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Research Article

Evaluation of Tropical Adaptive *Zea mays* L. Inbred Lines and Identification of Potential Heat Tolerant F1 Hybrids under Heat Stress Conditions

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ABSTRACT

A research study was led to estimate the genomic composition of five diverse forms of maize genotypes by investigating their combining abilities. The outcomes showed that General Combining Ability (GCA) in maize inbred lines exhibited significant variations across all characteristics under both heat and control environments. Specific Combining Ability (SCA) had significant variation in most characteristics under study, except for anthesis silk interval and number of ears per plant under heat stress. The genotypes ILC 276 and FM ILC 10 were recognized as decent general combiners for traits such as days to 50% tasseling, plant height, grain yield, number of kernels per ear, canopy temperature, cell membrane thermo-stability, pollen viability, and chlorophyll content. The crosses FM-ILC 10 x ILC 22, FM-ILC 10 x ILC 276, and FM-ILC 144 x ILC 255 were the best specific combiners for traits such as plant height, grain yield, number of kernels per ear, canopy temperature, pollen viability, and chlorophyll content. These hybrids are the best for high yield. The crosses ILC-22 x ILC-50, ILC-22 x ILC-276, ILC-50 x ILC-255, and ILC-50 x ILC-276 had high mid parent and better parent values for heterosis, demonstrating that they are the top-execution crosses with important positive heterosis for grain yield. Bi-plot investigation amid crosses of maize ILC 276 x ILC 22, ILC 276 x FM-ILC 10, and ILC 255 x FM-ILC 144 exhibited extreme genetic variability, and traits such as canopy temperature, cell membrane thermo-stability, plant height, anthesis silking interval and number of kernels per ear were positively associated with each other. Cluster analysis grouped the maize crosses into four and three clusters under normal and heat stress conditions respectively. These recognized crosses can be employed in further breeding plans to grow heat-tolerant hybrids for commercial seed production of maize.

Keywords: Maize inbred lines; Heat stress; Diallel analysis; GCA; SCA; Heterosis.

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INTRODUCTION

Maize (*Zea mays L.)* is a third most important cereal crop worldwide after wheat and rice [1]. It serves as human food, animal feed, and fuel for industry [2]. Maize contains vitamin C, A, and K, and a substantial amount of beta-carotene and selenium, that contribute to promote thyroid gland function and play a crucial role in inflammatory responses [3]. Maize is an important cereal crop with an average yield of 5.63 tons per hectare and approximately 1060.2 million tons of global production on 188-million-hectare area [4]. In Pakistan, maize is cultivated on a smaller scale, covering an area of approximately 1.4 million hectares, yielding 8.30 million metric tons of maize with an average yield of 5.93 metric tons per hectare [5]. Despite this, maize still contributes significantly to Pakistan's economy, with 0.6 percent of the GDP and 3.4 percent of the total value-added products [6]. The vast majority of maize production in Pakistan occurs in the provinces of KPK and Punjab [7,8]. It is predominantly cultivated in semi-arid regions, where it is often exposed to high temperatures, water scarcity, and a combination of these conditions in field settings [9]. Climate change has brought about one of its most notable effects- global warming. The increase in temperature has a significant impact on crop productivity, affecting yields in many regions [10,11]. Changes in climatic patterns over a period of time have a strategic role in the decline of agricultural productivity worldwide [12]. These epoch changes are the cause of divergence in precipitation, rise in temperature, and variation in frequency of rainfall, eventually causing floods, cyclones, and drought. Thus, the economic growth of the agriculture sector is intensely affected [13]. Heat and drought stress have been found to be the most intensifying factors, raising the negative influence on the production of many crops. High temperatures can reduce crop production in certain regions of the world [14,15]. In addition, extreme temperatures have led to a reduction in crop yield, while also promoting the growth of weeds and pests [16,17]. According to the Inter-governmental Panel on Climate Change [18], the risk posed by permanent changes in climatic conditions leads to temperature fluctuations [19]. The variations in temperature have resulted in a significant decrease in crop production in Pakistan.

High temperature exceeds the ideal condition for a prolonged period causes heat stress, affecting crop growth and plant development [20,21]. Maize development is optimal between 22°C and 30°C, which allows for the highest rate of photosynthesis. However, temperatures above 30°C decrease grain yields by reducing the photosynthetic rate and signaling pathway, as well as initiating protein degradation [22]. For every degree Celsius increase above 30°C, there is a 1% decrease in maize production under optimal conditions, 1.7% under water stress conditions, and approximately 40% or more due to combined stresses [22]. High temperatures can have detrimental effects on maize grain production by disrupting important physiological processes necessary for optimal growth and development. These effects include reduced biomass integration and grain abortion, which ultimately lead to a decrease in grain number. Heat stress at around 36 °C also reduces radiation use efficiency and metabolic activity, resulting in decreased dry matter production [23]. High temperatures during the reproductive stage can cause silk parchedness, pollen infertility, and low seed setting, resulting in a decrease in yield [24]. Heat stress can also reduce the number and mass of grains during the reproductive stage, leading to a decrease in maize yield. A daytime temperature of 35° C can lead to a 31% decrease in maize crop yield due to a declining number of grains and harvest index. Depending on the severity and duration of stress, the loss of maize grain yield can range from 15% to more than 80%. An increase of one degree Celsius in temperature leads to a 7.4% reduction in maize grain yield [25]. The heat stress during maize grain filling caused up to 29% yield losses [26].

In maize, it is crucial to evaluate inbred lines and their crosses under heat stress and normal conditions by determining morpho-physiological traits as indicators [27]. Maize crop hybrid crossing has been thoroughly researched, involving both conventional and molecular-based methods for developing cultivars [28]. The combining ability of inbred lines can be estimated to assess their usefulness in hybrid combinations and to create hybrids that can thrive in a range of environments. General combining Ability and Specific Combining Ability can improve the chances of obtaining the ideal combination of maize populations [29].

Plant breeding involves evaluating and using genetic variation, choosing desirable traits, and testing the selected superior genotypes [30]. The effectiveness of the selection process relies on the direction and strength of the relationship between yield and its components [31]. In addition, accurate estimations of genetic parameters are crucial in identifying the gene action involved in controlling quantitative traits. It is important to determine the key screening parameters when selecting tolerant cultivars. These parameters include days to 50% tasseling, days to 50% silking, Anthesis Silking Interval (ASI), number of ears per plant, plant height, ear height, number of kernel rows per ear, grain yield per plant, growing degree days (GDD), Chlorophyll Content (CC), Canopy Temperature Depression (CTD), pollen viability, and Cell Membrane Thermo-stability (CMTS). They have been proven to be useful in the screening of desired genotypes. Predominantly diallel analysis is used to assess the inbred lines against heat stress [32]. Several studies have used multivariate statistical analysis, such as principal component analysis (PCA), to assess the extent of genetic diversity in crop germplasm [33,34]. In this study, diallel cross was made to find hybrid plants that can endure heat stress during the reproductive and grain-filling phases create a new F_1 hybrid using selected parent lines and assess the yield-related characteristics of the resulting F_1 generation under heat stress.

MATERIALS AND METHODS

Germplasm Screening and Field Conditions

Twenty-seven maize inbred lines were obtained from the Maize, Sorghum, and Millet Program (MSM, CSI, NARC) for heat tolerance screening during 2021. Experiment was conducted at MSM field area (33°40'39.9"N 73°07'56.1"E) located in National Agricultural Research Centre, (NARC) Islamabad.

The experimental site soil analysis showed pH of 7.2-8.0, an organic matter of 0.3-0.5 %, available phosphorus 7-8 mg kg^{-1}, available potassium 60-90 mg kg^{-1} and soil EC between 0.7-0.8 dSm^{-1} in the topsoil layer of 20 cm.

Diallel Cross

Twenty-five hybrid varieties were developed by crossing five inbred lines using the full diallel mating design (Griffing's Method 4, Model 1) during the summer of 2022. Hybrid varieties with heat tolerance were chosen based on their SCA and GCA. Randomized Complete Block Design (RCBD) was used with three replications and two treatments (heat and control), the heat stress was applied by suspending the date of sowing. Metrological data from 2021 and 2022 (Table 1) were taken into account, based on the experiment location. Row-to-row and plant-to-plant distances were set at 75 cm and 25 cm, respectively. In 2022, all possible combinations of the inbred lines were crossed, and the seed from each cross was collected separately and evaluated for heat tolerance in the following season.

Table No. 1 Metrological data of temperature and precipitation during year 2021 and 2022

	Year-2022			**Year-2021**		
Month	Temperature (°C)		Precipitation	Temperature (°C)		Precipitation
	Mini	Maxi	Total (mm)	Mini	Maxi	Total (mm)
April	21.2	35.3	5.3	16.9	30	39.4
May	23.8	36.9	41.8	21.6	34.7	37.7
June	25.3	38	194.6	24.4	37.5	138.6
July	24.7	32.1	481.9	25.4	35.2	251.5

Data Collection

Data on various morpho-physiological attributes was collected, including the time it took for 50% tasseling and silking, the interval between anthesis and silking, the number of ears per plant, plant height (cm), ear height (cm), the number of kernel rows per ear, the grain yield per plant (in grams), and the measurement of Growing Degree Days (GDD) by starting from the date of sowing to the end of the anthesis stage [35]. The chlorophyll content, which plays a crucial role in stress response, was determined by using an SPAD-502 meter on randomly selected plants [36]. Canopy Temperature Depression (CTD) was measured with an infrared thermometer (Raynger II) on sunny days at approximately noon, with the thermometer positioned obliquely towards the canopy at an angle of approximately 30° relative to the horizontal. The ambient temperature was measured using a digital thermometer (Boneco 7041), and CTD was calculated as CTD = Ta – Tc, following standard protocols by [37].

The standard procedure for pollen viability was used for the tetrazolium test as described by [38]. We added 0.05 ml of fresh pollen grains to a microtube containing 5 ml of 0.75 percent 2,3,5-triphenyltetrazolium chloride. The microtubes were then covered with aluminum foil and placed in an incubator at 25°C for one hour. After incubation, we examined the pollen grains under a light microscope at 10x magnification. Pollen grains that showed red staining were considered viable, while those without staining were deemed unviable. Finally, we calculated the percentage of pollen viability as

Pollen Viability (%)=(stained pollen/total number of pollen)×100

Cell Membrane Thermo-Stability (CMTS) was determined using an Electrical Conductivity (EC) meter (Hanna Instruments, Model HI 2300). First step was submerged leaf discs in test tubes filled with 10 ml of deionized water. Then, placed the tubes in a water bath at 40°C for 1 hour and left them overnight at 22°C. The next day, measured the electric conductivity using an EC meter. Afterwards, heated the samples for 15 minutes at 121°C and left them overnight at 22°C. Repeated the measurements the next day. This process helped us assess the membrane integrity in normal and stressful conditions through electrolyte leakage. Following formula was used to calculate the cell membrane thermos-stability.

$$CMTS_{40} = (1 - C1_{40}/C2_{40}) \times 100$$

Statistical Analysis

The collected data underwent further analysis using Statistix 8.1 (Analytical Software) for ANOVA and Least Significant Difference (LSD), as outlined by [39]. Agro-physiological traits were compared using Griffing's Method 4, with Model 1 [40] analyzed through AGD-R software to determine General combining ability (GCA) and Specific combining ability (SCA). For Principal Component Analysis

(PCA) and Cluster Analysis (CA), the 'R' software (Development Core Team, 2015) was used in conjunction with the 'Agricolae' package [41].

RESULTS AND DISCUSSION

Phenotypic Performances

All inbred lines experienced significant variability in their attributes under normal and heat stress conditions. Morpho-physiological attributes such as DFT, Days to 50% Tasseling; DFS, Days to 50% Silking; ASI, Anthesis Silking Interval; PH, Plant Height; EH, Ear Height; GY, Grain Yield; NEP, Number of Ear Plant^{-1}; NKE, Number of Kernel Ear^{-1}; CC, Chlorophyll Content; PV, Pollen Viability; CMT, Cell Membrane Thermo-stability, all decreased significantly ($p \leq 0.05$) in all inbred lines except for CTD under heat stress when compared to the control (refer to Table No. 2 and 3). GY also experienced a significant drop under heat stress, with mean values ranging from 5.1 to 174.7 g compared to control conditions of 14.2 to 220.9 g (refer to Tables No. 2 and 3). This was attributed to the expressed heat stress on secondary attributes. A wide range of secondary attributes such as DFT (54.3-88.3 days), DFS (57-90.3 days), ASI (2-3.7 days), PH (101-217 cm), EH (53-108 cm), NKE (10-20), NEP (1-3.7), CC (37.7-61), CTD (29.3-33.1), PV (25.7-92 %) and CMTS (68.1-97.1 %) showed significant variability under control conditions. Under heat stress, DFT (54-87.7 days), DFS (57-89 days), ASI (2.3-4.3 days), PH (112-223 cm), EH (53-131 cm), NKE (8-14.7), NEP (1-3.3), CC (15.9-51.5), CTD (31.5-36), PV (5.2-66. %) and CMTS (64.1-95.1 %) revealed significant genetic variability for these attributes (refer to Table No. 2 and 3).

Analysis of Variance

The analysis of variance (ANOVA) for diallel analysis of maize inbreds exhibited significant genetic variability in all attributes, under both heat and control conditions (as mentioned in Table No. 4 and 5). This suggests that the genetic variation in the parents was sufficient for all the traits evaluated. In maize inbreds, General Combining Ability (GCA) showed significant variability in all attributes, under both heat and control conditions. Mean square values for GCA indicated that all attributes were influenced by additive gene action [42,43]. Specific combining ability (SCA) mean squares were highly significant for DFT, PH, EH, NKP, GY, CC, CMTS, and PV, while non-significant for ASI, DFS, and NEP under control conditions. In heat stress, SCA showed highly significant variability in all attributes except ASI and NEP. SCA estimated that DFT, PH, EH, NKP, GY, CC, CMTS, and PV showed non-additive gene action. In contrast, the remaining attributes indicated that both additive and non-additive gene actions were equally important. Similar findings have been reported for almost all traits [44,45].

Estimation of GCA, SCA and Heterosis

The genotype of parents FM ILC 10 showed positive results as a general combiner for various traits such as DFT, DFS, PH, CMTS, and CC under normal conditions. However, under heat stress, FM ILC 10 proved to be a good general combiner for more traits such as DFT, DFS, PH, GY, NKE, CT, CMTS, PV, and CC (as mentioned in Table No. 6 and 7). Likewise, the parent FM ILC 144 showed positive results as a general combiner for traits like DFS, EH, GY, NKE, CT, and PV under normal conditions. But under heat stress, it showed good results for DFT, DFS, NKE, and PV. These lines exhibited positive GCA effects, indicating that they possessed genes that responded to the given traits under both normal and heat stress conditions. On the other hand, parents ILC 22 and ILC 255 had poor results as general combiners for most of the traits under both conditions. However, the parent ILC 276 had showed higher GCA for DFT, DFS, GY, and PV under normal and heat stress conditions. The findings suggested that the additive genetic effects played a significant role in the performance of the offsprings. Previous studies have also reported similar findings, where the additive gene action was the primary regulator for traits like the number of kernels per row and plant height in maize genotypes, while dominance was prevalent for traits such as 100-grain weight, seed yield, ear length, and ear height [46, 47]. Researchers also reported that the number of kernels per ear was controlled by the additive effect [48]. These findings are in line with the results of Kambegowda [42].

Specific combining ability (SCA) estimates (Tables No. 8 and 9) indicated that the crosses FM-ILC 10 x ILC 22, FM-ILC 10 x ILC 276, FM-ILC 144 x ILC 22, and ILC 255 x ILC 276 were the best combiners for plant height, ear height, pollen viability and grain yield under control condition. These combinations showed significantly positive specific combining ability with various attributes, which indicated that these hybrids were considered best for high yielding. Under heat stress, the crosses FM-ILC 10 x ILC 22, FM-ILC 10 x ILC 276, FM-ILC 144 x ILC 255, FM-ILC 144 x ILC 276, and ILC 255 x ILC 276 were the best combiners for plant height, cell membrane thermo-stability, pollen viability, canopy temperature depression, chlorophyll content and grain yield (Table No. 8 and 9). All these combinations were more tolerant to heat stress, and best performs for yield. The cross FM-ILC 144 x ILC 255 under control conditions and ILC 22 x ILC 255 from heat stress were the best combinations for the development of dwarf and early maturing varieties. Specific combining ability suggests non-additive gene action.

The crosses had shown higher SCA values, which indicated non-additive effects, either dominance or epistatic effects. A non-additive effect on plant yield in maize. Number of kernels per ear, ear height, and grain yield per plant of maize hybrids studied. Similar findings have been reported by Muraya [49]. Higher values of SCA suggested that non-additive genes had more of an impact on these attributes. The current results agree with findings of Seyoum [50].

Table No. 2 Mean value and descriptive statistics of morpho-physiological attributes under control and heat stress condition.

	DFT		**DFS**		**ASI**		**PH**		**EH**		**NKE**	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat
Mean	72.1	72.3	75.3	75.2	2.9	3.6	160.6	169.6	84.5	85.1	13.5	12.1
MS	201.3	188.8	187.6	173.3	0.5	0.8	1840.4	1530.0	859.7	849.3	28.2	10.4
CV	0.6	0.7	0.7	0.8	19.1	10.5	5.8	5.9	13.2	11.9	17.7	10.9
P	**	**	**	**	**	**	**	**	**	**	**	**
Max	88.3	87.7	90.3	89.0	3.7	4.3	217.0	223.0	108.0	131.0		14.7
Min	54.3	54.0	57.0	57.0	2.0	2.3	101.0	112.0	53.0	53.0		8.0

Table No. 3 Mean value and descriptive statistics of morpho-physiological attributes under control and heat stress condition.

	NEP		**GY**		**CC**		**CTD**		**PV**		**CMTS**	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat
Mean	2.1	1.9	74.3	50.2	48.9	38.0	30.5	33.8	50.6	27.9	83.0	79.3
MS	1.2	1.2	8884.1	6809.1	73.9	301.7	2.2	4.8	649.3	849.7	212.5	195.9
CV	21.6	22.3	22.0	19.3	3.0	3.2	2.0	2.1	0.6	0.3	0.4	0.3
P	**	**	**	**	**	**	**	**	**	**	**	**
Max	3.7	3.3	220.9	174.7	61.0	51.5	33.1	36.0	92.0	66.5	97.1	95.1
Min	1.0	1.0	14.8	5.1	37.7	15.9	29.3	31.5	25.7	5.2	68.1	64.0

DFT, Days to 50% Tasseling; DFS, Days to 50% Silking; ASI, Anthesis Silking Interval; PH, Plant Height; EH, Ear Height; GY, Grain Yield; NEP, Number of Ear Plant^{-1}*; NKE, Number of Kernel Ear*$^{-1}$*; CC, Chlorophyll Content; CTD, Canopy Temperature Depression; PV, Pollen Viability; CMT, Cell Membrane Thermostability.*
*** Highly significant (P≤0.01), *Significant (P≤0.05),* ^{NS} *Non-significant (P>0.05).*

Table No. 4: Mean squares from diallel analysis for different attributes under control and heat stress condition in maize (Griffing's Method 4, Model I).

	DF	**Mean Square**											
		DFT		**DFS**		**ASI**		**PH**		**EH**		**NEP**	
		Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat
REP	2	19.35	19.36	19.36	19.36	9.4	0.09	8.44	19.36	45.17	122.61	0.01	0.03
Cross	24	88.1**	90.7**	85.8**	85.8*	0.3**	0.7**	1012**	814**	220.8**	244.7**	0.2*	0.3*
GCA	4	156.4**	149.1**	152.1**	156.8**	0.7**	0.6**	806.2**	354.4**	144.6**	254.9**	0.1*	0.7*
SCA	5	0.2**	1.3**	0.2^{NS}	0.5**	0.1^{NS}	0.1^{NS}	1499.8**	1089.4**	315.5**	358.3**	0.2NS	0.1^{NS}
Residual	48	0.1	0.1	0.1	0.1	0.1	0.2	2.1	0.1	0.3	0.7	0.3	0.2

Table No. 5: Mean squares from diallel analysis for different attributes under control and heat stress condition in maize (Griffing's Method 4, Model I).

	DF	**Mean Square**											
		NKE		**GY**		**CT**		**CMS**		**PV**		**CC**	
		Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat
REP	2	2.3	6.5	45.2	19.4	5.4	4.2	19.4	30.6	19.4	38.7	1.2	7.5
Cross	24	12.3**	7.6**	650**	315.3**	6.01**	8.4**	167.8**	163**	28.3**	246.2**	66.7**	69.1**
GCA	4	9.6**	10.7**	621.1**	123.6**	7.1**	9.8**	185.08**	180.6**	41.4**	199.4**	22.7**	22.5**
SCA	5	15.7**	4.2**	681.5**	263.3**	4.5**	5.3**	82**	80.5**	41.4**	472.3**	78.5**	120**
Residual	48	2.4	1	0.2	0.2	1.4	0.04	0.1	0.06	0.1	0.07	0.7	0.007

Table No. 6: General Combining Ability analysis for 5 inbred lines of maize for different heat tolerance related attributes.

Cross	**DFT**		**DFS**		**ASI**		**PH**		**EH**		**NEP**	
	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat
FM ILC 10 x FM ILC 10	1.83*	1.67*	1.73*	1.67*	-0.13	-0.17	10.09*	7.13*	-5.42*	-2.91*	0.09	0.44*
FM ILC 144 x FM ILC 144	4.33	4.67*	4.23*	4.33*	-0.02	0.00	-3.47*	-6.20*	3.24*	-0.91*	-0.13	0.00
ILC 22 x ILC 22	-6.17*	-5.67*	-6.27*	-6.33*	-0.24*	-0.33*	3.53*	-2.53*	3.91*	5.42*	0.09	-0.33*

ILC 255 x ILC 255	-2.17*	-2.50*	-1.77*	-1.83*	0.48*	0.38*	4.42*	6.30*	1.08*	5.26*	-0.13	0.00
ILC 276 x ILC 276	2.17*	1.83*	2.07*	2.17*	-0.08	0.11	-14.58	-4.70*	-2.81*	-6.86*	0.09	-0.11

Table No. 7: General Combining Ability analysis for 5 inbred lines of maize for different heat tolerance related attributes.

Cross	**GY**		**NKE**		**CT**		**CMT**		**PV**		**CC**	
	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat
FM ILC 10 x FM ILC 10	-3.22*	2.75*	0.40	0.80*	-0.88*	0.93*	4.24*	4.19*	0.05	1.22*	2.68*	2.11*
FM ILC 144 x FM ILC 144	2.58*	-0.01	1.51*	1.24*	1.39*	-1.71*	-0.18*	-0.51*	1.98*	2.81*	-1.36*	-0.27*
ILC 22 x ILC 22	-1.42*	-2.97*	-0.71	-1.42*	-0.15	-0.26*	3.43*	3.46*	-1.24*	-2.02*	0.13	0.60*
ILC 255 x ILC 255	-10.26*	-4.28*	-0.04*	-0.76*	-0.61*	0.55*	-0.25*	-0.04	-2.91*	-7.06*	-0.58*	-0.17*
ILC 276 x ILC 276	12.32*	4.50*	-1.16*	0.13	0.25	0.49*	-7.24*	-7.11*	2.12*	5.04*	-0.87*	-2.26*

Table No. 8: Specific Combining Ability analysis for 10 inbred lines of maize for different heat tolerance related attributes.

Cross	**DFT**		**DFS**		**ASI**		**PH**		**EH**		**NEP**	
	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat
FM ILC 10 x FM ILC 144	-0.17	-0.33	-0.17	0.00	0.06	-0.08	-4.56*	0.67*	-8.89*	-10.61*	0.11	-0.28
FM ILC 10 x ILC 22	0.33	-0.50*	0.33	0.17	-0.22	0.25	10.11*	-0.50*	5.61*	11.89*	-0.11	0.06
FM ILC 10 x ILC 255	-0.17	0.33	-0.17	-0.33	0.06	0.03	-6.78*	-3.33*	-8.89*	-4.61*	-0.22	0.06
FM ILC 10 x ILC 276	0.00	0.50*	0.00	0.17	0.11	-0.19	1.22	3.17*	12.17*	3.33*	0.22	0.17
FM ILC 144 x ILC 22	-0.17	1.00	-0.17	0.00	0.17	-0.25	20.33*	23.33*	3.78*	4.06*	-0.22	0.17
FM ILC 144 x ILC 255	0.33	-0.17	0.33	0.50*	-0.22	0.03	6.78*	-4.50*	6.61*	0.22	0.00	0.17
FM ILC 144 x ILC 276	0.00	-0.50*	0.00	-0.50*	0.00	0.31	-22.56*	-19.50*	-1.50*	6.33*	0.11	-0.06
ILC 22 x ILC 255	-0.17	-0.33	-0.17	-0.33	0.17	0.03	-25.89*	-15.67*	1.78*	-0.94*	0.44	-0.17
ILC 22 x ILC 276	0.00	-0.17	0.00	0.17	-0.11	-0.03	-4.56*	-7.17*	-11.17*	-15*	-0.11	-0.06
ILC 255 x ILC 276	0.00	0.17	0.00	0.17	0.00	-0.08	25.89*	23.50*	0.50	5.33*	-0.22	-0.06

Table No. 9: Specific Combining Ability analysis for 10 inbred lines of maize for different heat tolerance related attributes.

Cross	**GY**		**NKE**		**CT**		**CMT**		**PV**		**CC**	
	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat	Cont	Heat
FM ILC 10 x FM ILC 144	-7.22*	-6.68*	0.56	0.22	-0.55	-1.33*	-4.38*	-4.41*	-3.95*	-14.15*	4.34*	5.51*
FM ILC 10 x ILC 22	11.04*	12.01*	-1.89*	-1.11*	1.08	-0.38*	0.91*	0.79*	4.87*	13.96*	-4.78*	-5.85*
FM ILC 10 x ILC 255	-7.86*	-8.04*	0.78	0.89	-0.32	0.91*	1.29*	1.35*	-0.63*	1.11*	2.33*	2.82*
FM ILC 10 x ILC 276	4.03*	2.71*	0.56	0.00	-0.22	0.80*	2.18*	2.27*	-0.28	-0.93*	-1.88*	-2.48*
FM ILC 144 x ILC 22	13.98*	-1.10*	0.33	0.44	-1.55*	-0.27*	-1.61*	-1.55*	1.27*	7.93*	4.45*	5.31*
FM ILC 144 x ILC 255	-0.69*	3.41*	1.67*	0.44	0.85	0.72*	7.20*	7.08*	1.81*	3.96*	-4.87*	-5.91*
FM ILC 144 x ILC 276	-6.07*	4.36*	-2.56*	-1.11*	1.25	0.88*	-1.21*	-1.12*	0.88*	2.26*	-3.92*	-4.91*
ILC 22 x ILC 255	-9.26*	0.39	-1.44	-0.89	0.48	0.36*	-3.41*	-3.26*	-3.35*	-12.81*	-1.46*	-1.88*
ILC 22 x ILC 276	-15.77*	-11.31*	3.00*	1.56*	-0.02	0.30*	4.12*	4.02*	-2.78*	-9.07*	1.79*	2.41*
ILC 255 x ILC 276	17.80*	4.24*	-1.00	-0.44	-1.02	-1.98*	-5.08	-5.17*	2.18	7.74*	4.01*	4.98*

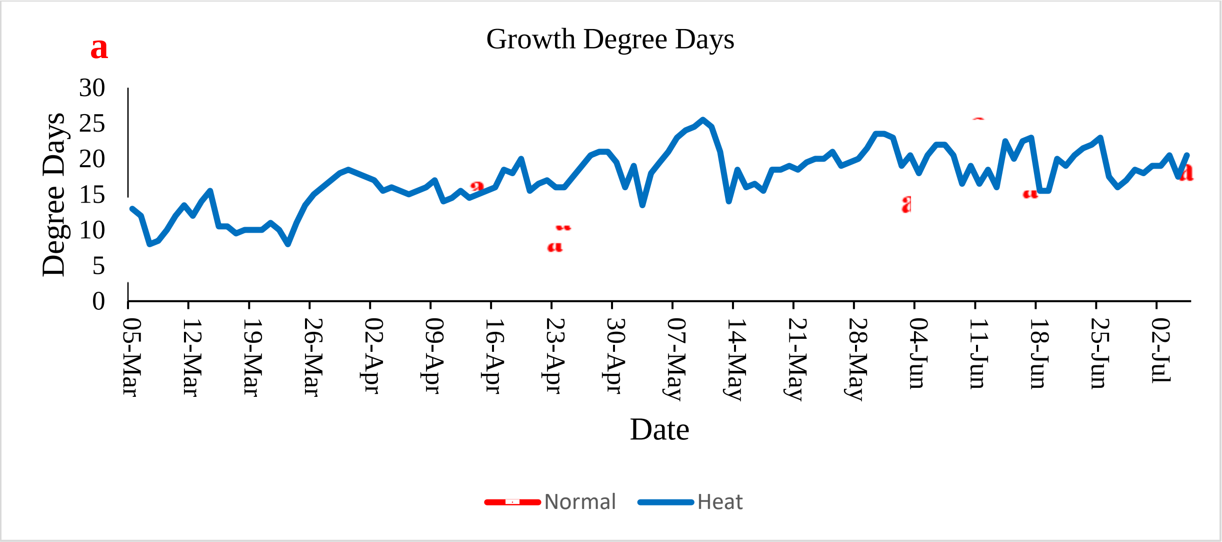
Table No. 10: Better Parent Heterosis and Mid Parent Heterosis for grain yield of maize under heat stress condition.

Female Parents	Male Parents	Grain Yield	
		Mid Parent Heterosis	Better Parent Heterosis
ILC-22	ILC-50	1.9	1.46
ILC-22	ILC-255	0.7	0.37
ILC-22	ILC-276	1.6	1.52
ILC-22	ILC-144	0.6	0.18
ILC-50	ILC-255	1.2	0.54
ILC-50	ILC-276	1.6	1.14
ILC-50	ILC-144	0.6	0.04
ILC-255	ILC-276	0.6	0.35
ILC-255	ILC-144	0.1	0.04
ILC-276	ILC-144	0.6	0.19

Moreover, [52] suggested that both additive and non-additive were significant for the genetic effect of various characters studied in maize. According to the heterosis table, the most successful crosses exhibited positive heterosis for grain yield. These crosses included ILC-22 x ILC-50, ILC-22 x ILC-276, ILC-50 x ILC-255, and ILC-50 x ILC-276. These crosses showed high MP and BP values for heterosis under heat stress, as shown in Table No. 10. The best combinations for heterosis in terms of grain yield were those that had high SCA values, indicating the best heterosis combination, as noted by [53]. The results suggest that both GCA and SCA contributed to heterosis for their exceptional performance [54]. Many researchers have suggested that maize shows high heterosis for grain yield [55,56,57].

Growth Degree Days

The growth degree days (GDD) for 27 inbred lines showed that the transition from vegetative to reproductive phase occured between 645 to 830-degree days under normal conditions (Figure 1a). However, under heat stress conditions, the transition occurs between 590 to 740-degree days during screening trial. During crosses, Growth Degree Days shifted from vegetative to reproductive phase between 630 to 810-degree days, while in heat stress conditions, the change occurs between 630 to 790-degree days (Figure 1b). To reach physiological maturity, maize requires 1,460-1,520-degree days. Before and after flowering, the grain yields vary significantly with significant variations in growth degree days (GDD). Higher growth degree days in maize lines have been established to produce high yields in diverse environments. High temperatures can cause decline of vegetative growth period in maize and turn to an advanced flowering stage, as reported by [58,59]. High-temperature effects on maize may be associated with abnormal development of males and females, such as sterile tassels, tassel blasts, and non-silked ears [60, 61].



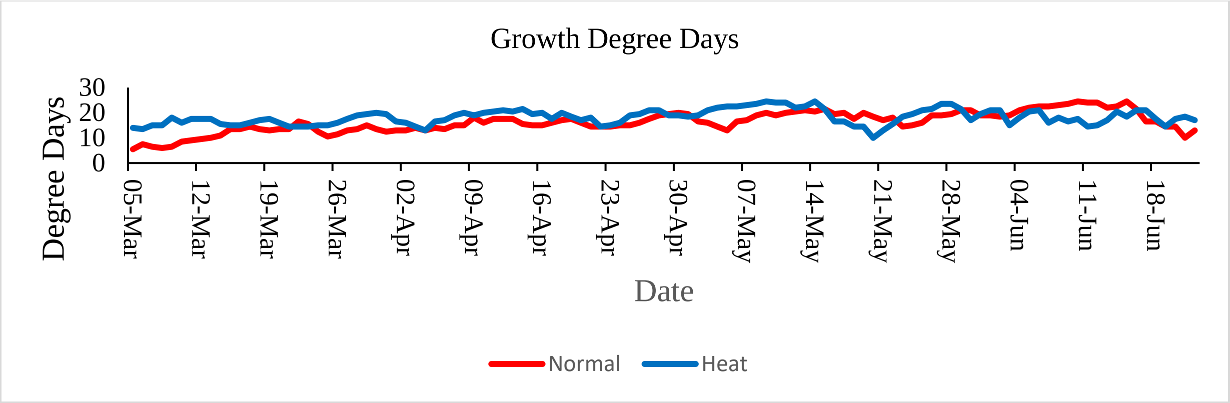


Figure 1. Graph Depicting Growth Degree Days of maize inbred lines under Control and Heat Stress Conditions (a) Growth degree days of parental inbred line of maize (b) Growth degree days of diallel analysis of maize.

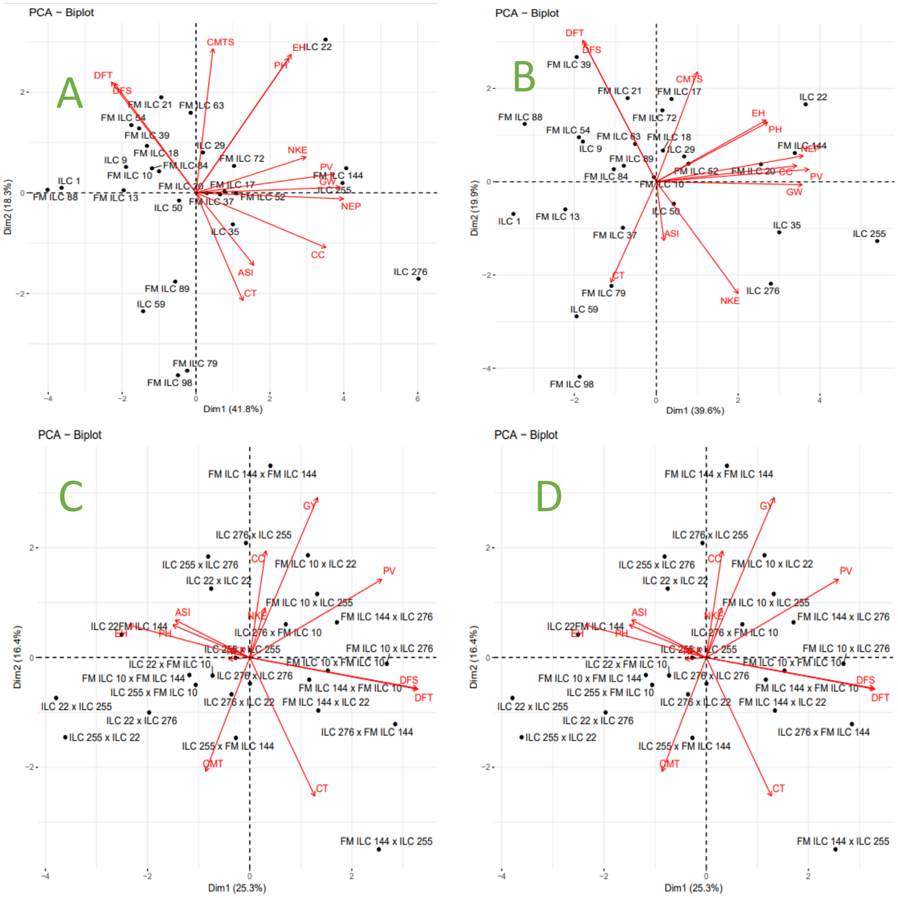


Figure 2. Principle Component Biplot Analysis for maize inbred lines a) Biplot Analysis for maize inbred lines under control conditions b) Biplot Analysis for Maize Inbred Lines under heat conditions c) Biplot Analysis for diallel crosses under control conditions d) Biplot Analysis for diallel crosses under Heat conditions

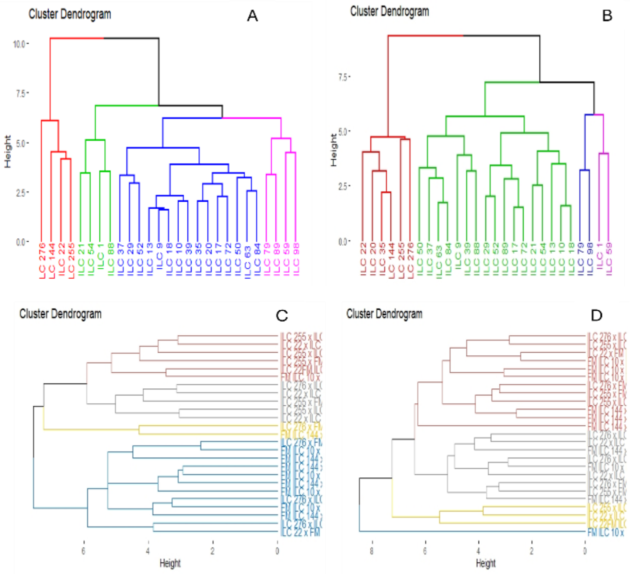


Figure 3. Hierarchical Cluster Analysis for maize inbred lines a) under control conditions b) under heat conditions c) Hierarchical Cluster Analysis for diallel crosses under control conditions d) Hierarchical Cluster Analysis for diallel crosses under Heat conditions

PCA, Cluster analysis

An analysis was conducted using PC-biplot on 27 maize inbred lines under normal and heat stress conditions (Figure 2a and b). The inbred lines FM-ILC 98, FM-ILC 88, FM-ILC 79, ILC 144, and ILC 276 exhibited maximum genetic variability. Meanwhile, FM-ILC 20, FM-ILC 37, and FM-ILC 52 had minimum genetic variability under control conditions. In contrast, during heat stress, ILC 255, ILC 88, ILC 1, FM-ILC 63, and ILC 276 showed maximum genetic variability, while FM-ILC 37, FM-ILC 52, and FM-ILC 20 had minimum genetic variability. Under control conditions, the traits CMT, PH, NKE, GY, and PV were positively correlated with each other. However, under heat stress, CMT, PH, GY, and PV were positively correlated with each other, while the other traits were negatively correlated with these attributes.

A bi-plot analysis was conducted on the crosses of maize under control and heat stress conditions (Figure 2c and d). The crosses ILC 276 x ILC 22, ILC 276 x FM-ILC 10, and ILC 255 x FM-ILC 144 had minimum genetic variability, while FM-ILC 144 x ILC 255 and FM-ILC 10 x FM-ILC 144 had maximum genetic variability. During heat stress, ILC 276 x ILC 22, ILC 276 x FM-ILC 10, and ILC 255 x FM-ILC 144 had minimum genetic variability, while FM-ILC 144 x ILC 255 and ILC 22 x ILC 255 had maximum genetic variability (Figure 2c and d). Under control conditions, PH, EH, ASI, and NKE were positively correlated with each other, while under heat stress, CMT, PH, EH, CC, ASI, NKE, and CT were positively correlated with each other, and the other traits were negatively correlated with these parameters. Cluster analysis was performed based on the diversity contributed by different traits, and the maize genotypes were divided into four clusters based on their diversity under both conditions. The cluster membership of various crosses of maize inbred lines was grouped into four clusters based on the mean values under both conditions (Figure 3a, b, c, and d).

Conclusion

Studies have found that the most effective maize hybrids are created through various parent combinations. The success of a hybrid can be measured by the presence of additive, non-additive, and epistatic effects. Non-additive effects, such as dominance and over-dominance, and epistatic genetic factors contribute to the superior performance and vigor of hybrids over their parent plants. Specifically, parents with high GCA values, such as ILC 276 and FM ILC 10, can be used to breed synthetic cultivars. Meanwhile, hybrids with high SCA values, such as FM-ILC 10 x ILC 22 and FM-ILC 10 x ILC 276, can be utilized in the development of heat-tolerant hybrids for commercial maize seed production.

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