Assessment of Climate-resilient Cotton Genotypes Exhibiting High-temperature Tolerance

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RESEARCH

Assessment of Climate-resilient Cotton Genotypes Exhibiting High-temperature Tolerance

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Abstract
Cotton is an important natural fiber worldwide. Abiotic factors such as high temperature stress reduce seed cotton production and fibre quality. The current research aims to screen cotton germplasm for high temperature tolerance. For this, 60 cotton genotypes were tested for two years with varying sowing dates. Split plot design was used to plant these genotypes after RCBD. The main plot sowing dates and sub-plot cotton genotypes were considered for both years. We recorded boll weight (BW), chlorophyll content (CC), cell membrane thermal stability (CMT), canopy temperature (CT), node number to first fruiting branch (NNFFB), and seed cotton yield (SCY). Heat-stress-tolerant cotton germplasm can be created by analyzing genetic variation, genotype-environment interaction, and characteristics associated with SCY. Studying the Genotype × Environment Interaction (GEI) of cotton genotypes for specified agro-physiological parameters using GGE biplot analysis. The genotypes FH Lalazar, MNH-1016, PB-76, MNH-992, and FH-458 are either stable or show positive interaction with high temperature stress conditions for most traits under study, suggesting they can be used in future breeding programs to develop heat stress-tolerant varieties. The correlation coefficients also showed that all traits except node number to first fruiting branch and canopy temperature were positively and significantly correlated with seed cotton yield under heat stress, suggesting that using one or more of these traits as selection criteria could increase cotton yield under heat stress.

Keywords *Gossypium hirsutum* L. · Breeding · Seed cotton yield · Heat stress · Cell membrane thermostability · Chlorophyll contents · Canopy temperature

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Introduction

The cotton plant is affected by several biotic and abiotic stresses in the field. High temperature is a major abiotic stress that limits cotton yield and quality (Zafar et al. 2024). In the cotton-growing regions of southern Punjab and Sindh, Pakistan, summer temperatures reach approximately 50 °C, adversely affecting seed cotton output and fibre quality (Khan et al. 2010; Majeed et al. 2021; Nasaruddin et al. 2024). Whereas the thermal kinetic window for cotton plants ranges from 24 to 32 °C (Bibi et al. 2008), the desired temperature for boll development and maturation ranges from 27 to 32 °C (Ekinci et al. 2017). The primary cause of yield reduction under high temperatures (46 °C) is fewer seeds per boll, which accounts for 45% of the yield loss against a 1 °C rise over the mean (Pettigrew 2008). The cotton plant exhibits maximum photosynthetic activity at 33 °C, which starts to decrease at 36 °C and above. There is a lower photosynthetic rate because the thylakoid membrane is breaking down and solutes are leaking out (Bibi et al. 2008). It is because high temperatures slow down photosynthesis, which means that fewer carbohydrates are made and can't be sent to sinks that are growing. This leads to a lower yield (Pettigrew 2008). High temperature stress during peak flowering negatively affects cotton yield; however, lower temperatures have to some extent a positive effect on seed cotton yield (Sarwar et al. 2011). Heat stress also decreases the level of total carbohydrates in pollen grains and ATP in stigmatic tissues (Snider et al. 2009). This results in decreased flowers per plant, a low number of fruit set per plant, and ultimately a low yield per plant in many crop species (Peet et al. 1998; Sato et al. 2002). The growth rate of cotton plants depends upon the number of buds on the main stem, the number of fruiting branches, and fruiting branch buds, which start to show symptoms of stress as the temperature goes over 30/22 °C. Heat stress reduces yield due to decreased photosynthetic activity, low development rate, and increased photorespiration (Polley 2002).

Adjusting the planting date is an effective method to mitigate heat stress during the peak blooming phase of the cotton crop, which usually occurs in mid to late summer (Reddy and Kumarı 2004). Likewise, an excessively early sowing date may prevent the peak blooming phase of the crop from commencing with heat stress. However, late sowing dates under sub-optimal weather conditions will result in less seed cotton yield and poor fiber quality (Zhao et al. 2012). Many researchers have used different parameters for screening germplasm against heat stress (Meldebekova et al. 2024; Kyere et al. 2024; Mahatthanaphatcharakun and Taratima 2025). The infrared thermometer and canopy temperature are powerful tools for monitoring and quantifying drought and heat stress in crops (DeJonge et al. 2015). Infrared thermometers are handheld thermometers and are preferred for measuring plant temperature in individual fields for their low cost and greater portability. In the recent past, researchers have become more interested in canopy temperature (CT) and canopy temperature depression (CTD) due to their ease of calculation and usefulness for screening large amounts of material over a very short period (Kebede et al. 2012).

The assessment of the correlation between seed cotton yield and its related features is essential for selecting plants to enhance yield, and this relationship can be utilized in breeding initiatives to generate high-yielding cotton strains. The correlation coefficient shows the extent to which one variable is associated with other variables. A strong and positive correlation shows that two or more variables are strongly associated with each other and can be improved simultaneously, whereas a weak and negative correlation represents that the variables under study are less related to each other, and a rise in one character will cause a decline in another variable. Hence, the correlation coefficient represents the strength of the alliance among various plant parameters and helps to find out the component traits that can be used for the selection of genotypes for the enhancement of fiber quality traits and yield of *Gossypium hirsutum* L. (Ali et al. 2020). The fiber length and fineness (micronaire) decreased as canopy temperature exceeded 31 and 32 °C, respectively (Conaty et al. 2015). The SPAD value (chlorophyll contents) is another physiological trait that might be utilized for rapid screening of genetic materials against heat and drought stress (Karademir Karademir et al. 2012). Probably the most effective and rapid technique to minimize the loss of cotton yield and quality due to high-temperature stress is to recognize and/or evolve heat stress-tolerant genotypes.

The objective of this research is to assess the various physio-morphological characteristics associated with high-temperature tolerance in cotton.

Materials and Methods

Experimental Material

A genetically diverse germplasm consisting of sixty cotton genotypes was obtained from various public and private sector cotton research institutes (Table 1). The study plan was conducted in 2022 and 2023 using a randomized full block design with three replications in a split plot layout. Sowing dates were maintained in the main plots, while cotton genotypes were allocated to the subplots, with a net plot size of 3 m × 5 m, maintaining a gap of 75 cm between rows and 30 cm within rows. All recommended agronomic, intercultural and management practices of early and late cotton

Table 1 List of sixty cotton genotypes evaluated for heat stress tolerance

Coding	Genotype	Institute	Coding	Genotype	Institute
1	FH—LALAZAR	CRS Faisalabad	31	BT. 802	Ali Akbar Group
2	ALLEPO-1	South Africa	32	CRIS-342	CCRI Sakrand
3	FH-942	CRS Faisalabad	33	BT-703	Ali Akbar Group
4	FH-326	CRS Faisalabad	34	A-555	Warble Private
5	MNH-1016	CRI Multan	35	PB-87	UAF
6	CIM-599	CCRI Multan	36	CIM-473	CCRI Multan
7	MNH-886	CRI Multan	37	BLIGHT MASTER-5	USA
8	FH-312	CRS Faisalabad	38	FH NOOR	CRS Faisalabad
9	GH-99	CRS Ghotki	39	CIM-496	CCRI Multan
10	KOPAL (A-2106)	Turkey	40	SITARA-008	Aziz Group Multan
11	VH-259	CRS Vehari	41	CIM-497	CCRI Multan
12	GOMAL-93	Gomal Uni. D I Khan	42	SLH-279	CRS Sahiwal
13	BABDAL	USA	43	NIAB L-1	NIAB Faisalabad
14	FH-114	CRS Faisalabad	44	BABSHAH	USA
15	CIM-511	CCRI Multan	45	BT-333	Ali Akbar Group
16	FH-412	CRS Faisalabad	46	NIAB-999	NIAB Faisalabad
17	CIM-616	CCRI Multan	47	MNH-988	CRI Multan
18	CRIS-134	CCRI Sakrand	48	CIM-573	CCRI Multan
19	GH-114	CRS Ghotki	49	CIM-506	CCRI Multan
20	PB-76	UAF	50	CRIS-121	CCRI Sakrand
21	H-1365-5	Private	51	BT-141	Ali Akbar Group
22	BH-160	CRS Bahawalpur	52	GP-112	CRI Multan
23	MNH-992	CRI Multan	53	CIM-499	CCRI Multan
24	DELTA QUEEN	USA	54	CRIS-9	CCRI Sakrand
25	BT-101	Ali Akbar Group	55	SLH-333	CRS Sahiwal
26	PB-86	UAF	56	A-1	BZU Multan
27	IMP-32	USSR	57	FH-113	CRS Faisalabad
28	VH-282	CRS Vehari	58	PB-896	UAF
29	CYTO-124	CCRI Multan	59	SLH337	CRS Sahiwal
30	CIM-602	CCRI Multan	60	FH-458	CRS Faisalabad

CRS Cotton Research Station; NIAB Nuclear Institute for Agriculture and Biology; NIBGE National Institute for Biotechnology and Genetic Engineering; CRI Cotton Research Institute; CCRI Central Cotton Research Institute; CRS Cotton Research Station; CEMB Centre for Excellence in Molecular Biology; BZU Bahauddin Zakrya University; UAF University of Agriculture Faisalabad

crop under Faisalabad, Pakistan conditions were adopted during the course of the experiment. Nitrogen, phosphorus, and potash fertilizers were applied at rates of 200, 60, and 100 kg ha^{-1}, respectively, to sustain the nutrient status of the experimental site. Nitrogen was applied at sowing at a rate of one-fourth, accompanied with the complete dosage of phosphorus and potash, while the remaining nitrogen was applied in split applications: one-fourth after 30 days, one-fourth at flowering, and one-fourth at peak boll formation. The experiment was thoroughly observed for insect pest infestations and was treated with appropriate chemicals as necessary (Karademir et al. 2012). The initial irrigation was given immediately after seed planting, followed by a second irrigation after 4–5 days to facilitate germination. A 7–10-day interval was established for the third and fourth irrigations, while subsequent irrigations followed at 15-day intervals or as required by the crop.

Application of Treatments

The induction of heat stress under field conditions is a difficult task. This was achieved through adjustment of sowing dates. Sowing in the first week of April aligns the crop's peak flowering phase with June-July's highest temperature (~38–39 °C) while sowing in June links it with October's optimal temperature (~24–32 °C). Thus, heat stress was applied at the peak flowering phase (75–80 days after planting) and compared to the control in the field. Climate data for two consecutive years (2022 and 2023) of the experiment were obtained from the observatory of Plant Physiology Section, Agronomic Research Institute, Faisalabad, Pakistan, which is situated near the experimental site (Fig. 1).

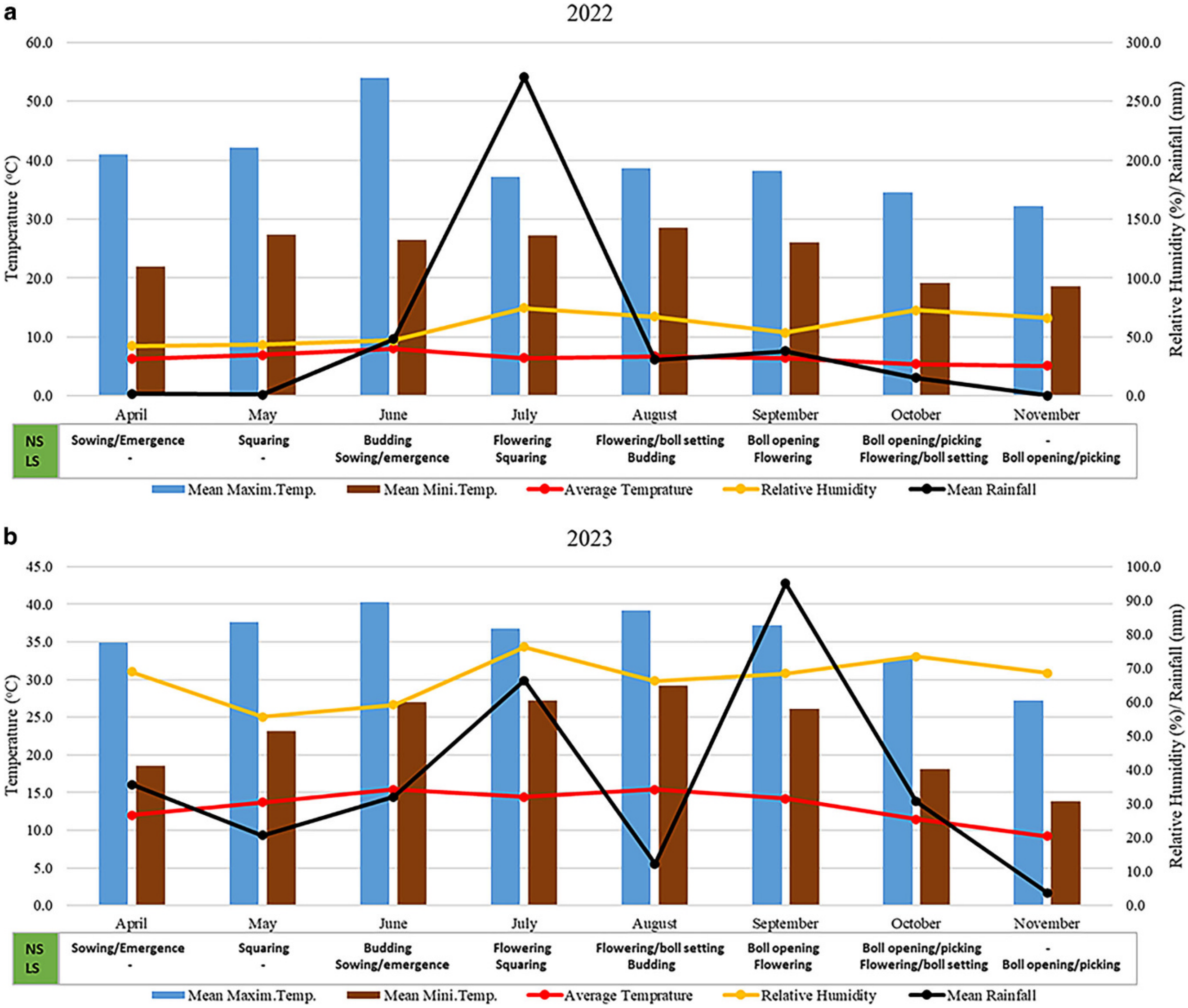

Fig. 1 Monthly weather data and crop growth stages for normal sown (NS) and late sown (LS) cotton at experimental site during (**a**) 2022 and (**b**) 2023

Observations Recorded

Cell Membrane Thermostability

Young, fully developed leaves from five plants per genotype were selected after 70–80 days of planting, during the peak flowering phase under both heat-stressed and normal sowing conditions. The leaves were thereafter chopped into uniform circles and placed in falcon tubes containing distilled water at 25 °C following two or three washes with distilled water. Samples were extracted in pairs from the midrib of the leaf on both sides. One group was employed as a control, while the other was subjected to heat treatment. One set of falcon tubes was submerged in a water bath at 50 °C for one hour, while the control set was maintained at 25 °C continuously. The two sets were heated to 25 °C to facilitate electrolyte diffusion and maintained at that temperature for the whole day. Following a complete day, the electrolytes were completely homogenized in the vials, and the electrical conductivity (EC) was assessed utilizing an EC meter (Model: HI-933300, Hanna Instruments, USA). The electrical conductivity of the heat-treated samples was designated as T_1, whereas the control's electrical conductivity was designated as C_1. The vials were subsequently autoclaved for 10 min at 0.01 MPa to thoroughly obliterate the tissues and liberate the electrolytes. The ECs of treatment (T_2) and control (C_2) were assessed when the falcon tubes reached room temperature. The following formula was utilized to compute the CMT% (Blum and Ebercon 1981).

$$\mathrm{CMT\%} = [\{1-(\mathrm{T1}/\mathrm{T2})\}/\{1-(\mathrm{C1}/\mathrm{C2})\}] \times 100 \quad (1)$$

Canopy Temperature

The heat stress tolerance of plants can be best measured by looking at the canopy temperature. An Infrared Thermometer (IRT, model: DT-8811H) was used to record the canopy temperatures of five plants per genotype under both treatments from 11:00 AM to 4:00 PM.

Chlorophyll Content

The Minolta SPAD-502 chlorophyll meter was used to measure the amount of chlorophyll in the fifth fully grown leaf below the plant's terminal during the peak flowering period (Chen et al. 2005).

Node Number to First Fruiting Branch

The node number associated with the first fruiting branch for five selected plants was determined, using the cotyledon node as the reference point (zero node).

Boll Weight

To find the average boll weight, we divided the total yield by the number of bolls, and we randomly selected 25 completely developed mature bolls.

Seed Cotton Yield

The primary aim of every breeding program is yield; however, biotic or abiotic stress results in reduced productivity. Upon maturation, five plants from each genotype were selected and harvested twice following the evaporation of dew. The average seed cotton yield per plant was determined by dividing the total yield by the total number of plants.

Statistical Analysis

Analysis of variance technique was used to observe significant differences among cultivars and treatments (RGD and S. 1997). To conduct GGE biplot, year-treatment combinations were considered as four environments i.e NS2022 and NS2023 for April sowing (stress) and LS2022 and LS2023 for June sowing (control) condition during 2022 and 2023, respectively. The analysis was performed using R software.

Table 2 Descriptive statistics of agro-physiological traits for 60 cotton genotypes under four environments

	Mean				Min				Max			
Environ.	NS2022	LS2022	NS2023	LS2023	NS2022	LS2022	NS2023	LS2023	NS2022	LS2022	NS2023	LS2023
CMT	50.03	43.04	50.22	45.57	35.02	30.80	38.57	31.67	70.58	61.37	69.43	69.67
CC	40.08	37.59	42.50	38.39	33.17	31.60	33.97	23.23	50.97	49.47	52.93	52.50
CT	32.61	36.10	31.85	35.17	30.93	32.37	27.87	29.40	34.50	42.93	34.13	42.63
NNFFB	6.76	6.58	6.63	7.30	4.33	4.30	4.97	4.90	9.63	9.23	8.00	10.33
BW	2.79	2.63	3.08	2.86	1.97	1.87	1.97	1.87	3.93	3.83	3.97	3.87
SCY	54.36	42.57	78.39	63.28	10.67	6.67	17.33	16.33	130.33	110.33	151.33	145.33

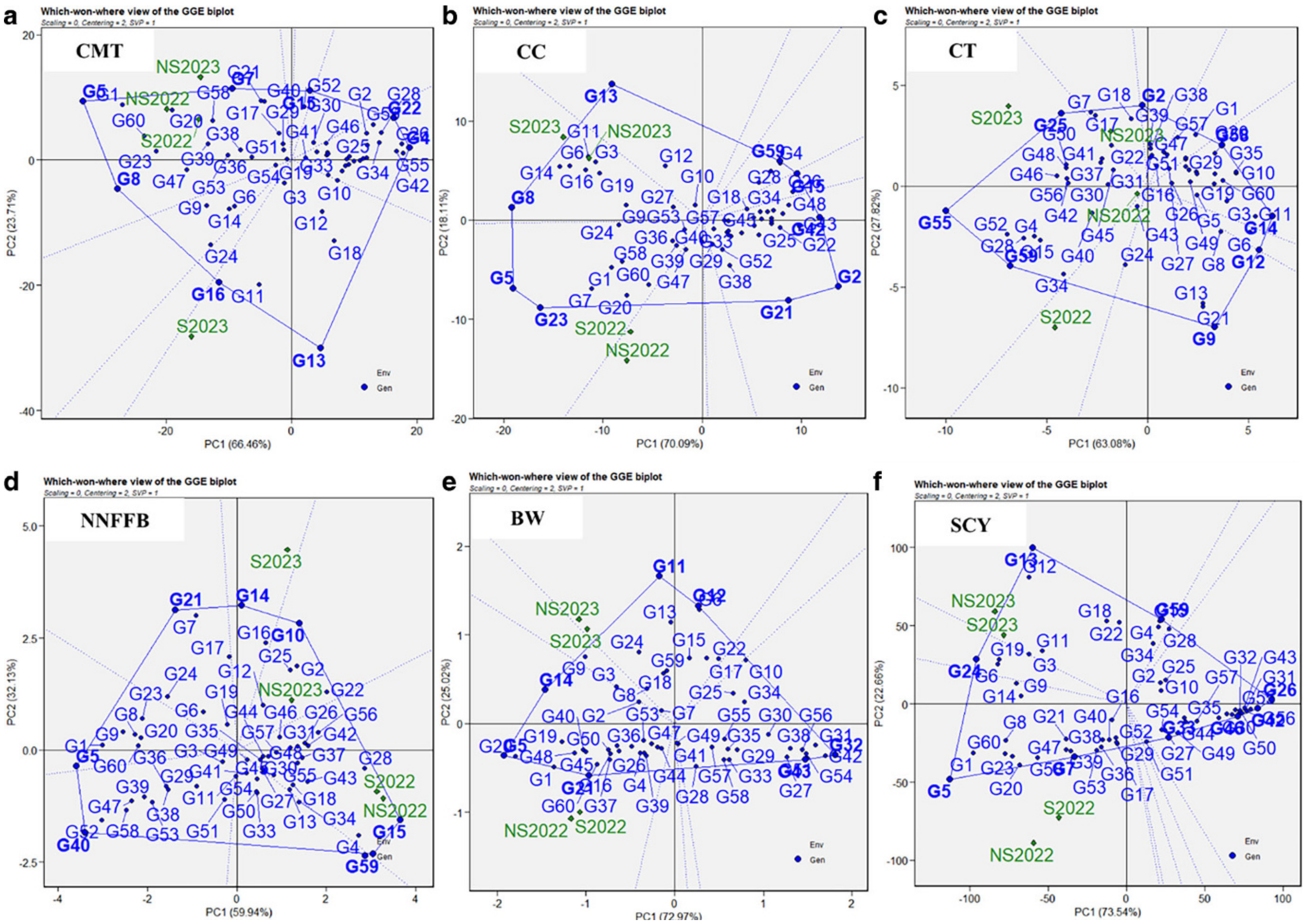

Fig. 2 "Which-won-where" pattern based on GGE biplot analysis. **a** *CMT* cell membrane. **b** *CC* chlorophyll contents. thermostability. **c** *CT* canopy temperature. **d** *NNFB* node numbers to first fruiting branch. **e** *BW* boll weight. **f** *SCY* seed cotton yield

The genotypes coding was used to perform the GGE biplot analysis instead of complete name (Table 1).

The GGE biplot was executed using the "metan" package in R programming. The GGE biplot graphic method was employed to analyze multi-environment trial data, assessing the adaptability and stability of cultivars, as well as the impacts of genotype, environment, and G × E interaction.

Results

Analysis of variance revealed substantial variations in mean squares for genotypes, environments, and the genotype × environment interaction for every trait (Table 2). Traits exhibiting notable variations across genotypes and settings were subsequently examined using GGE biplot analysis.

GGE Biplot Analysis

"Which-won-where" Pattern Based On GGE Biplot Analysis

The polygon view ("which-won-where" pattern) of the GGE biplot for various traits (Fig. 2) revealed distinct environments and optimal cotton genotypes for each environment.

The peripheral varieties were linked to form a polygon, which was divided into several sectors by perpendicular lines stretching from the origin to various edges. The genotypes located at the polygon's vertex demonstrated the highest average performance across the testing combinations in each polygon view.

Four cotton genotypes 1, 5, 20 and 23 were the best-performing genotype on BW, CC, CMT and SCY under two environments NS2022 and LS2022 whilst for CT and NNFB, theses genotypes attained the lowest values for these traits. The cotton genotypes 5, 20 and 21 showed the highest values for BW and CMT and the lowest values for CT and NNFB. In contrast for SCY, the cotton genotypes 6, 19 and

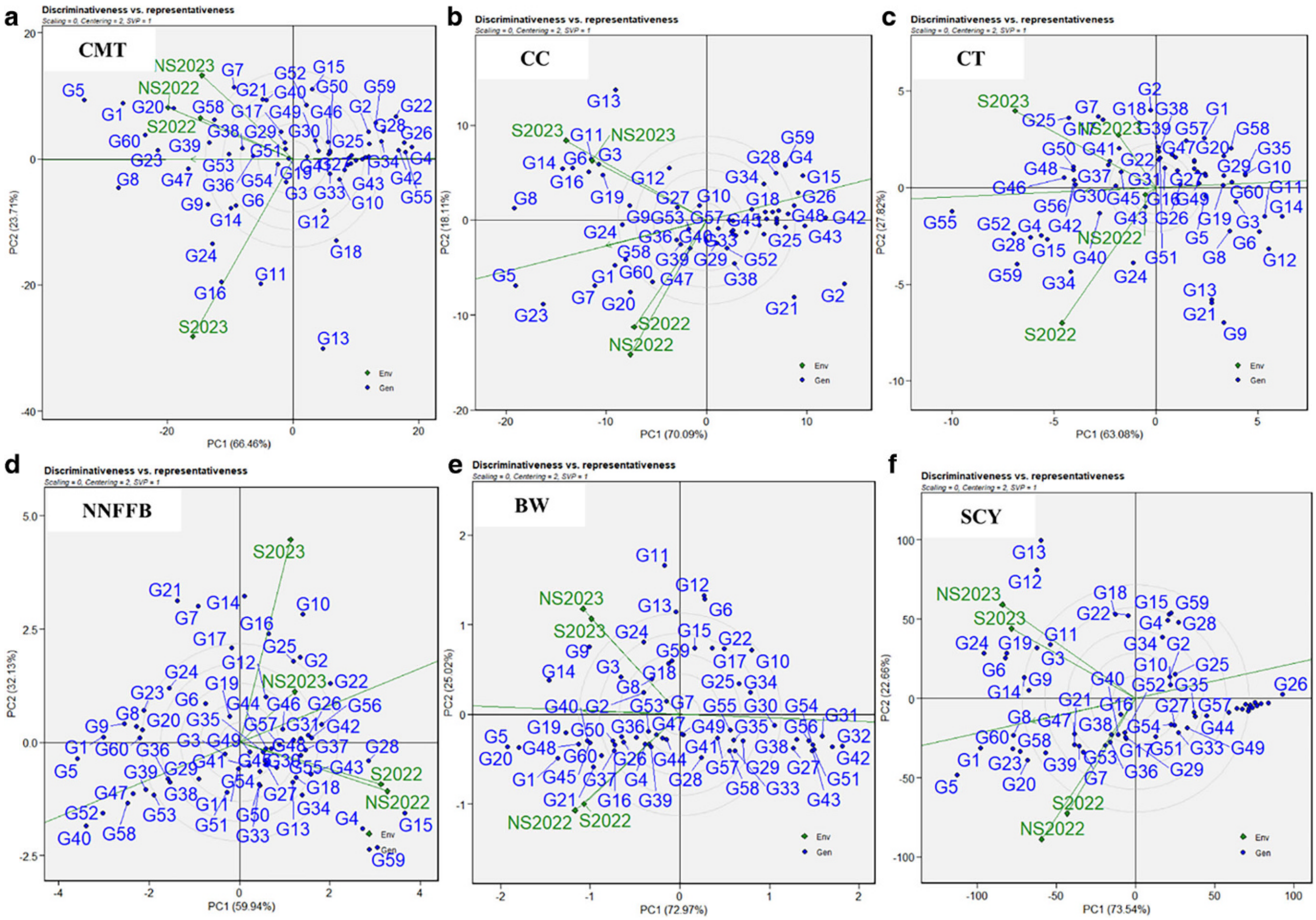

Fig. 3 The representativeness and discrimination ability based on genotype plus genotype × environment interaction (GGE) biplot analysis. **a** *CMT* cell membrane. **b** *CC* chlorophyll contents. Thermostability. **c** *CT* canopy temperature. **d** *NNFB* node numbers to first fruiting branch. **e** *BW* boll weight. **f** *SCY* seed cotton yield

24 showed stable performance for both environments i.e NS2023 and LS2023 (Fig. 2a, b, c, d, e, f).

The Representativeness and Discrimination Ability

The representativeness and discriminability of four testing conditions concerning several agro-physiological variables were evaluated using GGE biplot to find varieties with stable performance (Fig. 3). The discriminatory ability of the experimental site was directly related to the length of the environmental vector, defined as the line from the origin to the testing environment point. The representativeness of the target location was determined by the angle between the testing location vector and the average environmental coordination (AEC) horizontal axis, depicted by the single-arrowed line extending from the biplot origin. A narrower angle enhances the representativeness of the location.

NS2022 and NS2023 were the most discerning and representative of the test conditions for six agro-physiological variables (Fig. 3a, b, c, d, e, f).

The Stability and Mean Performance

Figure 4 illustrates the stability and mean performance of 60 cotton genotypes in relation to agro-physiological factors and distinct genotype-by-environment interactions, as analyzed by GGE biplot methodology. The projection of cotton genotypes on the AEC horizontal axis was oriented in the positive direction, indicating high values of the related attributes. The stability of each genotype was evaluated by the length of perpendicular line segments from the cotton genotype to the AEC, with the most stable genotype located close to the AEC horizontal axis and demonstrating a near-zero projection onto the AEC vertical axis.

The cotton genotypes with stable above- or below-average of different agro-physiological traits under different environments were displayed in figures (Fig. 4a, b, c, d, e, f). Of these, genotypes 5, 20 and 23 were considered stable with the highest phenotypic value of CC, CMT and SCY and lowest phenotypic values for CT and NNFB under two environments NS2022 and LS2022. The genotypes 11, 12 and 13 performed better for BW, CMT and

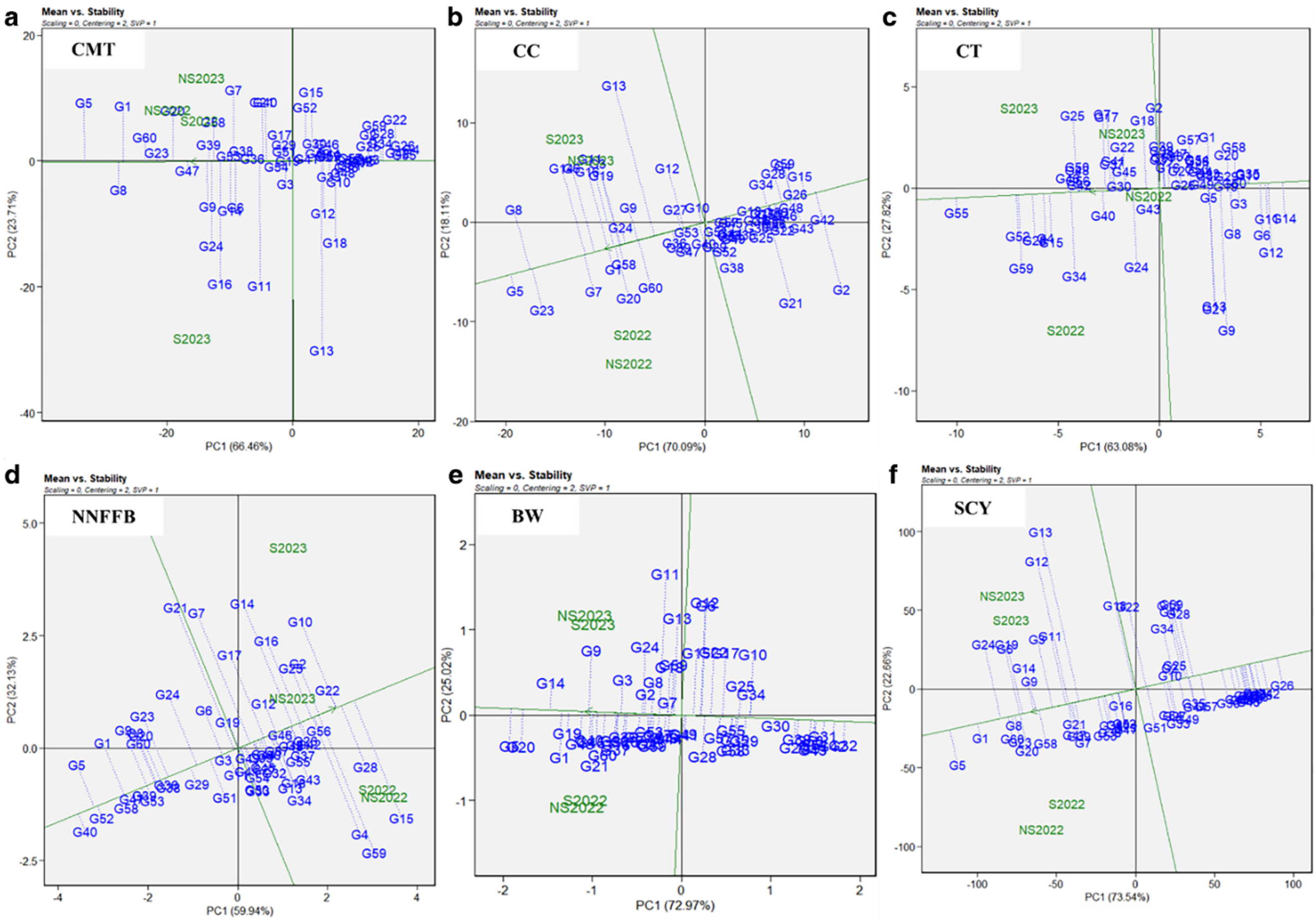

Fig. 4 The stability and the mean performance of 50 cotton genotypes across environment based on GGE biplot analysis. **a** *CMT* cell membrane. **b** *CC* chlorophyll contents. thermostability. **c** *CT* canopy temperature. **d** *NNFB* node numbers to first fruiting branch. **e** *BW* boll weight. **f** *SCY* seed cotton yield

Table 3 Degrees of freedom and mean squares for environment and genotype effects on agro-physiological traits

SOV	DF	CMTT%	CC	CT	NNFFB	BW	SCY
Env	3	2226.84**	848.76**	738.78**	19.61**	6.24**	41053**
Rep (Env)	8	2.35	7.93	2.89*	0.42	0.10**	53
Gen	59	461.01**	195.32**	27.34**	8.02**	2.48**	9227**
Gen:Env	177	82.32**	33.21**	11.92**	2.27**	0.31**	1290**
Residuals	472	2.55	9.67	1.12	0.29	0.02	39

SCY whereas for CT and NNFB, the genotypes 20 and 60 showed the lowest desired values under NS2023 and LS2023 (Fig. 4a, b, c, d, e, f).

Descriptive Statistics of Different Agro-physiological Traits

Cell Membrane Thermostability

Cell membrane thermostability showed decreasing trend under heat stressed condition as compared to normal sowing. CMT ranged from 35.02 to 70.58 with a mean of 50.03 under normal sowing date during 2022 while under heat stress, it ranged from 30.80 to 61.37 with an average of 43.04. Similarly, during 2023, CMT under normal sowing date ranged between 38.57 and 69.43 with a mean of 50.22 whereas under stress condition, the range of CMT was from 31.67 to 69.67 with an average of 45.57 (Table 3). The highest cell membrane thermo-stability was observed for selected tolerant parents under normal and stress conditions in 2022 and 2023.

Chlorophyll Contents

Sowing during month of April 2022 and 2023 (stressed condition) caused discoloration/scorching of leaves due to severe high temperature stress at peak flowering time and showed decreased chlorophyll contents. Under normal sowing date during 2022, the chlorophyll contents were ranged from 33.17 to 50.97 with a mean of 40.08 and under stressed condition it ranged from 31.60 to 50.97 with a mean of 42.50. During 2023, the genotypes showed a range of 33.97 and 52.93 with an average of 42.50 under normal sowing and from 38.39 to 52.50 with a mean of 38.39 under stress condition (Table 3). The higher chlorophyll contents were considered as high temperature tolerance indicator and genotypes showing higher chlorophyll contents under normal and stressed sowing during consecutive years were selected as tolerant parent for further breeding program.

Canopy Temperature

Canopy temperature (°C) was higher under stressed sowing due to higher air temperature and affected canopy shape by shedding of plant parts as compared the normal sowing. Under NS (normal sown), canopy temperature ranged from 32.37 to 34.50 with an average of 32.61 and from 27.87 to 34.13 with a mean of 31.85 during 2022 and 2023 respectively. under heat stress, canopy temperature ranged from 32.37 to 42.93 having mean of 36.10 and from 29.40 to 42.63 with a mean of 35.17 during 2022 and 2023 respectively (Table 3). Low canopy temperature is associated with high temperature stress tolerance in cotton and thus the genotypes showing lower canopy temperature under normal as well as stressed condition during both years of study were taken as tolerant parent in future breeding.

Node Number to First Fruiting Branch

Low number to first fruiting branch is an indication to high temperature tolerance and early maturity in cotton. Cotton genotypes under study showed a range of 4.33 to 9.63 with an average of 6.76 under normal sowing during 2022 and from 4.97 to 8.00 with an average of 6.63 during 2023 for this trait. Under high temperature stress during 2022, node number to first fruiting branch ranged from 4.30 to 9.23 with a mean value of 6.58 while during 2023, it ranged from 4.90 to 10.33 with an average of 6.63. The genotypes with low node number to first fruiting branch were selected for further crossing (Table 3).

Boll Weight (G)

Genotypes displayed higher boll weight under normal sowing as compared to stressed condition. The range of boll weight under normal condition during 2022 was 1.97 (g) to 3.93 (g) with a mean value of 2.79 (g) while during 2023, it ranged from 1.97 (g) to 3.97 (g) with a mean value of 3.08 (g). As for as stress condition was concerned, boll weight ranged from 1.87 (g) to 3.83 (g) with an average of 2.63 (g) during 2022 and from 1.87 (g) to 3.87 (g) with an average of 2.86 (g) during 2023 (Table 3).

Seed Cotton Yield (G)

The Seed cotton yield per plant (g) showed increasing trends because of extended period of favorable temperature at peak flowering under normal sowing date whereas decreasing trend was observed under high temperature stress. Seed cotton yield under NS ranged from 10.67 (g) to 130.33 (g) with an average value of 54.36 during 2022 and from 17.33 (g) to 151.33 (g) with a mean of 78.39 (g) during 2023. Under heat stress, seed cotton yield varied from 6.67 (g) to 110.33 (g) with mean of 42.57 (g) during 2022 and from 16.33 (g) to 145.33 (g) with an average of 63.28 (g) during 2023 (Table 3). Selection of tolerant genotypes was based on high seed cotton yield under normal and high temperature stress condition for two consecutive years.

Correlation Studied Among Various Traits Under Normal and High Temperature Stress Condition During 2022 and 2023

Correlation was studied among all traits under normal and high temperature stress during consecutive years of 2022 and 2023. Under NS2022, BW had significant positive correlation with CMT%, CC, CT and SCY but highly significant negative correlation with NNFFB and CT. Similarly, CC showed highly significant positive correlation with CMT% and SCY, however highly significant but negative correlation was found between CC and CT and NNFFB. CMT% was positively associated with SCY but negatively correlated with NNFFB and CT. CT showed positive correlation with NNFFB and negative association with SCY where as NNFFB also showed negative association with SCY (Fig. 1). Similarly, under high temperature stress during 2022, BW showed highly positive correlation with SCY, CMT% and CC but highly significant negative correlation with NNFFB and CT. CC was positively correlated with CMT% and SCY but negatively correlated with CT and NNFFB. CMT% un-der high temperature stress was positively associated with SCY but negatively correlated with NNFFB and CT. CT had negative correlation with SCY and positive with NNFFB while NNFFB also showed negative association with SCY (Fig. 2).

During 2023, BW showed highly positive correlation with CC, CMT% and SCY while highly significant but negative correlation was observed for CT and NNFFB under

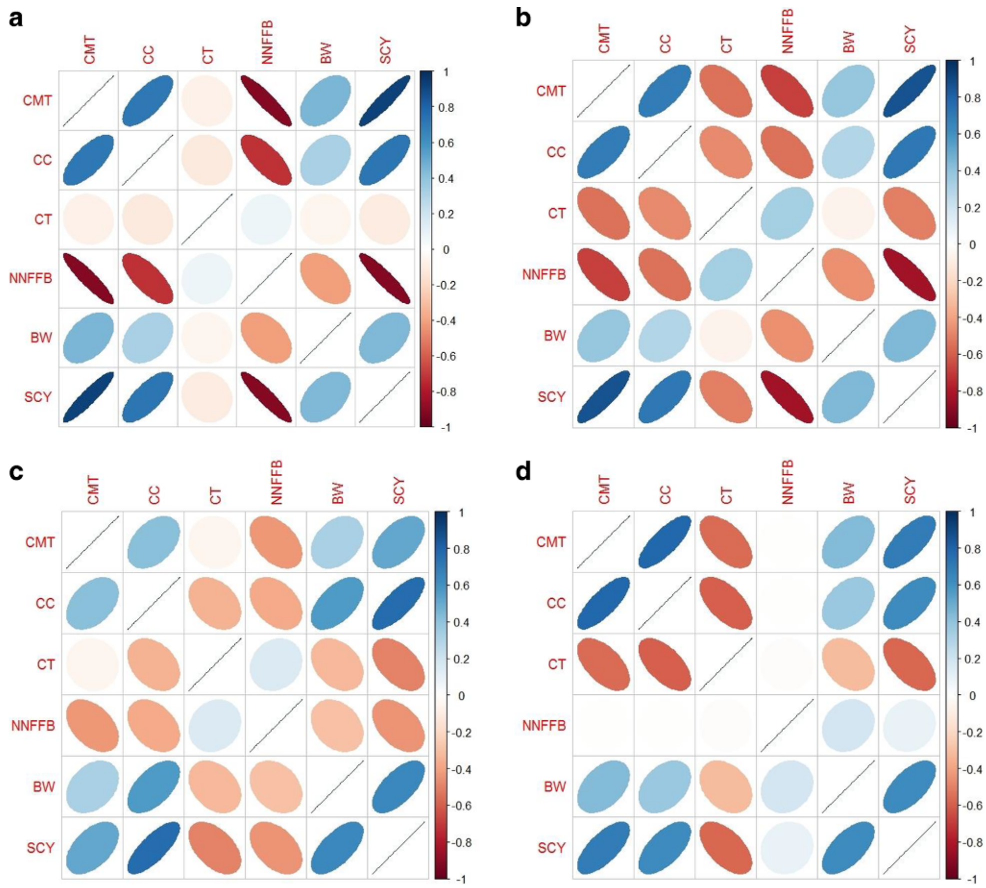

Fig. 5 Corrplot (Correlation analysis) of various agro-physiological traits in 60 cotton genotypes under **a** normal sowing 2022 (NS2022); **b** late sowing (LS2022); **c** normal sowing 2023 (NS2023); **d** late sowing 2023 (LS2023). Corrplot's color indicates association. Red indicated a positive association and blue a negative one. Furthermore, the intensity of these colors shows the level of relationships; darker demonstrated high correlation and lighter showed low correlation

normal sowing condition. CC had highly significant positive correlation CMT% and SCY but negative correlation with CT and NNFFB. CMT% showed highly significant negative correlation with CT and NNFFB but highly positive with SCY. CT and NNFFB showed significant negative correlation with SCY under normal sowing (Fig. 3). Under high temperature stress during 2023, BW was positively correlated with CC, CMT%, NNFFB and SCY but negatively correlated with CT. CC had significant positive association with CMT% and SCY but was negatively associated with CT and NNFFB. CMT% showed positive correlation SCY and negative correlation with CT which was also negatively correlated with SCY (Fig. 5).

Discussion

High-temperature stress (>36 °C) negatively impacts cotton production and quality, especially during the peak flowering period/reproductive phase. A diverse cotton genotypes was evaluated for high temperature stress by employing multiple sowing dates to align the peak flowering period of the crop with fluctuating temperature conditions in the field. The current study also provided valuable information about genetics of the morphological and physiological traits which could effectively be used for screening of cotton germplasm against high temperature stress (Zahid et al. 2016). Temperature rise (>33 °C) during present experiment at reproductive phase, decreased flowers, bolls and other yield components thus reducing economic yield conferring the previous observations (Brown et al. 2003). Cell membrane thermostability was measured to determine membrane leakage at cellular level which significantly differentiates cotton genotypes into susceptible and tolerant genotypes. The CMT% was observed to be lower during the NS of cotton genotypes at elevated temperatures (38–39 °C) during peak flowering compared to the optimal temperature range (18–31 °C) for the reproductive stage (Table 3). Elevated temperatures resulted in damage to the chloroplast membranes, which decreased CO2 uptake and impeded photosynthesis. Likewise, CC and CT were adversely impacted by elevated temperature stress. The results of the current study indicated that cotton in the Indo-Pak subcontinent typically encountered elevated temperatures during the peak flowering stage, which directly influenced physiological traits. These physiological parameters could be effectively utilized for the screening of cotton germplasm in response to high-temperature stress (Cottee et al. 2010; Salman et al. 2016). CT identified substantial genotypic variations in response to high-temperature stress and was utilized for the selection of tolerant genotypes, as evidenced in numerous studies addressing abiotic stresses (Purushothaman and Krishnamurthy 2014). Four cotton genotypes namely FH Lalazar, NNH-1016, PB-76 and MNH-992 were the best-performing genotype for BW, CC, CMT% and SCY under two environments NS2022 and LS2022 whilst for CT and NNFB, theses genotypes attained the lowest values for these traits. The cotton genotypes MNH-1016, PB-76 and H-1365-5 showed the highest values for BW and CMT and the lowest values for CT and NNFB under NS2023 and LS2023. In contrast for SCY, the cotton genotypes CIM-599, GH-114 and Delta queen showed stable performance for both environments i.e NS2023 and LS2023. Cotton genotypes MNH-1016, PB-76 and MNH-992 were considered stable with the highest phenotypic value of CC, CMT% and SCY and lowest phenotypic values for CT and NNFB under two environments NS2022 and LS2022. The genotypes VH-259, Gomal-93 and Badal performed better for BW, CMT and SCY whereas for CT and NNFB, the genotypes PB-76 and FH-458 showed the lowest desired values under NS2023 and LS2023.

Positive correlation of CMT% with yield under normal conditions is reported by early researchers (Azhar et al. 2009) however, the relationship between CMT% and crop yield under heat stress may vary with plant spp. and it is necessary to study individual crop before using CMT% as significant physiological selection criteria against heat stress. The results of present investigation showed that tolerant genotypes possessed higher CMT% while lower CMT% was observed for susceptible strains. Hence, it may be concluded that cell membranes of tolerant strains resist high temperature while those of sensitive genotypes become leaky at high temperature. The cell membrane structure under high-temperature stress got altered which increased the membrane permeability and leakage of cell electrolytes and eventually resulted in cell death. CMT was found efficient and rapid screening technique which can reliably be used for the assessment of genetic material at early growth stage (Azhar et al. 2009). In general, greenish color represents more CC with higher photosynthetic activities. In current study, the heat tolerant genotypes showed higher CC than heat sensitive genotypes. Previous researchers utilized chlorophyll content measurements to identify the optimal parent for stress breeding, concluding that a reduced chlorophyll ratio in plants subjected to stress correlates with enhanced tolerance to heat stress, and conversely (Rana et al. 2011). A significant and positive correlation was observed between CT and seed SCY. CT showed strong and positive correlation with seed cotton yield under normal conditions but negative correlation under heat stress. Positive correlation of CT at peak flowering period with seed cotton yield has been already determined by many researchers (Bahar et al. 2008) (Alam 2014). The study revealed that genotypes with higher CT, due to more closed stomata, produced the highest yields under normal condition as plant retains adequate water for various metabolic processes taking place within the plant. Nevertheless, a negative association between seed cotton output and CT. The time of recording CT may be another important factor for these different findings. The present findings of positive correlation of yield with its contributing traits are in accordance with the previous findings (Ahsan et al. 2015; Shao et al. 2016). This information will help the breeders in identification of gene action and to design appropriate breeding strategy for the development of heat tolerant genetic material in *Gossypium hirsutum* L.

Conclusions

Under changing climate scenario especially the increasing temperature, regular screening of existing genetic material against heat stress and development of heat tolerant cotton genotypes is necessary for a better yield of good quality seed cotton. Thus, the present re-search project was designed to explore the genetic variability in cotton germplasm against high temperature stress as well as to demonstrate the association of different yield related traits with SCY. FH-Lalazar, MNH-1016, PB-76, MNH-992, and FH-458 were stable under high temperature stress for most characteristics. These genotypes could be used to develop heat-stress-tolerant cultivars. The scientific information collected through this project will serve as mile stone for cotton breeders in future and genetic material developed through this project will be the country’s asset.

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Author Contribution S. Ali and W. Shafqat Chattha designed the project and wrote the final draft. M. Afzal and A.-H. Ghazy prepared figures. A. Ullah, W. Shafqat and M. Zahid Ihsan reviewed and edited the manuscript. A. Ullah and B. Ayub recorded the data and administrated the field experiment.

Data Availability Statement All the research data included in the article; further inquiries can be directed to the corresponding authors.

Conflict of interest S. Ali, W. Shafqat Chattha, A. Ullah, M. Zahid Ihsan, W. Shafqat, A.-H. Ghazy, M. Afzal and B. Ayub declare that they have no competing interests.

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