the mean yield of the cross between the ith tester and jth line, Xi denotes the mean yield of the ith tester, and Xj represents the mean yield of the jth line. The calculated HSGCA values were subjected to three classification steps outlined by Fan et al. (2009).

Table 1. List of inbred lines and testers with their pedigrees used in the study.

Inbred	Pedigree	Source
CLWN 349	HTBAB9 138·5-1.2TL-I-4-2TL-B-ITL-B_	CIMMYT
CML 494	LP~C"F·7-1-2-Z.2.2-8BB	CIMMYT
CLWN 364	SAHCl-5·1·1-5-3-B	CIMMYT
CLWN 341	LP SEQC3-H1-2-2·2-1-1-.a-B	CIMMYT
CLWN 238		CIMMYT
CLRCW 36		CIMMYT
ZM 523B-29-2-1-1-B*6	ZM 523B-29-2-1-1-B*6	CIMMYT
CLWN 359	SA3C4IiC(16X25)-2-4-3-1-B	CIMMYT
CLWN 247	(CL-FAWW11 x CML494)-B-24-2-2-B-B-1-B-8-B-B	CIMMYT
CML 442	CIMMYT M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BBBB	CIMMYT
CML 444	P43C9-1-1-1-1-1-BBBBB	CIMMYT
CML 198/LPSC	CML198/LPSC3H144-1-2-2-2-2-#-BB]-1-4-1-1-4-B*4-B-B-B	CIMMYT
CML 395/ CML 444	[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-BB-B-B-B	CIMMYT
ZM521B-66-4-1-1	ZM521B-66-4-1-1-BB-B-B-B	CIMMYT
CML 444/CML 395/ DTPWC8F31	[CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-BB-B-B-B	CIMMYT
Laposta Seq C7-F71-1-2	La Posta Seq C7-F71-1-2-1-1-B-B-B	CIMMYT
CML 254	TUXSEQ.149-2-BBB"II#'1·BB-f	CIMMYT
Laposta Seq C7-F18-3-2-1	La Posta Seq C7-F18-3-2-1-1-B-B-B-B-B	CIMMYT
J-16-1	Zm 523-16-2-1-1-B*4	CIMMYT
P43SRCq Fs100-1-1-8	P43SRCqFs100-1-1-8#1-B-13-B1	CIMMYT
TZM 501XKU1414XTZM501		CIMMYT
TZL Comp 3	TZL Comp 3-C2-S2-34-4-1-B	CIMMYT
CZL 068	[LZ956441/LZ966205]-B-3-4-4-BB	CIMMYT
CZL 0713	[SYN-USAB2/SYN-ELIB2]-12-1-1-1-BBB	CIMMYT
CLWN 240		CIMMYT
CZL 00001	INTA-191-2-1-2-B*6	CIMMYT
TZD II 68	TZE-W POP STR 104 S6 40/160-2/3	IITA
TZD II 134	TZE-W POP STR 107 S6 238/254-2/2-3/3-2/4-2/2	IITA
TZD II 140	TZE-W POP STR 105 S6 53/253-1/2-2/3-3/4-2/3	IITA
TZD II 141	TZE-W POP STR 105 S6 53/253-1/2-2/3-2/4-2/3	IITA
CZL 03007	CML445/ZM621B]-2-1-2-3-1-BB	CIMMYT
M131		IRAD
87036*		IRAD
1368*	Across 7721 BC_2 × TZSR	IITA
9071*	N28 × TZSR	IITA

Source: Ribeiro et al. (2020).

Step 1: All inbred lines with negative HSGCA effects were grouped together with their respective testers.
Step 2: If an inbred line was assigned to multiple heterotic groups in Step 1, it would be retained in the heterotic group associated with the smallest HSGCA value (or largest negative value), while being removed from other heterotic groups.

Step 3: In order to avoid assigning any line with positive HSGCA effects across all testers to any heterotic group, such lines were not assigned to any specific heterotic groups since they might belong to distinct heterotic groups compared to the testers.

(1) The GCA effects of several traits were used by Badu-Apraku et al. (2013, 2015) to create their categorization approach (HGCAMT).

The HGCAMT approach uses the following statistical model to divide the inbreds into the heterotic groups:

$$Y = \sum_{i=1}^{n}((\mathrm{Y}_i - \bar{\mathrm{Y}}_i)/\mathrm{s}) + \varepsilon\mathrm{ij}$$

where Y is HGCAMT, which uses the GCA of several variables to measure the genetic relationship between genotypes, *i* to *n*; Y_i is the individual GCA effect of genotypes for trait $_i$; $\bar{Y}_i$ μ is the mean of GCA effects across genotypes for trait *i*. s_i is the standard deviation of the GCA effects of trait *i*; ε_{ij} is the residual of the model associated with the combination of inbred i and trait j.

To reduce the effects of different scales of the traits, the GCA effects of the traits that had significant mean squares for genotype under low N and high N growing conditions as well as across test environments were standardized (mean of zero and standard deviation of 1) in order to achieve the HGCAMT grouping. Using

Table 2. Line × tester analysis of grain yield and other agronomic traits across 11 environments.

Source of variation	DF	GY (kg/ha)	DTS	DTA	ASI	EHT (cm)	PHT (cm)	EPP	SG (1-9)	PASP (1-5)	EASP (1-5)	HC	CC
Envt	11	305193537**	4449.88**	3432.49**	176.36**	22496.40**	81219.37**	11.81**	104.98**	85.92**	24.89**	58.35**	14783.06**
Hybrid	95	3582219**	44.15**	35.07**	2.33**	1147.96**	1418.75**	0.06**	1.12**	0.60**	1.25**	0.55**	233.60**
Envt*Hybrid	1045	1285303**	4.65012**	3.22**	1.34**	146.6459**	339.7874**	0.05**	0.38*	0.32**	0.62**	0.32**	43.33**
Line(GCA)	31	5050710**	67.71**	57.82**	2.50**	1608.08**	2694.55**	0.08**	2.08**	0.77**	2.02**	0.62**	452.86**
Tester(GCA)	2	48688191**	1244.26**	924.61**	31.46**	23677.89**	16799.07**	0.27**	26.12**	5.60**	3.79**	3.78*	4061.10**
Envt*Line(GCA)	341	1527327**	6.54**	4.67**	1.57**	238.51**	557.89**	0.05**	0.49^{ns}	0.4^{ns}	0.86**	0.39**	53.51*
Envt*Tester(GCA)	22	4738603**	12.17**	8.05**	2.82**	416.62**	684.41**	0.13**	1.16**	0.97**	1.61**	1.23**	140.66**
Line*Tester(SCA)	62	1873596**	8.47**	6.54**	1.53**	343.30**	691.17**	0.05^{ns}	0.45^{ns}	0.49^{ns}	0.87**	0.45**	55.01^{ns}
Envt*Line*Tester(SCA)	682	1263499**	5.75**	3.91**	1.46**	155.17^{ns}	370.52^{ns}	0.05*	0.41^{ns}	0.36^{ns}	0.55^{ns}	0.34**	40.84^{ns}
Error	1151	529842	4.59	3.21	1.02	161.85	378.76	0.04	0.44	0.39	0.55	0.27	46.22

GY: Grain yield; DTS: days to silk; DTA: days to anthesis; ASI: anthesis silking interval; PHT: plant height; EHT: ear height; EPP: number of ears per plant; SG: Stay green; PASP: plant aspect; EASP: ear aspect; HC: husk cover; *, **, Significant at 0.05 and ns: not significant.
Source: Authors

SAS software version 9.3, Ward's minimal variance cluster analysis was then performed on the standardized GCA effects (SAS Institute, 2011).

Grouping efficiency

According to Fan et al. (2009), grouping efficiency is the proportion of superior, high-yielding hybrids produced across all inter-heterotic group crossings.

RESULTS

Analysis of variance of grain yield and other traits across contrasting environments

Statistically significant ($p < 0.05$) variations were observed in the mean squares of environments (E), hybrids (G), and hybrid × environment interactions (GEI) for grain yield and all agronomic traits measured across different environments, as presented in Table 2. The analysis involved partitioning the hybrid components of variation into two categories: general combining ability (GCA) of the line (GCA-line) and GCA of the tester (GCA-tester), as well as specific combining ability (SCA) mean squares. The results indicated significant improvements in GCA-line, GCA-tester, and SCA for grain yield and most agronomic traits across various environments. Moreover, the mean squares of GCA-line × E and GCA-tester × E interactions were also found to be significant for most measured traits. However, the mean squares of SCA × E interactions did not show significant differences for most traits. These findings suggest that the GCA effects of the lines and testers were more consistent across different environments compared to the SCA effects. This implies that utilizing GCA-based approaches in the selection of superior genotypes for yield enhancement may offer potential advantages.

Groupings based on SCA of grain yield

The process outlined by Menkir et al. (2003) was followed with minor adjustments to classify the inbred lines into heterotic groups under two different growing conditions and across various environments. This classification relied on the specific combining ability (SCA) effects and mean grain yields of test crosses between the lines and three different testers. In order to assign the lines to specific heterotic groups, certain criteria were applied. Lines displaying positive SCA with one tester and negative SCA with the other testers, while also exhibiting a mean grain yield equal to or higher than the best tester cross, were allocated to the heterotic group opposite to the group of testers. These opposite tester heterotic groups were designated as anti-groups, such as anti1368 group A, anti9071 group B, and anti87036 group C, since three testers were employed in the study. For instance, under high nitrogen (N) conditions, the highest mean grain yield of the best tester hybrid was 87036 × 9071 (4252.90 kg/ha). Since the line CLWN 247 showed a positive SCA with tester 1368 and a

mean grain yield of 4506.51 kg/ha, it was classified as anti1368, indicating that it belonged to a heterotic group different from 1368. Additionally, lines exhibiting positive SCA and yields greater than the best tester cross (hybrid) between two testers were categorized into heterotic groups opposing both of these testers. The SCA approach was utilized to group the inbred lines under low N, high N, and across different environmental conditions, and the results are presented in Table 3. Under low N, 15 and 3 inbred lines were assigned to the anti 9071 and anti87036 heterotic groups, respectively, while 9 inbred lines were allocated to the anti1368 (group A) heterotic group. Similarly, under high N, 10, 15, and 3 inbred lines were assigned to anti1368, anti9071, and anti87036 heterotic groups, respectively. When considering groupings across environments, 9 inbred lines were assigned to anti1368, 14 to anti9071, and 5 to anti87036. Out of the 32 inbred lines, five could not be classified under low N, four under high N, and four across environments. In terms of the placement of inbred lines into the same heterotic group, there was consistency across the different growing environments. For example, TZD II 68 and CML 395/CML444 were consistently assigned to anti1368 heterotic group in all growing environments, while CLWN 238, CLWN 364, CML 198/LPSC, CZL 00001, and Laposta Seq C7-F18-3-2-1 were consistently placed in the anti9071 group. It is worth mentioning that inbred line TZD II 68 exhibited the highest SCA effect with 1368 under low N conditions. Notably, among all the inbred lines, CLWN 247 was the only one classified into the anti87036 group across different growing environments.

Groupings based on heterotic group's specific and general combining ability (HSGCA)

Table 4 presents the results of the HSGCA-based heterotic groupings, demonstrating the following outcomes. When nitrogen levels were low, fifteen inbreds were categorized into heterotic group A, three inbreds into heterotic group B (9071), and fourteen inbreds into heterotic group C (87036). Under high nitrogen levels, sixteen inbreds were assigned to heterotic group A (1368), three inbreds to heterotic group B (9071), and twelve inbreds to heterotic group C (87036). Across various environments, eighteen inbreds consistently belonged to group A (1368), one inbred to group B (9071), and ten inbreds to group C (87036). Additionally, specific inbreds were consistently classified into particular heterotic groups across all growing conditions. Notably, CLWN 238, CLWN 247, CLWN 349, CML 444/CML395, CML 254, CML 444, and TZL Comp 3 consistently fell within heterotic group A (1368). Conversely, CML 395/CML 444, CML 494, Laposta Seq C7-F18-3-2-1, and TZD II 140 were consistently grouped together in heterotic group C (87036).

Heterotic grouping based on GCA of multiple traits (HGCAMT)

The dendrograms presented in Figures 1, 2, and 3 showcase the groupings based on HGCAMT for low, high, and across nitrogen (N) environments. In each of these environments, the HGCAMT method revealed the presence of three distinct groups. Under low nitrogen conditions, Tester 1 (1368) identified 16 inbreds, Tester 2 (9071) identified 4 inbreds, and Tester 3 (87036) identified 12 inbreds, all belonging to their respective groups (A, B, and C).

When nitrogen levels were high, Tester 1 (1368) grouped 18 inbreds, Tester 2 (9071) grouped 5 inbreds, and Tester 3 (87036) grouped 9 inbreds. Across all environments, Tester 1 (1368) grouped 13 inbreds, Tester 2 (9071) grouped 5 inbreds, and Tester 3 (87036) grouped 14 inbreds. The dendrograms visually depict the relationships and associations between the inbreds based on the HGCAMT analysis, providing valuable insights into the grouping patterns observed under different nitrogen environments.

Grouping efficiency

According to Fan et al. (2009), the efficiency of heterotic grouping can be assessed by the proportion of high-yielding hybrids produced through inter-heterotic group crossings. To evaluate the effectiveness of three different heterotic grouping methods, the 99 hybrids were ranked based on grain yield across various environments: low nitrogen, high nitrogen, and all research conditions combined. The ranking process involved dividing the hybrids into two main categories: inter-group crosses and intra-group crosses. These categories were further divided into three groups based on mean grain yield: high-yielding hybrids (yield group 1, comprising the top 33 ranked hybrids), intermediate hybrids (yield group 2, ranked between 34th and 66th), and low-yielding hybrids (yield group 3, ranked between 67 and 99th), as illustrated in Table 5. The effectiveness of a categorization approach can be determined by the extent to which the heterotic groups enable the production of superior hybrids through inter-heterotic group crosses compared to within-group crosses. In other words, the optimal heterotic grouping method should result in a higher number of superior hybrids generated through inter-heterotic group crossings rather than within-group crossings.

In accordance with the results, the SCA method identified 24 high-yielding hybrids, HGCAMT identified 20, and HSGCA identified 29 from the total intergroup crosses under low nitrogen conditions. Under high nitrogen conditions, the SCA method identified 18 high-yielding hybrids, HGCAMT identified 23, and HSGCA identified 30, from the total intergroup crosses as

Table 3. Classification of the 15 inbreds into heterotic groups based on SCA effects of grain yield under low, high and across N environments.

Group A (Anti 1368)	Group B (Anti 9071)	Group C (Anti 87036)
	Low	
CLWN 240	CLWN 238	CLWN 247
CML 198/ LPSC	CLWN 359	CLWN 364
CML 395/ CML 444	CLWN 364	TZD II 134
CZL 068	CML 198/ LPSC	
CZL 0713	CLWN 247	
Laposta Seq C7-F71-1-1-2	CML 444	
M131	CZL 00001	
TZDII 68	J -16-1	
ZM523B-29-2-1-1-B*6	Laposta Seq C7-F18-3-2-1	
	P43SCRq Fs100-1-1-8	
	TZD II 134	
	TZD II 140	
	TZD II 141	
	TZM501 X KU1414 X TZM501	
	ZM523B-29-2-1-1-B*6	
	High	
CLWN 247	CLWN 238	CLRCW 36
CLWN 341	CLWN 240	CLWN 240
CML 444/CML 395/DTPWC8F31	CLWN 341	CLWN 247
CML 395/ CML 444	CLWN 364	CLWN 349
CML 494	CML 198/ LPSC	CML 442
CZL 068	CLWN 247	CZL 0713
CZL 0713	CML 254	CZL 03007
P43SCRq Fs100-1-1-8	CML 444	Laposta Seq C7-F71-1-1-2
TZD II 134	CML 444/ CML 395/DTPWC 8F31	M131
TZD II 68	CZL 00001	P43SRCq Fs100-1-1-8
	J -16-1	TZD II 141
	Laposta Seq C7-F18-3-2-1	
	TZD II 134	
	TZD II 140	
	TZD II 141	
	Across	
CLWN 341	CLWN 238	CLRCW 36
CLWN 359	CLWN 240	CLWN 240
CML 395/ CML 444	CLWN 247	CLWN 247
CML 494	CLWN 359	CLWN 349
CZL 068	CLWN 364	TZD II 134
CZL 0713	CML 198/ LPSC	
M131	CML 254	
P43SCRq Fs100-1-1-8	CML 444	
TZD II 68	CZL 00001	
	J -16-1	
	Laposta Seq C7-F18-3-2-1	
	TZD II 134	
	TZD II 140	
	TZM501 X KU1414 X TZM501	

Source: Authors

Table 4. Classification of intermediate maturing maize inbreds into heterotic groups based on HSGCA effects of grain yield under low N, high N and across N environments.

1368	9071	87036
	Low	
CLRCW 36	CLWN 341	CLWN 240
CLWN 238	CZL 068	CML 198/LPsc
CLWN 247	TZD II 68	CML 395/ CML 444
CLWN 349		CML 442
CLWN 359		CML 494
CLWN 364		CZL 00001
CML 444/CML 395/DTPWC8F31		CZL 03007
CML 254		CZL 0713
CML 444		J-16-1
P43SRCq Fs100-1-1-8		Laposta Seq C7-F18-3-2-1
TZL comp 3		Laposta Seq C7-F71-1-1-2
TZM 501 X KU 1414 X43 TZM 501		M131
TZD II 134		TZD II 140
TZD II 141		ZM523B-29-2-1-1-B*6
ZM 521B-66-4-1-1		
	High	
CLWN 238	CLRCW 36	CLWN 341
CLWN 240	CZL 0713	CLWN 359
CLWN 247	P43SRCq Fs100-1-1-8	CLWN 364
CLWN 349		CML 395/ CML 444
CML 444/CML 395/DTPWC8F31		CML 494
CML 198/LPsc		CZL 00001
CML 254		CZL 068
CML 442		J-16-1
CML 444		Laposta Seq C7-F18-3-2-1
CZL 03007		TZD II 68
Laposta Seq C7-F71-1-1-2		TZM 501 X KU 1414 X43 TZM 501
M131		TZD II 134
TZL comp 3		TZD II 140
TZD II 141		
ZM 521B-66-4-1-1		
ZM523B-29-2-1-1-B*6		
	Across	
CLRCW 36	CZL 0713	CLWN 341
CLWN 238		CLWN 359
CLWN 240		CML 395/ CML 444
CLWN 247		CML 494
CLWN 349		CZL 03007
CLWN 364		CZL 068
CML 444/CML 395/DTPWC8F31		Laposta Seq C7-F18-3-2-1
CML 198/LPsc		M131
CML 254		TZD II 68
CML 442		TZD II 140
CML 444		
CZL 00001		
J-16-1		
Laposta Seq C7-F71-1-1-2		
P43SRCq Fs100-1-1-8		
TZL comp 3		

Table 4. Contd.

TZM 501 X KU 1414 X43 TZM 501
TZD II 134
TZD II 141
ZM 521B-66-4-1-1

Source: Authors

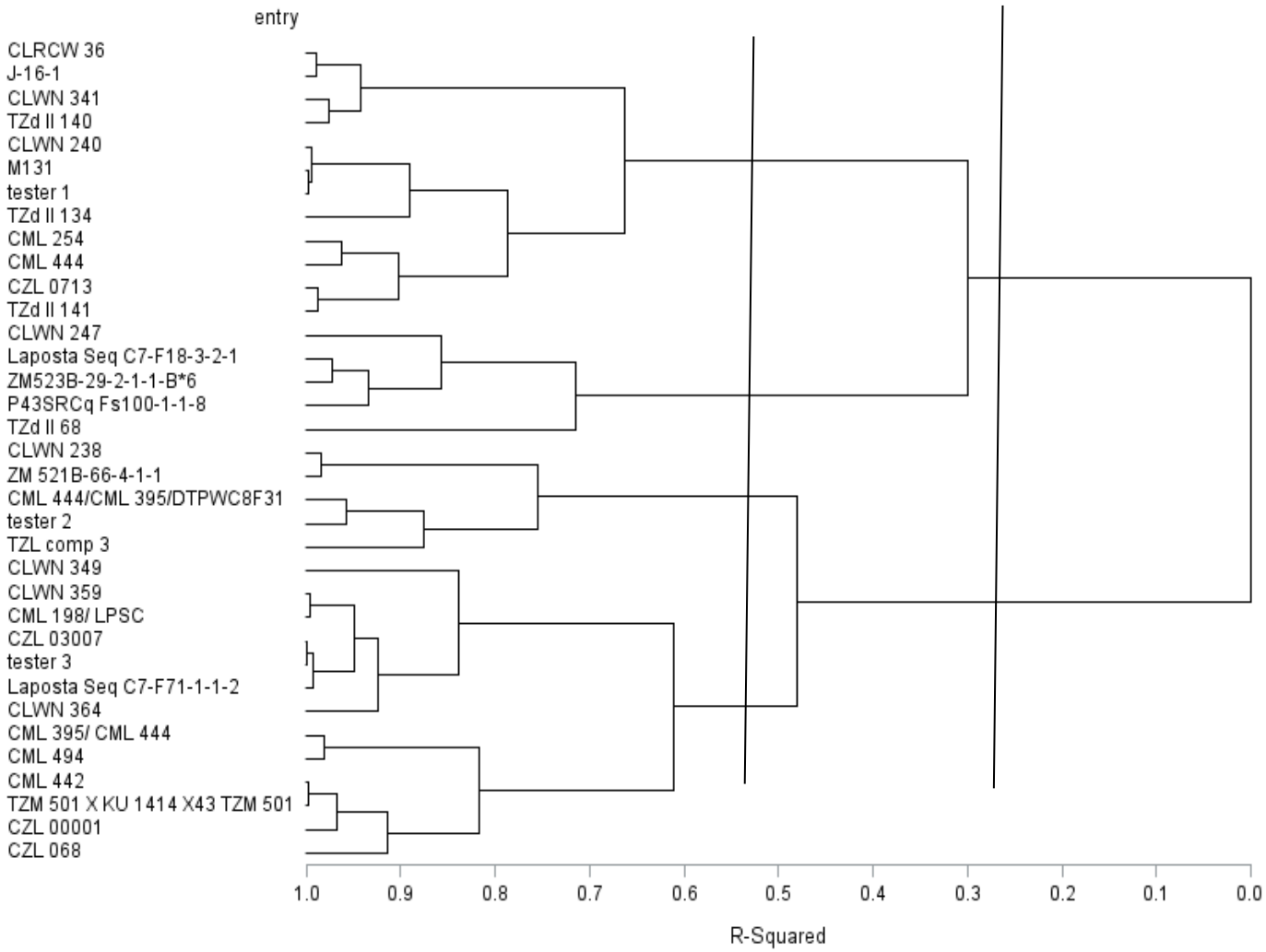

Figure 1. Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method under low N environment. Tester 1= 1368, tester 2= 9071, tester 3=87036.
Source: Authors

determined by the grouping methods (Table 5). Across different research environments, the SCA method revealed 17 high-yielding intergroup crosses, HGCAMT identified 20, and HSGCA identified 31. The breeding efficiency, as measured by the proportion of high-yielding hybrids produced through intergroup crosses, was found to be the highest for the SCA method under low nitrogen (57%), high nitrogen (56%), and across environments (53%) (Table 6). The HSGCA method exhibited the second-highest breeding efficiency across all growing environments, while the HGCAMT method had the lowest breeding efficiency. Considering the overall results, the SCA approach demonstrated the best breeding efficiency across all test environments, making it the most effective

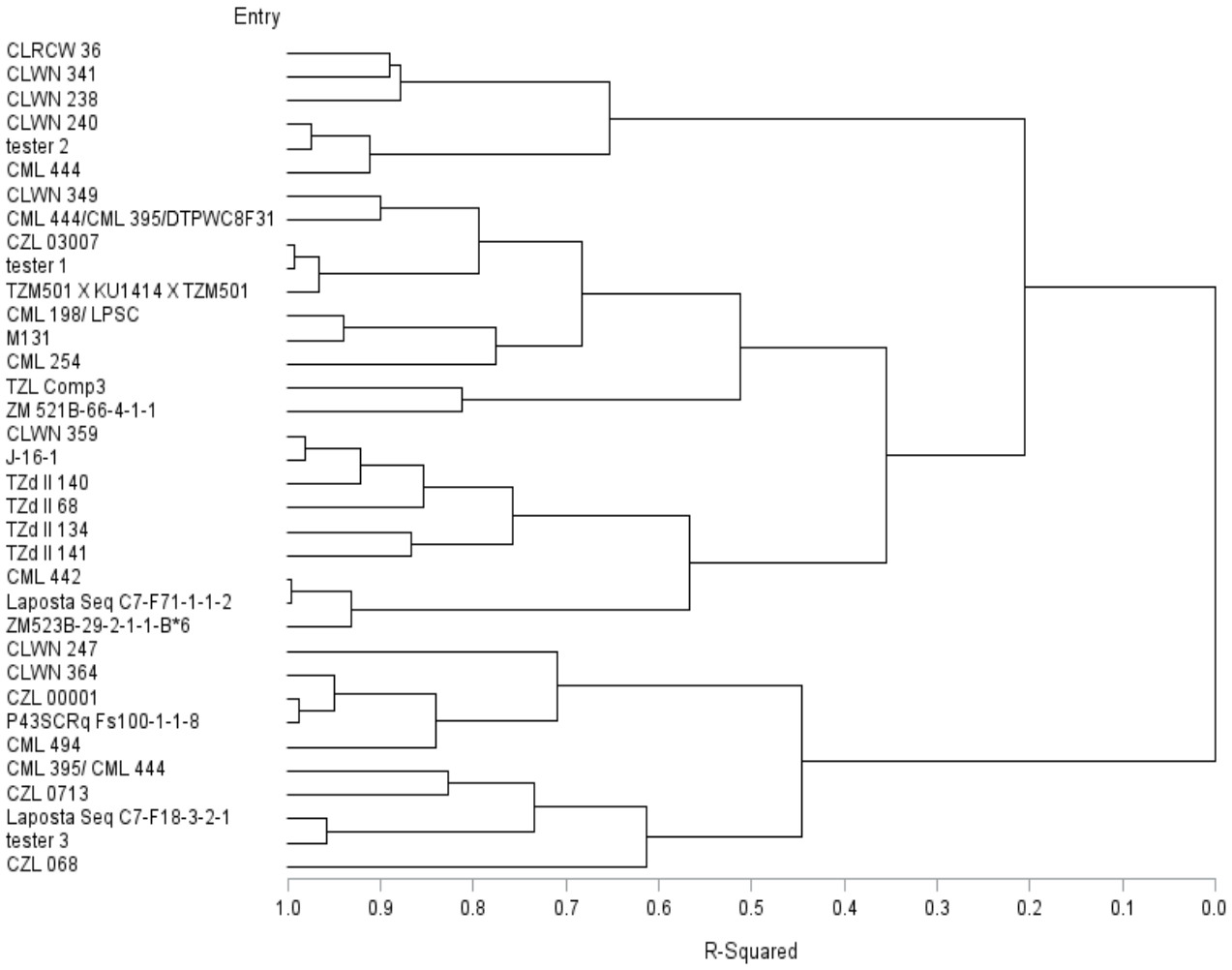

Figure 2. Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method under high N environments. Tester 1= 1368, tester 2= 9071, tester 3=87036.
Source: Authors

method for classifying inbreds into heterotic groups.

DISCUSSION

The analysis of grain yield and other agronomic variables across different environments revealed significant mean squares for E (environment), G (genotype), and GEI (genotype × environment interaction). This indicates that the test environments were diverse and that the hybrids exhibited sufficient genetic diversity, allowing for effective selection of the measured traits. It was also observed that the expression of grain yield and other traits varied across the different environments. These findings align with previous studies (Ifie, 2013; Obeng-Bio et al., 2020; Ribeiro et al., 2020) that reported significant genotype × environment interactions for maize grain yield and agronomic traits under low nitrogen conditions. In the present study, three distinct approaches (SCA, HGSCA, and HGCAM) were employed for heterotic grouping. These approaches yielded comparable but not identical grouping patterns. For instance, under low nitrogen conditions, CML 444, TZD II 134, and TZD II 141 were consistently classified into the same group (group A, 1368) by all three methods, while other inbreds such as CLRCW 36, CML 254, CLWN 247, and J-16-1 were placed in the same group by two of the three methods. Under high nitrogen conditions, five inbreds (CML198/ LPSC, CML 444/CML395, CZL 03007, and M131) were assigned to the same heterotic group A (1368). In heterotic group C (87036), under low nitrogen conditions, CML 395/CML444, CML 494, CZL 0001, and CML198/ LPSC were grouped together. The HSGCA and HGCAMT approaches exhibited greater similarity in terms of grouping inbred lines into the same group. Previous studies by Badu-Apraku and Oyekunle (2012) and Badu Apraku et al. (2015) reported similar findings, demonstrating agreement between categorization using

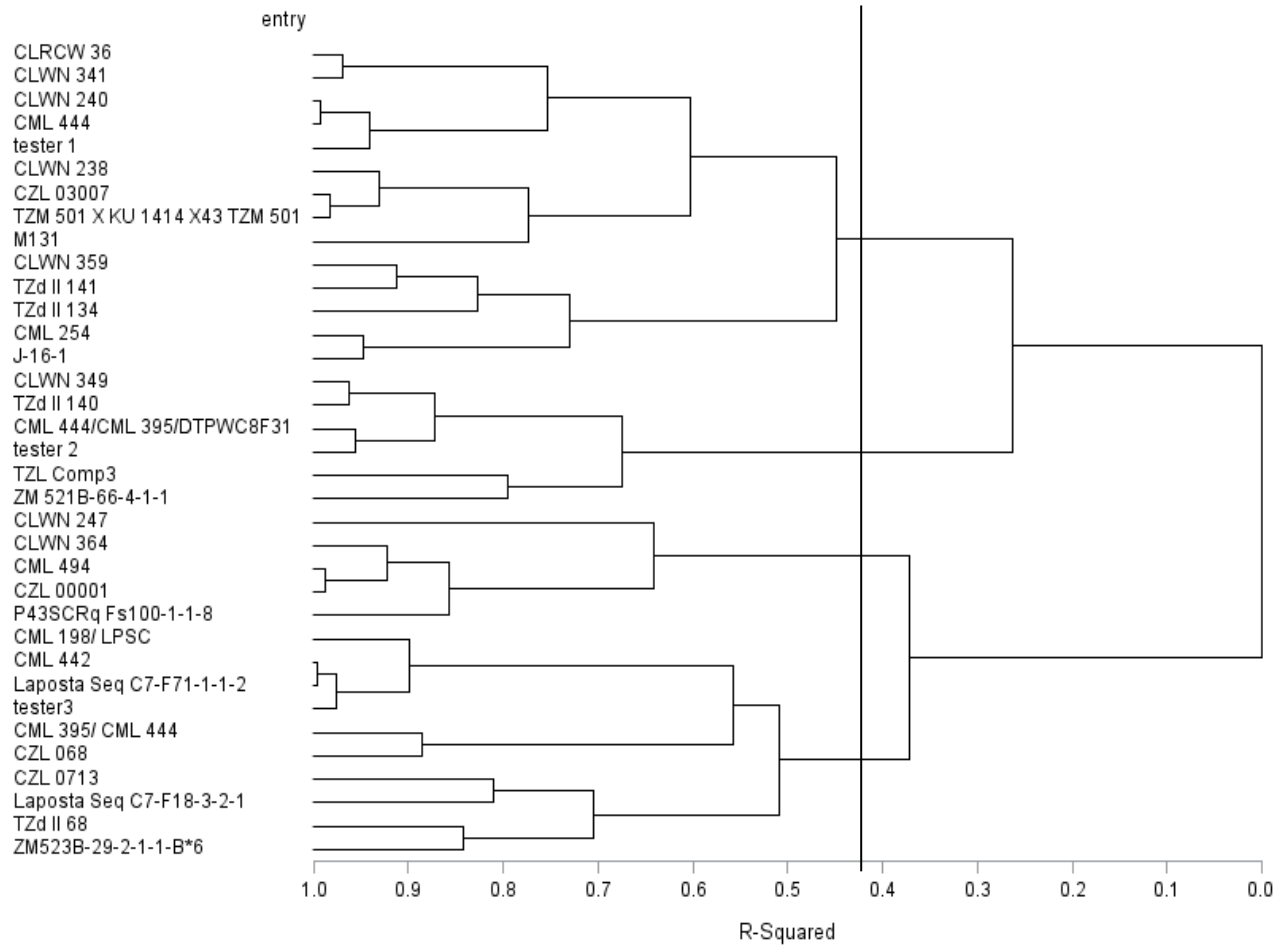

Figure 3. Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method across low and high N environments. Tester 1= 1368, tester 2= 9071, tester 3=87036
Source: Authors

the HGCAMT and HSGCA methods. However, compared to previous research, Badu Apraku et al. (2013) found that the HSGCA and molecular marker techniques showed greater similarity. Olayiwola et al. (2021) and Akinwale et al. (2014) also found a significant association between SCA and HSGCA classifications of inbred lines. Although there were slight variations, the three grouping methods exhibited reasonable consistency. A study by Badu-Apraku et al. (2013) reported a close correlation in the categorization of seven extra-early yellow inbreds using SCA, HGCAMT, and HSGCA techniques.

Accurate identification of the most effective heterotic grouping technique is essential for breeders to optimize their resources and achieve breeding goals efficiently. In all tested growing conditions, the SCA approach demonstrated the highest breeding efficiency, indicating its effectiveness in classifying inbreds. However, these findings contradict previous studies by Fan et al. (2009), Badu-Apraku et al. (2013, 2015), Akinwale et al. (2014), Amegbor et al. (2017), and Olayiwola (2021), which suggested that the HSGCA approach is more effective for inbred classification than the SCA method. The results, however, align with the conclusions of Laouali (2014), who found no advantages of the HSGCA approach over the SCA method and emphasized the effectiveness of the SCA method for inbred classification. Olayiwola et al. (2021) also reported high breeding efficiency for both HSGCA and SCA, indicating their effectiveness in grouping white inbred lines. It is important to note that the effectiveness of the HGCAMT technique may be compromised if only a few traits exhibit significant and positive GCA effects, as highlighted by Badu-Apraku et al. (2013). It is crucial to recognize that no heterotic grouping strategy is perfect due to the vast number of genetic combinations possible between any two inbred lines, resulting in the potential development of superior hybrids within a heterotic group (Akinwale et al., 2014).

The inconsistent groupings observed among the different methods across various environments underscored the sensitivity of these methods to growing

Table 5. Number of hybrids within the best 33 arranged in descending order of their yield (group 1), from 34th to 66th (group 2) and from 67th to 99th (group 3).

Yield group	Cross type	HSGCA	HGCAMT	SCA
Low-N environments				
1	Inter	29	20	24
1	Intra	3	7	2
2	Inter	25	23	9
2	Intra	7	7	5
3	Inter	9	15	9
3	Intra	22	14	13
High N environments				
1	Inter	30	23	18
1	Intra	3	7	11
2	Inter	22	22	9
2	Intra	11	10	10
3	Inter	15	19	5
3	Intra	18	13	10
Across low and high N environments				
1	Inter	31	20	17
1	Intra	2	5	9
2	Inter	22	16	7
2	Intra	11	8	12
3	Inter	14	19	8
3	Intra	19	13	13

Source: Authors

Table 6. Breeding efficiency (%) of HSGCA, HGCAMT and the SCA heterotic grouping methods under Low-N, High-N and across research environments.

Environment	HSGCA	HGCAMT	SCA
Low-N	46.03	34.48	57.14
High-N	44.77	35.94	56.25
Across	46.27	36.36	53.13

Source: Authors

conditions and highlighted the possibility of obtaining environment-specific heterotic patterns for improving grain yield. Categorizing these inbreds into distinct heterotic groups will facilitate the development of low-nitrogen-tolerant source populations. These inbreds within each heterotic group can then be recombined to form source populations, which can be further enhanced through recurrent selection and subsequent crosses with suitable testers to identify new hybrids.

Conclusion

The results obtained classified the inbreds into heterotic groups. Through extensive testing, two superior heterotic groups could be identified and lines in other groups could be further improved for incorporation into either group. The effectiveness of the heterotic grouping methods, namely SCA, HSGCA, and HGCAMT, varied depending on the genetic material being studied. For assigning inbred lines to groups within each environment, the SCA grouping approach was found to be the preferred method. Widely utilized, this classification technique combines specific combining ability, line-pedigree information, and field hybrid-yield data to allocate maize lines to heterotic groups. The SCA approach proved highly effective for heterotic grouping and provided an excellent opportunity for incorporating a large number of

intermediate inbreds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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