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Original Research Article

GENETIC CHARACTERIZATION AND PERFORMANCE EVALUATION OF ELITE COTTON STRAINS FOR MORPHOLOGICAL AND PHYSIO-CHEMICAL TRAITS UNDER HEAT STRESS CONDITIONS

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Abstract: *One of the key steps in developing heat-tolerant, climate-smart cotton genotypes is evaluating and screening available cultivated germplasm. The current study was designed to evaluate the key cotton strains developed for release in heat-prone areas of Punjab and Pakistan. Thirteen cotton strains, along with check variety BH-184, were sown under randomized complete block design (RCBD) in triplicates. The plant-to-plant and row-to-row distances were maintained at 30 cm and 70 cm, respectively. The results revealed the presence of highly significant variations among cotton strains for studied plant traits in 13 cotton strains. Correlation analysis unveiled the presence of highly significant and positive correlation of seed cotton yield with net photosynthetic rate (r = 0.982**), total chlorophyll contents (r = 0.976**), superoxide dismutase (r = 0.966**), bolls per plant (r = 0.786**), nodes per plant (r = 0.683**), plant height (r = 0.653**) and sympodia per plant (r = 0.623**) while strong negative correlation with superoxide dismutase (r = -0.952**). Cluster, principal component and biplot analysis classify cotton strains into groups based on their performance under heat stress conditions. These analyses verified the results obtained through correlations and further revealed that net photosynthetic rate, total chlorophyll contents, superoxide dismutase, plant height and sympodia per plant and superoxide dismutase were the most divergent traits and must be considered in developing a scheme to develop heat-tolerant cotton genotypes.*

Keywords: Correlation, Cluster Analysis, Photosynthesis, Antioxidants, CLCuV, Whitefly

Introduction

Cotton, scientifically known as *Gossypium*, is a versatile and indispensable crop that has played a pivotal role in shaping human civilization for centuries. Its significance lies not only in its economic value but also in its rich biological characteristics, historical importance, and global production impact. Cotton is a member of the Malvaceae family, a group of flowering plants known for their showy flowers and often fibrous fruits (Bayer and Kubitzki, 2003). Within the genus Gossypium, there are around 50 species, but only four are cultivated for their fibers: *Gossypium hirsutum* (Upland cotton), *Gossypium barbadense* (Pima cotton), *Gossypium arboreum*, and *Gossypium herbaceum* (Wendel and Grover, 2015). These species vary in their characteristics, such as fiber length, strength, and fineness, making them suitable for different applications. These fibers' length, fineness, and strength determine their quality and utility in various industries, particularly textiles.

The history of cotton traces back thousands of years, with evidence of its cultivation and use dating back to ancient civilizations such as those in the Indus Valley, Mesopotamia, and Egypt (Brite and Marston, 2013). Cotton played a central role in the economies and cultures of these early societies, serving as a symbol of wealth, prestige, and social status. The introduction of cotton to different parts of the world through trade routes facilitated cultural exchange

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and economic growth. Today, cotton is cultivated on a large scale in numerous countries across the globe, with major producers including China, India, the United States, and Pakistan. The production process involves several stages, from seed selection and planting to harvesting and ginning. Modern cotton cultivation often involves advanced agricultural practices, including mechanized planting and harvesting, irrigation systems, and applying fertilizers and pesticides. However, sustainable cotton production practices are gaining traction, emphasizing the use of organic farming methods, water conservation, and environmentally friendly pest management techniques to minimize the ecological footprint of cotton cultivation.

In Pakistan, cotton is mainly used in the textile and cooking oil industry. During 2022-23, the cotton crop was badly damaged in Pakistan due to the effects of climate change. The first damage was done by the raised temperature (7-10 ^{0}C) from the last few years from March till May, combined with the limited water supply and heatwaves, which affected cotton germination and initial crop growth. In 2022-23, the cotton crop contributed 0.3 per cent of GDP and 1.4 per cent in value added in agriculture. The cotton crop was sown on an area of 2.144 million hectares compared to 1.937 million hectares (ESP, 2022-23). However, severe floods in Sindh and Balochistan swept the whole crops, resulting in a severe yield reduction (4.910 million bales) compared to last year's production (8.329 million bales). Moreover, in the Punjab Province, Rajanpur, DG Khan and Taunsa were also badly affected by the floods and the insect pest infestation, especially pink bollworms, Whiteflies and Thrips (ESP, 2022-23).

Heat stress, coupled with drought stress, is one of the most important environmental factors responsible for lower crop yields worldwide and in Pakistan. Heat stress is responsible for poor crop germination, restrained initial crop growth, lower biomass production, lower boll formation and retention and ultimately, lower seed cotton yield. The optimum temperature for cotton crop growth and development is between 26 °C to 35 °C (Lokhande & Reddy, 2014). Compared to the other parts of the cotton crop, flowers and squares are the most susceptible parts under heat stress conditions (Salman *et al.,* 2019). Elevated temperatures may result in the loss of flowers and squares, decreased efficiency and duration of pollination, reduced boll weight, and, ultimately, reduced seed cotton yield (Xu et al., 2020). Likewise, the normal temperature at which pollen begins to grow is 28 degrees Celsius. Temperatures higher than this might hinder the growth of pollen, the elongation of pollen tubes, photosynthetic activity, the movement of gases via stomata, the shedding of cotton bolls, and eventually the amount of cotton produced (Salman et al., 2019). Reports indicated that each 1-degree Celsius increase above the optimal temperature in the field results in a reduction of 110 kilograms per hectare in production (Singh *et al.,* 2007).

Multiple factors may contribute to the progressive decline in cotton production over time, such as climate change, the emergence of various crop diseases, the use of inadequately inspected, unauthorized, and low-quality seeds, increased input costs and their availability, and the degradation of land resulting from intensive farming practices. The limited genetic diversity of recently developed cotton cultivars is responsible for many of these issues (Munir et al., 2020). Hence, the initial stage in the development of heat-tolerant cotton cultivars involves assessing the current cultivated germplasm under stressful conditions and choosing a range of parents for the hybridization program. To address this issue, various statistical methodologies can be employed. However, multivariate analysis offers the most optimal strategy for genotype selection. The evaluation of crop genotypes worldwide, under both stressed and non-stressed situations, is widely regarded as one of the most effective approaches (Munir et al., 2020, Yousaf et al., 2021, Rahman et al., 2022). Hence, multivariate model-based methods such as Principal Component Analysis and Cluster Analysis were utilized to evaluate cotton genotypes for their resistance to high temperatures.

Methodology

The current experimental study was carried out at the research farm of Cotton Research Station, Bahawalpur (29°23'10.4"N 71°39'08.8"E) during the crop year 2023. The study was consisted of thirteen cotton strains including one standard or check variety BH-184 and twelve elite strains i.e., BWS-1, BWS-2, BWS-3, BWS-4, BWS-5, BWS-6, BWS-7, BWS-8, BWS-9, BWS-10, BWS-11 and BWS-12. These strains were sown under randomized complete block design (RCBD) in three replications. The sowing of these strains was done in the third week of May, 2023 with the handheld seed drills @ 2 seeds per hill, which were thinned to 1 at initial seedling stage to keep one, healthy seedling to ensure optimum plant population. These seedlings experienced the elevated temperature stress (40°C-45°C) during his whole crop period, where occasionally temperature may reach up to 47°C - 48°C during the particular time of day. These strains were planted in four-rowed, 10 meters long rows with net plot size of 15cm^2. Fertilizers, pesticides, hoeing and other cultural/management practices were carried out to ensure plant health.

The water used for the irrigation purposes was get examined for its suitability and key traits and the values are given as under.

Table 1: Properties of Irrigation water used

Sr. #	Parameter	Value
1	Total Soluble Salts (TSS)	1170 ppm
2	Sodium Adsorption Ratio (SAR)	6.9
3	Residual Sodium Bicarbonate (RSB)	1.43
4	Chloride	4.3 meL^s

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Similarly, the soil was also tested for its properties so that optimum fertigation could be given to the soil to obtained good results.

Table 2s: Properties of Soil on which experiment was carried out

Sr. #	Parameter	Value
1	Soil Texture	Sandy Loam
2	pH	7.8
3	EC	4.7 dSm^s
4	Organic Matter	0.76
5	Available P	7.3 ppm
6	Available K	82 ppm
7	Zinc	1.3ppm

The data was recorded from ten randomly selected, guarded plants per replication per strain The data for several plant traits including plant populations (P. Pop), plant height (PH), nodes per plant (NP), monopods per plant (MP), sympods per plant (SP), bolls per plant (BP), CLCuV incidence percentage (CLCuV), whitefly attack (WF), net photosynthetic rate (Pn), total chlorophyll contents (Total Chl), hydrogen peroxide (H_2O_2), Superoxide dismutase (SOD) and seed cotton yield (SCY). Moreover, the CLCuV incidence percentage (CLCuV) was calculated as below according to the formula used by Aslam et al. (2022);

$$\text{CLCuV incidence percentage} = \frac{\text{Number of Infected Plant Units}}{\text{Total Number of Units Assesed (Healthy + Infected)}} \times 100$$

The whitefly attack was estimated by counting the adult whiteflies per 10 leaves per plant in July and August.

The obtained data were statistically subjected to analysis of variance and correlation coefficient analysis (Steel et al.,1997). Moreover, cluster and principal component analysis were performed to characterize cotton strains under heat stress conditions (Sneath & Sokal, 1973). To execute these analysis, Statistix 8.1 and XLSTAT 22.0. Statistical tool was used.

Results and Discussion

Analysis of Variance (ANOVA)

The analyzed data for analysis of variance showed highly significant variations among cotton strains for studied plant traits, including plant height (PH), nods per plant (NP), monopodia per plant (MP), sympodia per plant (SP), bolls per plant (BP), ClCuV incidence percentage (CLCuV %), whitefly attack percentage (WF), net photosynthetic rate (Pn), total chlorophyll contents (Total Chl), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD) and seed cotton yield (SCY) except plant population per hectares (P. Pop) for which variations were non-significant (Table 3). Moreover, variations among cotton strains for these parameters were non-significant in replications except for plant height, which showed highly significant diversity among cotton strains with respect to plant height. Similar findings were also reported by Aslam et al., 2022; Hussain et al., 2023a; Hussain et al., 2023b and Yousaf et al., 2023 who revealed significant variations among cotton genotypes for similar/same traits under heat stress conditions.

Table 3: Mean Square (MS) of selected traits of cotton strains under heat stress conditions

Source of Variation	Replications	Strains	Error
Traits/Df	2	12	24
Plant population per hectare (P.Pop)	745874^{NS}	$1.56E+07^{NS}$	1291760
Plant Height (PH)	42.64^{**}	1426.1^{**}	7.50
Nodes per Plant (NP)	2.85^{NS}	69.96^{**}	4.96
Monopodia per plant (MP)	0.077^{NS}	0.974^{**}	0.243
Sympodia per plant (SP)	1.10^{NS}	62.25^{**}	6.24
Bolls per plant (BP)	7.69^{NS}	51.89^{**}	4.69
CLCuV incidence (%) (CLCuV%)	4.99^{NS}	50.56^{**}	6.28
Whitefly Attack (WF)	0.25^{NS}	3.79^{**}	0.116
Net photosynthetic rate (Pn)	0.049^{NS}	48.82^{**}	2.43
Total Chlorophyll Contents (TCC)	0.11^{NS}	9.89^{**}	0.66
Hydrogen Peroxide (H_2O_2)	0.22^{NS}	4.32^{**}	0.26
Superoxide dismutase (SOD)	2.85^{NS}	891.53^{**}	67.79

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Seed Cotton Yield (SCY)	1481.9^{NS}	85686.1^{**}	3938.8

** = Highly significant at 0.01; * = Significant at 0.01; NS: Non-significant

Correlation Coefficient Analysis (CCA)

The correlation coefficient analysis was executed to understand the magnitude and direction of association between studied plant traits in upland cotton strains under heat stress conditions (Table 4). The results revealed the presence of highly significant and positive corelation of seed cotton yield with net photosynthetic rate (r = 0.982**), total chlorophyll contents (r = 0.976**), superoxide dismutase (r = 0.966**), bolls per plant (r = 0.786**), nodes per plant (r = 0.683**), plant height (r = 0.653**) and sympodia per plant (r = 0.623**) while strong negative correlation with superoxide dismutase (r = -0.952**). The highest positive correlation was observed between net photosynthetic rate and total chlorophyll contents (r = 0.997**), while the highest negative correlation was seen between net photosynthetic rate and superoxide dismutase (r = -0.983**) (Table 4). Similar findings were reported by Majeed et al., 2024, Salman et al., 2019, Thompson et al., 2022 and Lopez et al., 2003 who showed a significantly stronger association of morphological, physiological and biochemical traits with seed cotton yield and its quality under heat stress conditions. Therefore, these characters must be considered while selecting the parental material and subsequent generations to develop heat-tolerant, climate-smart cotton genotypes.

Table 4: Correlation between different plant parameters in upland cotton strains under heat stress conditions.

Variables	P. Pop	PH	NP	MP	SP	BP	CLCuV	WF	Pn	Total Chl	H_2O_2	SOD
PH	-0.289											
NP	-0.307	**0.878**										
MP	-0.417	0.123	0.012									
SP	-0.311	**0.861**	**0.966**	-0.016								
BP	-0.199	**0.641**	**0.693**	0.035	**0.665**							
CLCuV	-0.442	**0.653**	0.470	0.365	0.373	0.506						
WF	-0.079	0.293	-0.032	0.356	-0.047	0.275	0.541					
Pn	0.005	**0.642**	**0.696**	-0.174	**0.631**	**0.843**	0.363	0.236				
Total Chl	-0.042	**0.652**	**0.706**	-0.125	**0.639**	**0.835**	0.377	0.242	**0.997**			
H_2O_2	0.091	**-0.622**	**-0.702**	0.158	**-0.623**	**-0.812**	-0.387	-0.191	**-0.983**	**-0.983**		
SOD	-0.052	**0.651**	**0.739**	-0.211	**0.680**	**0.815**	0.357	0.216	**0.985**	**0.984**	**-0.979**	
SCY	0.105	**0.653**	**0.683**	-0.219	**0.623**	**0.786**	0.379	0.227	**0.982**	**0.976**	**-0.952**	**0.966**

Cluster Analysis (CA)

Cluster analysis is a multivariate analysis used to classify, categorize, or characterize crop varieties/genotypes based on their performance under specified circumstances. In the current study, cluster analysis was used to categorize upland cotton strains based on morpho-physiological and biochemical traits under heat stress conditions. The cluster analysis classified thirteen cotton strains into three groups: Class I, Class II, and Class III. Class-I was the biggest group comprised of nine cotton strains, i.e., BWS-1, BWS-3, BWS-4, BWS-5, BWS-6, BWS-8, BWS-9, BWS-10 and BWS-12 (Table 5 & Figure 1). This group was regarded as the intermediately productive group of cotton strains with an average seed cotton yield of 829.2 kg per ha. The comparatively higher yield of this group was due to the higher mean values of positively associated traits, i.e., plant height (99.37), nodes per plant (27.70), monopodia per plant (0.67), sympodia per plant (22.11), bolls per plant (19.22), net photosynthetic rate (23.27), total chlorophyll contents (7.27) and superoxide dismutase (161.9) while the lowest value for hydrogen peroxide (6.67) (Table 5). Class II was the second biggest group comprised of three cotton strains, i.e., BWS-2 and BWS-11, including check variety BH-184. This group was the group of genotypes with the highest heat tolerance and productivity, with an average yield of 851.5 kg per ha. The third and last group, Class-III, comprised only one cotton strain, BWS-7, characterized as the most heat-sensitive and least productive cotton strain. Its least production lies in its yield-associated traits, which showed minimum values under heat stress (Table 5). Several researchers used cluster analysis in categorising cotton genotypes under different circumstances and find it very useful in classifying the genotypes, based on their performance (Hussain et al., 2023b, Zafar et al., 2023, Yousaf et al., 2023, Aslam et al., 2022; Zafar et al., 2022 and Manan et al., 2022).

Table 5: Class means of three clusters through agglomerative hierarchical clustering in cotton genotypes

Plant Traits/Classes	Class-1	Class-2	Class-3
Plant population per hectare (P.Pop)	32149.4	31812.9	363485.6
Plant Height (PH)	99.37	79.56	73.33

Nodes per Plant (NP)	27.70	23.44	21.33
Monopodia per plant (MP)	0.67	0.00	0.00
Sympodia per plant (SP)	22.11	17.67	16.33
Bolls per plant (BP)	19.22	16.00	14.00
CLCuV incidence (%) (CLCuV%)	8.04	5.00	1.62
Whitefly Attack (WF)	5.23	5.57	4.20
Net photosynthetic rate (Pn)	23.27	23.09	19.03
Total Chlorophyll Contents (TCC)	7.27	7.13	4.97
Hydrogen Peroxide (H_2O_2)	6.67	6.88	8.10
Superoxide dismutase (SOD)	161.9	161.6	140.7
Seed Cotton Yield (SCY)	829.2	851.5	688.9

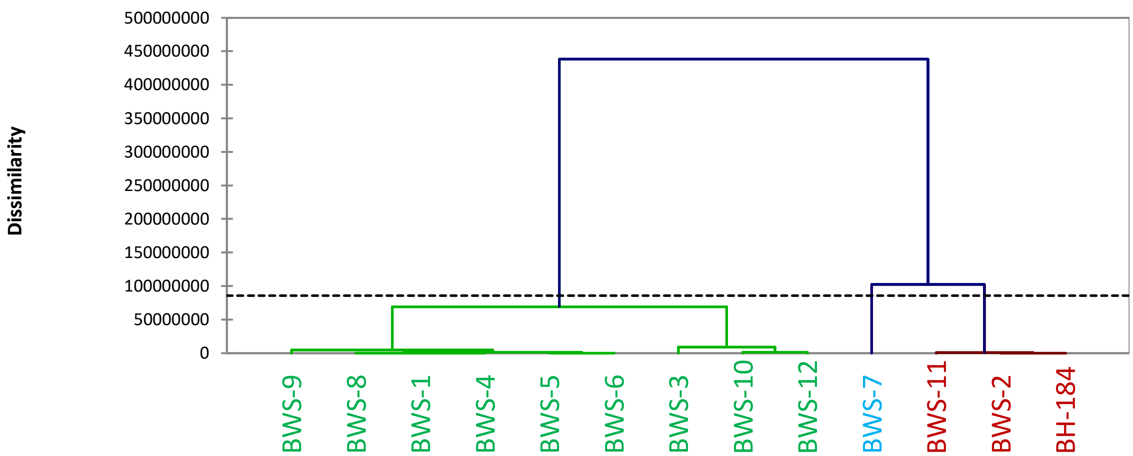
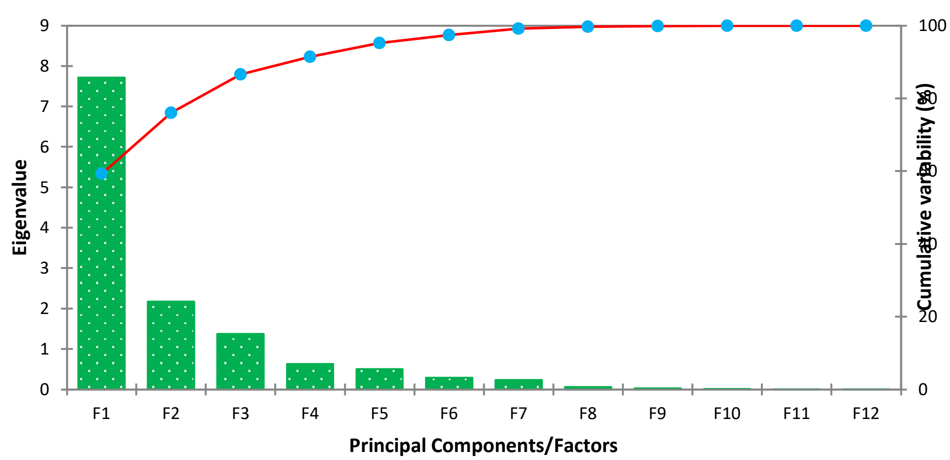


Figure 1: Dendrogram of cotton genotypes based on agglomerative hierarchical clustering

Principal Component Analysis

The principal component analysis (PCA) is another multivariate analysis that is used in the characterization of treatments/genotypes. In the current study, PCA was used to classify the cotton strains under heat stress conditions. The results obtained through PCA revealed that three principal components (PCs)/factors (Fs) out of twelve showed eigen more than 1 and contributed 86.56 % to the variability in the data, cumulatively. The PC1/F1 showed the highest contribution (59.3%) to the total variability in the data. The second PC/F2 gave an eigenvalue 2.174 and contributed 16.72 % variability in the total data (Table 6 & Figure 2).



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The PCA also revealed that correlations between variables and factors in Table 6. The PCA results showed that in PC1, plant height, nods per plant, sympodia per plant, bolls per plant, net photosynthetic rate, total chlorophyll contents, hydrogen peroxide, superoxide dismutase, and seed cotton yield showed maximum variability and correlation. Similarly, in PC2/F2, plant population per hectare, monopodia per plant, and ClCuV incidence percentage were the key traits in contributing to variation and diversity. Lastly, in PC3/F3, only whitefly attack percentage showed a significant correlation and contributed to variation.

Table 6: Eigenvalues, variability %, and Cumulative variability % in cotton genotypes through Principal Component Analysis

Principal Components	Eigenvalue	Variability (%)	Cumulative %
F1	7.71	59.308	59.308
F2	2.174	16.72	76.028
F3	1.37	10.537	86.565
F4	0.627	4.821	91.387
F5	0.498	3.829	95.215
F6	0.287	2.209	97.425
F7	0.232	1.787	99.212
F8	0.06	0.465	99.676
F9	0.026	0.201	99.877
F10	0.012	0.093	99.97
F11	0.003	0.026	99.996
F12	0.001	0.004	100

Table 7: Factor Loading/ Correlation between Plant traits and Principal Components in cotton genotypes under heat stresss

Correlations between variables and factors:

Plant Traits	**F1**	**F2**	**F3**
Plant population per hectare (P.Pop)	-0.185	-0.705	0.360
Plant Height (PH)	0.825	0.317	-0.197
Nodes per Plant (NP)	0.857	0.123	-0.449
Monopodia per plant (MP)	-0.051	0.803	0.134
Sympodia per plant (SP)	0.804	0.112	-0.508
Boll per plant (BP)	0.881	0.054	0.081
CLCuV incidence (%) (CLCuV%)	0.544	0.653	0.192
Whitefly Attack (WF)	0.262	0.461	0.761
Net photosynthetic rate (Pn)	0.950	-0.236	0.161
Total Chlorophyll Contents (TCC)	0.953	-0.194	0.150
Hydrogen Peroxide (H_2O_2)	-0.940	0.199	-0.112
Superoxide dismutase (SOD)	0.954	-0.228	0.091
Seed Cotton Yield (SCY)	0.932	-0.276	0.177

Similarly, PCA also unveiled the contribution of cotton strains under heat stress. In PC1/F1, five cotton strains i.e., BWS-4, BWS-7, BWS-8, BWS-11 and BWS-12 contributed 11.194%, 12.368%, 23.178%, 14.306% and 25.385% to the total variability (Table 7). In PC2/F2, four other cotton strains BWS-2, BWS-3, BWS-7, and BWS-10 contributed 14.268%, 16.841%, 16.242%, and 13.244% to the variability in the data. Lastly, three cotton strains BH-184, BWS-8, and BWS-12 contributed 11.896%, 47.193%, and 16.063% to the variations (Table 8). Several researchers used principal component and biplot analysis in categorising cotton genotypes under different circumstances and found it very useful in classifying the genotypes based on their performance (Zafar et al., 2023, Yousaf et al., 2023, Aslam et al., 2022; Zafar et al., 2022, Manan et al., 2022 and Zafar et al., 2021)

Table 8: Factor Loading/ Correlation between Plant traits and Principal Components in cotton genotypes under heat stress

Cotton Strains	**F1**	**F2**	**F3**
BWS-1	1.209	1.063	3.398
BWS-2	2.325	14.268	8.875

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BWS-3	0.301	16.841	0.647
BWS-4	11.194	9.658	10.044
BWS-5	2.616	6.905	0.556
BWS-6	3.287	3.263	0.332
BH-184	0.004	6.096	11.896
BWS-7	12.368	16.242	0.119
BWS-8	23.178	2.984	47.193
BWS-9	3.423	6.563	0.732
BWS-10	0.404	13.244	0.124
BWS-11	14.306	0.913	0.022
BWS-12	25.385	1.958	16.063

The PC1/PC2-based biplot analysis also confirms the results obtained through cluster analysis, correlation analysis and principal component analysis. The biplot showed that seed cotton yield has a significantly positive association with key yield-contributing traits, including net photosynthetic rate, superoxide dismutase, total chlorophyll contents, bolls per plant, sympodia per plant, nodes per plant and plant height as their corresponding lines lay near to the corresponding line of seed cotton yield while the highest negative association with hydrogen peroxide as its corresponding line was in completely opposite direction (Figure 3). With respect to cotton strains, BWS-8, BWS-5, BWS-2, BWS-1, and BH-184 were among the most productive and heat-tolerant cotton strains as they lay away from the base and are in line with the correspondence line of the seed cotton yield.

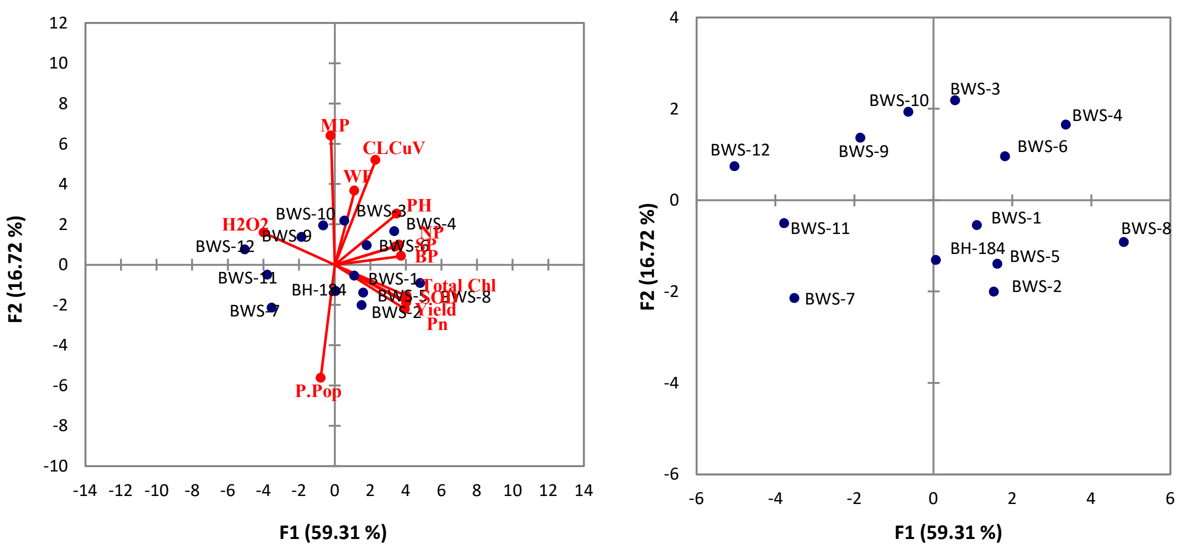

Figure 3: PC1/PC2 Cumulative Biplot between plant traits and cotton genotypes under heat stress

Conclusion

The genetic evaluation of the existing germplasm is preliminary step in the development of heat tolerant cotton genotypes. In current study, highly significant differences were observed among cotton strains for key yield associated traits under heat stress conditions. The correlation coefficient analysis unveiled the significantly positive correlation of seed cotton yield with net photosynthetic rate, total chlorophyll contents, superoxide dismutase, bolls per plant, nodes per plant, plant height and sympodia per plant while strong negative correlation with superoxide dismutase. Cluster analysis classify cotton strains into three groups, with Group-II being the group of most productive and heat tolerant cotton strains. The principal component and biplot analysis revealed that net photosynthetic rate, total chlorophyll contents, superoxide dismutase, plant height and sympodia per plant and superoxide dismutase were the most divergent traits and must be considered in framing a strategy to develop heat tolerant cotton genotypes.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department Concerned.
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Conflict of interest

The authors declared absence of conflict of interest.

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