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Research article

Estimation of Combining Ability in Field Maize Inbred Lines for Adaptive and Yield Component Traits Using Half-Diallel Crosses Under Well-Watered and Water-Stressed Conditions

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Abstract

Climate variability and prolonged droughts pose significant threats to field maize production, emphasizing the urgent need for drought-tolerant varieties to reduce yield losses and enhance resilience. This study aimed to identify superior maize hybrids by evaluating the general combining ability (GCA) and specific combining ability (SCA) of inbred lines. Genetic diversity analysis using 523 SNP markers identified 240 markers with a minor allele frequency (MAF) ≥ 0.05. The results categorized inbred lines into six clusters based on genetic distance, which ranged from 0.01 to 0.46. Ten lines from each cluster were selected for half-diallel crosses, generating 45 F1 hybrids. Field trials under well-watered and water-stressed conditions were conducted during the 2022 and 2023 dry seasons using a split-plot in randomized complete block design with two replications. Combining ability analysis showed that GCA, reflecting additive genetic effects, significantly influenced adaptive traits such as anthesis-silking interval (ASI), stay-green (SG), SPAD (chlorophyll content), and plant height (PH) traits associated with photosynthetic efficiency, reproductive success, and drought tolerance. Key parental lines, including Ki58, Nei582046, Kei1508, and Kei1618, were identified as strong donors for drought-adaptive traits, while DTMA192 was a significant contributor to yield component traits. Promising crosses, DTMA192 x Kei1618 and Ki58 x DTMA192, exhibited substantial SCA effects for adaptive and yield component traits, supporting a dual breeding approach. Integration of drought-adaptive trait selection based on parental GCA effects and hybrid performance on SCA provides an effective strategy to develop resilient, high-yielding maize hybrids, which are critical for regions facing water scarcity and climate change.

Keywords: drought tolerance; additive and non-additive effects; genetic diversity; SNP markers; diallel mating design

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1. Introduction

The development of maize varieties with enhanced drought tolerance is a critical focus in agricultural research, given the increasing frequency and severity of droughts due to climate change. Maize, a staple crop globally, is particularly vulnerable to water stress, which can significantly impact yield and food security. Drought stress can occur at any growth stage of maize, but it is most critical during the flowering and grain-filling stages, causing severe yield losses of up to 50% and 21%, respectively (Sheoran et al., 2022). During these stages, drought inhibits ear development, causes abnormal spikelet differentiation, changes hormone signaling involved in cell division and growth, and leads to asynchronous development in the tassel and ear. This results in a significant extension of the anthesis-silking interval (ASI) and reduction in silk receptivity, adversely affecting pollination and kernel set (Khan et al., 2022). Maize is a water-sensitive crop, with most grown in rain-fed areas that receive 300-500 mm of precipitation, which is near or below the critical level for good yields. In India, maize requires 500-600 mm of rainfall, but 80% of wet-season maize is rain-fed, facing erratic rains and water deficits from pre-flowering to late grain-filling stages. Dry-season maize also faces water shortages, with losses linked to irrigation shortages (Sah et al., 2020). To mitigate the negative impacts of drought on maize production, developing drought-tolerant maize varieties using various breeding approaches is essential. Leveraging genetic diversity and combining ability presents a promising approach to improving drought tolerance in maize. The importance of heterosis in plant breeding is significant and multifaceted, as evidenced by the sources provided. Heterosis, also known as hybrid vigor, plays a crucial role in developing plant varieties with higher yields, improved quality, and enhanced tolerance to environmental stresses like drought and temperature extremes. By harnessing heterosis, maize lines could be classified into specific heterotic groups, facilitating the development of new high-performing hybrids that surpass existing commercial varieties in key agronomic traits. (Xiao et al., 2021). The sources emphasize the significance of heterosis in maize breeding programs, highlighting its role in enhancing hybrid performance, predicting hybrid vigor, and accelerating the development of superior maize varieties. Through the exploitation of heterosis, breeders can create hybrids with increased adaptability to adverse environmental conditions, ultimately contributing to improved agricultural productivity, farmer incomes, and food security. The sources provide insights into the genetic mechanisms underlying heterosis in maize, emphasizing the importance of combining ability, genetic diversity, and heterotic patterns in breeding programs (Yu et al., 2020). Genetic diversity is a key factor in breeding for drought tolerance in maize. Diverse germplasm sources, including landraces, wild relatives, and exotic materials, harbor a wide range of alleles that can contribute to drought resistance mechanisms. Evaluating and characterizing the genetic diversity of maize germplasm for drought tolerance using high-throughput single nucleotide polymorphism (SNP) markers is the first step in identifying superior parents for hybridization (Menkir et al., 2024). The utilization of genetic diversity through SNP markers in maize breeding has been a significant focus in recent research studies. SNP markers, which are single nucleotide polymorphisms, offer a high-resolution tool for assessing genetic diversity and population structure in maize inbred lines. Studies have employed SNP markers to evaluate genetic purity, parentage verification, and diversity among maize inbred lines, providing valuable insights for breeding programs (Josia et al., 2021). Heterotic groups allow breeders to exploit the genetic diversity present in maize germplasm more effectively. By categorizing inbred lines into distinct heterotic groups based on their genetic backgrounds and combining abilities, breeders can strategically select parental lines with complementary traits for hybridization, leading to increased genetic variation in the resulting hybrids (Fan et al., 2018). Combining ability analysis is a powerful tool for assessing the potential of maize lines to produce superior hybrids for drought tolerance. General combining ability (GCA) estimates the average performance of a line in hybrid combinations and reflects the additive genetic effects, while specific combining ability (SCA) measures the deviation of a hybrid from the expected performance based on the GCA of its parents, indicating the presence of non-additive gene action. Identifying lines with high GCA for drought tolerance and those that exhibit high SCA in specific hybrid combinations can guide the selection of parents and the development of superior drought-tolerant hybrids (Sedhom et al., 2024). The integration of genetic diversity assessed by SNP markers and combining ability analysis is a promising approach for developing drought-tolerant maize varieties. By exploiting the available genetic resources and identifying superior combining lines, the development of high-yielding maize hybrids that can withstand drought stress could be successful and ensure food security in drought-prone regions. The present study aimed to assess the genetic diversity of maize inbred lines and evaluate their combining ability for developing drought-tolerant maize hybrids.

2. Materials and Methods

2.1 Sources of plant materials

In this study, fifty maize inbred lines were evaluated, comprising twenty lines sourced from the National Corn and Sorghum Research Center and the remaining lines from the Nakhon Sawan Field Crops Research Center (Table 1).

2.2 Extraction of genomic DNA

All fifty maize inbred lines were planted, and young leaf tissues were sampled from each line for DNA extraction using the cetyltrimethylammonium bromide (CTAB) method, following the protocol of Doyle (1987). The DNA concentration was measured at 260 nm with a NanoDrop™ 8000 Spectrophotometer, and DNA quality was verified based on A260/230 and A260/280 absorbance ratios. The extracted DNA was adjusted to a final concentration of 15 ng/µL for subsequent analyses.

2.3 Genotyping with Single Nucleotide Polymorphism (SNP) marker

The genotyping process for this study involved screening with 523 SNP markers designed based on published genes associated with drought tolerance traits as shown in Figure 1, including hormone signaling, ROS scavenging, stomatal regulation, root development, photosynthesis, and sugar metabolism (Shikha et al., 2017; Van Gioi et al., 2017; He et al., 2018). Genotyping followed the Kompetitive allele-specific polymerase chain reaction (KASP™) method as described by Semagn et al. (2014), using high-throughput genotyping equipment. Quality control, filtering, and data imputation were performed using TASSEL software v.5 (Bradbury et al., 2007). SNP markers with a minor allele frequency (MAF) below 5% and with more than 10% missing data were excluded. Following quality control, 240 SNP markers from the original 523 were retained, distributed across all 10 maize chromosomes: chromosome 1 (34), chromosome 2 (17),

Table 1. List of inbred lines used in this study

No.	Name	Pedigree
1	Ki3	Suwan 1(S)C4-S_8-5-3(2007)
2	Ki11	Suwan 1(S)C4-S_8-18-7(2025)
3	Ki45	{(Ki21xTzi15)-S_2xKi21}-S_8-36-2-2-2(Kei9304)
4	Ki47	KS6(S)C3-F_2-S_8-554-2-1-2-1
5	Ki48	Pioneer3013-S8-57-2
6	Ki53	KS27(S)C1-S8-39-1-1(Kei0703)
7	Ki58	(Agron8xKi50)-S12-10-1-1-2-1(Kei1023)
8	Ki59	(Agron35xKi45)-S8-6-1-2-1-3(Kei1024)
9	Ki60	(Agron12xKi49)-F2-S9-2-1-3-1-1(Kei1102)
10	Kei1420	Suwan5(S)C6-F2-S9-030-2-1-2-1-B
11	Kei1421	(Ki49xKei9806)-F2-S7-4-1-1-BxKei0703}-F2-S8-9-1-1-1-1-1
12	Kei1508	Suwan1(S)C15-F2-S7-222-1-1-3-1-1-B
13	Kei1509	Suwan1(S)C15-F2-S7-231-2-1-1-1-1-B
14	Kei1519	Suwan3(S)C8-F3-S6-142-1-1-1-1-B
15	Kei1533	KS28(S)C2-F2-S9-350-2-1-1-1-1-B
16	Kei1608	(Pac224-F2xPac999N-F2) F2-S8-6-1-1-6-B-B
17	Kei1615	(30B80-F2x30A33-F2)-S7-56-3-1-3-B-B
18	Kei1618	Suwan1(S)C13-F2-S9-224-3-1-1-1-2-B
19	Kei1715	(Kei1019xKi60)-F2-S8-12-1-3-1-4-2-3-B
20	Kei1719	(Kei1019xKi60)-F2-S8-34-1-1-1-1-1-1-B
21	Nei9008	(DA9-1(S)-7-3-1xSW1C9)-S9-19-11-1-B
22	Nei9202(T)	Pop.28(HS)C6-S9-5-2-1-B
23	Nei412019	DK888-S6-14-1-2-1-BB
24	Nei422006	KS23(S)C2-285-2-3-2-11-B-B
25	Nei422007	KS23(S)C2-285-2-3-2-14-B-B-#
26	Nei452004	KS23(S)C2-190-1-2-1-BBBBBB(LT)
27	Nei452006	Big939-59-2-B-1-2-2-BBBB
28	Nei452008(TF1)	Pio.3003-3-2-B-3-1-4-BBBB
29	Nei452009	C-5124001-57-1-B-2-2-3-BBBB
30	Nei452013	C-5124001-14-1-B-1-1-1-BBBB
31	Nei452015(TF3)	C-5124001-21-2-B-2-1-2-BBBB
32	Nei452026	C-5124001-57-1-B-1-1-3-BBBB
33	Nei452029	LY-AL-TO(S)C1-22-2-2-BBBB
34	Nei462013	(KS23(S)C2-190-1-2-1-BBBBxPIONEER3006-4-1-3-1-BBB)-37-1BBBBB
35	Nei492006	XPT9774-BBBBB-1
36	Nei492022	NS-KS23(S)C0F2-B-62-BBB-2
37	Nei492024	(Nei402020xNei402003)-BBBBBB-2
38	Nei502002	CA00388/KTX3752F2-7-1-1-2-BBBB-B-B-B-B-B
39	Nei502007	CTS013058/(AMATLC0HS167-1-1-1-2F/R)-BBBBB/Nei402011-BB-B-B
40	Nei512008	NP99202(RRS)C0-376-B-2-B-B-B-B
41	Nei512013	(Nei9202xNei422004)-BBBBBBB
42	Nei532003	NP99202(RRS)C1-28-B-1-B-B-B
43	Nei532005	NP99202(RRS)C1-35-B-1-B-B-B
44	Nei541017	NSEYP1(RRS)C1F2-157-2-1-B-B-B
45	Nei542010	[(KS23(S)C2-190-1-2-1-BBBBxPIONEER3006-4-1-3-1-BBB)-40-3-BBBBBxNei452008]-F2-B-B-B-10-1-B-B-B
46	Nei582016	KS23(S)C4-289-B-B-1-B-B-B
47	Nei582046	(Nei9202(T)xNei422004)-BBBBBB-1-B-B-B
48	DTMA192	CLQ-RCYQ54=(CML176xCL-G2501)-B-3-1-B-B-B
49	DTMA193	CL-RCY015=(CML-285*CL-00356)-B-1-1-B*9-B
50	DTMA202	CL-RCY031=(CL-02410*CML-287)-B-9-1-1-2-B*7-8

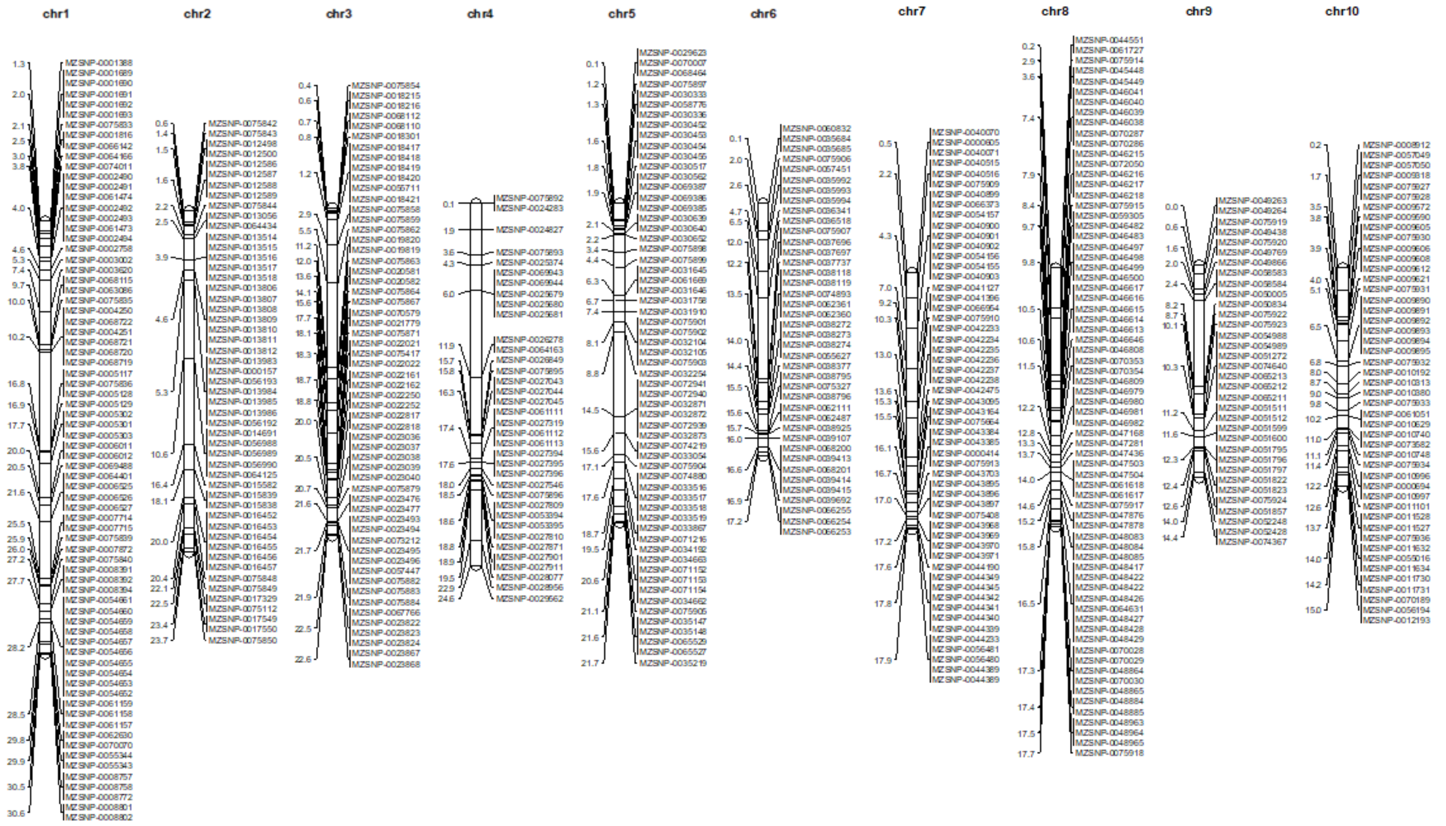

Figure 1. The distribution of 523 SNP markers that were initially designed to assess the genetic diversity of the 50 maize inbred lines.

chromosome 3 (17), chromosome 4 (18), chromosome 5 (39), chromosome 6 (20), chromosome 7 (24), chromosome 8 (35), chromosome 9 (16), and chromosome 10 (20). Only SNP markers that met these filtering criteria were selected for diversity analysis.

2.4 Diversity analysis

SNP genotypic data of 50 maize inbred lines and 240 markers were calculated for genetic distances, following the identity-by-state (IBS) similarity coefficient concept (Powell et al., 2010), using TASSEL software v. 5. (Bradbury et al., 2007). Subsequently, a cladogram was constructed using the neighbor-joining algorithm (Saitou & Nei 1987). Clusters were established using this phylogenetic tree among the inbred lines.

2.5 Field experiments

2.5.1 Experimental design and management

Ten maize inbred lines, representative of each cluster, were chosen and crossed in a 10 x 10 half-diallel mating scheme (Sprague & Tatum, 1942) to generate 45 F1 hybrid combinations. Those parental lines were Ki58 (P1), Nei582046 (P2), Nei492006 (P3), Nei492024 (P4), Ki11 (P5), DTMA192 (P6), Kei1508 (P7), Kei1618 (P8), Nei512008 (P9), and Nei532003 (P10). The 10 parental lines and 45 F1 hybrids were cultivated and evaluated twice at the field station of Hortigenetics Research S.E. Asia Ltd., Sansai, Chiang Mai, Thailand, during the dry season of 2022/2023 (October 2022-January 2023) and 2023/2024 (November 2023-February 2024). Soil properties for the experimental plots were as follows: pH 6.48, organic matter 0.92%, total nitrogen 0.046%, and available phosphorus 212 ppm. The experiment was conducted over two years using a split-plot design within a randomized complete block design (RCBD), with two replications. The main plot factor was water management, consisting of two levels, well-watered (WW) and water-stressed (WS) conditions. An irrigation system was applied twice daily, once in the morning for 10 min, delivering approximately 280 mL of water, and once in the afternoon for 20 min, providing around 550 mL of water. This resulted in a total daily water application of approximately 830 mL. Water stress was implemented by withholding irrigation from 2 weeks before silking until the end of the flowering period. The subplot consisted of 45 F1 crosses and their parental lines, with a total of 220 experimental plots (2 years × 2 water management treatments × 55 genotypes). Agronomic practices included spacing of 0.50 m between rows and 0.30 m between plants. Each plot consisted of 10 plants.

2.5.2 Data collection

Five plants in the middle of the row were selected from each plot during the vegetative stage. The following phenotypic traits were evaluated: plant height (PH) at the tasseling stage, days to 50% silking (SD), days to 50% anthesis (AD), anthesis–silking interval (ASI) as estimated from the difference between SD and AD, SPAD readings at 10 days after water withholding (SPAD), and percentage of stay-green at 10 days after water withholding (SG). For yield component traits, the top ears from all 10 plants in each plot were harvested to measure ear weight (EW), kernel weight (KW), and 100-kernel weight (100-KW). Data for all nine traits were collected across both years.

2.6 Statistical analyses

A combined analysis of variance (ANOVA) was conducted on all data to assess the variability among parents and their hybrids by using R version 4.3.1 statistical software (R Core Team, 2025). The mean performances of hybrids and parental inbred lines were compared using the least significant difference (LSD) method at a 5% level of significance. Griffing’s analysis for Method 2, Model 1 was used for estimating the effects of general combining ability (GCA) of the parents and the effects of specific combining ability (SCA) of the hybrids, which were obtained from the following equations.

$$g_i = \bar{y}_{i.} - \bar{y}_{..}$$

$$s_{ij} = y_{ij} - \bar{y}_{..} - g_i - g_j$$

Where g_i and g_j are the GCA effects for *i*-th and *j*-th parental lines, respectively; s_{ij} is the SCA effect for *ij*-th hybrid; y_{ij} is the trait value of *ij*-th hybrid; $\bar{y}_i.$ is the average of the hybrids among the *i*-th line crossed with a series of parents; $\bar{y}_i..$ is the overall mean.

3. Results and Discussion

3.1 Cluster analysis using SNP markers

The fifty maize inbred lines were genotyped using 240 high-quality SNP markers. According to the genotypic data, chromosome 5 was found to have the highest number of SNP markers, which was 39 in total whereas the lowest number was observed on chromosome 9 (16 SNP markers). This is a 47% decrease for chromosome 9 relative to the number of SNPs before filtering. Thus, a low SNP marker count does not reduce the total genome coverage needed for a large, even distribution of markers and thus highly accurate diversity index calculations. The clustering analysis with IBS identified 6 distinct clusters as shown in Figure 2. In a phylogenetic tree, groups are typically separated based on genetic differences, with differentiation achieved through genetic distance, branching patterns, and clustering algorithms. Genetic distance measures the number of differences in DNA sequences, grouping more similar sequences closer together while placing those with greater differences in separate groups. The branching structure of the tree, where each branch point or node represents a divergence event, indicates that sequences sharing a more recent common ancestor are grouped. Additionally, clustering algorithms like Neighbor-Joining are often used to analyze genetic data, clustering sequences based on their similarities and differences to form distinct groups. A clustering analysis of the 50 inbred maize lines based on genetic distances (GD) showed six major clusters, corresponding with GD values ranging from 0.01 to 0.46 and an average distance of approximately 0.33 (Figure 2). Inbred lines Nei422007 and Nei422006 which were obtained from the Nakhon Sawan Field Crops Research Center shared a genetic relationship (GD = 0.01). Nei422007 and Nei42006 were specifically derived from pedigree KS23(S)C2-285-2-3-2-14-B-B-#, while Nei422006 was derived from KS23(S)C2-285-2-3-2-11-B-B. This close genetic relationship aligns with their shared source population and similar selection histories, suggesting limited diversification during selective breeding. The contrast was greatest when considering the maximum genetic

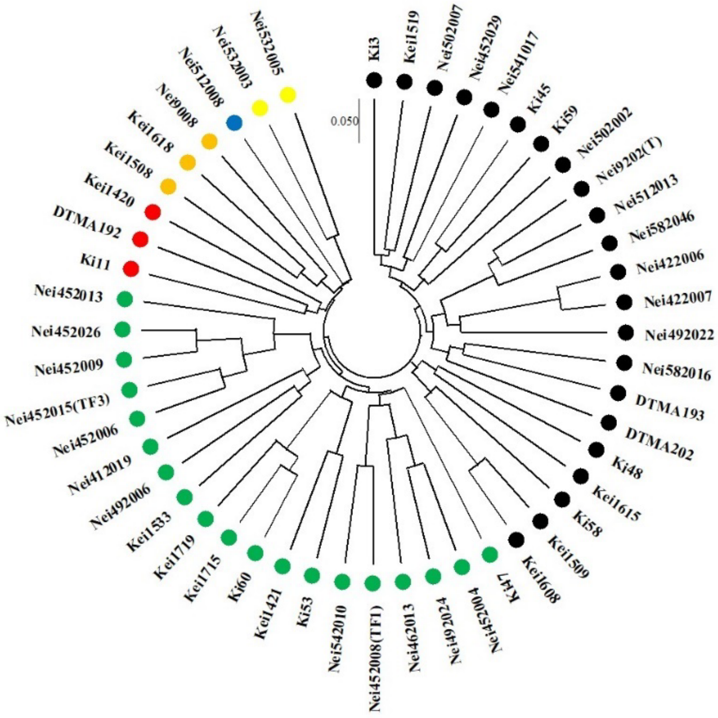

Figure 2. A cluster of the 50 maize inbred lines was evaluated according to the Neighbor-Joining method, considering the data of 240 SNP markers. Genetic distances between inbred lines were calculated using the identity-by-state (IBS) coefficient with TASSEL (version 5.0).

distance (GD = 0.46) between Nei452004 and Ki3, which were lines from totally different breeding backgrounds. Nei452004 (Nakhon Sawan Field Crops Research Center) had the pedigree KS23(S)C2-190-1-2-1-BBBBBB(LT), whereas Ki3 obtained from the National Corn and Sorghum Research Center, originated from Suwan 1(S)C4-S8-5-3(2007). This high GD reflects divergent breeding goals and source populations. These findings highlight the genetic diversity within the analyzed inbred lines and support previous research underscoring the advantages of crossing genetically diverse parents to enhance hybrid vigor by crossing parents of high genetic diversity. A narrower range of GD is possible if specific SNPs are chosen, as it focuses the genetic diversity assessment on the traits that these particular SNPs represent. For example, if SNPs are selected based on genes associated with drought tolerance, most GD values will reflect genetic differences directly or indirectly linked to traits associated with drought resilience. Consequently, the GD range may be narrower than the result of using a larger set of genome-wide SNPs, which captures broader genetic variation. There are a couple of notable benefits to this targeted approach. First, genetic distance is measured more efficiently, as the analysis focuses on SNPs directly related to trait variance in the data. This allows for a clearer understanding of the type and extent of genetic diversity that may be useful for contrasting phenotypes. Second, it improves the accuracy of heterotic grouping because while little direct knowledge has been gained about GD values correlating with genetic differences important for breeding objectives related to drought tolerance or root traits, focusing on trait-associated SNPs provides meaningful insights. This trait-focused approach is highly valuable for selecting parent lines and designing crosses to produce elite hybrids with desirable agronomic traits. However, although the GD range can be reduced by focusing on SNPs, this refinement makes the analysis more meaningful, as it directly aligns with desired target or breeding objectives. This is consistent with previous studies that showed the great advantages of SNP-based evaluation and group-wise association mapping in breeding programs to promote heterotic grouping and appropriate parental selection for targeted traits (Semagn et al., 2014; Van Gioi et al., 2017; Adu et al., 2019; Dube et al., 2023).

3.2 Descriptive statistics of all 9 traits

The statistical analysis of 9 adaptive and yield component traits revealed significant differences between well-watered (WW) and water-stressed (WS) conditions. Overall, mean trait values were consistently lower under WS conditions, underscoring the adverse effects of water stress on plant growth and yield (data not shown). For example, the average plant height (PH) under WW condition was 219.83 cm, which decreased slightly to 218.65 cm under WS, suggesting a marginal impact of water stress on vegetative growth. Similarly, reproductive traits, such as SD and AD, were delayed under WS conditions. The mean SD was 58.32 days under WW compared to 58.33 days under WS, while AD decreased slightly from 60.06 days under WW to 59.45 days under WS. Notable differences were observed in drought-adaptive traits. The ASI, a critical indicator of reproductive stress, increased under WS, averaging -1.12 days compared to -1.75 days under WW. This increase in ASI under WS conditions suggests extended anthesis-silking intervals due to water stress, potentially affecting pollination and kernel set. The results underscore the substantial impact of water stress on both adaptive and yield traits, demonstrating the critical need for breeding drought-tolerant varieties. Consistent with previous studies, the reductions observed in plant height, anthesis-silking interval, and stay-green traits highlight how water limitation disrupts both vegetative and reproductive processes, ultimately affecting yield components (Blum, 2011; Araus & Cairns, 2014). The delayed anthesis-silking interval observed under WS condition aligns with research indicating its association with reproductive stress and pollination efficiency under drought (Edmeades et al., 1997). Significant reductions were also recorded in SPAD chlorophyll content (SPAD) and stay-green (SG), which are essential indicators of photosynthetic efficiency and drought tolerance. The average SPAD dropped from 51.27 under WW to 41.89 under WS, while SG decreased markedly from 77.77 under WW to 61.72 under WS, indicating accelerated leaf senescence and reduced chlorophyll retention under drought conditions. Decreased chlorophyll content, as seen in the reduction of SPAD values, similarly reflects stress-induced limitations on photosynthetic efficiency, consistent with findings by Lobell and Gourdji (2012). Lower stay-green values suggest accelerated senescence, which is often linked to reduced water use efficiency and drought sensitivity, supporting findings by Tardieu (2012) that emphasized the importance of stay-green as a drought tolerance trait. Yield component traits also showed marked declines under WS condition (Figure 3). For instance, EW decreased from 2.38 g to 2.09 g, and KW dropped from 1.67 g to 1.41 g. The reduction in 100-KW, from 36.31 g under WW to 34.16 g under WS, further underscores the negative impact of water stress on yield components. These findings highlight the substantial effects of water stress on both adaptive and yield traits, emphasizing the need to develop drought-tolerant varieties.

3.3 Analysis of variance

All nine phenotypic traits plant height (PH), days to 50% silking (SD), days to 50% anthesis (AD), anthesis-silking interval (ASI, calculated as the difference between AD and SD), SPAD readings at 10 days after drying (SPAD), stay-green at 10 days after drying (SG), ear weight of 10 plants with the top ear (EW), kernel weight of 10 plants with the top ear (KW), and 100-kernel weight (100-KW) were analyzed using two-way ANOVA (Table 2). This analysis provided valuable insights into the contributions of environmental and genetic factors on the adaptive and yield component traits in maize. The year effect was highly significant for both adaptive and yield traits ($P<0.001$), indicating substantial variability between growing seasons from 2022 to 2024. This variability was likely influenced by environmental conditions, primarily temperature fluctuations and humidity levels. Table 3 presents the environmental conditions recorded in the experimental area during the 2022/2023 and 2023/2024 maize growing seasons. During the vegetative (October to December) and reproductive (January to February) stages, the 2023/2024 season experienced average temperatures 1-4°C higher than those in 2022/2023, along with slightly lower relative humidity, particularly in December and February. Rainfall was minimal in both seasons; however, 2023/2024 found some light rain in such cultivation months, while 2022/2023 recorded no rainfall from November to February. The harvest in March 2023/2024 also recorded marginally higher temperatures and lower relative humidity than 2022/2023. Overall, these data indicated that the 2023/2024 season was warmer and somewhat drier, conditions which may have affected maize growth and stress responses. The significant year effect ($P<0.001$) for both adaptive and yield traits highlight the need to consider annual climatic variability in trait assessments, as environmental conditions change notably across seasons (Challinor et al., 2014). The warmer, drier conditions in 2023/2024 likely influenced maize growth, affecting both vegetative and reproductive phases. These findings align with Chaves et al. (2003), who emphasized that increased temperatures and reduced humidity can exacerbate drought stress in maize, ultimately impacting productivity. The effect of water condition was significant, being deleterious in most cases to the adaptive and yield traits considered especially those associated with reproductive timing. However, its negative impact was partially mitigated due to its relationship with photosynthetic efficiency and output. Traits such as AD, ASI, SPAD, SG, EW, KW, and 100-KW were significantly affected by water availability, underscoring the critical role of water management in maintaining crop performance under drought conditions. The genotype effect was highly significant across all traits in both adaptive and yield component groups, reflecting the genetic diversity present among the evaluated maize lines. This result underscores the importance of selecting genotypes with stable performance across various environments, which is fundamental in breeding programs focused on drought tolerance. The significant genotype effect across traits highlights the need for such stability, as genotypes that consistently perform across diverse conditions are essential for reliable yield outcomes in drought-resilient breeding programs (Hallauer et al., 2010). Water condition effects were also significant, impacting reproductive traits such as AD and ASI, which are essential for pollination and grain set in maize. Similar findings by Barker et al. (2005) noted that water stress tends to delay reproductive timing and reduce yield in drought-prone environments. The decrease in photosynthetic efficiency, as reflected in SPAD and SG values, further emphasizes the importance of managing water availability to mitigate the adverse effects

Table 2. Mean squares from the analysis of variance for combining ability in maize for adaptive and yield component traits under well-watered and water-stressed conditions in 2022 and 2023

SOV	df	Mean square								
		PH	**SD**	**AD**	**ASI**	**SPAD**	**SG**	**EW**	**KW**	**100-KW**
Year	1	4802.60***	545.68***	1393.38***	195.11***	1555.21***	6564.90***	63.67***	2.89*	1524.90***
Block	1	532.00	61.10***	47.80***	0.82	58.00	24.00	1.18***	0.02	144.30***
Water condition	1	153.00	0.00	41.40***	42.66***	9680.00***	28332.00***	8.90***	7.72***	509.20***
Year x Block	1	11238.00***	10.50*	4.20	1.42	2192.00***	1237.00***	1.68***	0.00	143.20***
Year x Water condition x Block	2	10.00	5.30	4.20	2.68	110.00*	123.00*	0.01	0.67	20.80
Genotype	54	6256.90***	41.85***	46.20***	13.37***	94.84***	104.90***	2.26***	1.71***	79.04***
GCA	9	29231.40***	105.66***	133.97***	119.01***	567.83***	745.20***	4.81***	3.06***	292.50***
SCA	45	9170.40***	79.31***	84.08***	8.30***	114.04***	102.70	4.46***	3.50***	131.19***
Year x Water condition	1	1115.00*	0.00	11.50*	10.82*	306.00**	390.00***	0.18	0.15	6.10
Year x Genotype	54	354.80***	4.20**	4.25***	4.79***	36.25	42.70*	0.31***	0.71	19.08**
GCA x Y	9	1057.20***	4.29	20.81***	19.79***	135.12*	136.3	1.71***	1.64**	46.07***
SCA x Y	45	640.20***	9.21***	6.05***	7.53***	59.97	75.2	0.40***	1.38***	36.58***
Water condition x Genotype	54	134.00	2.30	2.40	1.83	18.00	30.00	0.06	0.64	9.40
Year x Water condition x Genotype	54	133.00	2.20	2.30	1.67	21.00	40.00*	0.05	0.54	10.10
Residuals	216	191.00	2.40	1.90	1.97	28.00	28.00	0.07	0.60	10.60
GCA/SCA		3.19	1.33	1.59	14.34	4.98	7.26	1.08	0.87	2.23

*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively. Adaptive traits include plant height (PH), days to 50% anthesis (AD), days to 50% silking (SD), anthesis-silking interval (ASI), SPAD, and stay-green (SG). Yield component traits include ear weight of the top ear (EW), kernel weight (KW), and 100-kernel weight (100-KW).

Table 3. The average values of temperature, humidity, and rainfall in the experimental area during maize growth in 2022/2023 and 2023/2024

Month	Stage of Maize	Temperature (°C)		Rain (mm)		Humidity (%)	
		2022/2023	2023/2024	2022/2023	2023/2024	2022/2023	2023/2024
October	Vegetative stages	23.94	25.72	0.13	0.17	89.32	89.54
November		22.83	24.80	0.00	0.00	87.40	84.19
December		20.76	24.47	0.02	0.00	87.48	83.19
January	Reproductive stages	18.71	21.08	0.00	0.03	81.48	82.96
February		21.27	21.61	0.03	0.00	75.88	74.26
March	Harvesting	23.47	24.63	0.00	0.08	77.42	70.45

on crop performance. This observation is in line with Fukai and Cooper (1995), who found that chlorophyll retention and leaf senescence are directly influenced by water stress and are crucial for breeding drought-tolerant varieties. In terms of interaction effects, the year × water condition interaction was significant for several adaptive traits, including PH, AD, ASI, and SG, indicating that the impact of water availability varied by year. This interaction underscores the need to consider the combined effects of annual climatic variations and water stress on trait performance. Additionally, the year × genotype interaction was significant for most traits, reflecting variability in genotype stability across years. This finding emphasizes the importance of selecting genotypes with consistent performance across different environmental conditions, supporting Hammer et al. (2005), who highlighted the critical role of genotype-environment interactions in breeding resilient crops. Moreover, the significant year × water condition × genotype interaction for SG and other adaptive traits indicates that breeding for drought tolerance must account for multiple environmental stressors. These findings illustrate the substantial influence of both environmental variability and genetic diversity on trait performance in maize. The complexity of interactions among years, water conditions, and genotypes emphasizes the need for genotypes with robust adaptability and drought resilience. This aligns with Cooper et al. (2009), who highlighted the importance of multi-environment trials to identify genotypes with stable performance across varied conditions. Collectively, these results suggest that breeding for drought tolerance in maize requires selecting genotypes capable of withstanding multi-stress conditions, supporting crop adaptation to climatic variability, and enhancing yield potential under water-limited environments.

3.4 Estimates of combining abilities

The combining ability estimates for adaptive and yield traits revealed the significance of both general combining ability (GCA) and specific combining ability (SCA) in maize breeding programs (Table 2), underscoring the genetic complexity involved in trait inheritance. The strong GCA estimates observed across both adaptive and yield component traits highlighted the importance of additive genetic effects in transmitting desirable traits from parent to offspring, providing a stable basis for trait inheritance across diverse environments (Hallauer et al., 2010). High GCA values for adaptive traits, such as PH and SG, suggested that these traits are primarily governed by additive effects, making them heritable and stable, and essential for breeding drought-resilient maize varieties. This aligns with findings by Bänziger et al. (2000), who emphasized the role of additive genetic effects in enhancing drought tolerance through consistent parental contributions. For yield component traits, high GCA values for EW and 100-KW indicated that additive effects are instrumental in achieving yield stability across various growing conditions, a priority in breeding programs aiming for reliable productivity under stress (Bernardo, 2020). Selecting parental lines with strong GCA for these traits can contribute to stable yield performance, which is especially crucial for food security in drought-prone areas. Conversely, SCA estimates, capturing non-additive genetic effects from specific parent combinations, were significant across many traits but generally lower than GCA estimates. Significant SCA effects for adaptive traits like PH and reproductive traits such as SD and AD reveal the role of non-additive interactions (dominance and epistasis) in enhancing hybrid adaptability, contributing to heterosis or hybrid vigor under stress conditions (Duvick, 1999). Notably, the significant SCA effects observed for EW and 100-KW suggest that specific hybrid combinations yield performance gains beyond additive effects alone, reinforcing the value of SCA-driven selection to maximize heterosis and yield enhancement (Melchinger, 1999). The GCA/SCA ratios provide information into the relative influence of additive and non-additive effects. High GCA/SCA ratios for traits like ASI and SG indicated that additive effects dominate, making these traits well-suited for selection based on GCA to achieve stable drought tolerance. In contrast, the lower GCA/SCA ratio observed for KW underscores the importance of non-additive effects, guiding breeding programs to focus on hybrid combinations that optimize heterosis for yield improvements (Sprague & Tatum, 1942). These results highlight the importance of selecting parental lines with strong GCA for traits influenced by additive effects, such as PH and SG, while targeting specific hybrid combinations with strong SCA for traits where non-additive effects enhance yield performance, such as EW and KW. Together, these findings emphasize the dual role of additive and non-additive effects in achieving stable, high-yielding maize varieties suitable for drought-prone environments.

3.5 General combining ability effects

This study revealed the significance of general combining ability (GCA) effects on adaptive and yield component traits, which are essential for identifying optimal parental lines for high-yield and drought-tolerant maize varieties (Table 4 and Figure 3). Observations revealed a broad range of GCA effects, particularly for PH. Nei582046 (P2) displayed the highest positive GCA value (45.45), indicating its potential to increase PH, a trait linked to yield stability under drought conditions (Campos et al., 2004). Conversely, Ki11 (P5) demonstrated the lowest GCA for PH (-37.22), suggesting a trend toward reduced height, which may be less advantageous for resilience under water-limited conditions. Negative GCA values were advantageous for flowering traits, such as SD, days to anthesis AD, and ASI, as they promote flowering synchrony under drought stress. Lines like Kei1508 (P7) and Nei492006 (P3) demonstrated shorter ASI, contributing to synchronized flowering, which is crucial for kernel set in drought conditions, supporting reproductive success. Positive GCA effects for photosynthetic traits, including SPAD and SG, in lines like Ki58 (P1) and Kei1618 (P8), reflect better chlorophyll retention and delayed senescence, both vital for maintaining photosynthetic efficiency and sustaining growth under drought stress. These findings are consistent with previous studies on the

Table 4. General combining ability estimates of ten parental lines for both adaptive and yield components across two water conditions and two years (2022 and 2023)

Parent	Adaptive trait						Yield component trait		
	PH	SD	AD	ASI	SPAD	SG	EW	KW	100-KW
P1	-20.97**	-1.05**	0.01	-1.06	3.48*	6.22**	-0.11	-0.10	0.98
P2	45.45**	1.55**	0.51	1.05	5.00**	-0.67	0.00	-0.02	-1.31
P3	-4.10	-0.76*	0.74	-1.50*	0.26	-4.09*	-0.14	-0.09	1.18
P4	9.95*	1.14**	1.20*	-0.06	-1.01	-3.47*	0.39*	0.06	1.69
P5	-37.22**	-0.05	-3.35**	3.30**	1.45	-0.82	0.22	0.28	-3.76**
P6	5.84	-0.43	-1.12	0.69	-0.91	2.43	0.64**	0.45*	2.57*
P7	7.43	-2.99	-0.78	-2.20**	-3.11*	-5.35	-0.20	0.18	0.73
P8	20.80**	2.03**	2.07**	-0.04	3.07*	4.62**	-0.29	-0.23	-4.48**
P9	3.47	0.99**	1.99**	-1.00	-1.63	3.26*	-0.30	-0.41*	2.56*
P10	-30.66**	-0.45	-1.26*	0.82	-6.61**	-2.13	-0.21	-0.13	-0.16

*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively. Adaptive traits include plant height (PH), days to 50% anthesis (AD), days to 50% silking (SD), anthesis-silking interval (ASI), SPAD, and stay-green (SG). Yield component traits include ear weight of the top ear (EW), kernel weight (KW), and 100-kernel weight (100-KW)

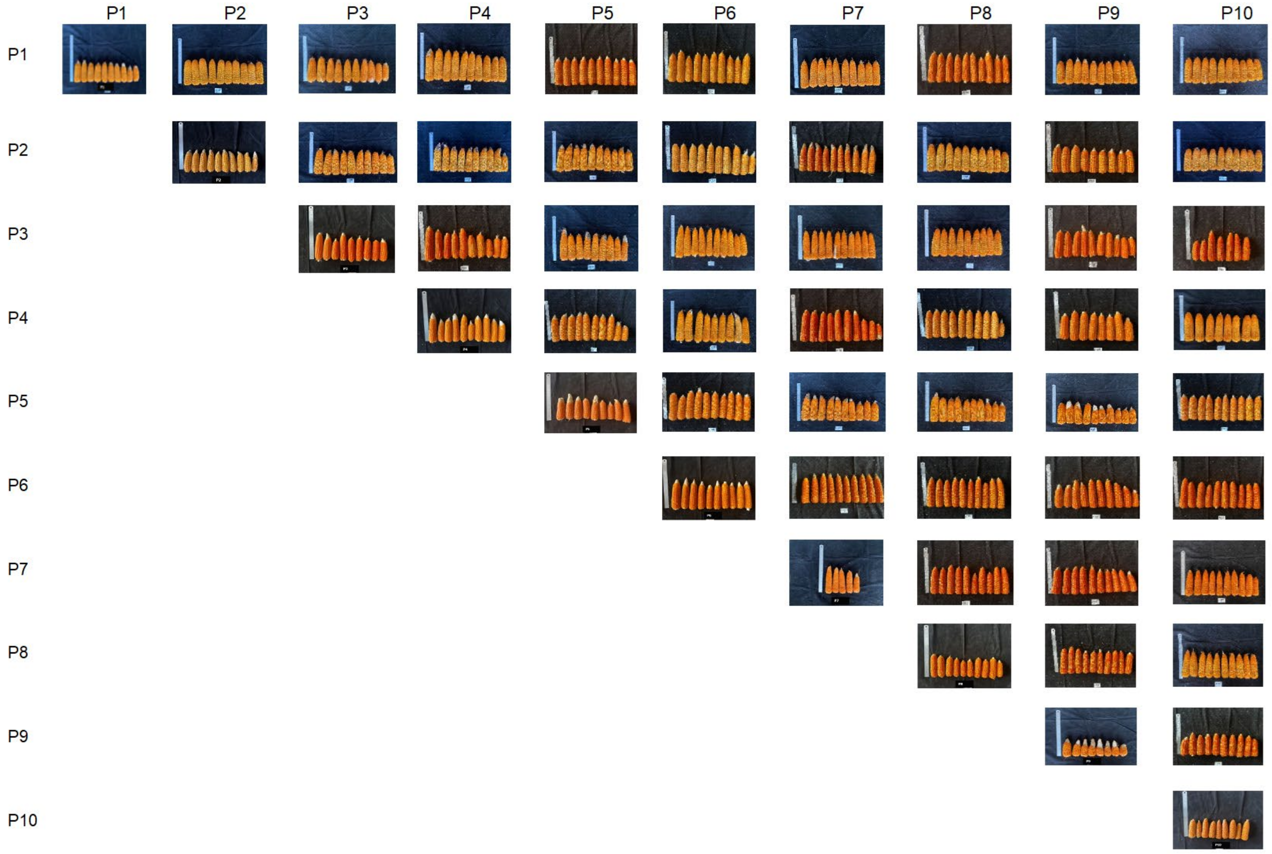

Figure 3. Ear characteristics of 10 parental lines and their 45 F1 crosses, obtained from a half-diallel mating design and tested under water-stressed conditions

role of chlorophyll retention in promoting drought tolerance through prolonged photosynthesis (Blum, 2011). Lines such as Nei582046 (P2) and Kei1618 (P8) exhibited strong GCA across several adaptive traits, including PH and SG, indicating their value in drought-resilient breeding programs. Similarly, Ki58 (P1) and Kei1508 (P7) demonstrated favorable GCA values for flowering synchrony and chlorophyll retention, enhancing their potential as adaptive parental lines. In contrast, yield component traits benefit from positive GCA effects, where DTMA192 (P6) showed significant GCA for EW, KW, and 100-KW. This indicates its potential for yield enhancement, particularly under well-watered conditions, as high GCA values for kernel traits directly impact yield stability (Tester & Langridge, 2010). While DTMA192 (P6) stood out for yield-related traits, Nei512008 (P9) also displayed strong positive GCA for 100-KW, suggesting its potential to improve kernel weight, a trait vital for grain yield. On the other hand, lines such as Ki11 (P5) and Kei1618 (P8) showed negative GCA estimates for KW and 100-KW, indicating a limited role in yield improvement. DTMA192 (P6) is thus a promising candidate for breeding programs aimed at yield enhancement, especially in favorable environments, while pairing it with drought-resilient lines like Nei582046 (P2) or Kei1618 (P8) could yield hybrids combining high productivity with resilience to water stress.

3.6 Specific combining ability effects

The specific combining ability (SCA) effects in this study provided critical insights into non-additive genetic contributions in maize hybrids, highlighting the unique performance potential of specific parental combinations (Table 5 and Figure 3). Unlike GCA, which represents additive genetic effects, SCA reflects dominance and epistatic interactions that contribute to hybrid vigor or heterosis, offering substantial benefits for traits related to drought resilience and yield (Briggs & Knowles, 1967). For adaptive traits, positive SCA effects in parameters like PH, SPAD chlorophyll content (Table 5), and SG indicate that certain hybrids can maintain growth and photosynthesis under drought stress. Notably, hybrids such as P4×P5, P2×P8, and P2×P10 displayed significant positive SCA effects for PH, indicating the potential for increased height, a trait associated with better water use efficiency and drought adaptation (Setter et al., 2001). Conversely, hybrids like P5×P8 with negative SCA for PH (Table 5) may have reduced adaptability in stress-prone environments, as decreased height can be less favorable for water-limited conditions. In reproductive traits, negative SCA effects for AD, SD, and ASI are desirable, as they indicate early flowering and improved synchrony, critical for pollination success under drought. Hybrids such as P1×P4 and P6×P8 showed negative SCA effects for AD, reflecting earlier flowering, while P1×P6 and P1×P8 displayed early silking. Shorter ASI values in crosses like P4×P9 and P6×P9 provide better synchronization between pollen shed and silking, reducing the risk of reproductive failure under drought stress (Edmeades et al., 1997). Positive SCA effects are particularly desirable for yield component traits as shown in Table 5, directly enhancing grain yield. Significant positive SCA effects for EW, KW, and 100-KW indicated that specific hybrids exceeded expectations for yield, making them highly suitable for productivity in diverse environments. For example, P1×P6 exhibited strong positive SCA effects for EW and KW, highlighting its potential for heavier ears and kernels under water stress. Hybrids such as P1×P3, P2×P10, P4×P5, and P6×P8 also showed positive SCA for EW and 100-KW, aligning with the desirable trait of increased kernel size and weight, essential for yield improvement (Chapman et al., 2000).

Table 5. Specific combining ability estimates of the F1 hybrid crosses for adaptive traits include plant height (PH), days to 50% anthesis (AD), days to 50% silking (SD), anthesis-silking interval (ASI), SPAD, and stay-green (SG). Yield component traits include weight of the top ear (EW), kernel weight (KW), and 100-kernel weight (100-KW) across water conditions in two years (2022 and 2023)

Crosses	**Adaptive traits**						**Yield component traits**		
	PH	**SD**	**AD**	**ASI**	**SPAD**	**SG**	**EW**	**KW**	**100-KW**
P1xP2	29.12**	-2.28*	-2.40*	-0.12	-1.84	-2.83	0.58*	0.55	1.59
P1xP3	29.92**	-0.51	-1.84	-1.33	4.24	0.23	0.61*	0.49	5.50*
P1xP4	2.54	-4.97**	-2.48*	2.49*	3.31	1.01	0.86**	0.71	4.22
P1xP5	27.21*	-2.93**	-4.05**	-1.12	5.13	8.23*	0.83**	0.43	-4.83*
P1xP6	7.90	-3.91**	-5.42**	-1.52	-1.27	-5.55	1.21**	0.94*	1.38
P1xP7	12.97	1.76	0.89	-0.87	7.47*	7.88*	0.12	-0.11	2.46
P1xP8	21.69*	-4.34**	-4.13**	0.21	1.36	0.96	-1.02**	-0.77	10.59**
P1xP9	23.60*	-2.76**	-1.59	1.17	2.88	0.42	0.06	0.04	5.70*
P1xP10	16.48	-0.51	0.10	0.61	-8.74**	-13.05**	0.10	0.10	-1.37
P2xP3	9.75	-0.26	-0.94	-0.68	-8.03*	-6.23	0.22	0.28	2.00
P2xP4	37.36**	-1.47	-1.34	0.13	7.26*	6.74*	0.53*	0.49	-3.62
P2xP5	-10.88	-2.93**	-1.65	1.28	-3.46	-5.13	0.52*	-0.03	2.26
P2xP6	5.65	-1.66	-1.03	0.63	2.61	-4.06	0.22	0.21	5.77*
P2xP7	39.05**	0.01	-0.71	-0.72	-5.54	-4.44	0.18	-0.10	4.56
P2xP8	43.18**	-1.09	-1.73	-0.64	-1.82	-0.12	0.65*	0.41	0.34
P2xP9	40.09**	-4.01**	-2.44*	1.57	-3.92	-5.53	0.33	0.42	-0.65
P2xP10	42.14**	-1.26	-1.75	-0.49	6.57*	2.40	0.69*	0.57	5.12*
P3xP4	-12.25	0.30	0.48	0.17	-5.23	-1.03	-0.01	0.03	0.93
P3xP5	41.16**	0.60	-1.34	-1.93	-1.11	-1.15	0.29	-0.23	1.07
P3xP6	35.61**	-2.88**	-0.96	1.92	4.38	-0.44	1.05**	0.75	3.63
P3xP7	8.60	-2.47*	-1.90	0.57	-3.23	-1.93	0.41	0.06	-2.97
P3xP8	26.06*	-4.32**	-2.92*	1.40	-1.27	-1.13	0.35	0.47	2.49
P3xP9	-19.94*	-2.49*	-1.38	1.11	1.48	1.16	-0.13	-0.02	2.50
P3xP10	22.52*	-1.99*	0.56	2.55*	4.70	2.48	0.55*	0.43	0.58

Table 5. Specific combining ability estimates of the F1 hybrid crosses for adaptive traits include plant height (PH), days to 50% anthesis (AD), days to 50% silking (SD), anthesis-silking interval (ASI), SPAD, and stay-green (SG). Yield component traits include weight of the top ear (EW), kernel weight (KW), and 100-kernel weight (100-KW) across water conditions in two years (2022 and 2023) (continued)

Crosses	**Adaptive traits**						**Yield component traits**		
	PH	**SD**	**AD**	**ASI**	**SPAD**	**SG**	**EW**	**KW**	**100-KW**
P4xP5	43.79**	-1.61	-0.23	1.38	-1.40	0.61	0.93**	0.22	5.44*
P4xP7	10.38	-2.18*	-2.55*	-0.37	-4.39	-6.76*	0.36	-0.03	3.75
P4xP8	22.43*	-3.03**	-2.07	0.96	4.17	1.32	0.70*	0.63	2.49
P4xP9	15.59	0.30	-3.03*	-3.33**	-4.20	-4.54	0.43	0.32	3.95
P4xP10	40.14**	0.55	0.66	0.11	-7.64*	-3.29	0.09	0.01	5.46*
P5xP6	5.40	-2.55*	-2.92*	-0.37	3.75	6.61	0.42	0.20	4.51
P5xP7	5.48	-1.88	-1.11	0.78	-0.62	-3.79	0.10	3.47**	3.61
P5xP8	-21.65*	-1.49	-2.63*	-1.14	-4.14	-6.66	0.21	-0.07	-2.73
P5xP9	26.93*	-4.41**	-3.34*	1.07	0.30	-3.29	0.27	-0.21	3.40
P5xP10	21.48*	-1.66	-1.65	0.01	7.69*	5.90	0.65*	0.22	2.91
P6xP7	17.00	-2.36*	-1.98	0.38	5.62	5.99	0.84**	0.25	4.24
P6xP8	7.38	-4.47**	-3.25*	1.21	-2.46	-1.57	0.57*	0.60	5.67*
P6xP9	23.04*	-0.63	-3.71*	-3.08**	4.86	0.34	0.25	0.30	-4.30
P6xP10	37.17**	0.62	-0.03	-0.64	-1.89	-1.34	0.12	0.11	-3.15
P7xP8	-16.30	0.95	-1.44	-2.39*	3.37	4.35	0.59*	0.08	1.07
P7xP9	-2.72	1.03	-0.90	-1.93	0.06	-1.61	0.25	-0.08	-4.89*
P7xP10	13.08	-4.47**	-2.96*	1.51	2.00	-2.66	0.07	-0.21	-1.21
P8xP9	23.49*	1.18	1.83	0.65	3.92	-0.01	0.71**	0.56	-0.53
P8xP10	26.38*	-2.57*	-2.73*	-0.16	-11.92**	-6.30	0.72**	0.38	1.52
P9xP10	39.95**	-2.24*	-3.19*	-0.95	-8.52*	0.81	0.93**	0.61	1.68

*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively.

These SCA effects emphasized specific hybrid combinations with high potential for both drought resilience and yield enhancement. Hybrids such as P4×P5, P2×P8, and P2×P10 are strong candidates for adaptive traits, while P1×P6 and P6×P8 stand out for yield components, particularly in EW, KW, and 100-KW. The findings underscore the importance of selecting hybrids with strong SCA effects to maximize heterosis, as these combinations exhibit enhanced performance under challenging environmental conditions (Johnson, 2004). Harnessing hybrids with advantageous non-additive interactions through SCA can lead to high-yielding, drought-tolerant maize varieties, enhancing crop resilience in variable environments.

4. Conclusions

A combination of SNP markers with known relation to drought tolerance and the power of genetic diversity assessments facilitated a maize strategic breeding approach that achieved enhanced resolution for genetic clustering patterns detection in 50 inbred lines. Estimation of combining ability not only forms the basis for discrimination between lines from distinct clusters but also provides information on genetic potential to transmit adaptive traits especially those relating to drought tolerance. This plays a critical role in leveraging hybrids for drought-prone areas. Another advantage of hybrid stability in yield potential makes this trait especially appropriate under drought stress. The integration of SNP–based genetic diversity analysis and combining ability evaluation can be a more reliable strategy for developing drought resilient stress tolerant high-yielding hybrids in maize essential to accomplish food security under climate change and variability coupled with water shortage.

Key parental lines, including Ki58, Nei582046, Kei1508, and Kei1618, were identified as strong donors for drought-adaptive traits, while DTMA192 was a significant contributor to yield component traits. The F1 crosses DTMA192 x Kei1618 (P6xP8) and Ki58 x DTMA192 (P1xP8) demonstrated the highest SCA effects across most adaptive and yield component traits. These hybrids show strong potential as promising candidates for further utilization in our breeding program. Integration of the drought-adaptive trait selection based on parental GCA effects and hybrid performance on SCA provides an effective strategy to develop resilient, high-yielding maize hybrids, critical for regions facing water scarcity and climate change.

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6. Authors’ Contributions

Kamolrat Boonmawat performed the experiment; collected data; wrote and revised paper. Darush Struss facilitated laboratory resources; coordinated logistical support and maintained research facilities. Pattama Hannok conceptualized and designed the research study; provided guidance on data collection and supervised data analysis process; shaped main idea and structured the content; ensured the integrity and accuracy of paper.

7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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