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Efficiencies of Heterotic Grouping Methods for Classifying Early Maturing Maize Inbred Lines

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Abstract: The success of a hybrid breeding program is dependent on available heterotic patterns for exploitation of grain-yield heterosis. The efficiency of the assignment of germplasm lines into heterotic groups is a prerequisite for obtaining useful heterotic patterns among germplasm lines. A total of 256 maize hybrids, comprising 244 top crosses, six diallel cross hybrids, and six checks, were grown under *Striga* infestation, drought, and optimal conditions, from 2015 to 2017. The study determined the combining abilities of the parental inbreds, classified the inbreds into heterotic groups, and compared the efficiencies of the following four grouping methods for classifying the inbreds: specific combining ability (SCA) effect of grain yield; general combining ability (GCA) effects of multiple traits (HGCAMT); SCA and GCA (HSGCA) for yield; and single nucleotide polymorphism-based genetic distance (SNP-based genetic distance (GD)). Significant GCA and/or SCA mean squares were revealed for most measured traits in all test environments. Sums of squares (SS) due to GCA were higher than SCA SS for measured traits in all test environments. The HSGCA, SCA, and SNP-based GD methods identified four heterotic groups, whereas the HGCAMT identified three groups, in all environments. The additive gene effect was preponderant in the inheritance of most measured traits. The efficiencies of the grouping methods varied with the test environments. The HSGCA and SCA methods were the most efficient for grouping in all test conditions. For practical breeding purposes, the HGCAMT and HSGCA methods were recommended under *Striga* infestation and drought, respectively. The heterotic patterns, which were revealed in this study, were effective for planning hybridization schemes for developing high-yielding, *Striga*-tolerant/resistant, and drought-tolerant maize hybrids for stressful environments.

Keywords: breeding efficiency; cluster; combining ability; genetics; maize germplasm; hybrid; inbred

1. Introduction

The development of hybrid maize for the exploitation of grain yield heterosis has contributed to making maize (*Zea mays* L.) a crop of economic importance in sub-Saharan Africa (SSA), satisfying the growing requirement for a nutritional crop for the people in the subregion. Identification of distinct heterotic groups should help the development of outstanding hybrids and improve the efficiency of a hybrid development program. Several biotic and abiotic stress factors including drought and infestation by *Striga hermonthica*, a parasitic weed, have constrained maize cultivation in SSA. Concerted research efforts are continually required to identify parental resources for the development of hybrids adapted to areas experiencing the stresses.

Rainfall is perhaps the single most important climatic factor influencing agricultural production [1]. The effect of drought on plants is multidimensional, and crops are affected to varying extents depending on the stage of the life cycle during which drought occurs. According to Adebayo and Menkir [2], drought causes as high as 15 and 17% reductions in annual grain yield, respectively, in West and Central Africa and the tropics. Drought occurrence when the maize plant is most responsive has caused up to 90% reduction in grain yield [3].

Heisey and Edmeades [4] proposed the following two approaches to control the effects of drought on maize: (i) changing the maize plant through genetic improvement and (ii) changing the maize plants' environment. Genetic improvement of maize for drought tolerance can be achieved by breeding maize for drought escape through early maturity [5], or by breeding maize varieties that tolerate drought stress. A drought escape mechanism was the basis for earlier successes in the development of maize for dry areas. For instance, the R200 hybrid series of Zimbabwe [6] and the Katumani varieties of Kenya [7] were improved for drought tolerance through a drought escape mechanism. According to Heisey and Edmeades [4], drought escape through early maturity does not imply that a variety can tolerate erratic drought stress which occurs during a "short" season. Hence, there is a need to identify useful sources of genes to improve drought tolerance in tropical maize breeding programs using appropriate strategies. Substantial progress has been made in breeding subtropical and tropical maize for tolerance to drought. Over the decades, CIMMYT has established a maize breeding program that utilizes full-sib recurrent selection to increase grain yield and flowering synchronization with a recorded grain yield increase of up to 144 kg/ha/yr under drought stress [8]. To complement the efforts of the IITA maize program, the West and Central Africa Collaborative Maize Research Network (WECAMAN) began a *Striga* tolerance breeding program in Côte d'Ivoire, in 1994. The program aimed to develop maize germplasm that combined earliness and tolerance or resistance to the maize streak virus, *Striga* and drought [9].

Xu et al. [10] attributed the progress in crop improvement for drought tolerance to the use of appropriate test environments. Recent incessant climate changes have necessitated the simulation of drought environments to test maize candidate varieties for tolerance to drought. Campos et al. [11] described a managed drought stress environment as an environment with the severity and timing of drought controlled to depict natural environmental conditions. To simulate drought, good water management is required to maximize the expression of genetic variability for key drought tolerance traits to identify superior genotypes [12].

S. hermonthica is a parasitic weed that attacks maize, sorghum, and other cereal crops, and it constitutes the most important single biotic factor limiting crop production and productivity in SSA [13], where the majority of the diets are cereal-based. The prevalence of the weed in areas with the highest maize yield potentials, such as the lowland savannah and mid-altitude agro-ecologies, imposes severe limitations on productivity of the crop [14]. Depending on the level of *Striga* infestation, as much as 90% reduction in maize grain yield has been recorded, and farmers have reportedly abandoned their fields [13]. *Striga* control options are available but none of the methods can achieve total control of the parasite, warranting the use of an integrated management system [14]. The use of improved genotypes with inherent levels of resistance/tolerance to *Striga* has been recommended as the most practicable approach for minimizing yield reductions due to *Striga* infestation in SSA [15].

It is essential to have knowledge of the extent and pattern of genetic diversity among germplasm lines for identifying and selecting parents to develop heterotic F_1 hybrids and to identify sources of useful alleles for introgressive hybridization [16]. Information on the type of gene action controlling yield and related traits under stress is key for identifying useful parents and hybrids, as well as for designing suitable strategies to breed multiple stress-tolerant hybrids. This information is available through combining ability studies conducted by several researchers [17–20], who have reported conflicting observations. The inconsistencies observed in gene action which modulates the inheritance of grain yield and other agronomic traits of tropical maize have necessitated studies to establish the

type of gene action that controls grain yield and related traits in the set of elite maize inbreds used in the present study.

Shull [21] was the first to report heterosis in maize and Meena et al. [22] described the concept as the superiority of a progeny over the performance of its parents [23]. Stuber [24] credited the successes recorded in the commercial hybrid seed industry of many crops to the exploitation of heterosis. Fan et al. [25] described a "heterotic groupas" a group of genotypes, related or otherwise, that displayed similar combining ability and heterotic response upon crossing with genotypes from another distinct heterotic group. According to Lee [26], a heterotic group is a collection of germplasm exhibiting higher heterosis in combination with germplasm from an external group than when crossed with a member of its own group. Contrarily, a heterotic pattern is a specific pair of heterotic groups expressing high heterosis and hybrid performance upon crossing.

To satisfy the increasing demand for maize in SSA, there is a need to identify inbred combinations for the exploitation of heterosis for increased grain yield to enhance food availability in the subregion. According to Hallauer et al. [27], the temperate germplasm is more clearly classified into heterotic groups than tropical germplasm. Therefore, Wen et al. [28] proposed introgression and the use of distinct heterotic patterns to increase heterosis in tropical germplasm.

A heterotic group classification method is either quantitative or molecular based [29,30]. On the one hand, quantitative methods of classification, such as HGCAMT [31], HSGCA, and SCA [30] methods, employ combining ability estimates of pedigree lines and hybrid field data to classify inbreds. On the other hand, the molecular-based techniques classify lines based on genetic distance (GD) or genetic similarity (GS) from molecular markers [32] which is very useful for describing heterotic groups and studying associations among inbreds at the molecular level [33]. Results from both quantitative and molecular approaches vary depending on the environment, the test material or molecular technique used. For instance, Shieh and Thseng [34] and Benchimol et al. [35] found GDs unsuitable for predicting F1 hybrid performance or heterosis value of crosses from lines from genetically different heterotic groups. Lanza et al. [36] and Balestre et al. [37], on the contrary, reported the efficiency of the molecular marker-based approach in assigning maize inbreds into heterotic groups, and observed useful associations between hybrid yield and genetic distance. Menkir et al. [38] and Barata and Carena [39] recommended a molecular-based approach as an important preliminary tool in field evaluations for combining ability studies to create distinct heterotic groups having higher within-group than between-group genetic similarities.

An important demerit of the SCA method grouping is the significant influence of the interaction between two parents, as well as between genotype and environment, leading to assignment of the same lines into different heterotic groups in different studies [29,40]. To address the shortcomings of the SCA approach, Fan et al. [30] proposed the HSGCA method, which employed SCA and GCA effects of grain yield; both methods utilized grain yield data to assign inbred lines into heterotic groups. Contrarily, the HGCAMT method [31] utilizes significant GCA effects of multiple traits.

The efficiencies of various methods in classifying germplasm lines into heterotic groups have been studied for various crops. Liu et al. [41] evaluated the efficiencies of agronomic performance per se, GCA effects of agronomic traits, and GD from RAPD markers for classifying wheat lines. They observed higher average heterosis between groups defined by RAPD markers and other methods and recommended molecular markers as the first choice for defining heterotic groups in wheat. Akinwale et al. [42], Badu-Apraku et al. [43], Amegbor et al. [44] and Olayiwola [45] compared the efficiencies of different grouping methods for assigning maize germplasm lines into heterotic groups and found the HSGCA to be the most efficient method across contrasting environments. In contrast, Badu-Apraku et al. [33] observed the superiority of the HGCAMT method over the HSGCA and SNP-based GD methods, for defining heterotic groups among early-maturing yellow maize inbreds.

Fan et al. [30] and Badu-Apraku et al. [43] detailed the procedures for comparing the efficiencies of heterotic grouping methods, and these approaches have been employed by several workers to investigate the grouping of maize germplasm lines [42,44,46–48]. In addition, Akinwale et al. [42] used orthogonal contrasts to compare the efficiencies of SCA, HSGCA, and SSR-based GD methods for grouping early maturing yellow maize inbreds evaluated under *Striga*-infested and *Striga*-free growing conditions. They observed that the HSGCA method was the most efficient under the research conditions.

Discriminant analysis is used to develop a set of discriminant functions that are linear combinations of independent variables for discriminating among the categories of a dependent variable. This statistical tool is useful in assessing the accuracy of assigning members into known groups. With this approach, germplasm lines that have been misclassified can also be identified and reclassified appropriately, enhancing the separation of less-related individuals. Therefore, the chances of obtaining effective heterotic patterns within germplasm lines are enhanced. However, the use of discriminant analysis to compare the efficiencies of heterotic grouping methods is novel and, so far, has not been used in the classification of tropical maize inbreds.

During the past decade, the IITA Maize Improvement Program (IITA-MIP) has developed several early maturing yellow endosperm inbred lines with combined tolerance to drought and low soil nitrogen, as well as resistance to *Striga hermonthica*. However, inbreds in the newly developed panel of inbreds have not been classified into heterotic groups to facilitate and maximize their use in hybrid breeding programs in the tropics. The present study was conducted with the following aims: (i) to estimate the combining abilities of grain yield and other agronomic traits of selected IITA early-maturing yellow maize inbreds under drought, *Striga* infestation, and optimal growing environments; (ii) to classify the parental lines into heterotic groups using the HGCAMT, HSGCA, SCA, and GD from SNP markers; and (iii) to compare the efficiencies of the four grouping methods.

2. Materials and Methods

2.1. Description of Trial Environments

This study was conducted at the following four locations: Ikenne (6°87′ N 3°7′ E, 60 m a.s.l.), Kadawa (12°00′ N 8°22′ E, 580 m a.s.l.), Mokwa (9°18′ N 5°4′ E, 457 m a.s.l.), and Abuja (9°16′ N 7°20′ E, 300 m a.s.l.), in Nigeria. Ikenne is a test environment for the management of induced drought stress, whereas Kadawa is a terminal drought-prone site during the rainy season. Mokwa and Abuja are routinely used by the Maize Improvement Program of IITA to evaluate maize genotypes for reaction to *Striga* infestation.

2.2. Genetic Materials

A set of 61 early-maturing (90–95 days to physiological maturity) yellow maize inbreds (Table 1) were selected based on preliminary evaluation (data not presented) under drought and *Striga* infestation, at Ikenne (in 2014) and Mokwa (in 2015), respectively. The lines (as females) were crossed with four inbred testers (as males) to generate 244 top crosses. The testers were intermated to generate six diallel crosses. A total of 256 hybrids comprising the 244 top crosses, six diallel crosses, and six commercial hybrid checks (TZEI 124 × TZEI 25, TZEI 24 × TZEI 17, TZEI 11 × TZEI 25, TZE-Y Pop DT STR × TZEI 13, and TZE-Y Pop DT STR × TZEI 17) were evaluated in this study.

Table 1. Characteristics of inbred lines used in the study.

ID	Designation	Reaction to Drought	Reaction to *Striga*	ID	Designation	Reaction to Drought	Reaction to *Striga*
G1	ENT 13	Tolerant	Resistant	G34	TZEI 560	Susceptible	-
G2	TZEI 10	-	Resistant	G35	TZEI 561	Tolerant	-
G3	TZEI 124	-	-	G36	TZEI 562	Tolerant	-
G4	TZEI 129	Susceptible	-	G37	TZEI 563	Tolerant	-
G5	TZEI 23	Susceptible	Tolerant	G38	TZEI 567	Susceptible	-
G6	TZEI 25	-	-	G39	TZEI 571	Tolerant	-
G7	TZEI 416	-	Susceptible	G40	TZEI 572	Tolerant	-
G8	TZEI 422	-	Resistant	G41	TZEI 574	Tolerant	-
G9	TZEI 426	-	Susceptible	G42	TZEI 576	Tolerant	-
G10	TZEI 447	-	Susceptible	G43	TZEI 578	Tolerant	-
G11	TZEI 448	-	Resistant	G44	TZEI 582	Tolerant	-
G12	TZEI 459	-	Resistant	G45	TZEI 584	Tolerant	-
G13	TZEI 466	-	Susceptible	G46	TZEI 585	Tolerant	-
G14	TZEI 492	-	Resistant	G47	TZEI 586	Tolerant	-
G15	TZEI 496	-	Resistant	G48	TZEI 587	Tolerant	-
G16	TZEI 502	-	Resistant	G49	TZEI 594	Tolerant	-
G17	TZEI 503	-	Resistant	G50	TZEI 595	Susceptible	-
G18	TZEI 511	-	Resistant	G51	TZEI 597	Tolerant	-
G19	TZEI 517	-	Resistant	G52	TZEI 598	Tolerant	-
G20	TZEI 519	-	Susceptible	G53	TZEI 599	Tolerant	-
G21	TZEI 534	Tolerant	-	G54	TZEI 600	Tolerant	-
G22	TZEI 539	Tolerant	-	G55	TZEI 601	Tolerant	-
G23	TZEI 540	Tolerant	-	G56	TZEI 602	Tolerant	-
G24	TZEI 544	Tolerant	-	G57	TZEI 603	Tolerant	-
G25	TZEI 547	Tolerant	-	G58	TZEI 604	Tolerant	-
G26	TZEI 549	Susceptible	-	G59	TZEI 608	Tolerant	-
G27	TZEI 550	Tolerant	-	G60	TZEI 609	Tolerant	-
G28	TZEI 551	Tolerant	-	G61	TZEI 610	Tolerant	-
G29	TZEI 552	Tolerant	-	G62	TZEI 615	Tolerant	-
G30	TZEI 554	Tolerant	-	G63	TZEI 617	Tolerant	-
G31	TZEI 557	Susceptible	-	G64	TZEI 619	Tolerant	-
G32	TZEI 558	Tolerant	-	G65	TZEI 620	Tolerant	-
G33	TZEI 559	Tolerant	-				

2.3. Field Evaluation

The 256 hybrids were evaluated for their performance under drought at Ikenne in 2015 and Kadawa in 2017, *Striga*-infested conditions at Mokwa in 2016 and 2017 and Abuja in 2016, and optimal conditions at Ikenne in 2016 and 2017, as well as Mokwa and Abuja in 2016. Crop management practices were similar in all environments except for the imposition of stress treatments. An alpha-lattice design such as the randomized incomplete block design (RIBD) is more appropriate for heterogeneous conditions characteristic of tropical soils, and thus more efficient than the randomized complete block design for discriminating among large numbers of test materials. There was a large number of hybrids and an alpha-lattice design was, therefore, considered to be the most appropriate for this experiment. Thus, a 16 × 16 RIBD with two replications was used for this study. Each replicate was divided into 16 balanced blocks, each containing 16 entries. This minimized variation within blocks while maximizing variation among blocks and was expected to reduce the experimental error. Each experimental unit was a 3 m single-row plot, spaced 0.75 m apart. Three seeds were sown per hole at a spacing of 0.4 m and thinned to two plants per stand at two weeks after planting, to obtain a final population density of 66,667 plants per hectare.

2.3.1. Management of Drought and Optimal Environments

At Ikenne, under managed drought stress, plants were provided with water twice a week via a sprinkler irrigation system that supplied 17 mm of water weekly. Irrigation water was supplied from sowing, until 25 days after sowing (DAS). To achieve managed drought, irrigation water was withdrawn at 25 DAS, and therefore the plants depended on stored water in the soil for growth and development.

Additionally, under the managed drought, 60 kg N per hectare of NPK 15:15:15, a compound fertilizer, was applied at the time of sowing and top-dressed with an additional 60 kg N per hectare of urea at three weeks after sowing (WAS). Under the optimal environment, the same rates of NPK and urea fertilizers were applied at 3 and 5 WAS, respectively. Weeds were controlled using a blend of 5 L per hectare of atrazine and paraquat, and subsequently by hand-weeding.

2.3.2. Management of *Striga*-Infested Fields

Mature *S. hermonthica* seeds were collected and prepared as described by Ifie [49]. Suicidal germination of existing *Striga* seeds in the field was stimulated, and artificial infestation of the soil with the prepared seeds was done according to the procedure of Kim [50]. The rate of NPK 15:15:15 fertilizer was reduced, and application delayed until about 30 days after planting when 30 kg per hectare was applied. The delayed fertilizer application was to impose nutritional stress on the plants such that when the fertilizer was applied, the plants would produce strigolactone which stimulates the germination of *Striga* seeds. Weeds, other than *Striga*, were hand-weeded regularly, subject to field inspection.

2.3.3. DNA Extraction and Genotyping

Leaf samples were taken from about 10 plants per inbred, at 2 weeks after planting. Bulked leaf samples were lyophilized at the Bioscience Centre, IITA, Ibadan, Nigeria. Extraction of genomic DNA from the lyophilized leaf samples was done at the Molecular Breeding Laboratory, CIMMYT, Mexico using the CIMMYT protocol available online: (http://www.generationcp.org/capcorner/chile_wksp_2005/manuals/manual_01.pdf). Genotyping-by-sequencing (GBS) was done using the protocol of Elshire et al. [51]. A sequencing library was prepared according to the GBS protocol using the restriction enzyme ApeKI. Calling of the GBS library reads was done in TASSEL 3.0 [52], followed by filtering in Burrows–Wheeler alignment tool [53]. Aligning the obtained sequences to the maize reference genome B73 RefGen v1 produced 51,009 SNPs distributed across the 10 maize chromosomes. Approximately 9% (4841) of the SNP loci had minor allelic frequency above 0.05 without missing data and were selected using TASSEL 5.0. The 4841 SNPs were utilized for the genetic diversity analysis of the 65 inbred lines. Estimates of Rogers' [54] genetic distance among pairs of inbreds were obtained via PowerMarker version 3.25 [55].

2.4. Data Collection

In all growing conditions, data collection began at flowering. Agronomic observations and their mode of determination are presented in Table 2. Field-dry harvested ears from each plot were shelled manually and grain moisture determined using Kett grain moisture tester PM-450. Under drought stress at Ikenne, all ears were shelled using a machine sheller and grain weight (kg) per plot was determined using a sensitive scale. In other environments, the field weight of the ears in each plot was determined using a spring balance. A shelling percentage of 80 was assumed for all hybrids, for determination of grain weight. In both cases, grain weight obtained per plot was adjusted to 15% moisture content and converted to grain yield (kg) per hectare using the following equation:

$$GY = GWT \times \left(\frac{100 - m}{85}\right) \times \frac{10000}{A} \quad (1)$$

where GY = grain yield in kg/ha, GWT = grain yield at harvest moisture content per plot, m = grain moisture content at harvest, and A = plot area.

Table 2. Plant observations and their mode of determination for 256 maize hybrids from 2015 to 2017, under individual and across environments.

S/N	Observation	Mode of Determination
1	Anthesis-silking interval (ASI)	Positive difference between DYS and DYA.
2	Number of ears per plant (EPP)	Dividing the total number of ears per plot by the number of plants harvested.
3	Ear aspect (EASP)	Composite trait that assesses the general appeal of the ears encompassing ear size, uniformity of size, color and texture, extent of grain filling, insect and disease damage, rated on a scale of 1–9 where, 1 = clean, uniform, large, and well-filled ears and 9 = only one or no ears or ears with undesirable features such as diseases, small ears, and ears with poorly filled grains [18,31].
Specific to Optimal Environment		
4	Plant aspect (PASP)	Rated on a scale of 1–9, where 1 = excellent and 9 = poor [18,31].
Specific to Drought Environment		
5	Stay-green character (otherwise called leaf death)	Plants in each experimental unit were rated together at 70 DAS, on a scale of 1–9 where 1 = 0–10% dead leaf area taken upwards from the base of the plant and 9 = 90–100% dead leaf area [31].
Specific to *Striga*-Infested Environment		
6	Host-plant damage syndrome rating	Rated at 10 WAS, on a scale of 1–9 where 1 = no damage, indicating normal plant growth, and 9 = complete collapse or death of the maize plant [48].
7	Number of emerged *Striga* plants	Physical counting of the number of emerged *Striga* plants associated with plants in an experimental unit taken at 10 WAS [56].

WAS, weeks after sowing; DYS, number of days to silking; DYA, number of days to anthesis; DAS, days after sowing.

2.5. Data Analyses

Environment-specific analysis of variance (ANOVA) was performed for hybrid data collected under each of the following three research conditions: *Striga*-infested, drought, and optimal. Data on traits common to the three research conditions were pooled for combined ANOVA across environments. Analysis of variance was performed using PROC GLM in SAS [57] using a RANDOM statement with the TEST option [57] where block nested within replicate × environments, replicate within environments, and environments were random effects while genotypes were fixed effects. Means obtained from each ANOVA procedure were used for the line × tester analysis, as described by Singh and Chaudhary [58]. General (GCA) and specific combining ability (SCA) were computed for grain yield and other measured traits under each research condition, via SAS [57]. For each trait, hybrid mean squares were partitioned into line, tester, and line × tester components, whereas the hybrid × environment was partitioned into line × environment, tester × environment, and line × tester × environment. The GCA effect of each of the four testers was obtained based on its performance in hybrid combinations with all 61 lines (females). Similarly, the GCA effect of each line was determined based on the performance in combination with the four testers. The effects of both GCA and SCA were determined for each trait under each growing condition and across research environments. The general linear model for line × tester mating design is:

$$Y_{ijbkl} = \mu + \alpha_l + \beta_{b(k)} + \Gamma_{k(l)} + g_i + g_j + s_{ij} + (\alpha v)_{ijl} + \varepsilon_{ijbkl} \quad (2)$$

where Y_{ijbkl} = observed value of the progeny between the ith line and the jth tester in the bth block within the kth replication in the lth environment, μ = population mean, α_l = average effect due to environment, $\delta_{b(k)}$ = effect of block nested within replication, $\Gamma_{k(l)}$ = effect of replication nested within environment, g_i = general combining ability (GCA) of the ith line, g_j = general combining ability (GCA) effect of the jth tester, s_{ij} = specific combining ability (SCA) of the ijth testcross, $(\alpha v)_{ijl}$ = effect of interaction between the ijth testcross and lth location, and ε_{ijbkl} = residual effect. The relative importance of GCA and SCA sums of squares for measured traits were determined according to the method proposed by Baker [59].

The inbred lines were classified into heterotic groups under *Striga*-infested and drought conditions and across research environments based on SCA for grain yield, HGCAMT, HSGCA, and SNP-based GD. The HSGCA values were estimated as HSGCA = Cross mean (X_{ij}) + Tester mean (X_i) = GCA + SCA [42,60], where X_{ij} is the mean yield of the cross between ith tester and jth line, Xj is the mean yield of the ith tester. Grouping by HGCAMT was achieved by standardizing the GCA effects (mean of zero and standard deviation of 1) of the traits that had significant genotype mean squares under *Striga*-infested, drought, and combined environments, to minimize the effects of different scales of the traits. The assignment of inbreds into heterotic groups by the HGCAMT followed the statistical model:

$$Y = \sum_{i=1}^{n} \left[\frac{(Gi - gi)}{s} \right] + \varepsilon ij \quad (3)$$

where Y is HGCAMT, which is the genetic value measuring relationship among genotypes based on the GCA of multiple traits i to n; G is the individual GCA effects of genotypes for trait I; g 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. The SNP data and HSGCA values, as well as the standardized GCA effects of HGCAMT, were converted to Euclidean distance estimates using PROC DISTANCE in SAS [57]. Then, the Euclidean distance estimates were subjected to Ward's minimum variance cluster analysis, also in SAS [57]. For HSGCA, HGCAMT, and SNP-based GD methods, the inbreds were assigned to clusters at 65% level of dissimilarity (R^2 = 65%) under *Striga*-infested environment and 55% level of dissimilarity (R^2 = 55%) under drought and combined environments). Since the inbred testers used in the study were of known, and different, heterotic groups, the assignment of the inbreds into heterotic groups based on the SCA effects of their hybrids was done using the procedure of Fan et al. [30] as follows:

Step 1 All inbreds that formed hybrids with negative SCA effects on crossing with a tester were assigned to the heterotic group of that tester, whereas inbreds with hybrids that had positive SCA effects with all the testers were regarded as "not classified" since they belonged to an unknown heterotic group;

Step 2 Since some inbreds were found to belong to two or more heterotic groups, the values of the SCA effects with the different testers were considered, and the inbreds were retained in the group for which the SCA effect was least (highest negative).

The following four methods were used to assess and compare the efficiencies of the grouping methods for classifying the inbreds: (1) ANOVA with cluster as a source of variation rather than genotype [33], (2) T-test pairwise comparison of the mean grain yield of all possible pairs of clusters defined by each grouping method, (3) classification error rate from discriminant analysis, and (4) a modified version of the procedure described by [30,43].Thus, the 244 top crosses were arranged from the highest to the lowest based on mean grain yield, and divided into yield groups 1, 2, and 3 (YG1, YG2 and YG3), where YG1 represented hybrids with the highest grain yields and YG3 were hybrids with the lowest grain yields. The range of observed grain yields was determined as the arithmetic difference between the highest and the lowest yields. Thereafter, the range was divided into three equal portions and added twice, cumulatively to the minimum yield value. The first addition created the upper boundary for YG3, while adding the second addition created the lower boundary for YG1.

Hybrids between boundaries automatically fell into YG2. Hybrids in each group were further divided into inter- and within-group crosses to compute breeding efficiencies as the percentage of inter-group crosses that belonged to YG1. Lastly, the breeding efficiencies of the four grouping methods were estimated by subtracting the classification error count (the percent estimate of misclassified inbred lines in clusters) from unity (i.e., 100%). Error counts estimates were obtained from the discriminant analysis using SAS "proc discrim" [57].

3. Results

3.1. Performance of Hybrids under Striga-Infested, Drought, and Combined Research Environments

Analysis of variance (ANOVA) revealed significant ($p < 0.05/0.01$) mean squares (MS) of hybrid (G), environment (E), and hybrid × environment interaction (G × E) for measured traits under *Striga*-infested, drought, and the combined environments except E and G × E for ear aspect (EASP) under *Striga* and G for the number of ears per plant (EPP) under optimal environments. The line × tester analysis used to decompose the G effects into its GCA_{Line}, GCA_{Tester}, and SCA components, and the G×E into GCA_{Line} × E, GCA_{Tester} × E, and SCA × E components showed significant ($p < 0.05/0.01$) GCA and SCA mean squares for all measured traits under *Striga*-infested, drought, and combined environments with the exception of GCA_{Line} for yield, anthesis-silking interval (ASI), EASP, and EPP under *Striga*, EPP in all environments, GCA_{Tester} for yield, EPP, and EASP under *Striga*, yield, ASI and EASP under drought, ASI and EPP under optimal, EPP under combined environments, and SCA for EASP under *Striga*, ASI under optimal, and EPP in all environments. In individual and combined environments, significant ($p < 0.05/0.01$) GCA_{Line} × E, and SCA×E mean squares were observed for all traits except GCA_{Line} × E for EASP under *Striga*, ASI under optimal, and EPP in all environments. There were significant ($p < 0.05/0.01$) GCA_{Tester} × E for measured traits in all environments except for ASI and EASP under *Striga*, and EPP under drought. In addition, significant ($p < 0.05/0.01$) SCA × E mean squares were observed for measured traits except for EASP under *Striga* and drought, EPP under drought and optimal, and ASI under combined environments (Tables 3 and 4). The percent contributions of GCA_{Tester} sums of squares in total variation among the hybrids were consistently higher than those of GCA_{Line} and SCA for measured traits in all research environments, and ranged from ≈71 (for EASP), 75 (for ASI), 75 (for EPP), and 78 (for EPP) to 96 (for number of emerged *Striga* plants (ESC)), 93 (for EASP), 99 (for EASP), and 97 (for grain yield (GY)) under *Striga*, drought, optimal, and combined environments, respectively (Table 5). Estimates of GCA effects for GY and other measured traits under *Striga*, drought, optimal, and combined environment revealed significant GCA effects for measured traits in all environments (Tables S1 and S2). With respect to GY, there were significant positive GCA effects for inbreds G47, G43, G51, and G7 under *Striga* while inbreds G10, G37, G50, G58, and G60 showed positive and significant estimates of GCA effects under drought. Inbreds G1, G3, G24, G41, G45, G46, G53, and G54 showed positive and significant estimates of GCA effects under optimal environment. Significant negative GCA effect estimates were observed for inbred G19 for ASI under drought and optimal environments, whereas G6, G10, G12, and G55 showed significant negative GCA effect estimates under drought. Inbred G5 had negative and significant GCA effect estimates for ASI under *Striga*-infested conditions.

Table 3. Mean squares from the line × tester analysis of variance of maize topcrosses evaluated under *Striga*-infested and drought environments.

SOV	DF	Yield (× 10^5)	ASI	EPP	EASP	SDR	ESC	STGR
				***Striga*-Infested Environment**				
Blk (Rep × E)	90	30.74 ($p < 0.01$)	2.52 ($p < 0.01$)	0.02 ($p < 0.05$)	2.90 (NS)	0.64 ($p < 0.01$)	71.11 ($p < 0.01$)	-
Rep (E)	3	39.20 ($p < 0.05$)	4.92 ($p < 0.05$)	0.09 ($p < 0.01$)	4.73 (NS)	1.87 ($p < 0.01$)	831.03 ($p < 0.01$)	-
Environment	2	6784.15 ($p < 0.01$)	189.04 ($p < 0.01$)	1.00 ($p < 0.01$)	147.22 ($p < 0.01$)	1997.14 ($p < 0.01$)	18216.81 ($p < 0.01$)	-
Hybrid	243	23.45 ($p < 0.01$)	3.16 ($p < 0.01$)	0.03 ($p < 0.01$)	2.57 (NS)	1.17 ($p < 0.01$)	84.85 ($p < 0.01$)	-
Hybrid × E	486	18.56 ($p < 0.01$)	2.44 ($p < 0.01$)	0.03 ($p < 0.01$)	2.76 (NS)	1.10 ($p < 0.01$)	79.44 ($p < 0.01$)	-
GCA_{Line}	60	31.03 (NS)	3.87 (NS)	0.03 (NS)	2.80 (NS)	1.03 ($p < 0.01$)	94.28 ($p < 0.01$)	-
GCA_{Tester}	3	110.45 (NS)	4.09 ($p < 0.05$)	0.21 (NS)	3.32 (NS)	9.92 ($p < 0.01$)	1339.13 ($p < 0.01$)	-
GCA_{Line} × E	120	24.94 ($p < 0.05$)	3.18 ($p < 0.01$)	0.03 ($p < 0.01$)	2.98 (NS)	0.79 ($p < 0.01$)	92.35 ($p < 0.01$)	-
GCA_{Tester} × E	6	75.62 ($p < 0.01$)	1.14 (NS)	0.19 ($p < 0.01$)	3.32 (NS)	6.70 ($p < 0.01$)	522.53 ($p < 0.01$)	-
SCA	180	19.29 ($p < 0.01$)	2.88 ($p < 0.05$)	0.03 (NS)	2.45 (NS)	1.06 ($p < 0.01$)	59.06 ($p < 0.01$)	-
SCA × E	360	15.14 ($p < 0.01$)	2.30 ($p < 0.01$)	0.03 ($p < 0.01$)	2.64 (NS)	1.07 ($p < 0.01$)	67.16 ($p < 0.01$)	-
Error	639	10.83	0.89	0.01	2.41	0.31	31.57	-
				Drought Environment				
Blk (Rep × E)	60	32.39 ($p < 0.01$)	1.54 ($p < 0.01$)	0.14 (NS)	2.60 ($p < 0.01$)	-	-	1.82 ($p < 0.01$)
Rep (E)	2	11.90 (NS)	11.98 ($p < 0.01$)	0.22 (NS)	13.65 ($p < 0.01$)	-	-	5.77 ($p < 0.01$)
Environment	1	7193.95 ($p < 0.01$)	291.89 ($p < 0.01$)	19.20 ($p < 0.01$)	228.52 ($p < 0.01$)	-	-	874.31 ($p < 0.01$)
Hybrid	243	14.39 ($p < 0.01$)	1.69 ($p < 0.01$)	0.14 ($p < 0.01$)	1.22 ($p < 0.01$)	-	-	0.98 ($p < 0.01$)
Hybrid × E	486	11.93 ($p < 0.01$)	1.16 ($p < 0.01$)	0.13 ($p < 0.01$)	0.96 ($p < 0.01$)	-	-	0.90 ($p < 0.01$)
GCA_{Line}	60	20.23 ($p < 0.05$)	2.44 ($p < 0.01$)	0.14 (NS)	1.16 ($p < 0.01$)	-	-	1.37 ($p < 0.01$)
GCA_{Tester}	3	72.12 (NS)	1.70 (NS)	0.33 ($p < 0.05$)	12.69 (NS)	-	-	0.89 (NS)
GCA_{Line} × E	60	11.60 ($p < 0.01$)	1.28 ($p < 0.01$)	0.11 (NS)	0.90 (NS)	-	-	1.14 ($p < 0.01$)
GCA_{Tester} × E	3	191.05 ($p < 0.01$)	2.74 ($p < 0.01$)	0.20 (NS)	15.02 ($p < 0.01$)	-	-	6.12 ($p < 0.01$)
SCA	180	11.42 ($p < 0.05$)	1.40 ($p < 0.05$)	0.14 (NS)	1.05 ($p < 0.01$)	-	-	0.83($p < 0.05$)
SCA × E	180	8.91 ($p < 0.05$)	1.06 ($p < 0.05$)	0.13 (NS)	0.72 (NS)	-	-	0.71 ($p < 0.01$)
Error	425	6.91	0.84	0.12	0.71	-	-	0.42

SOV, source of variation; ns, not significant; GCA_{Line}, general combining ability due to line; GCA_{Tester}, general combining ability due to tester; SCA, specific combining ability; Blk, block; Rep, replication; E, Environment; ASI, anthesis-silking interval; PASP, plant aspect; EPP, number of ears per plant; EASP, ear aspect; SDR, *Striga* damage rating; ESC, emerged *Striga* count; STGR, stay-green character.

Table 4. Mean squares from line × tester analysis of variance of maize topcrosses evaluated under optimal and combined growing environments.

SOV	DF	Yield ($\times 10^5$)	ASI	PASP	EPP	EASP
Optimal Environment						
Blk (Rep × E)	120	36.25 ($p < 0.01$)	1.92 (NS)	0.64 ($p < 0.01$)	0.09 (NS)	0.59 ($p < 0.01$)
Rep (E)	4	715.16 ($p < 0.01$)	1.03 (NS)	1.62 ($p < 0.01$)	0.10 (NS)	1.97 ($p < 0.01$)
Environment	3	8148.05 ($p < 0.01$)	641.12 ($p < 0.01$)	39.80 ($p < 0.01$)	2.72 ($p < 0.01$)	82.02 ($p < 0.01$)
Hybrid	243	53.84 ($p < 0.01$)	2.87 ($p < 0.01$)	1.23 ($p < 0.01$)	0.08 (NS)	1.88 ($p < 0.01$)
Hybrid × E	486	15.72 ($p < 0.01$)	2.24 ($p < 0.01$)	0.50 ($p < 0.01$)	0.09 ($p < 0.01$)	0.55 ($p < 0.01$)
GCA_{Line}	60	79.98 ($p < 0.01$)	4.32 ($p < 0.01$)	1.39 ($p < 0.01$)	0.06 (NS)	3.02 ($p < 0.01$)
GCA_{Tester}	3	1331.17 ($p < 0.01$)	15.99 (NS)	35.61 ($p < 0.01$)	0.18 (NS)	47.08 ($p < 0.01$)
GCA_{Line} × E	180	17.33 ($p < 0.01$)	2.29 ($p < 0.05$)	0.50 ($p < 0.01$)	0.08 (NS)	0.70 ($p < 0.01$)
GCA_{Tester} × E	9	77.79 ($p < 0.01$)	5.04 ($p < 0.01$)	2.59 ($p < 0.01$)	0.19 ($p < 0.01$)	3.48 ($p < 0.01$)
SCA	180	22.57 ($p < 0.01$)	2.11 (NS)	0.59 ($p < 0.05$)	0.08 (NS)	0.71 ($p < 0.01$)
SCA × E	540	13.90 ($p < 0.01$)	2.20 ($p < 0.01$)	0.46 ($p < 0.01$)	0.09 (NS)	0.44 ($p < 0.05$)
Error	852	10.04	1.81	0.33	0.08	0.37
Combined Environment						
Blk (Rep × E)	270	33.55 ($p < 0.01$)	2.02 ($p < 0.01$)	0.90 ($p < 0.01$)	0.08 ($p < 0.05$)	1.80 ($p < 0.01$)
Rep (E)	9	333.57 ($p < 0.01$)	4.55 ($p < 0.01$)	2.62 ($p < 0.01$)	0.12 (NS)	5.48 ($p < 0.01$)
Environment	8	6744.69 ($p < 0.01$)	413.28 ($p < 0.01$)	1064.49 ($p < 0.01$)	4.60 ($p < 0.01$)	107.60 ($p < 0.01$)
Hybrid	243	51.05 ($p < 0.01$)	2.93 ($p < 0.01$)	1.30 ($p < 0.01$)	0.09 ($p < 0.01$)	2.68 ($p < 0.01$)
Hybrid × E	486	17.30 ($p < 0.01$)	2.21 ($p < 0.01$)	0.84 ($p < 0.01$)	0.08 ($p < 0.01$)	1.40 ($p < 0.01$)
GCA_{Line}	60	76.19 ($p < 0.01$)	4.57 ($p < 0.01$)	1.53 ($p < 0.01$)	0.09 (NS)	3.69 ($p < 0.01$)
GCA_{Tester}	3	886.36 ($p < 0.01$)	10.55 (NS)	13.44 (NS)	0.19 (NS)	41.41 ($p < 0.01$)
GCA_{Line} × E	480	21.07 ($p < 0.01$)	2.59 ($p < 0.01$)	0.81 ($p < 0.01$)	0.07 (NS)	1.54 ($p < 0.01$)
GCA_{Tester} × E	24	149.67 ($p < 0.01$)	3.92 ($p < 0.01$)	7.45 ($p < 0.01$)	0.21 ($p < 0.01$)	6.75 ($p < 0.01$)
SCA	180	28.03 ($p < 0.01$)	2.23 ($p < 0.01$)	1.04 ($p < 0.01$)	0.08 (NS)	1.67 ($p < 0.01$)
SCA × E	1440	13.33 ($p < 0.01$)	2.06 (NS)	0.72 ($p < 0.01$)	0.08 ($p < 0.01$)	1.23 ($p < 0.05$)
Error	1917	9.60	1.47	0.34	0.07	1.13

SOV, source of variation; ns, not significant; GCA_{Line}, general combining ability due to line; GCA_{Tester}, general combining ability due to tester; SCA, specific combining ability; Blk, block; Rep, replication; E, Environment; ASI, anthesis-silking interval; PASP, plant aspect; EPP, number of ears per plant; EASP, ear aspect; SDR, *Striga* damage rating; ESC, emerged *Striga* count; STGR, stay-green character.

Table 5. Percentage of GCAs and SCA sums of squares (SS) to total genotypic SS of grain yield and other agronomic traits of maize hybrids under *Striga*-infested, drought, optimal, and combined environments.

Trait	*Striga*-Infested			Drought			Optimal			Combined Environment		
	% of Total SS			% of Total SS			% of Total SS			% of Total SS		
	GCA_L	GCA_T	SCA	GCA_L	GCA_T	SCA	GCA_L	GCA_T	SCA	GCA_L	GCA_T	SCA
Grain yield	19.3	68.7	12	19.49	69.5	11.01	5.58	92.85	1.57	7.69	89.48	2.83
ASI	35.7	37.73	26.57	44.04	30.69	25.27	19.27	71.32	9.41	26.34	60.81	12.85
PASP	-	-	-	-	-	-	3.7	94.73	1.57	9.56	83.95	6.5
EPP	11.11	77.78	11.11	22.95	54.1	22.95	18.75	56.25	25	25	52.78	22.22
EASP	32.67	38.74	28.59	7.79	85.17	7.05	5.94	92.66	1.4	7.89	88.54	3.57
SDR	8.58	82.6	8.83	-	-	-	-	-	-	-	-	-
ESC	6.32	89.73	3.96	-	-	-	-	-	-	-	-	-
STGR	-	-	-	44.34	28.8	26.86	-	-	-	-	-	-

GCA_L, line GCA; GCA_T, tester GCA; ASI, anthesis-silking interval; PASP, plant aspect; EPP, number of ears per plant; EASP, ear aspect; SDR, *Striga* damage rating; ESC, emerged *Striga* count; STGR, stay-green character.

Inbreds G29 and G54 had significant and negative GCA effect estimates for *Striga* damage (SDR) while G2 and G26 had negative and significant GCA effect estimates for ESC. Inbreds G10, G12, and G55 had negative and significant estimates of GCA effects for stay-green characteristic (STGR), whereas inbreds G1, G3, G4, G45, G49, G51, and G54 had negative and significant estimates of GCA effects for plant aspect (PASP) under optimal environments. Negative and significant estimates of GCA effect were observed for inbred G37 for EASP under drought and optimal research growing conditions. The inbreds G47 and G42 had significant and positive GCA effects for EPP under *Striga* and drought, respectively, whereas inbreds G33 and G65 showed positive and significant GCA effects for EPP under optimal × environment.

3.2. Heterotic Grouping of Inbreds and Relationship among Grouping Methods

Table 6 summarizes the groupings of the 65 maize inbred lines based on dendrograms constructed from HGCAMT, HSGCA, SCA, and SNP-based GD grouping methods. Under the individual or combined growing conditions, four distinct heterotic groups were identified by all the methods, except the HGCAMT, which consistently assigned the inbreds into three clear-cut heterotic groups. Grouping using the SCA of GY per se was effective in assigning all the inbreds into heterotic groups in all test environments, except inbred G47 which could not be classified under the combined environments. In the same vein, the HGCAMT method could not classify the inbreds G29, G42, and G49 under drought, and inbred G24 under combined environment. The HSGCA method could not classify inbreds G7, G33, G47, G51, and G53 under *Striga*-infested environment; inbreds G30, G37, G50, and G55 under drought; and inbreds G3, G 24, G30, G46, G49, G51, and G52 under combined environments. The SNP-based GD method could not classify inbreds G8, G14, G16, G20, G38, G40, G50, and G58 under the combined environments.

Comparable patterns were observed in the groupings by the different methods. For instance, under *Striga*, both SCA and HGCAMT methods classified together, all four inbreds (G7, G43, G47, and G51) with significant and positive estimates of GCA for GY in a group, with the exception of G47 which was placed in a different group by the SCA method. In contrast, the HSGCA could not classify three of the inbreds (as earlier mentioned). Under drought, the HGCAMT method classified together in a group, G10, G12, G37, G50, and G60, which were inbreds with significant and positive estimates of GCA for GY, whereas inbreds G9, G20, and G58 with significant and negative GCA were classified together in a different group. There were close associations among the grouping methods in the grouping of the same inbreds together under *Striga* and drought growing conditions. Notably, the SCA and HSGCA methods consistently placed inbreds G11, G15, G16, G19, G20, G28, and G41 in a group, whereas inbreds G6, G9, G18, G36, and G46 were also grouped together; both the SCA and HGCAMT methods consistently grouped inbreds G2 and G51 together, whereas inbreds G32 and G34 were also grouped together. The HGCAMT and HSGCA methods grouped the pair of inbreds G27 and G65 in a group. Groupings using the SCA, HGCAMT, and HSGCA methods placed inbreds G57 and G58, G25 and G44, G22, and G56 in a group while G15, G16, and G19 were also placed together.

The chances of selecting parental genotypes to obtain useful heterotic patterns are increased when genetically diverse genotypes are placed in different heterotic groups. The pairs of inbreds G25 and G44 and inbreds G15 and G19 were consistently classified into different groups under *Striga* infestation, whereas the pairs of inbreds G57 and G58, and G15 and G19 were consistently placed in different groups under drought. The SNP-based GD method also consistently classified the pair of inbreds G15 and G19 into different heterotic groups from G25, G24, and G57.

Table 6. Summary of the classification of maize inbred lines by SCA, HGCAMT, HSGCA, and SNP-GD methods under *Striga*-infested, drought, and combined environments.

Method	Cluster 1	Cluster 2	Cluster 3	Cluster 4
		***Striga*-infested**		
SCA	G1, G3, G10, G12, G24, G29, G33, G35, G37, G39, G45, G59, G60, G64	G4, G8, G26, G27, (G25, G44) ††, G47, G50, G53, G54, (G57, G58) †††	G2, G14, G31, G38, G40, G42, G48, G52, (G22, G56) ††, G61, G62, (G7, G43, G51) †, (G6, G9, G18, G36, G46) Ψ	G5, G13, G17, G21, G23, G30, G32, G34, G49, G55, G63, G65, (G11, G15, G16, G19, G20, G28, G41) ʊ
HGCAMT	G14, G17, G18, G26, G29, G31, G32, G34, G36, G39, G40, G55, (G57, G58, G59) ††, (G22, G56) †††	G4, G3, G8, G9, G12, (G15, G16, G19) ††, G20, G27, G30, G35, G37, G41, G42, G45, G49, G50, G60, G61, G64, G65	G1, G2, G5, G6, G10, G11, G13, G21, G23, G24, G28, G33, G38, G46, G47, G48, G52, G53, G54, G62, G63, (G7, G43, G51) †, (G25, G44) †††	
HSGCA	G3, G10, G12, G14, G24, G29, G35, G37, G39, G45, G59, G60, G64	G31, G38, G40, G42, G43, G48, G61, G62, (G6, G9, G18, G36, G46) Ψ, (G22, G56) †††	G8, G26, G27, G50, G54, (G57, G58) ††, G65, (G25, G44) †††	G13, G17, G21, G23, G30, G32, G34, G49, G52, G55, G63, (G11, G15, G16, G19, G20, G28, G41) ʊ
		Drought		
SCA	G1, G13, G17, G21, G24, G27, G31, G35, G43, G45, G52, G54, G55, (G57, G58) ** ††, G62, G65, (G22, G56) †††	G2, G3, G7, G14, G29, G32, G33, G34, G37, G47, G50, G51, G53, G59, (G6, G9, G18, G36, G46) Ψ	G4, G8, G12, G23, G26, G38, G39, G42, G48, G49, G63, G64	G5, G10, G30, G40, G60, G61, (G11, G15, G16, G19, G20, G28, G41) ʊ, (G25, G44) †††
HGCAMT	G4, G11, G21, G26, G30, G33, G36, G38, G43, G46, G55, G64, (G10, G12, G37, G50, G60) ⫛, (G22, G56) †††	G6, G7, G14, G17, G41, G52, G57, G62, G63, (G9, G20, G58) ε, (G57, G58) ** †††	G1, G2, G5, G3, G8, G13, (G15, G16, G19) ††, G18, G23, G24, G27, G28, G31, G32, G34, G35, G39, G40, G45, G47, G48, G51, G53, G54, G59, G61, G65, (G25, G44) ** †††	
HSGCA	G13, G14, G17, G21, G24, G27, G31, G35, G43, G45, G52, G54, (G57, G58) ** ††, G62, G65, (G22, G56) †††	G3, G7, G29, G32, G33, G47, G51, G53, G59, (G6, G9, G18, G36, G46) Ψ	G8, G12, G23, G26, G34, G38, G39, G42, G48, G49, G63, G64	G10, G40, G60, G61, (G11, G15, G16, G19, G20, G28, G41) ʊ, (G25, G44) †††

Table 6. *Cont.*

Method	Cluster 1	Cluster 2	Cluster 3	Cluster 4
		Combined environment		
SCA	G1, G12, G14, G17, G22, G29, G34, G35, G37, G45, G48, G55, G59, G64	G2, G3, G6, G9, G21, G30, G31, G32, G33, G36, G38, G39, G43, G44, G53, G56, G61, G62	G4, G8, G24, G25, G26, G27, G42, G46, G49, G50, G51, G54, G57, G58, G60, G63, G65	G5, G7, G10, G11, G13, G15, G16, G18, G19, G20, G23, G28, G40, G41, G52
HGCAMT	G1, G4, G3, G7, G10, G13, G16, G23, G30, G33, G37, G42, G43, G45, G46, G47, G49, G50, G51, G53, G54, G60, G63, G64, G65	G2, G6, G11, G21, G22, G25, G26, G27, G28, G29, G31, G32, G34, G35, G36, G39, G40, G41, G44, G48, G52, G55, G56, G57, G58, G59, G62	G5, G8, G9, G12, G14, G15, G17, G18, G19, G20, G38, G61	
HSGCA	G12, G14, G17, G22, G29, G34, G35, G37, G45, G48, G55, G59, G64	G6, G9, G21, G31, G32, G33, G36, G38, G39, G44, G47, G53, G56, G61, G62	G8, G25, G26, G27, G42, G50, G54, G57, G58, G60, G63, G65	G7, G10, G11, G13, G15, G16, G18, G19, G20, G23, G28, G40, G41, G43
SNP-GD	G1, G5, G6, G3, G4	G2, G7, G9, G10, G11, G12, G13, G15, G17, G18, G19	G39, G41, G21, G42, G22, G43, G23, G44,G24, G45, G25, G46, G26, G47, G27, G48, G28, G49, G29. G59, G30, G60, G31, G61, G32, G33, G62, G34, G63, G35, G64, G36, G65, G37	G51, G52, G53, G54, G55, G56, (G57)

†, inbreds with positive and significant GCA effects for grain yield grouped together by the SCA and HGCAMT methods under *Striga*-infested environment; ⫛, inbreds with positive and significant GCA effects for grain yield grouped together by the HGCAMT method under drought; ε, inbred with negative and significant GCA effects for grain yield grouped together the HGCAMT method under drought; ʊ, inbreds consistently grouped together in one group by the SCA and HSGCA methods while Ψ were those in another group; Yellow color, inbreds grouped together by SCA and HGCAMT methods under *Striga*-infested and drought environments; Purple color, another set of inbreds grouped together by SCA and HGCAMT methods, but not necessarily together with those in yellow under *Striga*-infested and drought environments; Green color, inbreds grouped together by HSGCA and HGCAMT methods under *Striga*-infested and drought environments; ††, inbreds grouped together by SCA, HSGCA, and HGCAMT methods in both *Striga*-infested and drought environments; **, pairs of inbreds consistently separated by SCA, HSGCA, and HGCAMT methodsunder drought.

3.3. Efficiencies of Heterotic Grouping Methods

Mean squares of GY generated from ANOVA with cluster as a source of variation (Table 7) revealed that the HGCAMT grouping method showed significant ($p < 0.01$) between-group differences under *Striga* and combined environments, whereas the HSGCA and SCA methods revealed significant ($p < 0.05$) mean squares under drought.

Table 7. Mean squares of grain yield of clusters identified by different grouping methods under drought, *Striga*, and combined environments, between 2015 and 2017.

Environment	HGCAMT	HSGCA	SCA	SNP-GD
Striga	1,483,220.26 **	171,213.79 ns	89,577.59 ns	746,154.17 ns
Drought	97,634.09 ns	353,589.42 *	273,962.47 *	175,796.37 ns
Combined	1,680,620.89 **	201,214.88 ns	80,858.69 ns	201,568.36 ns

* and **, significant at 5 and 1% probability levels respectively; ns, not significant; SCA, specific combining ability; HGCAMT, general combining ability of multiple traits; HSGCA, heterotic groups specific and general combining ability; SNP-GD, SNP-based genetic distance.

From the pairwise comparison of mean GY of clusters defined by each grouping method (Table 8), under *Striga*-infested growing conditions, Cluster 2 × 3 defined by the HGCAMT were statistically different, whereas Cluster 1 × 3 and 1 × 4 defined by SNP-based GD method were statistically different. Under drought, significant differences were revealed for Clusters 1 × 3 and 1 × 4 defined by the HSGCA method, whereas Cluster 2 × 3 defined by the SNP-based GD were statistically significant. Similarly, statistical significance was observed for Clusters 2 × 3 and 2 × 4 defined by the HGCAMT and SCA grouping methods, respectively, under combined environments. There were also significant differences observed for Clusters 1 × 2, 2 × 4, and 3 × 4 defined by the HSGCA grouping method under combined environments.

As shown in Table 9, the HSGCA and SCA grouping methods were consistent in obtaining higher mean GYs of crosses obtained from parents selected from different groups than within the same groups. In contrast, grouping with HGCAMT- and SNP-based GD methods revealed higher mean GYs of crosses obtained from parents within the same heterotic groups than from different group GYs. The HSGCA method had the highest efficiency under *Striga*-infested (65.96%) and drought (37.94%) environments which was comparable with the SCA method with breeding efficiencies of 64.32 and 37.70%, in the respective environments. In addition, across environments, the SCA and HSGCA methods had the highest, and comparable, breeding efficiencies of 46.45%and 46.07%, respectively. Furthermore, the SNP-based GD method had the lowest breeding efficiency of 28.57% under drought, whereas the HGCAMT method had the lowest efficiencies of 48.41 and 33.33% under *Striga*-infested and across growing conditions, respectively.

The estimates of the efficiencies of the grouping methods in all environments, based on the classification error rates obtained from discriminant analysis are presented in Table 10. The HGCAMT method had the lowest error rate of 61.57% (i.e., 38.11% efficiency) under *Striga*, whereas the HSGCA method had the lowest error rates of 67.50% (that is, 32.50% efficiency) and 42.46% (25.85% efficiency) under drought and across research environments, respectively.

Table 8. Heterotic patterns obtained from heterotic groups defined by HGCAMT, HSGCA, SCA, and SNP-GD grouping methods.

Heterotic Pattern	*Striga*				Drought				Combined			
	HGCAMT	HSGCA	SCA	SNP-GD	HGCAMT	HSGCA	SCA	SNP-GD	HGCAMT	HSGCA	SCA	SNP-GD
1 × 2	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns
1 × 3	ns	ns	ns	**	ns	**	ns	ns	ns	ns	ns	ns
1 × 4	-	ns	ns	**	-	**	ns	ns	-	ns	ns	ns
2 × 3	**	ns	ns	ns	ns	ns	ns	**	**	ns	ns	ns
2 × 4	-	ns	ns	ns	-	ns	ns	ns	-	**	**	ns
3 × 4	-	ns	ns	ns	-	ns	ns	ns	-	**	ns	ns

**, significant at 1% probability level; ns, not significant; 1, 2, 3, and 4, Clusters 1, 2, 3, and 4, respectively; SCA, specific combining ability; HGCAMT, general combining ability of multiple traits; HSGCA, heterotic groups specific and general combining ability; SNP-GD, SNP-based genetic distance.

Table 9. Efficiencies of heterotic grouping methods in classifying lines into heterotic groups as compared using standard procedure.

Yield Group	Cross Type	Number of Hybrids			
		HGCAMT	HSGCA	SCA	SNP-GD
***Striga*-infested environment**					
1	Intergroup	76	124	119	107
1	Within group	55	7	12	9
2	Intergroup	43	45	47	51
2	Within group	21	19	17	5
3	Intergroup	38	19	19	38
3	Within group	11	30	30	2
Inter-group crosses mean (kg/ha grain yield)		3463.75	3753.39	3738.25	3559.4
Within-group crosses mean (kg/ha grain yield)		3754.84	2943.6	3032.27	3803.1
Number of intergroup crosses		157	188	185	196
Number of within-group crosses		87	56	59	16
Breeding efficiency (%)		48.41	65.96	64.32	54.59
Drought					
1	Intergroup	41	71	69	56
1	Within group	30	0	2	7
2	Intergroup	70	100	99	103
2	Within group	56	26	27	6
3	Intergroup	25	16	15	37
3	Within group	22	31	32	3
Intergroup crosses mean (kg/ha grain yield)		3101.64	3316.92	3318.02	3109.47
Within-group crosses mean (kg/ha grain yield)		3111.76	2414.55	2470.41	3320.56
Number of intergroup crosses		136	187	183	196
Number of within-group crosses		108	57	61	16
Breeding efficiency (%)		30.15	37.97	37.7	28.57
Across research environments					
1	Intergroup	52	88	85	71
1	Within group	38	2	5	8
2	Intergroup	54	61	56	61
2	Within group	17	12	17	5
3	Intergroup	50	42	42	64
3	Within group	29	39	39	3
Intergroup crosses mean (kg/ha grain yield)		3742.94	3907.55	3909.38	3760.42
Within-group crosses mean (kg/ha grain yield)		3779.46	3204.19	3290.94	3920.02
Number of intergroup crosses		156	191	183	196
Number of within-group crosses		84	53	61	16
Breeding efficiency (%)		33.33	46.07	46.45	36.22

SCA, specific combining ability; HGCAMT, general combining ability of multiple traits; HSGCA, heterotic groups specific and general combining ability; SNP-GD, SNP-based genetic distance.

Table 10. Efficiencies (%) of heterotic grouping methods in classifying lines into heterotic groups as compared with using discriminant analysis.

Environments	HGCAMT	HSGCA	SCA	SNP-GD
Striga	38.43	27.48	18.11	37.58
Drought	30.00	32.50	31.70	28.32
Combined	34.70	42.46	28.53	25.85

SCA, specific combining ability; HGCAMT, general combining ability of multiple traits; HSGCA, heterotic groups specific and general combining ability; SNP-GD, SNP-based genetic distance.

4. Discussions

Significant genotype mean squares (MS), which were observed for most traits in all growing conditions, indicated the existence of substantial genetic differences among the hybrids for improvement through selection under the research conditions. Significant environment MS observed for the measured traits across the research conditions indicated that each environment was distinct and suggested the need for multi-environment evaluation of the hybrids. The significant genotype × environment MS for most traits measured indicated differential hybrid performance in the contrasting test environments. This implied that different hybrids should be selected for specific growing conditions [33,42,61].

Significant variability observed for the measured traits provided evidence of inherent genetic differences among parents and progenies and necessitated further assessment through line × tester analysis. The significant GCA and SCA MS for GY and other measured traits in individual and across environments implied that there were differences in the performance of the inbreds and testers as parents in crosses, and that additive or non-additive gene actions controlled the inheritance of the traits. Therefore, appreciable progress could be made using appropriate breeding methods (such as backcrossing, hybridization, and recurrent selection) for population improvement and varietal development. Additionally, the results indicated that the inbreds were genetically diverse and could be classified into discrete heterotic groups under the different growing conditions. Thus, inbreds with superior, useful combining abilities for improvement could be identified [42,43,62]. Elmyhun et al. [17] investigated the gene action modulating grain yield and related traits in eight maize inbreds crossed to two testers. The results revealed significant GCA or SCA MS for most measured traits including grain yield, flowering, plant and ear aspects, and number of ears per plant. Furthermore, Badu-Apraku et al. [18] reported higher SCA than GCA mean squares for grain yield of early yellow maize inbreds under diverse growing conditions and highlighted the major role of non-additive gene effects in the expression of the traits in the progeny. In contrast, Badu-Apraku et al. [16] observed a major role of additive gene action for determining grain yield via diallel analysis of a different set of maize inbreds under diverse growing conditions. Additionally, Oyekunle and Badu-Apraku [19] reported comparable results for early-maturing white and yellow maize inbreds. Derera et al. [20] also reported greater roles of additive and non-additive genetic effects for grain yield in maize inbreds under drought and well-watered conditions, respectively.

The non-significant GCA_{Tester} and GCA_{line} MS for GY, whereas SCA MS was significant under *Striga*-infested environment, suggested the possibility of trait improvement by exploiting non-additive gene effects through hybridization. This result differed from the reports of Derera et al. [20], Amegbor et al. [44], Kim [63] and Akanvou [64] that GY was mainly influenced by additive gene effect under *Striga* infestation. The significant GCA_{Tester}, GCA_{Line}, and SCA MS for SDR and ESC indicated that both additive and non-additive gene actions were important in the expression of the traits in the hybrids. Therefore, these traits could be improved through selection or hybridization. The fact that the GCA (GCA_{Tester} + GCA_{Line}) sums of squares for the traits were several times larger than the SCA sums of squares indicated that additive gene action played a major role in the expression of the traits. These findings were consistent with those of Yallou et al. [14], Badu-Apraku et al. [18] and Arifin et al. [49]. However, contrary reports have been presented by Gethi and Smith [65] and Badu-Apraku et al. [66] who indicated that non-additive genetic effects played a more significant role than the additive genetic effects in the inheritance of SDR in maize. These contrasting observations corroborated the report of Tengan et al. [67] which indicated that both the additive and the non-additive gene actions influenced several traits in maize, depending on the type of genetic materials.

The significant GCA and SCA MS for GY and other measured traits under drought suggested that both the additive and non-additive genetic effects significantly influenced the inheritance of the traits. Thus, there is the possibility of improvement via selection and exploitation of heterosis. Significant GCA_{Line} rather than GCA_{Tester} MS for most of the traits implied that the sources of the additive gene effects for these traits were the lines, indicating that selection to improve GY, ASI, EASP and STGR would be more effective if targeted on the lines rather than the testers. Badu-Apraku et al. [33] obtained

comparable results for similar traits, while Annor and Badu-Apraku [68] reported contrary results except for numbers of days to silking (DYS) and anthesis (DYA). The different results obtained from these studies could have arisen from the genetic differences of the test materials. This possibly led to the conflicting reports on the inheritance of these traits, thus, highlighting the important roles of both types of gene action in conferring drought tolerance. Values of GCA (GCA_{Tester} and/or GCA_{Line}) were larger than those of SCA for GY, further confirming the greater importance of the additive over the non-additive gene action in the control of GY among the inbred lines under drought. The non-significant GCA (GCA_{Tester} or GCA_{Line}) effects of some of the traits could be due to the high GCA (GCA_{Tester} or GCA_{Line}) × environment, as stated earlier. The differences in the results of the present study and those of earlier studies could be attributed to the different genotypes used. The chances are that the inbred lines used possessed genes with different modes of action for drought tolerance. Thus, appreciable progress could be made through both recurrent selection and hybridization for hybrid development and population improvement.

The comparison of the results of combining ability analysis under *Striga*-infested and drought showed similar trends in the gene action controlling resistance to *Striga* and tolerance to drought in the inbred lines, i.e., additive genetic effect was more important than the non-additive genetic effects for GY and most traits. Similar findings have been reported for resistance to *Striga* and tolerance to drought in early- and extra-early-maturing maize inbreds, respectively [18,69].

The significant GCA (GCA_{Tester} or GCA_{Line}) and SCA MS with respect to the measured traits, with the exception of EPP in the optimal environments, showed the important roles of the additive and non-additive gene actions in the transfer of the genetic resources for GY and other traits from parents to progenies under optimal conditions. Hence, there is a possibility of improvement of the traits using appropriate breeding methods. The GCA (GCA_{Tester} + GCA_{Line}) MS for GY was several times larger than SCA under optimal growing conditions, indicating the greater role of additive gene action in controlling GY under optimal growing conditions.The results obtained in the present study are consistent with those of Ifie [49] and Katsantonis [70] who indicated that maize GY was majorly controlled by additive gene action under optimal environments.

The significant GCA_{Tester}×E, GCA_{Line}×E, and SCA×E MS observed for measured traits in individual and across environments is an indication that inbred performance as parents in hybrid combinations varied significantly from one test environment to the other, and different breeding strategies should, therefore, be adopted to develop hybrids for contrasting environments. Badu-Apraku et al. [16] reported significant GCA × E and SCA × E mean squares for measured traits under diverse growing conditions. Thus, trait improvement is possible through selection or hybridization, with different parental inbreds selected for hybrid development for different environments. This observation corroborated the findings of Ariyo [71] and Kang [72] who reported on the significant impact of environmental components in the expression of phenotype. Thus, neglecting environmental components in field assessment would reduce effectiveness of selection.

The combining ability effect of a genotype represents the additive nature of that genotype and defines its usefulness for population improvement and varietal development [42,73]. The desirability of either positive or negative GCA for a trait is a function of the breeder's interest. Knowledge of the combining ability of available genotypes is vital for planning an effective hybrid program through identification of outstanding parents. The line × tester technique assists the breeder to classify and select parent genotypes in terms of their potential performance in hybrid combination [74]. General combining ability effects were positive and significant for inbreds G7, G43, G47, and G51 with respect to GY and other measured traits under *Striga*-infested environments, indicating that the inbred lines possessed yield-improving alleles to pass to their progenies for adaptation to *Striga*-endemic areas. In *Striga* research, a genotype is said to be resistant if it is able to induce *Striga* seeds to germinate, but prevents further development of the seedlings, either by disallowing attachment to its roots or by killing attached parasitic seedlings. Compared to the susceptible genotype, a *Striga*-resistant genotype results in the growth of fewer *Striga* plants and produces greater yield [75–77]. In contrast to resistance,

tolerance to *Striga* denotes the situation where, despite supporting equal levels of *Striga* infestation as the intolerant or sensitive genotype [78], the host plant shows no associated impairment of growth or GY losses [79,80]. The negative and significant estimates of GCA observed for inbreds G29 and G54 for SDR suggests that these inbreds could be sources of useful favorable alleles for population improvement and varietal development for tolerance to *Striga*. The negative and significant GCA estimates for ESC detected for G26 and G2 implied that *Striga* resistance genes were present in the genetic architecture of the inbreds. These lines could be sources of useful alleles for improvement of *Striga* resistance in tropical maize germplasm. Under drought, inbreds G10, G12, G37, G50, and G60 had positive and significant GCA estimates for GY, and these inbreds would likely contribute to higher GY of their progenies under the research conditions. The inbreds G10 and G12 both combined negative and significant estimates of GCA for STGR with positive and significant GCA estimates for GY, and both had two hybrids among the top 20 (data not presented) hybrids under drought. These inbreds will likely be sources of beneficial alleles for improvement of drought tolerance for improvement of tropical maize.

Different inbreds displayed desirable GCA for GY and SDR, or ESC, or STGR. Thus, multiple-stress tolerant single-cross hybrids can be developed through planned hybridizations among the materials evaluated in the present study. Inbreds G3, G24, G41, G45, G46, G53, G54, G1, and G4 with positive and significant estimates of GCA for GY under optimal environments would likely contribute higher GY production in their progenies in similar growing conditions. Furthermore, different inbreds were identified with superior GCA estimates for GY in the different growing conditions, suggesting the possibility of planned hybridization to develop multiple-stress tolerant/resistant hybrids with improved GY. In addition, the inbreds identified with superior GCA estimates for GY would likely transfer useful alleles to their progenies in a recurrent selection program. Therefore, new inbreds with improved favorable reactions to *Striga* and drought could be extracted from these materials.

According to Fan et al. [30], the efficiency of a heterotic grouping method is a function of how vigorous the inter-heterotic group crosses are as compared with the within-group crosses. The correspondence among the grouping methods in classifying the inbreds implied that some of the methods largely grouped the inbred lines similarly. The inconsistent groupings by each method from one environment to another suggested the sensitivity of the methods to the growing environments and underscored the possibility of obtaining environment-specific heterotic patterns for grain-yield improvement. For instance, the HSGCA grouping method was the most efficient for classifying the inbred lines under *Striga* infestation. This made it the most reliable for identifying the inherent genetic variability among the inbreds under *Striga* infestation. In the same vein, the SCA grouping method was superior for the same purpose under drought. Several studies have reported the superiority of HSGCA and SCA methods over other methods, in classifying maize inbreds [42–45,68].

The significant mean squares from ANOVA based on "cluster" as a source of variation implied that at least one cluster was different from the other clusters in mean GY, and that there was the possibility of obtaining useful heterotic patterns through careful selection of parents across clusters. Thus, the HGCAMT was the most reliable method under *Striga*-infested environment while the HSGCA and SCA methods were effective under drought. Similar trends were observed through the classification error rates from discriminant analysis with the HGCAMT grouping method having the highest efficiency under *Striga,* whereas the HSGCA method had the highest efficiency under drought and combined environments.

Information on which heterotic grouping method should be selected as the most effective for grouping of parental inbreds would save resources for the breeders and aid in timely achievement of breeding objectives. The results of pairwise comparison of cluster means for all the grouping methods revealed useful heterotic patterns under *Striga*, drought, and combined environments. Heterotic patterns 1 × 3 and 1 × 4 (via SNP-GD) and 2 × 3 (via HGCAMT) were useful under *Striga*, whereas 1 × 3 and 1 × 4 (via HSGCA) and 2 × 3 (via SNP-GD) were useful under drought. Comparable

results have been reported for HGCAMT and HSGCA methods in earlier studies on tropical maize germplasm [3,42,45].

Fan et al. [30] defined the breeding efficiency of a heterotic grouping method as the percentage of superior high-yielding hybrids obtained across the total number of inter-heterotic group crosses. However, the efficiency of a heterotic grouping method must also depend on the percentage of available inbred lines classified, the possibility of obtaining heterotic patterns among identified groups, and the ability to reveal the level of genetic variability among classified genotypes. A grouping method is efficient when between-group heterosis exceeds within-group heterosis for grain yield. In the present study, the HSGCA and SCA methods displayed higher between-group than within-group grain yield heterosis, in all test conditions, and therefore were more efficient than the HGCAMT and SNP methods. For practical purposes, the choice of a heterotic grouping method for a breeding program should not be based solely on estimates of within- and between-group heterosis for grain yield. Both the HSGCA and SCA methods also identified a higher number of heterotic groups than the HGCAMT, implying that they were more important in revealing the level of inherent genetic variability among the classified inbreds. However, there are numerous challenges involved in handling large numbers of heterotic groups in a breeding program. Fan et al. [25] suggested a maximum of three heterotic groups for maize programs, but strongly encouraged two. In addition, the HSGCA method classified fewer lines as compared with the HGCAMT, in all test conditions, implying that the HSGCA method would render more lines redundant or to be discarded, which could, if tested in hybrid combinations, have potentials for use in developing heterotic hybrids in the future. In addition, the fewer number of heterotic groups identified by the HGCAMT method could be easier to handle (due to reduced labor, cost, land area, and time of evaluation) in a breeding program. Additionally, the method employed the GCA effects of grain yield and other traits which are stable and have high heritability under stress. Although the HGCAMT method still identified fewer groups under drought, results of statistical tests revealed superiority of the HSGCA under this growing condition.

Based on the results obtained in the present study, we concluded that sufficient genetic variation existed among the inbreds to allow selection and classification into heterotic groups. Inbreds G7, G29, G43, G47, G51, and G54 would be useful genetic resources for maize improvement for *Striga*-endemic regions, whereas inbreds G10 and G12 would be invaluable resources for beneficial alleles for breeding more tolerance to drought. The significant influence of genotype × environment interaction is one of the demerits of the SCA grouping [30], prompting the development of the HSGCA approach. An important factor in genotype × environment interaction is the number of genotypes and the environments for testing, such that the larger the size of one or both components, the larger the size of the interaction. As expected, since the grouping methods required the performance estimates of the lines in the different environments, the groupings varied from one environment to another. This is beneficial because the breeding program can identify which grouping method is most suitable for germplasm improvement in a specific growing condition. The breeding efficiencies of the four grouping methods, HGCAMT, HSGCA, SCA, and SNP GD, varied with growing conditions. The HSGCA and SCA methods were the most efficient for grouping in all test conditions. However, based on the results of statistical tests (chances of obtaining heterotic patterns) and practicability (considerations of cost-efficiency, time, labor, and ease of handling), the HGCAMT and HSGCA methods would be more appropriate under *Striga*-infestation and drought environments, respectively. The heterotic patterns revealed in the present research would be useful for planning crosses for the development of high-yielding, *Striga* and drought-tolerant maize cultivars.

The ultimate objective of our research was to have, as few as, two useful heterotic groups. The results obtained in this study were useful because they classified the inbreds into heterotic groups, among which heterotic patterns were identified for each stress condition. Through extensive testing, two superior heterotic groups could be identified and lines in other groups could be further improved for incorporation into either group.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/8/1198/s1, Table S1: General combining ability (GCA) effects of 65 early-maturing maize inbred lines under *Striga*-infested and drought environments in Mokwa (2016, 2017) and Abuja (2016), Table S2: General combining ability (GCA) effects of 61 early-maturing maize inbred lines under optimal environments at Ikenne (2016, 2017), Mokwa (2016), and Abuja (2016).

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