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Original Research Article

GENETIC CHARACTERIZATION OF COTTON GENOTYPES BASED ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL, AND DISEASE-ASSOCIATED TRAITS THROUGH MULTIVARIATE APPROACHES

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Abstract: *The first step in creating new crop varieties is to evaluate current germplasm based on agro-morphological, physiological, biochemical, and molecular properties. The recent study compared ten cotton genotypes' key morpho-physiological and biochemical characteristics. Ten cotton genotypes, including BH-249, BH-617, BH-227, BH-248, BH-613, BH-244, BH-247, BH-606, BH-184, and BH-600, were arranged in triplicates under randomized complete block design (RCBD) with plant-to-plant and bed-to-bed distances of 30 cm and 75 cm, respectively. Data obtained from the mature, fully guarded plants were subjected to analysis of variance, and the results revealed the presence of significant variations in the studied plant traits. The correlation analysis revealed a significantly positive correlation of cotton yield with the plant height (r = 0.92**), transpiration rate (r = 0.79**), and ascorbic acid (r = 0.64**), while a significantly negative correlation with monopodial branches (r = -0.65**), virus effect plants (r = -0.59) and boll weight (r = -0.50). Similarly, seed cotton yield also showed a positive correlation with the number of bolls (r = 0.55) and peroxidase (r = 0.51), but these correlations were insignificant. Multivariate analysis approaches i.e., principal component, biplot, and cluster analysis, were used to classify and group cotton genotypes based on their performance. These analyses revealed that BH-247 and BH-606 were the most productive cotton genotypes. Therefore, these genotypes could be recommended for cultivation in core-cotton areas following extensive multilocation testing.*

Keywords: Net Photosynthetic Rate, Roxs, Peroxidase, Correlation, Fibre Quality, Proline, Ascorbic Acid

Introduction

Cotton holds profound importance as a versatile and indispensable global crop that intersects agriculture, industry, and commerce. Its significance is deeply rooted in its multifaceted utility, serving as a vital raw material for textiles, an essential source of income for millions of farmers, and a catalyst for economic growth in many regions (Najib et al., 2022). Cotton is the most significant industrial crop in natural fibre and oil production (Salimath et al., 2021). In 2020–21, it was grown on 31.42 million hectares and produced 111.48 million 480-pound bales, averaging 773 kg/ha globally (USDA, 2022). Cotton's remarkable fiber quality, recognized for its comfort, durability, and absorbency, has made it a staple in the textile industry, shaping fashion and clothing worldwide (Ravandi and Valizadeh, 2011). Furthermore, cotton cultivation has a substantial socio-economic impact, particularly in developing nations, where it provides livelihoods for numerous smallholder farmers and supports entire communities. The crop's economic value extends

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beyond its fibers, as cottonseed yields oil used in various food products and livestock feed, contributing to food security (Glin et al., 2014). Additionally, cotton has played a historic role in global trade, shaping relationships between nations and fostering cultural exchanges. Amid growing environmental concerns, efforts to develop sustainable cotton production practices underscore its importance as a model for responsible agriculture. Cotton's far-reaching influence, from fashion to livelihoods, trade to sustainability, highlights its pivotal role in the global fabric of society and commerce (Fletcher, 2013).

Cotton sustainability under changing climatic conditions is a critical concern, particularly in Pakistan, where the cotton industry holds immense socio-economic significance. Pakistan ranks among the world's major cotton-producing nations, with its economy heavily reliant on cotton cultivation and textile exports. However, the sector faces formidable challenges due to shifting climate patterns. Rising temperatures, erratic rainfall, and increased occurrences of extreme weather events pose substantial threats to cotton production. These changes can lead to reduced yields, decreased fiber quality, and heightened susceptibility to pests and diseases. Cotton cultivation provides livelihoods to a substantial segment of Pakistan's population, including farmers, ginners, spinners, and textile workers. Additionally, the cotton industry in Pakistan plays a vital role in attracting foreign investment and building industrial infrastructure. During 2021–2022, the cultivated area under cotton in Pakistan was 1.93 million hectares, and an average of 731 kilograms per hectare was produced, producing 8.329 million bales (ESP, 2021–2022).

Although Pakistan's per-hectare cotton production is very close to the global average, it lags far behind the major cotton-producing nations, such as Australia (2,217 kg^{-1}), China (19,765 kg ha^{-1}), Turkey (1804 kg ha^{-1}), Brazil (17,220 kg ha^{-1}), and the United States (957 kg ha^{-1}) (USDA, 2022). High input costs, inaccessibility of inputs, high disease and insect-pest infestation rates, drought stress, heat stress, lack of mechanized harvesting, and inaccessibility of quality seed are the primary causes of Pakistan's low cotton yield.

To create new crop varieties, existing crop germplasm must be evaluated since it offers a thorough grasp of the genetic diversity, traits, and potential present in existing crops. This knowledge is essential for spotting parameters that can be used in crop breeding to improve disease resistance, nutritional value, or other desired qualities. Statistical techniques, including ANOVA, correlation coefficient analysis, cluster analysis, and principal component analysis, are essential in this complex procedure. Therefore, the current study was created to assess both the potential candidate lines and the currently approved cotton kinds.

Methodology

Experimental Location and Site

The current investigation was conducted at Cotton Research Institute, Bahawalpur's research area during the 2022-23 crop year. The genotypes BH-249, BH-617, BH-227, BH-248, BH-613, BH-244, BH-247, BH-606, BH-184, and BH-600 were included in the study. The experiment was conducted with three replicates using a Randomized Complete Block Design (RCBD). With the assistance of a dibbler, two seeds were planted per hill. The crop was thinned to a single seedling at the seedling stage to ensure appropriate growth and development. Two 5-meter-long rows represented each genotype. The distance between plants and rows was maintained at 25 cm and 75 cm, respectively. Standard agronomic and plant protection practices were applied for each of the study genotypes.

Data Recording and Measurement

At maturity, several plants' morphological, physiological, and biochemical traits were measured including i.e., Plant Population (PP), Virus Attack (VP), Number of bolls (NB), Plant Height (PH), Plant Height (PH), Sympodial Branches (SB), Ginning out term (GOT%), Staple Length (SL), Boll Weight (BW), Net Photosynthetic Rate (Pn), Transpiration Rate (Tr), Proline (Pro), Hydrogen Peroxide (H2O2), Total Phenolic Contents (TPC), Ascorbic Acid (ASA), Flavonoids (Flv), Malondialdehyde (MDA), Peroxidase (POD), Catalase (CAT) and lint yield of cotton (Y). The physiological traits were recorded using Infrared Gas Analyzer (IRGA) CI-320 while biochemical traits were measured through photo spectrometry.

Statistical Analysis

To analyze the differences between cotton genotypes based on morphological, physiological, and biochemical features, the recorded data of measured traits were subjected to analysis of variance (ANOVA) (Steel et al., 1997). To assess the association between seed cotton yield and other relevant parameters, correlation coefficient analysis was also carried out as described by Steel et al., 1997. Principal component analysis (PCA) and cluster analysis (CA), two multivariate techniques, were also used to group cotton genotypes according to their performance under given conditions (Sneath & Sokal, 1973). Two statistical software, Statistix 8.1 and XLSTAT, were utilized to carry out the operations of statistical data analysis. Furthermore, the results were illustrated using Microsoft Excel and OriginPro.

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Results and Discussion

Analysis of Variance

The results of the analysis of variance (ANOVA) revealed the occurrence of highly significant differences ($p<0.01$) between cotton genotypes for the morphological, physiological, biochemical, and diseases related traits of the plants that were studied, including plant height (PH), net photosynthetic rate (Pn), hydrogen peroxide (H_2O_2), total phenolic contents (TPC), ascorbic acid (ASA), flavonoids (Flv), malondialdehyde (MDA), peroxidase (POD), catalase (CAT) and seed cotton yield per plot (Y). However, significant differences ($p<0.05$) among cotton genotypes were observed for the number of bolls (NB), monopodial branches (MB), sympodial branches (SB), ginning out term (GOT%), staple length (SL), boll weight (BW), transpiration rate (Tr) and proline (Pro). However, the changes for plant population and virus attack were non-significant at 1% and 5%, respectively. Similar findings were made by Bhatti et al. (2020a), Bhatti et al. (2020b), Aslam et al. (2022), Hussain et al. (2023), and Yousaf et al. (2023), who emphasized the importance of using a diversity of cotton germplasm to make the best selections and demonstrated the presence of significant variations in cotton genotypes for morpho-physiological and biochemical traits.

Table 1: Mean Square (MS) values of key cotton traits in ten cotton genotypes

SOV	Replication	Genotypes	Error
Degree of Freedom (df)	***2***	***9***	***18***
Plant Population (PP)	62.233	63.944^{NS}	114.233
Virus Attack (VP)	67.5	9.5^{NS}	10.6111
Number of bolls (NB)	1.9603	42.11^{*}	12.7596
Plant Height (PH)	2.977	503.2^{**}	113.11
Monopodial Branches (MB)	0.57733	0.348^{*}	0.10326
Sympodial Branches (SB)	2.169	9.433^{*}	2.86159
Ginning out term (GOT%)	1.2843	38.462^{*}	10.4991
Staple Length (SL)	25.327	32.272^{*}	10.3374
Boll Weight (BW)	0.11433	0.5809^{*}	0.26026
Net Photosynthetic Rate (Pn)	1.9363	65.36^{**}	17.8867
Transpiration Rate (Tr)	0.00042	0.0226^{*}	0.00935
Proline (Pro)	21.1578	15.3258^{*}	6.3778
Hydrogen Peroxide (H_2O_2)	0.30233	5.248^{**}	0.01715
Total Phenolic Contents (TPC)	0.482	549.9^{**}	0.122
Ascorbic Acid (ASA)	195.23	1996.9^{**}	14.01
Flavonoids (Flv)	1910	113019^{**}	73.78
Malondialdehyde (MDA)	9.94	4401.7^{**}	4.17
Peroxidase (POD)	43.3	26494.4^{**}	204.5
Catalase (CAT)	5.03	4465.9^{**}	122.59
Yield/plot	0.127	0.7757^{**}	0.15737

****: Significant at 1%, *: Significant at 5%, NS: Non-significant,**

Correlation Coefficient Analysis

Correlation coefficient analysis is one of the most popular statistical methods to evaluate the importance and exposure of the linear relationship between two quantitative variables. To illustrate the quantitative relationship between morpho-physiological and biochemical features in cotton genotypes, correlation coefficients were calculated in the current study (Figure 1). The results discovered the presence of a significantly positive correlation between cotton yield with the plant height ($r = 0.92^{**}$), transpiration rate ($r = 0.79^{**}$), and ascorbic acid ($r = 0.64^{**}$), while a significantly negative correlation with monopodial branches ($r = -0.65^{**}$), virus effect plants (r = -0.59) and boll weight (r = -0.50). Similarly, seed cotton yield also showed a positive correlation with the number of bolls (r = 0.55) and peroxidase (r = 0.51) but these correlations were not significant (Figure 1). Yousaf et al. (2023) also showed that seed cotton yield could be increased by making the selection based on highly correlated traits like net photosynthetic rate, transpiration rate, and nodes per plant. Similarly, traits like monopodial branches, virus-affected plants, and boll weight are negatively correlated because the more food utilized in the source, the less food reserve will be available to sink, consequently lowering the seed cotton yield (Hasanuzzaman et al., 2021).

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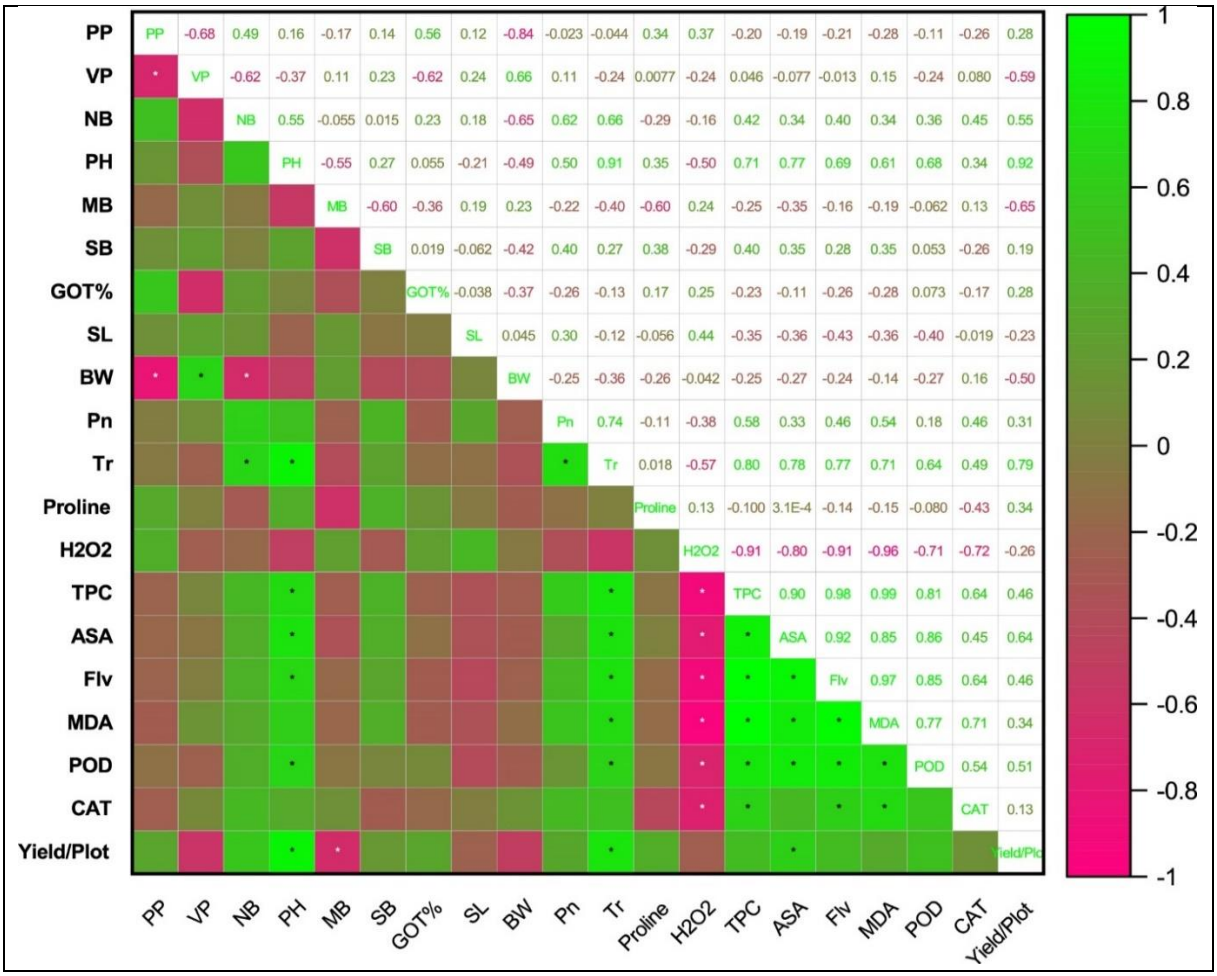

Figure 1: *Correlation coefficient analysis between key plant traits in cotton genotypes*

PP: Plant Population, **VA**: Virus attack (*%*), **NB:** No. of Bolls, **PH:** Plant Height (*cm*), **MB**: Monopodial Branches, **SB**: Sympodial Branches, **GOT**: Ginning out turn (*%*), **SL**: Staple length (*mm*), **BW**: Boll weight (*g*), **Pn**: Net Photosynthetic Rate (μ *mole* m^{-2} s^{-1}), **Tr**: Transpiration rate (*mmole* m^{-2} s^{-1}), **Pro**: Proline (*mg* g^{-1} *FW*), **H2O2**: Hydrogen peroxide (μ*mole* g^{-1}), **TPC**: Total phenolic contents (μ*mol* g^{-1} *FW*), **ASA:** Ascorbic acid (μ*g* g^{-1} *FW*), **Flv**: Flavonoids (μ*g* mL^{-1} *sample*), **MDA**: Malondialdehyde (μ*mol* g^{-1} *FW*), **POD**: Peroxidase (*Units* g^{-1} *FW*), **CAT**: Catalase (*Units* g^{-1} *FW*), **Y**: Yield/Plot (*Kg* $plot^{-1}$)

Cluster Analysis

Cluster analysis, a multivariate analysis, is frequently used in plant sciences to categorize, classify, or characterize genotypes based on how well they perform under specific conditions. Ten cotton genotypes were examined for cluster analysis in the current study. The cluster analysis organizes the 10 cotton genotypes into three groups or clusters (Figure 2).

Among the three clusters, Cluster-I, which included two genotypes only BH-249 and BH-613, is distinguished by the average performance (2.30 kg $plot^{-1}$) along with the highest values for sympodial branches (18.07), net photosynthetic rate (24.97 μ*mol* $m^{-2}s^{-1}$), transpiration rate (0.38 *mmol* $m^{-2}s^{-1}$), Total Phenolic Contents (82.13 μ*mol* g^{-1} *FW*), Ascorbic Acid (588 μ*g* g^{-1} *FW*), Flavonoids (988.33 μ*gmL*$^{-1}$ *sample*), Malondialdehyde (262.20 μ*mol* g^{-1} *FW*), Peroxidase (960.50 *Units*g^{-1} *FW*) and Catalase (715 *Units* g^{-1} *FW*) (Table 2/Figure 2). However, the lowest values were observed for plant population (41.0), ginning out turn (40.55%), staple length (26.95 mm) and hydrogen peroxide (5.05 μ*molg*$^{-1}$ *FW*). The second cluster, Cluster-II is the combined largest group of cotton genotypes comprised of four genotypes i.e., BH-617, BH-600, BH-227 and BH-184. This cluster is characterized by its lowest seed cotton yield (2.21 kg $plot^{-1}$). It has the lowest mean values for the number of bolls (41.35), plant height (150.64 cm), sympodial branches (16.71), net photosynthetic rate (23.05 μ*molm*$^{-2}s^{-1}$), and transpiration rate (0.29 *mmol* $m^{-2}s^{-1}$) (Table 2).

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The Cluster-III consisted of four cotton genotypes, including BH-248, BH-244, BH-247, and BH-606, and this cluster was regarded as the cluster of best performing and highest productive genotypes with average yield (2.44 kg plot^{-1}). The group is characterized by the highest values for traits including number of bolls (42.58), plant height (159.94 cm), sympodial branches (18.07), and Proline (*mg g^{-1} FW*), while the lowest values for virus attack (5.33 %), monopodial branches (0.59) and total phenolic contents (69.78 *µmol g^{-1} FW*) (Table 2). Several plant researchers utilized cluster analysis for characterizing and grouping cotton genotypes based on their performance and found it very effective for selecting parent material (Alabady et al., 2008; Sarwar et al., 2021).

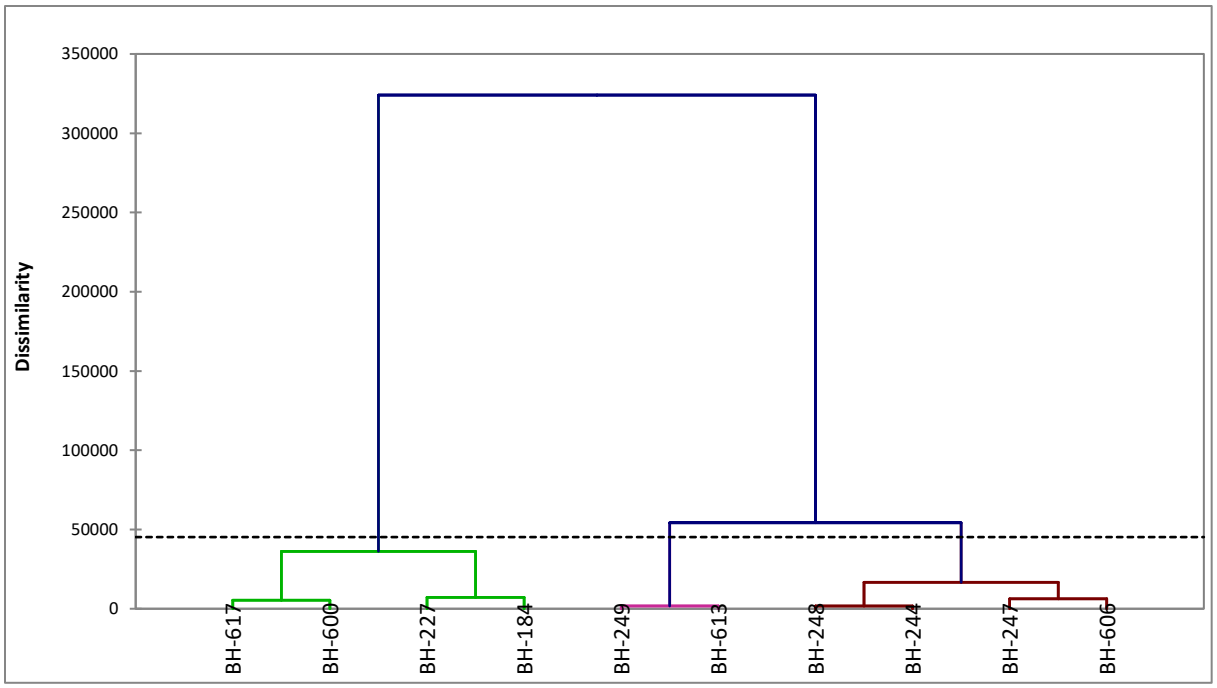

Figure 2: *Dendrogram of cotton genotypes based on cluster analysis*

Table 2: Cluster means for studied plant traits in cotton genotypes under study.

Plant Traits	Class-1	Class-2	Class-3
Plant Population (PP)	41.00	45.00	44.92
Virus Attack (VP)	6.00	5.42	5.33
Number of bolls (NB)	42.15	41.35	42.58
Plant Height (PH)	158.11	150.64	159.94
Monopodial Branches (MB)	0.96	1.00	0.59
Sympodial Branches (SB)	18.07	16.71	18.07
Ginning out term (GOT%)	40.55	41.88	41.50
Staple Length (SL)	26.95	29.99	29.36
Boll Weight (BW)	2.58	2.71	2.48
Net Photosynthetic Rate (Pn)	24.97	23.05	24.13
Transpiration Rate (Tr)	0.38	0.29	0.38
Proline (Pro)	6.06	6.39	7.40
Hydrogen Peroxide (H_2O_2)	5.05	7.84	6.24
Total Phenolic Contents (TPC)	82.13	51.27	69.78
Ascorbic Acid (ASA)	588.00	537.00	583.58
Flavonoids (Flv)	988.33	539.83	811.25
Malondialdehyde (MDA)	262.20	174.95	222.65
Peroxidase (POD)	960.50	768.67	877.42
Catalase (CAT)	715.00	673.25	689.42
Yield/plot	2.30	2.21	2.44

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Principal Component Analysis (PCA)

In the current study, the principal component analysis extracted nine principal components (PCs) based on their performance and diversity of plant traits related to morpho-physiology and biochemistry. The data given in Table 3 and Figure 3 displays that the first five of these nine PCs had an eigenvalue larger than 1, therefore these significant PCs will be discussed in detail. These five PCs accounted for 89.82% of the variance in the data as presented in Table 3.

Table 3: Eigenvalues, variability percentage and Cumulative variability percentage in cotton genotypes through Principal Component Analysis

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigenvalue	8.45	4.16	2.38	1.88	1.09	0.79	0.59	0.46	0.19
Variability (%)	42.2	20.82	11.91	9.38	5.45	3.94	2.95	2.32	0.97
Cumulative %	42.2	63.08	74.99	84.38	89.82	93.76	96.71	99.03	100.00

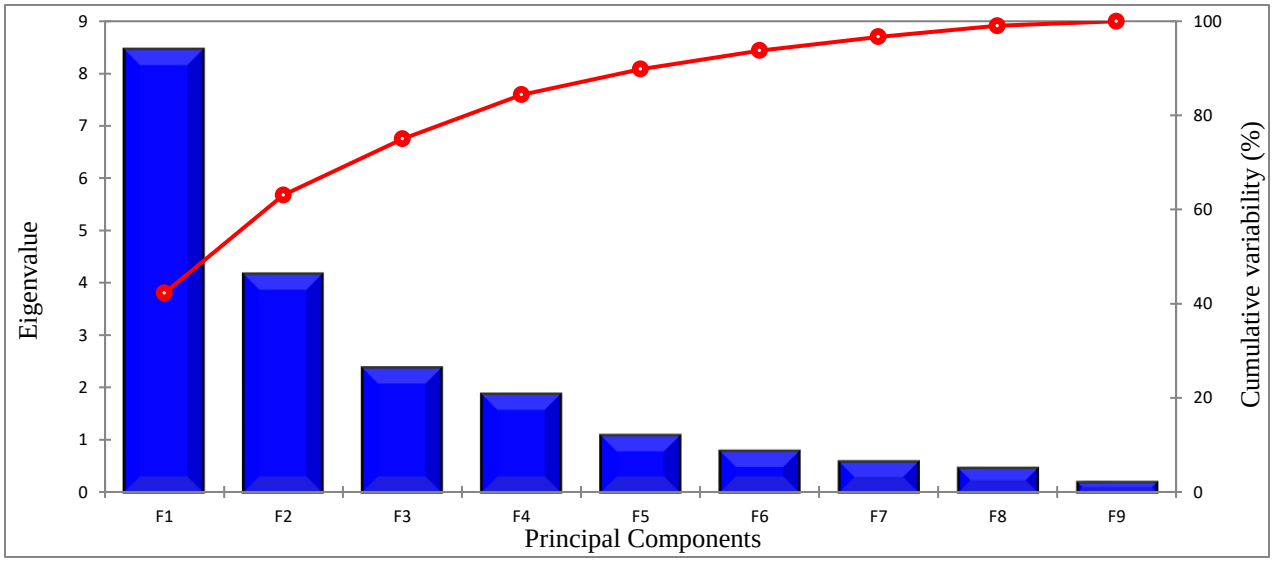

***Figure 3**: Scree plot of Principal Component Analysis in cotton genotypes*

The PCA exhibited that PC1 accounted for 42.2% of the total variation in the data and number of bolls per plant, plant height, net photosynthetic rate, transpiration rate, hydrogen peroxide, total phenolic contents, ascorbic acid, flavonoids, malondialdehyde, peroxidase, catalase, and seed cotton yield were the key contributing traits (Table 4). In principal component 2, which contributed 20.82% to the variability in the data and has an eigenvalue of 4.16, four plant traits, i.e., plant population, virus plants, ginning out turn, and boll weight were the major differentiating traits. Monopodial branches, sympodial branches, and proline were the key features of principal component 3, which account for 11.91% variability in the data and has an eigenvalue of 2.38. Similarly, PC4 contributed 9.38 % to the total genetic diversity with eigenvalues 1.88, and sike length was the only trait contributing significantly towards data variability (Table 4). The last significant principal component, PC-5, contributed 5.45% to the diversity in the data with an eigenvalue of 1.09, while no parameter could significantly impact this principal component. To observe the pattern of association between maize hybrids and investigated traits across cotton genotypes, PC1/PC2 biplot was generated using principal component analysis. The PC1/PC2 biplot analysis revealed hydrogen peroxide, plant population, seed cotton yield, plant height, transpiration rate, total phenolic contents, malondialdehyde, catalase, and boll weight to be the most distinguishing characteristics among cotton genotypes (Figure 4). The minimum variations were observed in staple length, sympodial branches, and net photosynthetic rate. The biplot also showed that the most productive hybrids are the three cotton genotypes BH-247, BH-606, and BH-244. Moreover, three cotton genotypes, including BH-249, BH-227, and BH-184, showed maximum contribution towards variability in the data (Table 5). Principal Component Analysis (PCA) plays a pivotal role in characterizing cotton genotypes by extracting essential information from complex and high-dimensional datasets (Westhues et al., 2021). In cotton genotyping, where numerous genetic markers and phenotypic traits contribute to the overall variability, PCA is a powerful tool for dimensionality reduction (Bhandari et al., 2017).

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Table 4: Correlations/Factor loading between plant traits and principal components as derived from PCA

	F1	F2	F3	F4	F5
Plant Population (PP)	-0.048	0.869	-0.193	0.066	0.318
Virus Attack (VP)	-0.171	-0.774	0.446	0.369	0.043
Number of bolls (NB)	0.563	0.433	-0.632	0.283	0.030
Plant Height (PH)	0.863	0.344	0.103	0.023	-0.300
Monopodial Branches (MB)	-0.382	-0.445	-0.647	-0.085	0.249
Sympodial Branches (SB)	0.352	0.193	0.642	0.390	0.468
Ginning out term (GOT%)	-0.081	0.686	-0.084	-0.316	0.017
Staple Length (SL)	-0.324	0.054	-0.262	0.748	-0.177
Boll Weight (BW)	-0.379	-0.771	0.122	-0.100	-0.442
Net Photosynthetic Rate (Pn)	0.591	-0.034	-0.133	0.744	-0.037
Transpiration Rate (Tr)	0.907	0.112	-0.072	0.216	-0.264
Proline (Pro)	-0.004	0.475	0.719	0.029	-0.163
Hydrogen Peroxide (H_2O_2)	-0.820	0.447	-0.134	0.133	-0.131
Total Phenolic Contents (TPC)	0.960	-0.229	0.041	0.015	0.153
Ascorbic Acid (ASA)	0.918	-0.091	0.131	-0.168	0.000
Flavonoids (Flv)	0.943	-0.251	-0.013	-0.121	0.150
Malondialdehyde (MDA)	0.910	-0.353	0.037	-0.005	0.187
Peroxidase (POD)	0.821	-0.077	-0.130	-0.395	0.040
Catalase (CAT)	0.602	-0.437	-0.461	0.026	-0.141
Yield/plot	0.687	0.562	0.083	-0.090	-0.412

Table 5: Contribution of the cotton genotypes (%) in different principal components

	F1	F2	F3	F4	F5
BH-249	17.873	0.039	4.440	0.871	4.219
BH-617	1.215	6.858	28.529	0.006	0.071
BH-227	24.513	0.511	28.133	0.190	2.989
BH-248	3.571	21.811	1.372	6.018	36.719
BH-613	5.476	29.081	4.458	4.064	9.303
BH-244	6.232	1.271	2.087	6.045	21.772
BH-247	2.526	22.288	14.944	18.870	0.245
BH-606	0.857	10.474	2.583	61.601	6.116
BH-184	35.647	7.359	5.522	0.192	3.020
BH-600	2.089	0.308	7.933	2.143	15.546

By transforming the original data into a new set of uncorrelated variables, or principal components, PCA simplifies the analysis while retaining the most significant patterns of variation (Lever et al., 2017). This reduction not only aids in visualization, allowing researchers to create insightful plots that capture the relationships and clusters among cotton genotypes but also helps identify the key markers or traits contributing to the observed differences. Moreover, PCA facilitates the detection of outliers and data errors, enhancing data quality control. In cotton breeding and genetic studies, PCA assists in assessing genetic diversity, guiding breeding decisions, and comparing different sets of genotypes. PCA empowers researchers and breeders to uncover the inherent complexity within cotton genotypes, enabling informed choices for crop improvement and contributing to the advancement of cotton agricultural practices (Van Dijk et al., 2021). Isong et al., 2017 and Sarwar et al. 2020 used principal component analysis to categorize cotton genotypes based on agronomically important traits. They found it convincing to group the cotton genotypes based on their performance.

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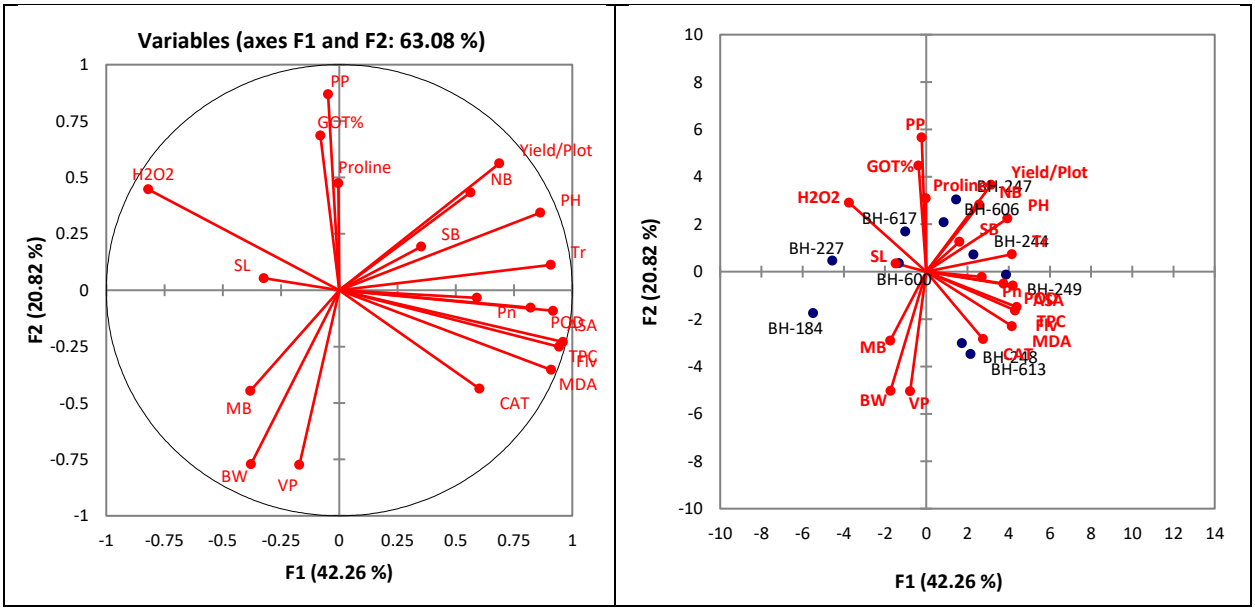

Conclusion

The experimental results indicated the presence of significant variations in the plant parameters studied in ten cotton genotypes. The correlation analysis revealed a significantly positive correlation of seed cotton yield with plant height, transpiration rate, ascorbic acid, number of bolls, peroxidase, flavonoids, and net photosynthetic rate. Two multivariate analysis approaches, i.e., principal component and cluster analysis, were used to categorize cotton genotypes based on their performance. These analyses revealed that BH-247 and BH-606 were the most productive cotton genotypes. Therefore, these genotypes could be recommended for cultivation in core-cotton areas following extensive multilocation testing.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

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Conflict of interest

The authors declared the absence of conflict of interest.

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