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Original Research Article

QUANTITATIVE EFFECTS OF HEAT STRESS ON FIBER RELATED AND AGRONOMICALLY IMPORTANT PARAMETERS IN COTTON (*Gossypium hirsutum* L.)

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Abstract: *Climate change is one of the biggest problems for growing crops in a sustainable way around the world. At the cotton research station in Bahawalpur, this experiment aimed to assess and classify cotton genotypes under conditions of heat stress. The study was done using RCBD with three replications. The distance between plants was 30 cm, and the distance between rows was kept at 75 cm. For key plant and fiber quality traits, data were taken from ten fully guarded plants and chosen randomly. Under conditions of heat stress, ANOVA showed that there were highly significant differences among the plant traits that were studied. The correlation coefficient analysis showed that seed cotton yield has a positive correlation with plant height (r = 0.46), plant population per hectare (r = 0.33), sympodial branches per plant (r = 0.27), number of bolls per plant (r = 0.27) and nodes per plant (r = 0.27) but a negative relationship with staple length (r = -0.35). The multivariate statistical methods of principal component and cluster analysis were used to describe cotton genotypes. Principal component analysis and cluster analysis showed that the most productive and heat-tolerant cotton genotypes were BH-200, BH-254, CIM-600, and BH-341. Also, BH-284 seemed more resistant to CLCuV than the other genotypes. So, rigorous, large-scale, and multilocation testing must be done on these cotton genotypes and plant traits to make cotton genotypes that can handle heat and CLCuV.*

Keywords: Abiotic stress tolerance, high temperature, biplot, cluster analysis, principal components, multivariate

Introduction

Climate change significantly impact crop production, as it affects various factors crucial for plant growth and productivity, such as temperature, precipitation, atmospheric CO_2 concentration, and the timing and intensity of extreme weather events (IPCC, 2014). Rising temperatures can increase extreme heat events' frequency, reducing crop yield and quality (Challinor *et al*., 2014). In addition, changes in precipitation patterns and more frequent and intense drought events can also negatively impact crop growth and productivity (Asseng *et al*., 2013). Moreover, a gradual increase in the concentration of CO_2 resulting from human activities i.e., combustion of fossil fuels, deforestation, and change in land use practices and industrial processes, could affect both ways, i.e., negatively and positively. The elevation in CO2 could stimulate the process of photosynthesis, resulting in increased crop yield; it can also negatively affect water use efficiency, nitrogen uptake, and grain quality (Asseng *et al*., 2013). Li *et al*. (2021) studied the metadata from 1980 to 2016 through a global crop growth model to analyze the impacts of climate change on crop yields and world food security and found that global crop yields have been negatively affected by climate change, with a decline of 2.6% on average, and threatened the food supply chain in many regions of the world.

Cotton (*Gossypium spp*.) is a fiber and oilseed crop that is widely cultivated throughout the world. Cotton is the fourth most important fiber crop globally, with over 25 million hectares of cotton cultivated globally (FAO, 2021). It is one of the most

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important fiber crops globally and has a long history of use in textile production (Shah *et al*., 2018). The origin of cotton is thought to be in the regions of the ancient civilizations of India and the Americas (Prasad, 2019). The domestication of cotton as a crop is thought to have occurred around 5000 years ago in India and the Indus Valley of Pakistan (Zerega & Jain, 2016). Cotton is an important source of fiber for clothing, bedding, towels, and other textiles. It is valued for its softness, absorbency, and durability (Prasad, 2019). In addition to fiber production, cotton is also grown for its oil and food value. Cottonseed oil is used in cooking, cosmetics, and as a biofuel, while the seed meal is used for animal feed (Zerega & Jain, 2016). Moreover, it is also used in the medical industry to manufacture surgical gowns, bandages, and swabs.

Heat stress is a major environmental constraint that affects cotton growth and development, leading to reduced plant performance and decreased fiber yield. Extreme temperatures can cause significant physiological and morphological changes in cotton plants, resulting in reduced growth, flower and boll development, and fiber quality (Liu *et al*., 2019). High temperatures can cause a reduction in photosynthesis and stomatal conductance, leading to reduced growth and yield (Rana *et al*., 2020). Heat stress can cause changes in morphological and physiological traits in upland cotton, including reduced plant height, reduced leaf area, reduced boll number and weight, and altered root structure and function, which ultimately result in lower fiber yield and quality (Lu *et al*., 2019; Zhang *et al*., 2019; Xu *et al*., 2020). In addition to affecting growth and yield, heat stress can significantly impact fiber quality. High temperatures can cause reductions in fiber length, strength, micronaire, and changes in fiber colour and uniformity (Liu *et al*., 2019). The impacts of heat stress on cotton can also be exacerbated by water stress, which can result in even greater reductions in plant performance and yield (Liu *et al*., 2019).

To mitigate the effects of heat stress, several management strategies could be employed, including genetic improvement, planting date and geometry, and irrigation and mulching. Improving the heat tolerance of cotton varieties through breeding is an important aspect of managing the effects of heat stress on cotton production. To start a breeding program, evaluating the existing germplasm for the search heat tolerant parents to be used in the hybridization program is pivotal. Therefore, the current study aimed to characterize the cotton germplasm for its heat tolerance based on agronomically important morphological and fiber-related traits.

Materials and Methods

Experimental material, location and layout.

The field trial was conducted at Cotton Research Station, Bahawalpur *(29°23'10.4"N 71°39'08.8" E)* during the 2022 growing season. Nineteen cotton genotypes, including elite lines and approved verities, were evaluated during the cotton growing season in 2022 (Table 1). The experiment was executed under RCBD in triplicates. The plantation was done in the fourth week of May 2022 with the help of a hand-driven seed drill @ 3 seeds per hill to ensure maximum germination and seedling stand. At the early seedling stage, the seedlings were thinned to one healthy seedling only to reduce crop competition. The reason for late sowing was to put the crop in a situation where it could experience high-temperature stress (40°C-45°C) at grand-growth, flower and square developmental stages. The sowing was done on four-rowed, three-meter-long raised ridges. Standard agronomic practices, i.e., fertilizer application, pesticide application and irrigation, were applied to all genotypes.

Table 1: Cotton genotypes used in the study

Sr.	Cotton Genotype	Genotype Status	Sr.	Cotton Genotype	Genotype Status
1	BH-341	Elite Line	**11**	BH-287	Elite Line
2	BH-377	Elite Line	**12**	BH-301	Elite Line
3	BH-247	Elite Line	**13**	BH-232	Elite Line
4	BH-254	Elite Line	**14**	BH-348	Elite Line
5	BH-283	Elite Line	**15**	BH-184	Approved Variety
6	BH-272	Elite Line	**16**	BH-320	Elite Line
7	BH-200	Elite Line	**17**	BH-333	Elite Line
8	BH-297	Elite Line	**18**	BH-350	Elite Line
9	BH-306	Elite Line	**19**	BH-284	Elite Line
10	CIM-600	Approved Variety			

Data Recording

Data was recorded for several agronomically important traits, i.e., plant height, number of nodes per plant, monopods per plant, sympodial per plant, bolls per plant, CLCuV incidence percentage, plant population per hectare, lint percentage, staple length, micronaire value, fiber strength and cotton yield. The data were recorded from ten different, fully guided plants per genotype per replication and then the average was calculated. The fiber or staple-related parameters were recorded through High-Volume Instrument (1 X USTER HVI 900 A).

Statistical Data Analysis

The recorded data were subjected to analysis of variance (ANOVA) and correlation coefficient analysis following the method recommended by

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Steel *et al.* (1997). Furthermore, principal component and cluster analysis were used to categorize and classify cotton genotypes under heat stress conditions (Sneath & Sokal, 1973). For their execution, two statistical tools i.e., XLSTAT 22.0 and OriginPro 22.0 were used. The illustration of data was done through Microsoft Excel 21.0.

Metrological Data

The experiment was sown in the cotton planting season of 2022 under field conditions in the 4th week of May 2022. The metrological data was recorded for various climate-related parameters, i.e., minimum temperature, maximum temperature, rainfall and relative humidity throughout the growing season. However, here data from sowing to boll formation is presented in Figure 1. The data revealed that during the days from sowing to harvesting, cotton genotypes experienced an average maximum temperature of around 40 °C - 41 °C. However, for some days, the temperature remained as high as 45 °C, much higher than the optimum temperature. Such a high temperature could drastically affect the growth and development of cotton genotypes.

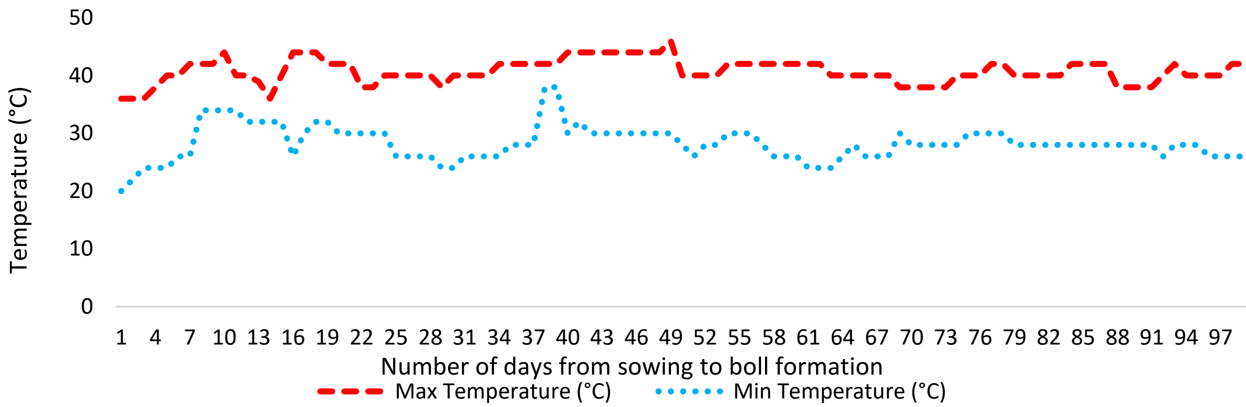

Figure 1: Variation in Temperature for the whole cotton growing season (2022)

Results

Analysis of Variance (ANOVA)

The ANOVA revealed that most of the evaluated traits, such as plant height (PH), sympodial branches per plant (SP), number of bolls per plant (BP), CLCuV incidence percentage (CLCuV), plant population per hectare (PP), lint percentage (%), fiber length (FL), fiber fineness (FF), fiber strength (FS), and seed cotton yield (SCY) under heat stress conditions, showed highly significant differences between cotton genotypes (Table 2). The changes in nodes per plant (NP) and monopodial branches per plant (MP) in cotton genotypes, however, were significant only (Table 2).

Correlation Coefficient Analysis

The correlation revealed a positive correlation of seed cotton yield with plant height (r = 0.46), plant population per hectare (r = 0.33), sympodial branches per plant (r = 0.27), number of bolls per plant (r = 0.27) and nodes per plant (r = 0.27) (Figure 2). However, staple length negatively correlated with seed cotton yield (r = -0.35). The highest positive correlation was found between nodes per plant and sympodial branches per plant (r = 0.96) followed by plant height (r = 0.75), respectively (Figure 2).

Table 2: Mean Square (MS) values of selected traits of cotton genotypes under heat stress conditions

Source of variation (SOV)	Replication (R)	Genotypes (G)	Error (E)
Degree of freedom (df)	2	19	35
Plant height (PH)	12.3	714.3**	103.7
Number of nodes per plant (NP)	1.25	18.9*	9.1
Monopods per plant (MP)	0.63	1.94*	0.85
Sympodial per plant (SP)	4.5	19.8**	7.6
Bolls per plant (BP)	55.3	73.7**	12.1
CLCuV incidence percentage (CLCuV)	11.9	23.6**	7.1
Plant population per hectare (PP)	7.90E+07	1.5E+07**	5.30E+06
Lint percentage (Lint)	0.032	13.9**	0.91
Staple length (SL)	0.002	1.94**	0.18
Micronaire value (FF)	0.02	0.48**	0.06
Fiber strength (FS)	2.95	47.7**	6.0
Seed cotton yield (SCY)	8391.4	75296.7**	14205.2

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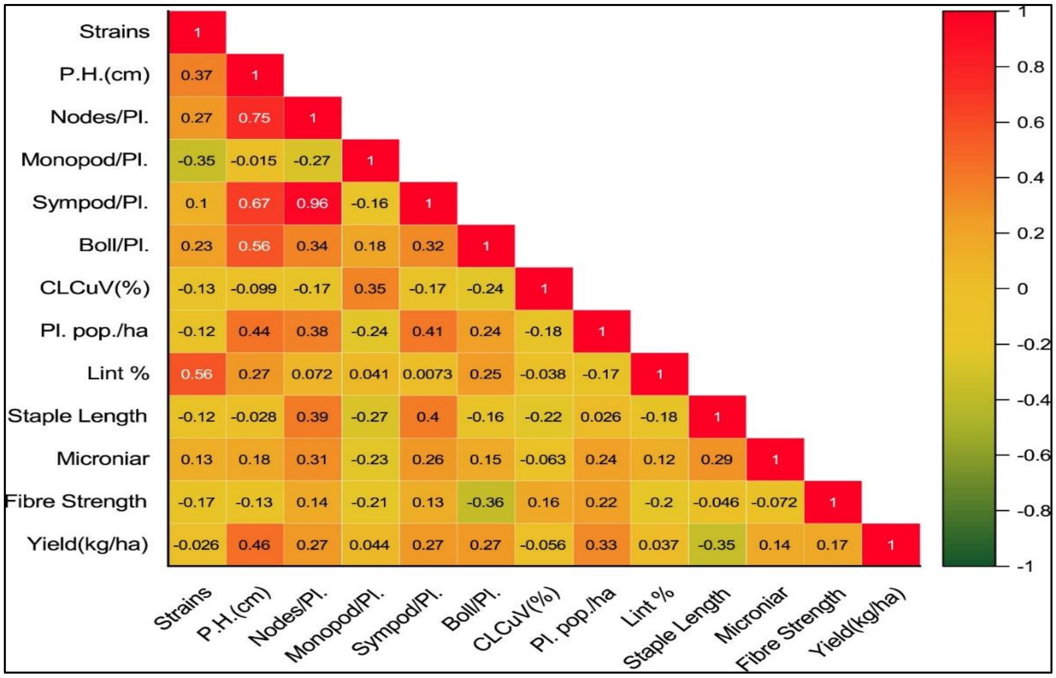

Figure 2: *Correlation coefficient analysis between key plant traits in cotton genotypes under heat stress conditions*

Agglomerative Hierarchical Clustering (AHC)/Cluster Analysis

Agglomerative Hierarchical Clustering (AHC), or cluster analysis, was executed to classify cotton genotypes into different groups based on their performance under heat stress conditions. The cluster analysis categorized cotton genotypes into three main classes/clusters (Cluster-I, II and III) (Table 3 & Figure 3). Cluster-1 comprises eight cotton genotypes, i.e., BH-333, BH-284, BH-350, BH-301, BH-232, BH-320, BH-184 and BH-348, all of them were among the lowest producers under heat stress conditions. The key factor for a lower yield in this cluster was lower plant height, a smaller number of nodes per plant, less sympodial per plant, a reduced number of bolls per plant, a smaller number of plants per hectare and ultimately, the reduced seed cotton yield. Cluster-2 comprised five cotton genotypes: BH-297, BH-200, BH-287, CIM-600, and BH-306. These cotton genotypes were modestly productive and, therefore could somewhat handle heat stress. The highest values for monopods per plant, sympodial per plant and lint percentage characterize this cluster. The third and last cluster was the cotton genotypes with the highest heat tolerance and productivity. The cluster consisted of six cotton genotypes, i.e., BH-377, BH-341, BH-272, BH-247, BH-283 and BH-254. The highest values in cluster-3 were demonstrated by plant height (109.0), nodes per plant (22.6), bolls per plant (23.2), CLCuV incidence percentage (9.7%), plant population per hectare (29930.0), fiber strength (99.3) and seed cotton yield (1369.5) (Table 3).

Table 3: Cluster means for morpho-agronomical and fiber-linked parameters in cotton genotypes under heat stress conditions

Cluster/Plant Traits	Cluster-1	Cluster-2	Cluster-3
Plant height (PH)	94.5	107.1	109.0
Nodes per plant (NP)	21.0	22.2	22.6
Monopods per plant (MP)	1.3	1.6	1.1
Sympodial per plant (SP)	16.2	17.9	17.7
Bolls per plant (BP)	19.8	22.4	23.2
CLCuV incidence percentage (CLCuV)	9.6	9.4	9.7
Plant population per hectare (PP)	25497.3	28201.7	29929.9
Lint percentage (Lint)	38.5	38.6	37.5
Staple length (SL)	28.4	28.2	28.4
Micronaire value (FF)	4.4	4.3	4.7
Fiber strength (FS)	97.8	95.7	99.3
Seed cotton yield (SCY)	1229.0	1263.0	1369.5

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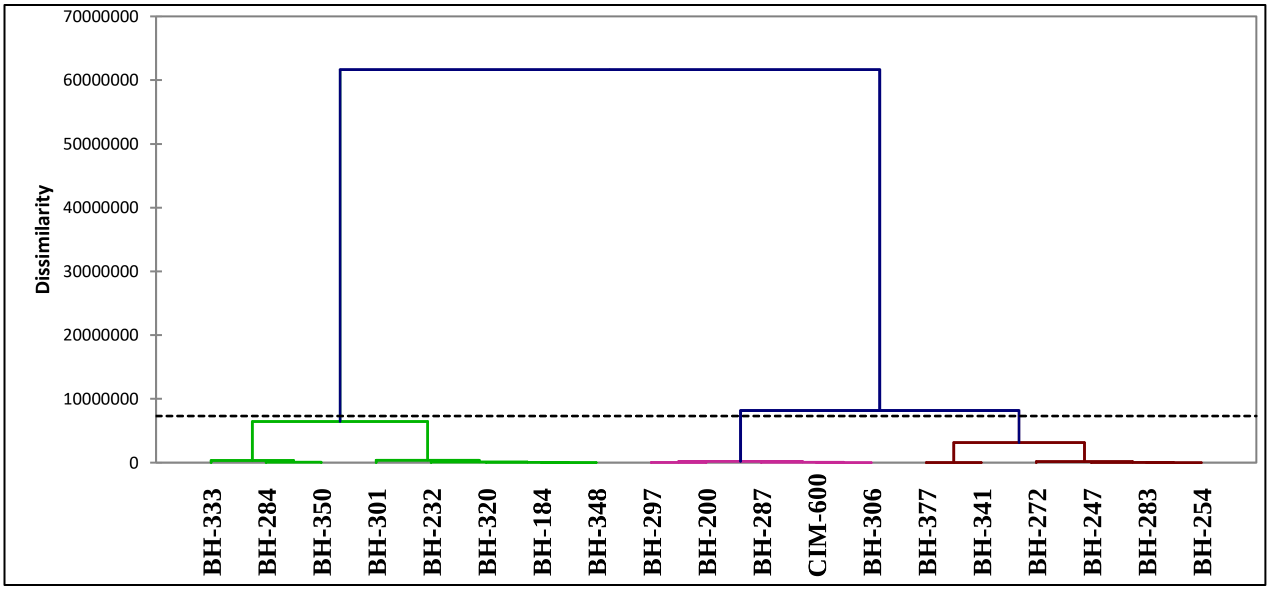

Figure 3: Dendrogram of cotton genotypes based on agglomerative hierarchical clustering

Principal Component Analysis (PCA)

Principal component analysis was done to characterize cotton genotypes based on genetic diversity under heat stress. The PCA extracted twelve principal components (PCs) for the genetic diversity present in the cotton genotypes based on their key morphological and fiber quality traits (Table 4). Among these 12 PCs, only four PCs had an eigenvalue greater than 1, having variations up to 69.35% of the overall data under heat stress conditions (Table 4 & Figure 4).

The factor loading/ correlation between significant PCs and plant traits are represented in Table 5. The PC-1 exhibited 3.6 eigenvalue score and a 30.1% contribution to the total variability in the data. The maximum contribution in genetic diversity was observed in plant height (0.8364), nodes per plant (0.9147), sympodial branches per plant (0.8795), plant populations per hectare (0.5966) and micronaire value (0.4309) (Table 5). In the PC-2, having 1.99 eigenvalues and 16.66% contribution to total variability in the data, monopodial branches per plant (-0.6103), bolls per plant (-0.5919) and staple length (0.6893). Similarly, PC-3 and PC-4 accounted for 13.08% and 9.5% variability in the data, respectively. Fiber strength (0.7404) and seed cotton yield (0.5245) were the main contributing traits in PC-3, while CLCuV incidence percentage (0.6739) was the major contributor to variability in PC-4 (Table 5).

Table 4: Eigenvalues, variability percentage and Cumulative variability percentage in nineteen cotton genotypes through Principal Component Analysis

	Eigenvalue	**Variability (%)**	**Cumulative %**
Principal Component-1 (PC-1)	3.6128	30.1066	30.1066
Principal Component-2 (PC-2)	1.9989	16.6577	46.7643
Principal Component-3 (PC-3)	1.5705	13.0877	59.8520
Principal Component-4 (PC-4)	1.1403	9.5029	69.3549
Principal Component-5 (PC-5)	0.9829	8.1904	77.5453
Principal Component-6 (PC-6)	0.8668	7.2233	84.7686
Principal Component-7 (PC-7)	0.5818	4.8483	89.6168
Principal Component-8 (PC-8)	0.4730	3.9419	93.5588
Principal Component-9 (PC-9)	0.3890	3.2418	96.8006
Principal Component-10 (PC-10)	0.2264	1.8869	98.6875
Principal Component-11 (PC-11)	0.1470	1.2248	99.9123
Principal Component-12 (PC-12)	0.0105	0.0877	100.0000

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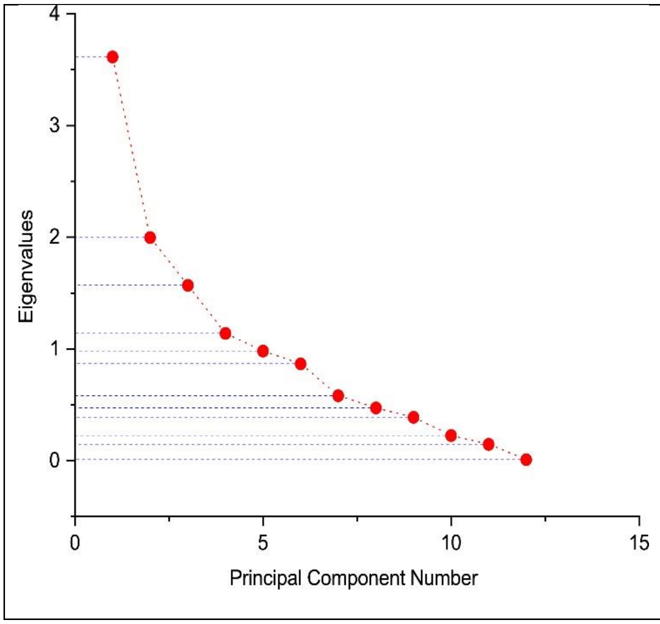

Figure 4: Scree plot of Principal Component Analysis in cotton genotypes

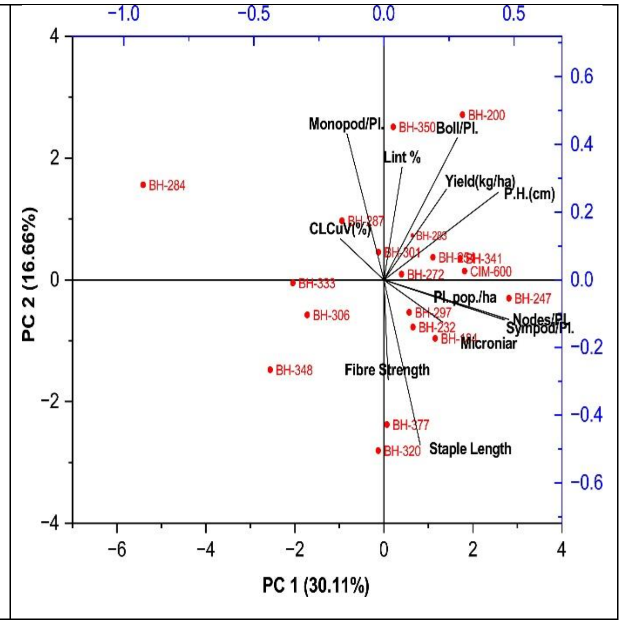

Figure 5: *PC1/PC2 Cumulative Biplot between plant traits and cotton genotypes under heat stress*

The principal component analysis-based biplots were plotted to identify the relationship between cotton genotypes and key plant traits under heat stress conditions (Figure 5). The PC-1/PC-2 biplot showed that the seed cotton yield was strongly linked to the number of bolls per plant, the height of the plant, the percentage of lint, the number of monopodial branches per plant, and the number of plants. In the same way, the most distinguishing characteristics were the number of monopodial branches per plant, the number of bolls per plant, the height of the plant, and the length of the staples. On the other hand, BH-200, BH-284, BH-348, BH-377, and BH-BH320 were some of the most distinctive cotton genotypes, while BH-272, BH-301, and BH-254 were some of the most sustainable cotton genotypes (Figure 5).

Table 5: Factor Loading/ Correlation between Plant traits and Principal Components in cotton genotypes under heat stress

	F1	**F2**	**F3**	**F4**
Plant height (PH)	0.8364	-0.3650	0.0340	0.1203
Nodes per plant (NP)	0.9147	0.1656	-0.0014	0.2778
Monopods per plant (MP)	-0.2699	-0.6103	0.0551	0.4843
Sympodial per plant (SP)	0.8795	0.1667	0.0210	0.3227
Bolls per plant (BP)	0.5367	-0.5919	-0.2446	-0.1184
CLCuV incidence percentage (CLCuV)	-0.3189	-0.1714	0.3946	0.6739
Plant population per hectare (PP)	0.5966	0.1143	0.3791	-0.2994
Lint percentage (Lint)	0.1335	-0.4708	-0.3541	-0.0271
Staple length (SL)	0.2659	0.6893	-0.4581	0.2994
Micronaire value (FF)	0.4309	0.1775	-0.2192	-0.0396
Fiber strength (FS)	0.0322	0.4169	0.7404	0.0372
Seed cotton yield (SCY)	0.4575	-0.3783	0.5245	-0.2425

Discussion

High temperatures can have significant impacts on cotton production. Cotton is a heat-sensitive crop; prolonged exposure to high temperatures can lead to reduced yields and quality. Heat stress could severely reduce photosynthesis, decrease boll bearing, increase flower, fruit and boll abscission, stunted growth, reduce fiber quality and increase insect-pest attack (Zong and Wang, 2017). One of the first steps in every new breeding programme is to estimate and study the genetic differences in the cultivated germplasm. Every new breeding

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programme depends on how much variation there is in the germplasm that is already there. It gives a chance to choose and use the best set of genes in a mixed population to improve a crop for a certain trait under various biotic and abiotic stresses, such as CLCuV, heat, and drought. Since cotton yield is a complicated, polygenic trait, direct selection can't improve it. So, it is very important to choose hybridizing parents from different germplasm sources based on the traits they share. Therefore, one of the most important things to improve crops is to look at the available germplasm for differences in key morphological and fiber quality attributes (Aslam *et al*., 2022). The present study was designed to evaluate existing cotton genotypes for the variations among their key plant traits under heat stress conditions.

The results obtained through ANOVA revealed the presence of significant variations for the studied key plant traits in cotton genotypes under heat stress conditions. The variations were highly significant for all the traits except the number of nodes per plant and monopods per plant, which were significant only. Similar observations were reported by several researchers who revealed the incidence of significant genetic variations in cotton genotypes under heat stress conditions Bhatti *et al.* (2020a), Bhatti *et al.* (2020b) and Yousaf *et al*. (2023). The correlation coefficient analysis was used to establish the relationship between studied plant traits in cotton genotypes under heat stress conditions. Plant scientist is widely using this statistical to conclude based on associations between plant traits in several crop species under different climatic conditions (Mumtaz *et al*., 2018; Yousaf *et al.* 2021a; Yousaf *et al.*, 2021b; Riaz *et al;* 2021 and Rahman *et al.*, 2022). The results revealed the presence of a positive correlation of seed cotton yield with plant height, plant population per hectare, bolls per plant, sympodial branches per plant and nodes per plant. This might be because plants with greater height will have more fruiting branches (sympodial branches), which will bear more fruit (bolls), ultimately increasing the seed cotton yield of the plant. Similar observations were reported by Jiang *et al*. (2015), Munir *et al*. (2017) and Yang *et al*. (2020) who revealed a positive correlation between plant height, bolls per plant and sympodial branches per plant with seed cotton yield suggesting that taller plants with more branches and bolls may be more resilient to heat stress and produce higher yields.

On the other hand, a negative correlation of seed cotton yield was found with fiber length. This might be since heat stress denatures the cellulose molecules making the cotton staple. Maqbool *et al*. (2019) and Waghmare *et al*. (2020) reported that high temperatures can disrupt the metabolic processes involved in cotton fiber development, resulting in a reduction in staple length. Heat stress can cause changes in the balance of hormones that regulate fiber elongation and secondary cell wall thickening, leading to premature termination of fiber elongation and shorter fibers. Heat stress can also affect the rate of cell division and expansion, resulting in a decrease in overall plant growth and productivity. Therefore, these traits must be considered while planning a breeding program to develop cotton genotypes for heat stress tolerance.

Characterizing germplasm under given circumstances is one of the key steps in developing cotton genotypes with specific objectives. Characterizing crop species through multivariate analysis-based approaches is one of the most frequently used statistical approaches in plant sciences (Yousaf *et al.*, 2017; Saeed *et al*., 2018; Yousaf *et al.* 2018; Yousaf *et al*., 2022; Ghani *et al*., 2023). The current experimental research used PCA and AHC to characterize and classify cotton genotypes under heat stress conditions. The PCA extracted 12 PCs, of which only four contributed 69.35% to the total variability in the data. The maximum variability in these PCs was contributed by plant height, nodes per plant, sympodial branches per plant, plant population per hectare and micronaire value. Therefore, transgressive segregants for these traits must be considered and selected to create more variability. The PC-1/PC-2 biplot unveiled and confirmed the results of the correlation coefficient analysis. Similarly, among cotton genotypes, BH-200, BH-350, BH-284, BH-247, BH-348, BH-377 and BH-320 were the major contributors to genetic variability in the data. Similar results were also reported by Khan *et al.* (2020) and Zafar *et al.* (2021) who showed the usage of multivariate analysis like PCA and AHC for the characterization and assessment of genetic variability in cotton genotypes under heat stress conditions.

Conclusion and Recommendations

Assessment through multivariate analytic techniques such as principal component analysis and cluster analysis gives an in-depth understanding of races with heterogeneous genetic composition. This might significantly aid breeders in selecting genotypes that align with their breeding goals. The current study revealed that an efficient parental selection for a breeding program must consider several correlated traits, i.e., *plant height, plant population per hectare, sympodial branches per plant, number of bolls per plant, nodes per plant and staple length.* Furthermore, six cotton genotypes, i.e., BH-200, BH-350, BH-284, BH-247, BH-348, BH-377 and BH-320 should be included in any breeding program to develop heat-tolerant cotton genotypes to broaden the genetic base of germplasm. Furthermore, after systematic, large-scale, multilocation testing, BH-200 and BH-247 could be recommended for heat-prone areas.

Conflict of interest
The authors declared the absence of a conflict of interest.

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