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Genetic Diversity and Combining Ability of Developed Maize Lines to Realize Heterotic and High Yielding Hybrids for Arid Conditions

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ABSTRACT

Developing high-yield maize hybrids is critical for sustaining maize production, especially in the face of rapid climate changes and the growing global population. Exploring the genetic diversity and combining ability in parental inbreds is needed for developing such high-yielding hybrids. Consequently, this study aimed at evaluating parental genetic diversity employing simple sequence repeats (SSR) markers, estimating effects of general (GCA) and specific (SCA) combining abilities for grain yield and yield contributing characters, identifying high yielding hybrids, and evaluating the association of SCA effects and performance of hybrids with genetic distance. Half-diallel mating scheme was utilized to develop 21 F₁ hybrids from seven diverse maize inbred lines. The F₁ hybrids along with check hybrid (SC-10), were investigated in a field trial over two growing seasons under arid conditions. The assessed F₁ hybrids displayed significant genetic variations across all recorded traits. The inbreds P₁ and P₃ were detected as effective combiners to develop early maturing hybrids. Additionally, P₃ and P₄ were recognized as better combiners for improving grain yield and yield attributed characters. The hybrids P₁ × P₅ and P₄ × P₇ displayed significant SCA effects coupled with favorable agronomic performance. These hybrids are recommended



for further evaluation and release as variety for arid environments to increase total maize production and contribute to food security. The alleles per locus differed between 2 and 5, with average of 3.5 alleles/locus. The polymorphic information content (PIC) altered between 0.21 to 0.74, with a mean of 0.56. Unweighted neighbor-joining tree grouped the inbred lines into three clusters, providing a valuable tool to decrease the crosses needed to be assessed in the trial field. Parental genetic distance varied from 0.63 to 0.90, averaging 0.79. The relationship between genetic diversity assessed through SSR markers and SCA effects was insignificant for all considered traits. Otherwise, SCA demonstrated a significant correlation with hybrid performance, suggesting that SCA serves as a reliable predictor for hybrid performance. The assessed maize inbred lines and developed hybrids revealed substantial genetic variability, offering valuable resources for enhancing maize productivity under arid conditions. The identified promising inbred lines (P_1 , P_3 , and P_4) might be regarded as effective combiners for developing early-maturing genotypes and excellent combiners for enhancing yield attributes. Notably, the developed hybrids $P_1 \times P_5$ and $P_4 \times P_7$ possessed significant SCA alongside superior yield traits. SCA demonstrated a significant correlation with hybrid performance, suggesting its potential as a reliable predictor for the performance of developed hybrids.

KEYWORDS

Arid environment adaptation; maize breeding; sustainable maize production; hybrid performance; combining ability; genetic diversity

1 Introduction

Maize is among the most vital cereal crops in total production and cultivated area [1]. Its grains contain about 70% starch, 8.5% dietary fiber, 10% protein, 4.8% fat, and 3.0% sugar without antinutrients [2]. Maize is globally cultivated for various purposes involving edible oil, animal feed, fuel, and human food [3,4]. The global maize cultivation covers approximately 206 million hectares, yielding around 1.21 billion tons of grain [5]. In Egypt, maize production is lacking in order to meet the growing population; accordingly, around nine million tons of maize are imported annually [5]. The disparity between production and consumption is widening due to current and projected climate fluctuations. Subsequently, breeding high-yielding hybrids has become essential for maintaining maize production, principally with climate change and population growth.

Identifying suitable parents and selecting desirable traits is critical for implementing appropriate breeding strategies [6–9]. Exploring the general (GCA) and specific (SCA) combining abilities and understanding inheritance patterns of important agronomic characters is vital. Diallel mating design provides valuable insights into the effects of both GCA and SCA and genetic mechanisms governing the inheritance of targeted attributes during early generations [10–13]. In addition, this analysis is useful for distinguishing between non-additive and additive effects of the analyzed traits. SCA is typically associated with dominance effects, while GCA is linked to additive effects [14–16]. Additive and non-additive gene actions are demonstrated to be vital in inheritance of yield traits of maize [17–19]. However, numerous previously published reports exposed the dominance of non-additive gene effects governing yield traits in maize hybrids [20–22]. Due to the contrasting results depicted in previous studies and utilizing different inbred lines for genetic analyses, it is necessary to further study inheritance mode of maize grain yield by exploiting diverse inbred lines with varying genetic backgrounds.

Understanding molecular genetic distance (GD) is crucial for maize breeders, as it helps accelerate the discovery of vigorous hybrids without assessing all potential parent combinations [23–25]. In recent years, many DNA markers have successfully uncovered maize inbred lines genetic distance (GD). Among the most

valuable molecular markers are simple sequence repeats [26,27]. The informative, multiallelic, codominant, and reproducible markers are vital characteristics of SSRs [28,29]. The association between SSR based genetic distance of parent and both combining ability and hybrid (F_1) performance especially for grain yield has contrasting results ranging from not significant [30,31] to significantly vital [32–34]. However, using molecular markers to determine the association between combining ability and hybrid performance in maize is still inconclusive in Egypt [35]. Therefore, this study was performed to (i) explore GCA for evaluated inbred and specific combining ability of their hybrids, (ii) depict inheritance nature of studied agronomic characters, (iii) identify high-yielding hybrids, and (iv) explore the genetic diversity of parental lines and association of genetic diversity with SCA and performance of hybrids.

2 Materials and Methods

2.1 Experimental Layout and Plant Materials

Seven genetically distinct maize inbred lines (*Zea mays* L.) were utilized in the current study, obtained from CIMMYT and the Agricultural Research Center Egypt. The source and pedigree information for these lines are detailed in Table A1. The parental inbreds were crossed applying half-diallel mating scheme to develop twenty one F_1 single crosses during summer 2021. The generated F_1 hybrids, along with commercial hybrid SC-10, were assessed in a two-year field trial conducted during the summer seasons of 2022 and 2023 at a private farm in El-Mahmoudia, Egypt (31°15'N, 30°29'E). The experimental site features an hot and arid climate (Fig. 1). In Egypt, where no summer rainfall, maize cultivation depends on irrigation. The soil characteristics were investigated and presented in Table A2 for both seasons. Randomized Complete Block Design was performed in three replications at each season. The plots contained 5 m long three rows with 0.65 m wide. Potassium, phosphorus, and nitrogen fertilization were added at 116 kg/K₂O, 76 kg P₂O₅/ha, and 290 N/ha.

2.2 Data Collection

The height of plants (cm) was recorded from soil level to top of first branch of tassel. Days to silking were documented when 50% of plants started in silk appearing. Ten ears were randomly collected at harvest from each experimental unit to estimate no. of rows/ear, ear length, weight of thousand kernels and no. of kernels/row. The plots were manually harvested, and grain yield/plant was estimated by adjusting the weight of the shelled grain at 15.5% moisture content.

2.3 Statistical Analysis

Variance analysis for obtained data was conducted using R software 4.1.1. The least significant difference (LSD) test ($p < 0.05$) was applied to explore significance of differences among averages of assessed hybrids. Combining abilities were estimated according to Griffing's method 4 model 1 [36]. Standard heterosis was determined by comparing the mean of F_1 hybrids over the standard check (SC) hybrid using the following formulae: Heterosis over the standard check = $[(F_1 - SC)/SC] \times 100$. Hierarchical cluster analysis was conducted to categorize assessed hybrids based on yield traits. In addition, principal components and heatmap analyses were performed to study interrelationships among evaluated characters.

2.4 Molecular Analysis

DNA was isolated from 200 mg of assessed lines leaves employing the CTAB protocol [37]. Nano Drop spectrophotometer was used to assess DNA consistency and quantity. Fourteen SSR primer pairs were used, with sequences detailed in Table A3, obtained from Maize-GDB database. PCR was conducted in 10 μ L reaction volume comprising 1 μ L of genomic DNA template (20 ng/ μ L), 1 unit of Taq DNA polymerase, 2 mM MgCl₂, 0.2 mM dNTPs, and 0.5 μ M each of forward and reverse primers. The PCR protocol included an initial denaturation at 94°C for 2 min, followed by cycles of 94°C for 30 s, 55°C for 30 s for

annealing, and 72°C for 30 s for extension, ending with a final extension at 72°C for 3 min. Amplified products were subjected to analysis on 1.5% agarose gel. Bands were recorded per SSR marker, creating a binary data matrix where band presence was marked as 1 and absence as 0. According to Jaccard [38], genetic distances of the parental lines were determined using PAST software.

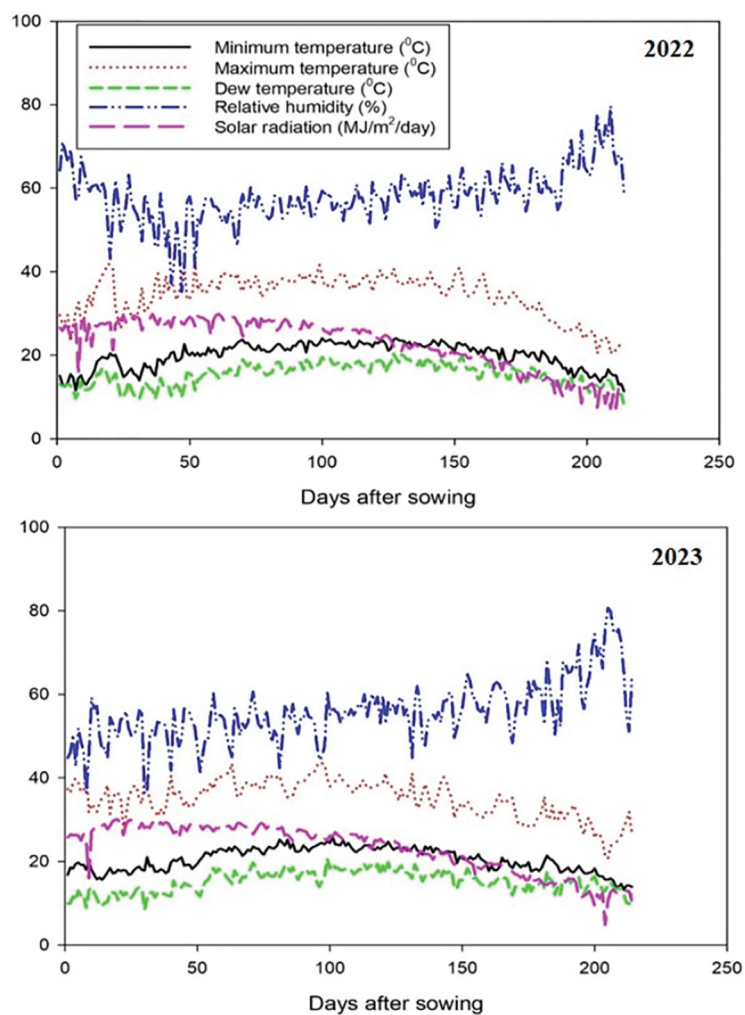


Figure 1: Meteorological characterization for the 2022 and 2023 growing seasons

3 Results

3.1 Variance Analysis

Analysis of variance exhibited an insignificant effect due to growing season (S) for most traits. In addition, the interaction of season with tested hybrids ($H \times S$), with GCA, and SCA were not significant for nearly all the recorded characters. Otherwise, the assessed hybrids displayed highly significant variation for all evaluated characters (Table 1). Moreover, the hybrid effect, specifically the separation into SCA and GCA components, revealed that SCA and GCA showed significant or highly significant impacts on all traits. The ratio of GCA/SCA was lower than one for all characters except days to silking. Besides, the magnitude of $GCA \times S$ interaction was less than $SCA \times S$ for all evaluated characters except 1000 kernel weight, ear length, number of rows/ear, and grain yield (Table 1).

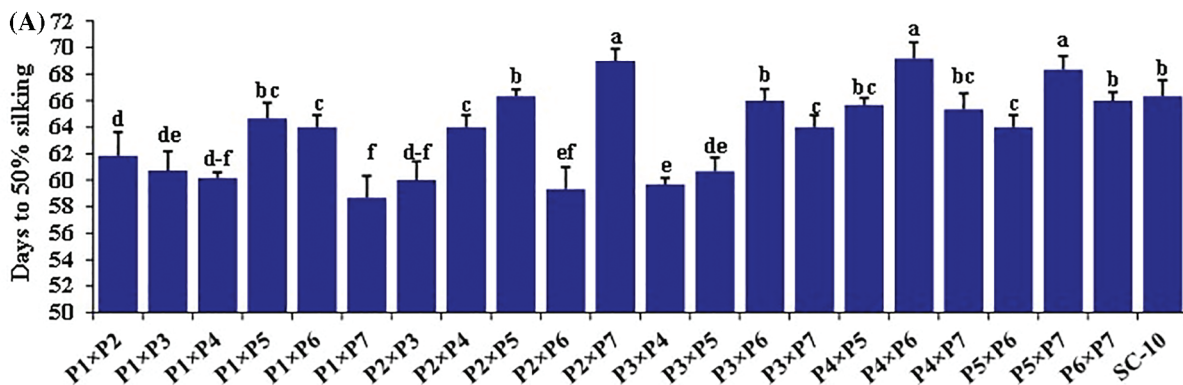
Table 1: Mean squares of variance analysis performed for the assessed traits across both seasons. The numbers are mean squares (has been clarified in the title) and the units have been added in the footnote

Source of variance	df	DS	PH	EL	NR/E	NK/R	TKW	GY
Seasons (S)	1	227.5	45573	250.6	74.52	1434	1629*	2136
Replication/S	4	18.81	5639	27.59	1.07	95.75**	100.1	12.87
Hybrids (H)	20	63.96**	1579**	14.43**	6.10**	119.7**	4874**	57.09**
GCA	6	91.38**	1113**	7.29**	4.54**	56.94**	3453**	47.76**
SCA	14	52.21**	1779**	17.49**	6.77**	146.6**	5483**	61.09**
H × S	20	9.01	199.2	1.12	1.37	19.77*	1901*	7.49
GCA × S	6	8.53	95.81	1.64	1.63	5.31	2997.09*	12.47
SCA × S	14	9.21	243.5*	0.90	1.25	25.96	1431.69*	5.35
Error	80	4.90	130.9	0.85	0.89	11.34	167.63	8.74
GCA/SCA		1.75	0.63	0.42	0.67	0.39	0.63	0.78
GCA × S/SCA × S		0.93	0.39	1.74	1.30	0.20	2.09	2.33

Note: df is degrees of freedom; DS—days to silking (day); PH—plant height (cm); EL—length of ear (cm); NK/R—number of kernels/row; NR/E—number of rows/ear; TKW—weight of thousand kernels (g); and GY—grain yield (g/plant). * and ** denote *p*-values below 0.05 and 0.01, in the same order.

3.2 Performance and Classification of Hybrids

The hybrids differed significantly for all the traits (Figs. 2 and 3). Days to silking fluctuated from 58.7 to 69.2 days with an average of 63.8 days. The earliest hybrid was $P_1 \times P_7$, while the latest hybrid was $P_4 \times P_6$. Plant height averaged 231.2 cm and varied from 195.8 to 261.7 cm (Fig. 2A). The hybrid $P_1 \times P_5$ obtained the uppermost values of plant height, whereas hybrid $P_1 \times P_7$ displayed the lowest values (Fig. 2B). Ear length fluctuated from 16.6 to 22.3, averaging 19.5 cm. The hybrid $P_4 \times P_6$ produced the most extended ear, but the hybrid $P_1 \times P_7$ had the shortest ear (Fig. 2C). Similarly, number of rows/ear fluctuated from 12.7 to 16.3, averaging 14.6. The cross $P_3 \times P_5$ resulted in the highest number of rows, whereas $P_3 \times P_6$ registered the minimum number (Fig. 3A). The average number of kernels/row was 36.9, varying from 29.0 to 44.0. $P_4 \times P_7$ exhibited the uppermost values, meanwhile, the lowest values were observed in $P_1 \times P_7$ (Fig. 3B). In the same context, the mean of 1000-kernel weight diverged from 251.7 to 361.0 g, averaging 322.7. The hybrid $P_4 \times P_7$ recorded the heaviest TKW, whereas $P_4 \times P_6$ had the lightest kernel weight (Fig. 3C). Grain yield per plant ranged from 117.7 to 129.5, averaging 124.7 g. The hybrids $P_4 \times P_7$, $P_1 \times P_5$, and $P_3 \times P_5$ produced the uppermost grain yield, but the hybrid $P_1 \times P_7$ displayed the lowermost grain yield (Fig. 3D).

**Figure 2:** (Continued)

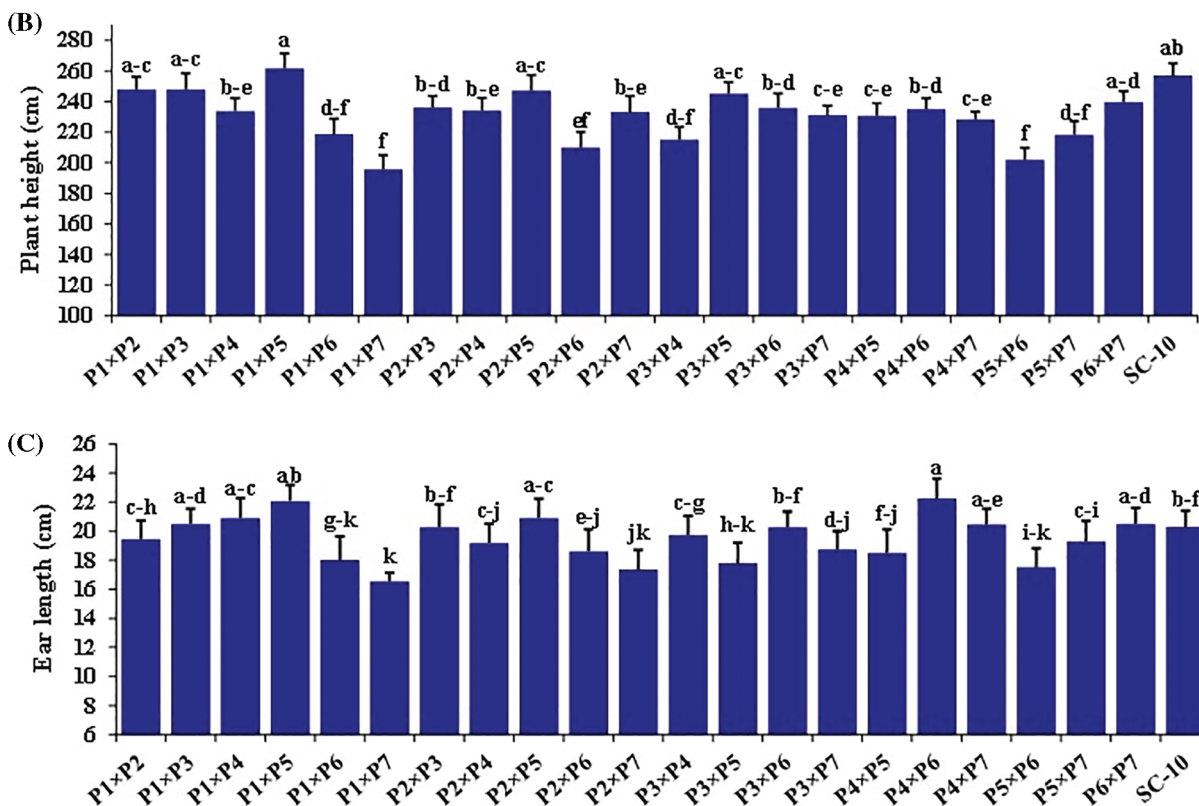


Figure 2: Performance evaluation of twenty one F₁ hybrids and check hybrid for (A) days to silking, (B) plant height and (C) ear length. Bars above columns indicate SE. Columns with different letters represent significant differences as determined by LSD with a *p*-value of less than 0.05

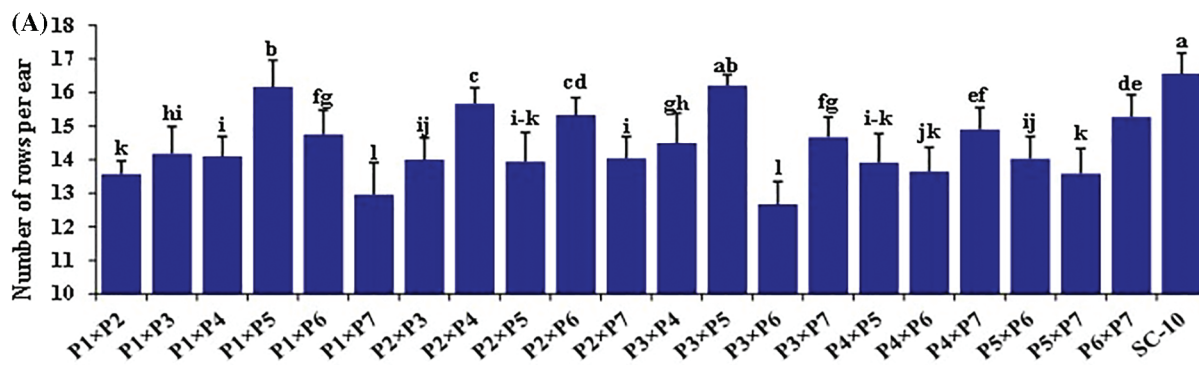


Figure 3: (Continued)

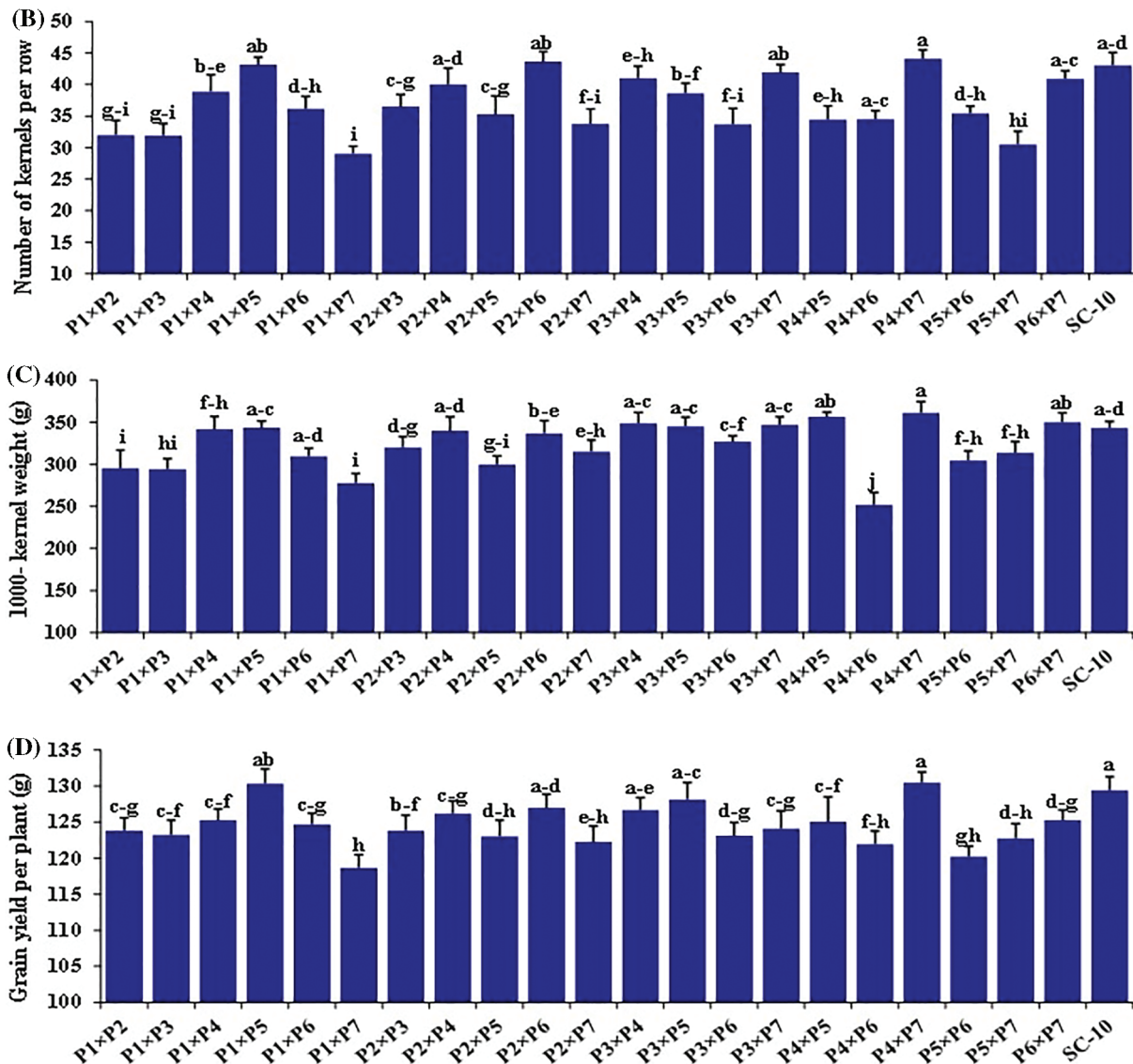


Figure 3: Performance evaluation of twenty one F_1 hybrids and the check hybrid for (A) Number of rows/ear, (B) Number of kernels/row, (C) 1000 kernel weight, and (D) grain yield per plant. Bars above columns indicate SE. Columns with different letters imply significant differences as determined by LSD with a p -value of less than 0.05

The F_1 hybrids, along with the check SC-10, were classified based on yield traits. These hybrids were grouped into five distinct clusters as illustrated in Fig. 4. Group-A contained three hybrids ($P_4 \times P_7$, $P_1 \times P_5$, and SC-10), which exhibited superior yield and contributing traits. Group-B comprised 4 hybrids ($P_3 \times P_5$, $P_3 \times P_4$, $P_2 \times P_6$, and $P_2 \times P_4$) depicted by high-yielding traits. Groups-C and D comprised seven and six hybrids, respectively, showing intermediate-high and intermediate-low yield traits. Finally, Group-E comprised the two hybrids with the lowermost agronomic performance ($P_5 \times P_6$ and $P_1 \times P_7$).

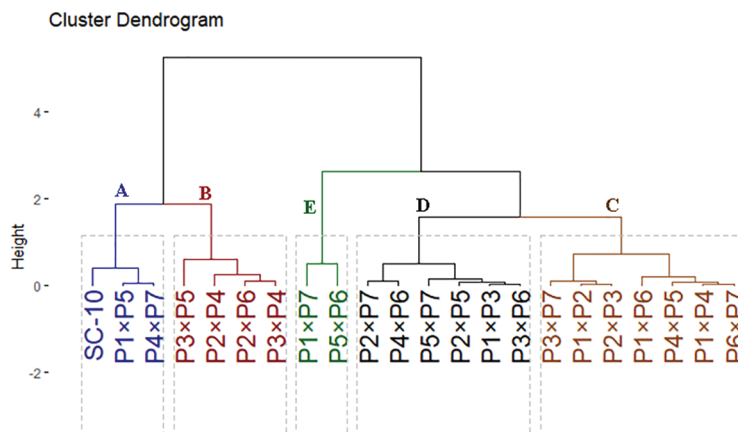


Figure 4: Dendrogram illustrating the phenotypic distances among the 21 F_1 hybrids and check SC-10, based on the studied yield characters

3.3 Standard Heterosis

Standard heterosis over the commercial check SC10 has been given in Fig. 5. Positive heterotic effects are required for evaluated characters excepting days to silking and plant height for which the negative heterotic effects are favorable. Earliness is a crucial factor that considerably affects maize productivity in arid environments. The cross combinations $P_1 \times P_4$, $P_1 \times P_3$, $P_1 \times P_7$, $P_1 \times P_2$, $P_2 \times P_6$, $P_2 \times P_3$, $P_3 \times P_4$, and $P_3 \times P_5$ recorded negative heterosis for days to silking. The negative and considerable heterosis for days to silking is required due to its association with earliness. Fifteen hybrids showed negative heterotic effects for plant height towards shortness. The most pronounced negative heterotic effects were detected in $P_1 \times P_6$, $P_3 \times P_5$, $P_1 \times P_7$, $P_2 \times P_6$, $P_2 \times P_7$, $P_5 \times P_6$, $P_3 \times P_7$, and $P_4 \times P_5$. In contrast, the uppermost positive substantial heterosis for ear length was illustrated by $P_1 \times P_5$ and $P_4 \times P_6$. The best heterotic combinations for number of rows/ear were $P_2 \times P_4$, $P_1 \times P_5$, and $P_3 \times P_5$. Likewise, two hybrids $P_2 \times P_6$ and $P_4 \times P_7$ manifested positive heterosis for no. of kernels/row. The crosses with the best heterotic effects for 1000-kernel weight were $P_4 \times P_7$ and $P_4 \times P_5$. The cross combinations $P_1 \times P_5$ and $P_4 \times P_7$ disclosed the superior positive heterosis for grain yield. Owing to their benefits for one or more associated features, these heterotic combinations appear superior in grain yield.

3.4 General Combining Ability (GCA)

All recorded agronomic characters except days to silking and plant height were desired to have highly substantial positive GCA effects. Table 2 explained the GCA effects of assessed inbreds. The lines P_1 and P_3 exhibited the superior negative GCA estimates for days to silking, while P_6 and P_7 displayed substantially negative GCA estimates for plant height. Otherwise, specific lines reflected positive GCA effects for diverse traits. Specifically, P_4 mirrored a notably superior positive GCA effect for ear length, number of kernels/row. In addition, inbred P_2 and P_5 had positive GCA effect for no. of rows per ear and both P_3 and P_4 manifested significant GCA estimates for grain yield and 1000-kernel weight.

3.5 Specific Combining Ability

The effects of SCA showed that the hybrids $P_3 \times P_4$, $P_5 \times P_6$, $P_3 \times P_5$, $P_2 \times P_6$, and $P_1 \times P_7$ obtained desirable substantial negative effects for days to silking (Table 3). Likewise, favorable substantial negative SCA estimates for plant height were assigned for $P_3 \times P_4$, $P_1 \times P_7$, $P_2 \times P_6$, and $P_5 \times P_6$. Otherwise, the highest positive SCA effects for ear length were displayed by $P_4 \times P_6$, $P_1 \times P_3$, $P_2 \times P_5$, $P_1 \times P_5$, $P_4 \times P_7$ and $P_6 \times P_7$. Moreover, hybrids $P_3 \times P_5$, $P_1 \times P_5$ and $P_6 \times P_7$ recorded substantially

positive SCA estimates for no. of rows/ear. Meanwhile, hybrids $P_2 \times P_6$, $P_3 \times P_7$, $P_1 \times P_5$, and $P_4 \times P_7$ displayed most substantial and positive SCA estimates for no. of kernels/row. The advantageous SCA effect for 1000-kernel weight was noticed in $P_1 \times P_5$, $P_2 \times P_6$, $P_1 \times P_4$, $P_1 \times P_6$, $P_4 \times P_7$, $P_4 \times P_5$, and $P_6 \times P_7$. The hybrids $P_1 \times P_5$, $P_2 \times P_6$, $P_4 \times P_7$, and $P_6 \times P_7$ displayed the topmost substantial positive SCA for grain yield. None of evaluated hybrids recorded desirable SCA estimates for all characters. However, certain hybrids demonstrated desirable effects for grain yield and beneficial SCA effects for its related traits. Notably, hybrids $P_1 \times P_5$ and $P_4 \times P_7$ exhibited favorable SCA effects for no. of grain/row, ear length, no. of rows/ear, grain yield and 1000 kernel weight.

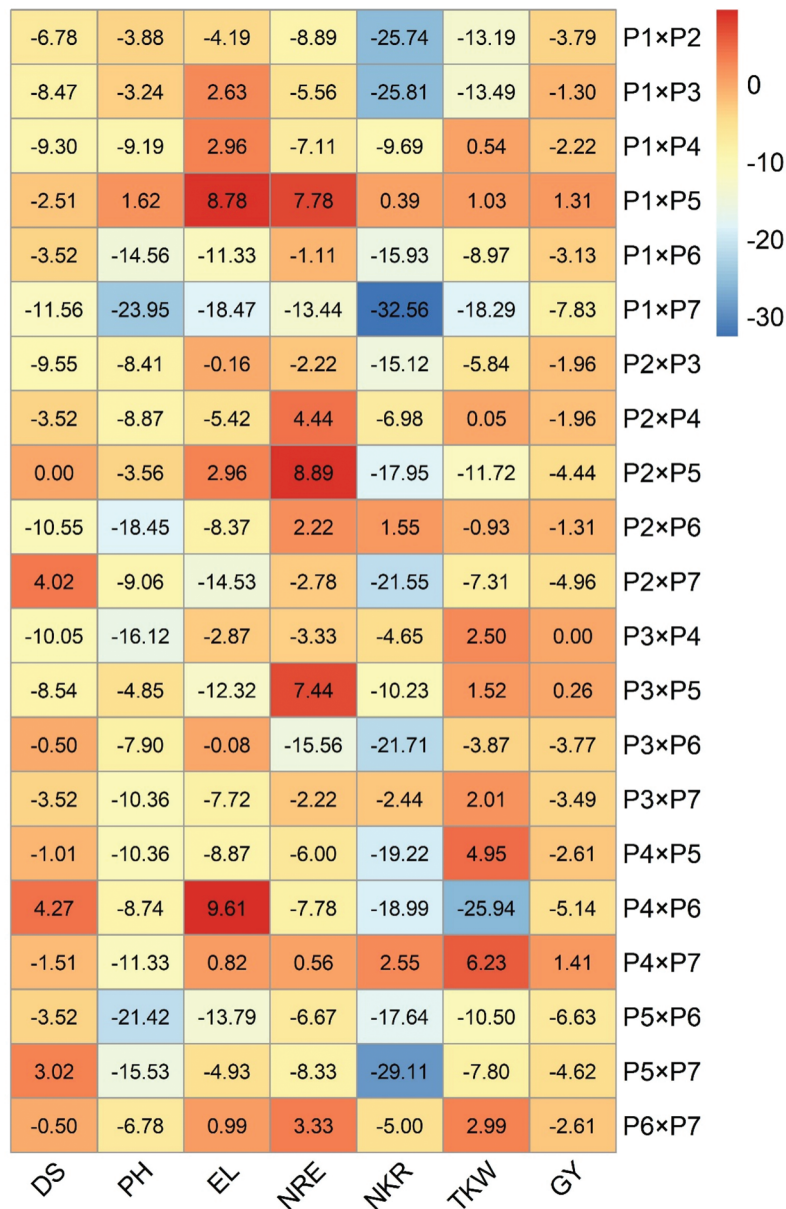


Figure 5: Heatmap depicting heterosis over the standard check (SH %)

Table 2: Estimated effects of GCA for the evaluated inbreds for all measured agronomic traits over two growing seasons. The units have been added in the footnote

Inbred line	DS	PH	EL	NR/E	NK/R	TKW	GY
P ₁	-2.42**	4.23	0.18	-0.37	-2.13**	-15.01**	-0.04
P ₂	-0.33	4.73	-0.23	0.53*	-0.11	-5.88	-0.41
P ₃	-2.22**	5.43	0.14	-0.16	0.39	8.92**	1.67*
P ₄	0.37	-1.64	0.82**	-0.09	2.37**	12.66**	1.60*
P ₅	1.50**	3.76	-0.17	0.58*	-0.79	5.29	0.02
P ₆	1.27*	-8.46**	0.04	-0.28	0.59	-11.51**	-1.48*
P ₇	1.83**	-8.03**	-0.80**	-0.20	-0.31	5.52	-1.35
LSD (gi) _{0.05}	1.05	5.44	0.44	0.45	1.60	6.16	1.41
LSD (gi) _{0.01}	1.40	7.22	0.58	0.60	2.12	8.16	1.86

Note: DS—days to silking (day); PH—plant height (cm); EL—length of ear (cm); NK/R—number of kernels/row; NR/E—number of rows/ear; TKW—weight of thousand kernels (g); and GY—grain yield (g/plant). * and ** denote *p*-values below 0.05 and 0.01, in the same order.

Table 3: The effects of SCA of the evaluated 21 crosses for evaluated traits over two growing seasons. The units have been added in the footnote

Hybrids	DS	PH	EL	NR/E	NK/R	TKW	GY
P ₁ × P ₂	0.89	7.40	0.01	-1.10*	-2.77	-6.79	-0.80
P ₁ × P ₃	1.67	8.37	1.02*	0.10	-3.30*	-22.59**	0.28
P ₁ × P ₄	-1.47	0.10	0.41	-0.20	1.66	21.34**	-0.82
P ₁ × P ₅	1.89	22.54**	2.58**	1.36**	9.15**	30.38**	5.27**
P ₁ × P ₆	1.46	-6.91	-1.71**	0.89*	0.76	13.18*	1.10
P ₁ × P ₇	-4.44**	-31.51**	-2.31**	-1.04*	-5.50**	-35.52**	-5.03**
P ₂ × P ₃	-1.14	-5.46	0.87*	-0.30	-0.73	-5.72	-0.18
P ₂ × P ₄	0.27	0.44	-0.88*	0.63	0.80	10.54	-0.12
P ₂ × P ₅	1.47	8.70	1.81**	0.63	-0.76	-22.09**	-1.70
P ₂ × P ₆	-5.30**	-17.41**	-0.70	0.49	6.25**	31.38**	3.80**
P ₂ × P ₇	3.80**	6.33	-1.10*	-0.34	-2.79	-7.32	-0.99
P ₃ × P ₄	-2.17*	-18.93**	-0.74	0.16	1.30	4.08	0.30
P ₃ × P ₅	-2.31*	4.67	-1.66**	1.10*	2.06	8.11	2.22
P ₃ × P ₆	3.26**	9.06	0.61	-1.49**	-4.25**	6.58	-1.43
P ₃ × P ₇	0.69	2.29	-0.09	0.43	4.93**	9.54	-1.19
P ₄ × P ₅	0.10	-2.43	-1.64**	-0.98*	-3.78*	16.04*	-1.38
P ₄ × P ₆	3.84**	13.96*	1.90**	-0.39	-5.06**	-72.16**	-3.11*
P ₄ × P ₇	-0.56	6.86	0.96*	0.78	5.09**	20.14**	5.13**
P ₅ × P ₆	-2.46*	-24.11**	-1.86**	-0.89*	-1.32	-12.29*	-3.43*
P ₅ × P ₇	1.30	-9.37	0.78	-1.22**	-5.35**	-20.16**	-0.99

(Continued)

Table 3 (continued)							
Hybrids	DS	PH	EL	NR/E	NK/R	TKW	GY
$P_6 \times P_7$	-0.80	25.40**	1.77**	1.39**	3.63*	33.31**	3.08*
LSDS _{ij0.05}	2.08	10.73	0.86	0.88	3.16	12.14	2.77
LSDS _{ij0.01}	2.75	14.23	1.14	1.17	4.19	16.10	3.68

Note: DS—days to silking (day); PH—plant height (cm); EL—length of ear (cm); NK/R—number of kernels/row; NR/E—number of rows/ear; TKW—weight of thousand kernels (g); and GY—grain yield (g/plant). * and ** denote p -values below 0.05 and 0.01, in the same order.

3.6 Interrelation among Hybrids and Characters

The association between developed hybrids and evaluated characters was analyzed employing principal component analysis. The first two PCAs displayed a considerable percentage of the total variance, with PC-1 explaining 52.27% and PC-2 accounting for 20.35%. Therefore, these PCs were utilized to create the biplot as shown in Fig. 6. Notably, PC-1 exhibited greater variation and was closely associated with the evaluated hybrids. This component effectively separated the hybrids into those on the negative and positive sides of PC-1. The evaluated traits were aligned with hybrids on positive side of PC-1, indicating that these hybrids, particularly $P_4 \times P_7$, $P_1 \times P_5$, and SC-10, demonstrated superior agronomic performance. Conversely, the hybrids on negative side of PC-1, such as $P_5 \times P_6$ and $P_1 \times P_7$, exhibited lower agronomic performance. Vectors proximity suggested a positive robust association among studied characters. The grain yield was associated positively with no. of kernels/row, 1000 grain weight, no. of rows/ear, and ear length. Furthermore, heatmap based on the evaluated agronomic traits categorized hybrids into distinctive groups (Fig. 7). Hybrids $P_1 \times P_5$, $P_4 \times P_7$, and SC-10 exhibited highest values across all characters (indicated in blue), whereas hybrids $P_5 \times P_6$ and $P_1 \times P_7$ had lowest performance (marked in red).

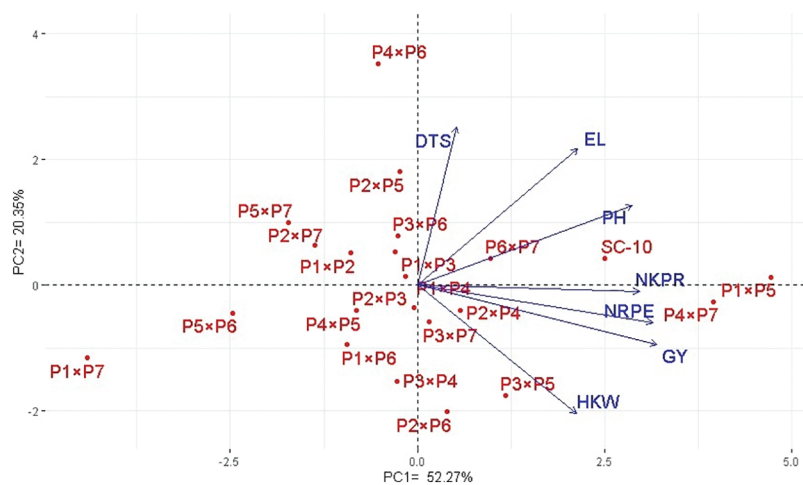


Figure 6: PC biplot for studied characters and the assessed maize hybrids

3.7 Genetic Diversity among Parental Lines

Genetic diversity was studied employing fourteen SSR markers to evaluate genetic variability among seven parental lines. Ten markers were found to be polymorphic and were used to further investigate the genetic diversity (Fig. A1). Number of alleles/locus varied, ranging from 2 (observed in markers phi-9610 and phi-453121) to 5 (observed in marker umc-1033), averaging 3.5 alleles per locus (as shown in

Table 4). The primary allele frequency averaged 0.45, ranging from 0.29 to 0.73. Gene variability varied between 0.24 (phi-96100) and 0.78 (umc-1033), with overall average of 0.62. Additionally, polymorphic information content averaged 0.56, with values ranging from 0.21 (phi-96100) to 0.74 (umc-1033).

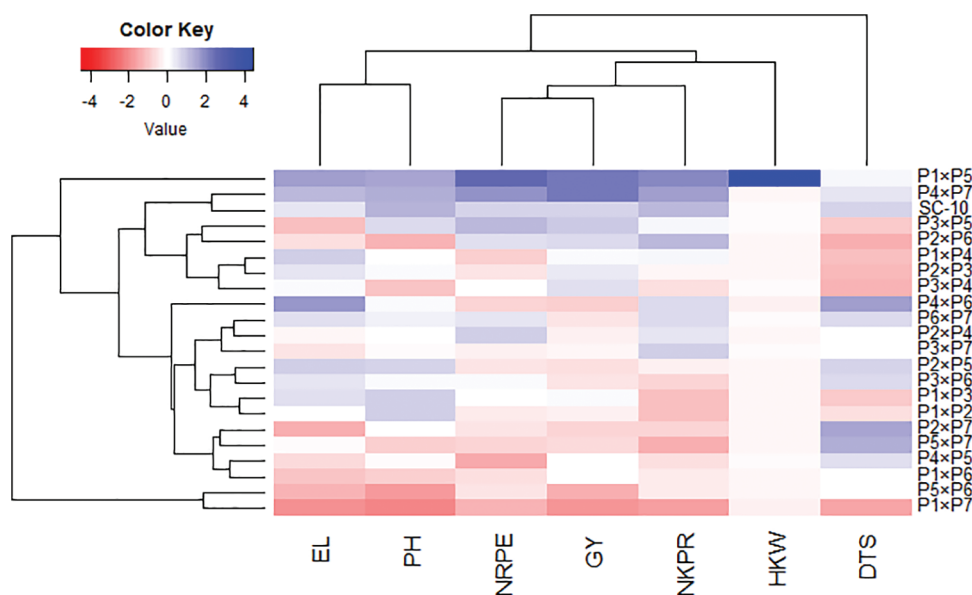


Figure 7: Heatmap categorized the assessed maize hybrids into distinct clusters based on the studied agronomic traits. In this representation, red denotes high values, while blue implies low values for the corresponding attributes

Table 4: The SSR markers employed in the present study

Markers	Chrom.	Repeat motifs	Alleles' number	Frequency of major allele	Gene diversity	PC
Phi96100	2	ACCT	2	0.73	0.24	0.21
Phi-453121	3	ACC	2	0.57	0.49	0.37
Phi-072	4	(GAC)4	3	0.43	0.61	0.53
Phi-024	5	CCT	4	0.43	0.66	0.60
Umc-1014	6	(GA)12	4	0.29	0.73	0.68
Umc-2332	7	(CTC)5	4	0.43	0.69	0.64
Phi-112	7	AG	4	0.43	0.69	0.64
Phi-233376	8	CCG	4	0.43	0.69	0.64
Umc-1033	9	(GA)25	5	0.29	0.78	0.74
Phi-301654	10	CCG	3	0.43	0.61	0.53
Mean			3.5	0.45	0.62	0.56

Genetic distance measured utilized SSR markers differed between 0.63 and 0.90, averaging 0.79 (Table 5). Lowest genetic distance was found between two lines P₁ and P₂ (0.63), while greatest distance was observed between P₆ and P₄ and also between P₄ and P₆ (0.90). The neighbor-joining tree constructed using a genetic distance matrix, divided the parental lines to three main groups with internal

subgroups that displayed variable levels of diversity (Fig. 8). Group-A contained P₆ and P₇, Group-B included P₃ and P₅, and Group-C comprised P₁, P₂, and P₄.

Table 5: Genetic distance among parental inbreds based on utilized SSR markers

Parents	1	2	3	4	5	6	7
1	-						
2	0.63	-					
3	0.78	0.63	-				
4	0.71	0.78	0.89	-			
5	0.89	0.76	0.69	0.83	-		
6	0.84	0.78	0.71	0.90	0.83	-	
7	0.71	0.90	0.84	0.90	0.83	0.71	-

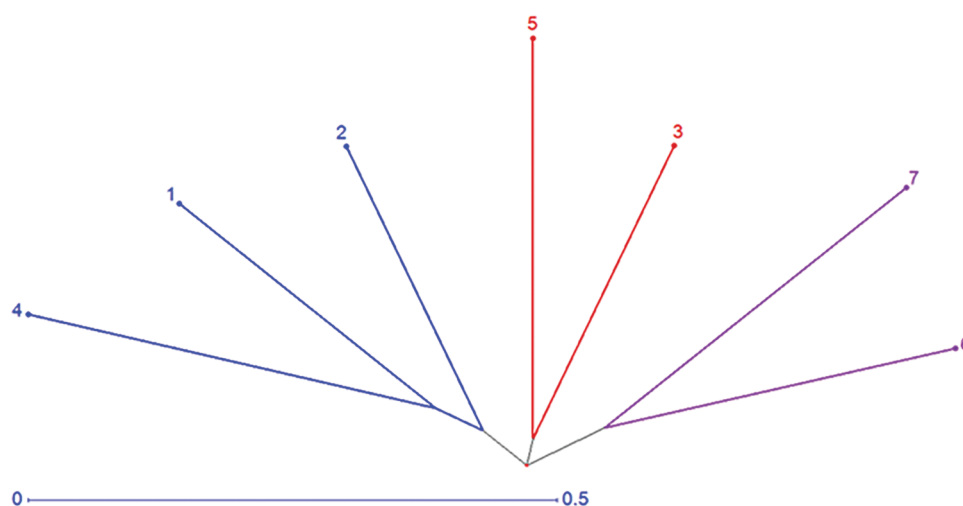


Figure 8: Neighbor-joining tree of the estimated lines based on applied SSR markers

3.8 Relationship between Genetic Diversity of Inbreds, SCA, and Hybrid Performance

The relationship between genetic diversity based on SSR markers and SCA effects was insignificant for studied agronomic traits (Table 6). Similarly, genetic diversity of parental lines marked a nonsignificant association with F₁ hybrid performance. Conversely, F₁ hybrid performance strongly correlated with SCA effects for all evaluated traits.

Table 6: Correlation between genetic diversity of inbreds as characterized by SSR markers with F₁ hybrid performance and specific combining ability estimates for the agronomic traits studied. The units have been added in the footnote

Correlation	DS	PH	EL	NR/E	NK/R	TKW	GY
GD and SCA	0.31	0.01	0.04	0.19	0.18	-0.07	0.15
F₁ and SCA	0.76**	0.89**	0.92**	0.88**	0.93**	0.89**	0.87**
GD and F₁	0.43	-0.15	0.08	0.13	0.28	0.08	0.13

Note: DS—days to silking (day); PH—plant height (cm); EL—length of ear (cm); NK/R—number of kernels/row; NR/E—number of rows/ear; TKW—weight of thousand kernels (g); and GY—grain yield (g/plant). ** indicate *p*-values less than 0.01.

4 Discussion

Maize production faces considerable challenges in arid environments attributed to diverse environmental stresses. The main problems encountered in arid environments include high temperatures, high evaporation rates, and poor soil fertility. Additionally, the unpredictable climatic conditions in arid regions pose further challenges to sustainable agriculture [39–41]. These harsh conditions often result in reduced crop yields and limit the cultivation of traditional maize hybrids. The aim of current study was to explore combining ability and genetic diversity of newly developed maize lines specifically adapted for arid environments. Using developed high-yielding maize hybrids adapted to arid climates could enhance maize production and enhance food security in arid environments.

The development of high-yielding maize hybrids largely depends on selecting suitable parental lines. Consequently, recognizing diverse parental inbred with advantageous alleles is essential to confirm their effective transfer to the offspring [35]. The favorable GCA estimates detected in inbred lines P_1 and P_3 for days to silking suggest their potential for enhancing earliness. Parental lines P_6 and P_7 were considered valuable combiners for reducing plant height as a critical factor in improving lodging tolerance. Additionally, P_3 and P_4 exhibited desirable GCA estimates for grain yield, 1000-kernel weight, ear length, no. of rows/ear, and no. kernels/row. This indicates that these inbreds possess valuable alleles that can be passed on to their offspring to produce high-yielding maize hybrids [42,43]. The derived hybrids displayed significant variations in days to silking, plant height, ear length, weight of thousand kernel, no. of rows/ear, no. of kernels/row, and grain yield. The detected significant genetic variation supports the selection of desirable hybrids. The crosses $P_1 \times P_5$ and $P_4 \times P_7$ reflected outstanding agronomic performance with grain yield greater than the check hybrid. This finding implies that these hybrids have the potential to compete with check hybrids and may serve as favorable hybrids for valuable utilization after additional evaluation. Additionally, they can be utilized in generating new and superior lines [44].

Similarly, previously published reports confirmed considerable genetic variability for different characters in developed maize hybrids such as Babic et al. [45], Oluwaseun et al. [46], Amegbor et al. [47], Ajala et al. [48]. The identification of desirable hybrids is accomplished through the use of SCA estimates. In this study, a significant number of evaluated hybrids demonstrated the favorable effects of SCA for at least one trait. The cross combinations $P_2 \times P_6$, $P_1 \times P_5$, and $P_4 \times P_7$ were determined as the greatest particular combiner for breeding high yielding maize hybrids compared to the others. The great SCA effect appeared as a result of nonadditive gene effects. Two hybrids of the assessed crosses, $P_1 \times P_5$ and $P_4 \times P_7$, marked favorable SCA coupled with superior grain yield. Consequently, these maize hybrids could be exploited in breeding to enhance maize productivity under arid conditions.

Understanding inheritance pattern of agronomic characters is fundamental for breeding programs [49,50]. According to considerable GCA and SCA effects, additive and nonadditive gene actions were shown to be proportionately relevant in inheritance of assessed agronomic character in the current work. However, ratio of GCA to SCA was less than one for nearly all measured characters except for days to silking. This suggests that non additive gene action substantially regulates inheritance of evaluated traits [51–53]. Therefore, crossing method is greatly efficient in elevating these traits and employing heterosis effects. These findings are in consonance with Makumbi et al. [54], Derera et al. [55], which also depicted significance of nonadditive gene action in inheritance of agronomic characters in maize. Otherwise, Annor et al. [56] and Oyetunde et al. [57] elucidated that additive gene action played decisive role in inheritance of yield characters in maize. The nonsignificant GCA \times S interaction and also SCA \times S interaction indicated a lack of environmental influence on determining gene expression of the studied traits [35].

Molecular markers are an effective tool for exploring genetic variability among maize lines [2,58,59]. These markers can assist maize breeders in recognizing parental lines with desirable characters for generating high yielding hybrids. The results presented genetic variability levels among parental lines employing SSR markers. The genetic diversity analysis utilizing SSR markers indicated an average of 3.5 alleles/locus, fluctuating from 2 to 5 alleles. This coincides with previous results of Wegary et al. [60], and Kamara et al. [35]. Variations in allele numbers across studies may be influenced by factors such as the specific SSR markers employed and the population size. The PIC values of SSR markers were employed to evaluate their ability to discriminate between parental genotypes. The markers umc-1014 and umc-1033 exhibited high PIC values of 0.68 and 0.74, respectively, indicating their effectiveness in differentiating parental inbreds based on their genetic associations. The SSR markers used in the analysis divided the parental lines into three different clusters. Crossing distinct lines from distinct groups can produce improved high yielding hybrids. The information obtained from cluster analysis might assist in decreasing crosses needed to be assessed in field trials. The lack of association between genetic diversity through SSR markers with SCA effects and hybrid performance for evaluated traits might be ascribed to small number of markers employed. This is proven by the results reported by Dhliwayo et al. [61].

Contrarily, Phumichai et al. [34] and Reif et al. [62] elucidated a substantial relationship between genetic diversity and performance of maize hybrid. In the study of Phumichai et al. [34] seven parental maize genotypes were evaluated employing a set of sixty-four SSR loci. Also, Reif et al. [62] assessed 336 maize genotypes (48 individuals from seven populations) using eighty-five SSR markers. The increasing number of markers used in these studies enhanced the resolution of genetic diversity assessments. The higher number of markers amplified more loci and provided a broader view of genetic markers across the genomes of the parental lines and more genetic differences. The present findings indicated that the SCA effects are substantially associated with performance of maize hybrids. These results concur with the previous results of Mageto et al. [21], Kamara et al. [35].

5 Conclusions

The current investigation exhibited substantial genetic variability among the assessed maize hybrids for all evaluated agronomic characters. Nonadditive effects were more prevalent for yield characters. The lines P_1 and P_3 were determined as effective combiners for developing early maturing genotypes. Besides, P_3 and P_4 were determined as superior combiners to improve grain yield and most of its contributing attributes. The hybrids $P_1 \times P_5$ and $P_4 \times P_7$ possessed favorable SCA along with desirable superior grain yield. These promising hybrids would be additionally assessed for potential commercial exploitation. In the current investigation, SSR-based genetic diversity did not significantly predict SCA effects or performance of hybrids. Notwithstanding, SCA could be substantially exploited to expect performance of developed hybrids.

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Appendix A

Table A1: Code, pedigree and source of the seven maize lines

Code	Pedigree	Source
P ₁	Locally developed	ARC Egypt
P ₂	Locally developed	ARC Egypt
P ₃	Locally developed	ARC Egypt
P ₄	Locally developed	ARC Egypt
P ₅	[7480{TZVAR}/TZSR]-Y-1-345-1.1.1.1.S5.2.5.4.4.4.b.b	CIMMYT-Mexico
P ₆	[7794]. SELF.4.1.S9.1.4.7.4.5.b.b	CIMMYT-Mexico
P ₇	[EMSR] #B# bF101 sr.2.1.sr.3.2.4.b.b	CIMMYT-Mexico

Table A2: Some characteristics of the soil in the experimental site during the two growing seasons

Properties	Season 1	Season 2
Soil texture	Clay	Clay
Sand (%)	15.83	14.40
Silt (%)	33.77	32.80
Clay (%)	50.40	52.80
pH (1:2.5 water suspension)	8.3	7.90
EC (dSm ⁻¹)	3.35	3.46
Organic matter	1.76	1.52
Available nitrogen (mg kg ⁻¹)	34.56	31.75
Available phosphorus (mg kg ⁻¹)	10.42	10.20
Available potassium (mg kg ⁻¹)	270.63	265.80

Table A3: SSR primers and their sequences used in the present study

Marker	Forward primer	Reverse primer
Phi308707	GCAACAAGATCCAGCCGAT	GTCGCCCTCATATGACCTTC
Phi96100	AGGAGGACCCCAACTCCTG	TTGCACGAGCCATCGTAT
Phi453121	ACCTTGCCTGTCCTTCTTTCT	CAAGCAAGACTTTTGATCAGCC
Phi072	ACCGTGCATGATTAATTTCTCCAGCCTT	GACAGCGCGCAAATGGATTGAACT
Umc2038	ACAGAAACCAATGCATGTGATGAG	ACAGAAACCAATGCATGTGATGAG
Phi024	ACTGTTCCACCAAACCAAGCCGAGA	AGTAGGGTTGGGGATCTCCTCC
Umc1014	GAAAGTCGATCGAGAGACCCTG	CCCTCTCTTCACCCCTTCCTT
Umc2332	GTCGGAGAAGGAGCTACTGAGCTA	CACAGGTACGTCTGGATGCTGT
Phi233376	CCGGCAGTCGATTACTCC	CGAGACCAAGAGAACCCTCA
Phi015	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG
Umc1033	CTTCTTCGTAAAGGCATTTTGTGC	GTGCGGGATTCTTAGTTTGC
Phi108411	CGTCCCTTGGATTTTCGAC	CGTACGGGACCTGTCAACAA
Phi301654	GAATGCATGCTTTTCAAGGAC	CGCACAGAGAGCAGAACG

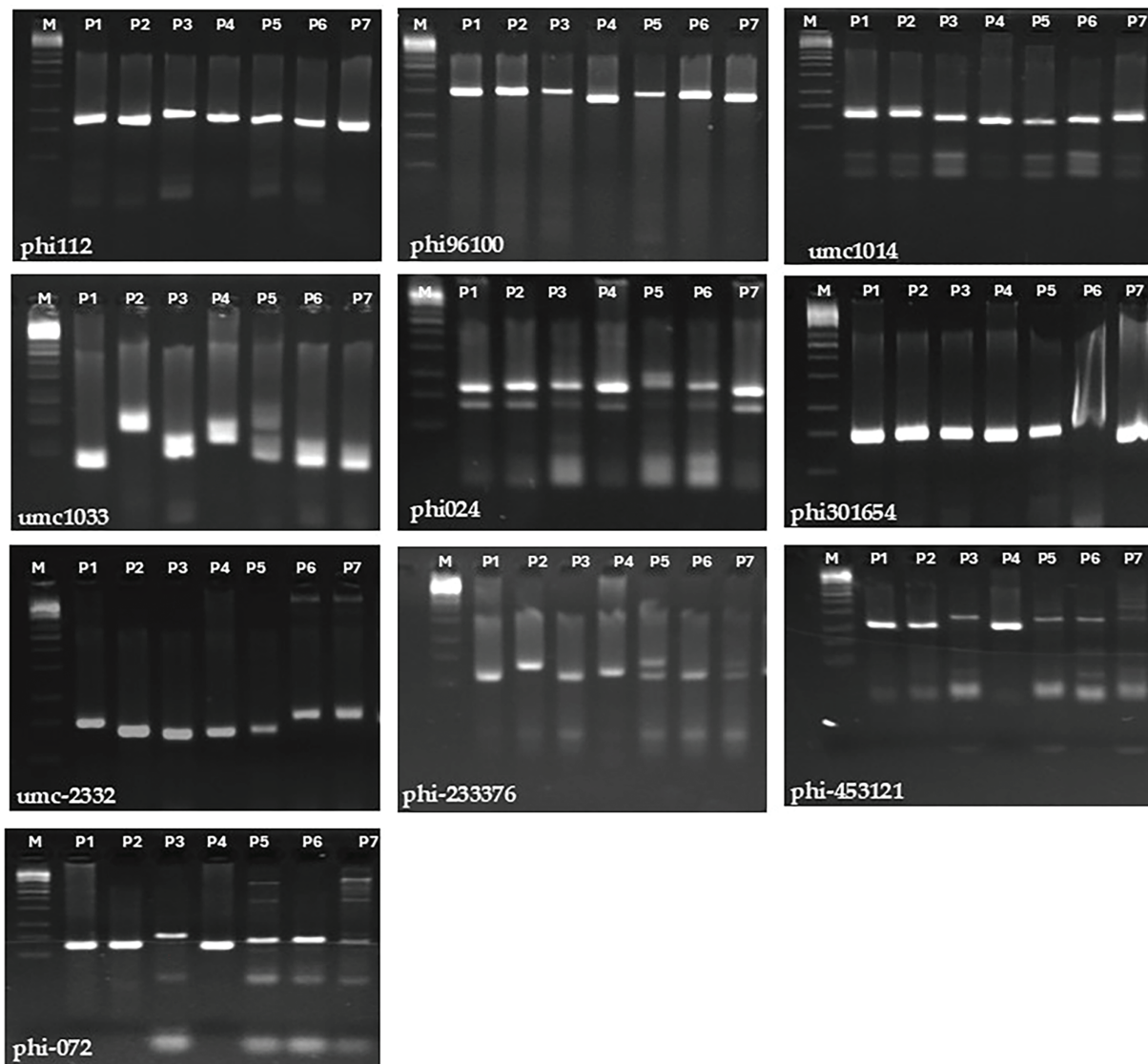


Figure A1: SSR-PCR amplification patterns of seven maize inbred lines using ten SSR primers