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Original Research Article

Genetic Association of Plant Physiological Parameters with Agronomically Important, Yield-Related Traits in American Cotton (*Gossypium Hirsutum L.*)

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Abstract: *Cotton (Gossypium hirsutum L.) productivity in semi-arid regions like Pakistan is severely constrained by water scarcity and heat stress. With an average yield of 570.99 kg/ha, Pakistan lags behind major cotton-producing countries such as China (1,992 kg/ha), emphasizing the urgent need for identifying climate-resilient, high-yielding genotypes.* ***Objective:*** *To evaluate the physiological and yield-related performance of ten elite cotton genotypes under semi-arid conditions, and to identify superior candidates for breeding programs to improve cotton yield.* ***Methods:*** *A field trial was conducted at the Cotton Research Station, Bahawalpur, using a Randomized Complete Block Design (RCBD) with three replications. Ten cotton genotypes (BH-188, BH-226, BH-291, BH-403, BH-407, BH-410, BH-423, BH-563, BH-184, and CIM-600) were assessed for physiological traits including photosynthetic rate, stomatal conductance, and transpiration rate, as well as yield-related parameters such as boll weight, number of sympodial branches, and seed cotton yield. Statistical analyses included ANOVA, Pearson correlation, Principal Component Analysis (PCA), and cluster analysis to determine genotypic variation and trait associations.* ***Results:*** *ANOVA revealed highly significant genotypic differences ($p \leq 0.01$) for most traits: seed cotton yield ($F = 2.89245$), photosynthetic rate ($F = 15.793$), and sympodial branches ($F = 7.4963$). Correlation analysis demonstrated strong positive relationships between yield and sympodial branches ($r = 0.75^*$) and boll weight ($r = 0.25$). PCA showed that the first two principal components (PC1 = 33%, PC2 = 21.6%) explained 54.6% of the total variation, primarily influenced by yield and physiological traits. Cluster analysis grouped genotypes into three distinct clusters, with Cluster 1 (BH-188, BH-226, BH-410) exhibiting the highest yield (4.56 t/ha), driven by superior photosynthetic rate (30.33 $\mu mol\ m^{-2}\ s^{-1}$) and sympodial branching (21.66).* ***Conclusion:*** *This study highlights BH-226 and BH-410 as promising candidates for breeding programs to enhance cotton yield under semi-arid conditions. Their superior physiological efficiency and yield-related traits present viable solutions to overcoming productivity challenges in water-stressed environments. These genotypes can be pivotal in developing climate-resilient cotton varieties tailored to Pakistan's agro-climatic conditions.*

Keywords: Upland Cotton, Multivariate Analysis, Principal Component Analysis, Correlation

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Introduction

Cotton (*Gossypium hirsutum* L.), often termed "white gold," is a cornerstone of global agriculture, serving as the primary natural fiber for the textile industry and a vital source of seed oil and livestock feed, supporting millions of livelihoods worldwide. Globally, Upland cotton accounts for over 90% of production, cultivated across 33–35 million hectares, yielding more than 70 million tons of seed cotton annually, with an average lint yield of 800 kg/ha across diverse agroecological zones (1) Major producers like China (2,089 kg/ha), Brazil (1,911 kg/ha), and the United States (1,992 kg/ha) leverage advanced technologies to sustain high outputs, contributing over 50% of the global supply (2). In Pakistan, cotton is a linchpin of the agrarian economy, grown on approximately 2.14 million hectares, primarily in Punjab and Sindh, producing 4.91 million tonnes annually; however, the national average yield of 472 kg/ha reflects challenges posed by water scarcity, pests, and suboptimal management, lagging far behind global benchmarks (3, 4). This disparity underscores the urgent need to enhance cotton productivity in Pakistan to meet both domestic industrial demands and export potential.

Agronomic traits such as boll weight, number of bolls per plant, and sympodial branches, alongside physiological parameters like net photosynthetic rate, stomatal conductance, and transpiration rate, play pivotal roles in cotton improvement by directly influencing seed cotton yield. Boll weight and sympodial branches, the primary fruiting structures, enhance yield through increased lint and seed production, with studies showing a 10–15% yield boost in genotypes with optimized assimilate partitioning (5). Physiological traits drive these outcomes by regulating carbon assimilation and water use; for instance, higher photosynthetic rates have been linked to a 15–20% increase in seed cotton yield under stress conditions by supporting boll filling (6). Fiber quality traits—length, strength, and fineness—are equally critical, as they determine industrial value and are positively associated with photosynthetic efficiency. However, finer fibers may reduce ginning out-turn, presenting a yield-quality trade-off (7). These traits collectively shape cotton's genetic potential, making their integration essential for breeding high-yielding, quality-focused varieties.

Different statistical approaches, such as principal component analysis (PCA), cluster analysis, and correlation studies, are powerful tools for dissecting the complex relationships between physiological and agronomic traits in cotton, aiding in identifying superior genotypes for breeding. PCA reduces trait dimensionality, revealing primary sources of variation; for example, it has been used to explain over 50% of yield variance in cotton through physiological and yield-related traits, guiding trait prioritization (8). Cluster analysis groups genotypes based on trait similarity, enabling the selection of diverse parents for hybridization, as demonstrated in studies identifying yield-focused versus quality-focused cotton clusters under stress (9). Correlation studies quantify trait interdependencies, such as the strong positive link between photosynthetic rate and boll weight, offering insights for indirect selection to enhance seed cotton yield without measuring all traits directly (10). These techniques collectively enhance the precision of genetic association studies, supporting targeted improvements in cotton productivity and adaptability.

Given the critical role of physiological and agronomic traits in cotton improvement and the challenges faced in Pakistan's semi-arid regions, this study aims to elucidate the genetic associations between plant physiological parameters—net photosynthetic rate, stomatal conductance, and transpiration rate—and agronomically important, yield-related traits—boll weight, number of bolls, sympodial branches, and seed cotton yield—in *Gossypium hirsutum*. By employing multivariate analysis techniques like PCA, cluster analysis, and correlation studies, the research seeks to identify genotypes with optimal trait combinations for high yield and fiber quality, providing a foundation for breeding programs to develop resilient, productive cotton varieties suited to diverse environmental conditions.

Methodology

The experiment was conducted at the Research Area of the Cotton Research Station, Bahawalpur, Pakistan (29.39°N, 71.69°E, elevation 116 m), characterized by a semi-arid climate with annual rainfall ranging from 200–300 mm and temperatures between 25–45°C during the cotton growing season, featuring clay-loamy soils (35% clay, 40% silt, 25% sand, pH 7.8–8.2). Ten cotton genotypes were assessed, comprising eight elite strains (BH-188, BH-226, BH-291, BH-403, BH-407, BH-410, BH-423, and BH-563) and two check varieties (BH-184 and CIM-600), arranged in a Randomized Complete Block Design (RCBD) with three replications. Experimental plots were designed to standardize plant density across all treatments with a uniform plant-to-plant distance of 30 cm and a row-to-row distance of 75 cm. Consistent agronomic and pathological practices were applied to all genotypes, including irrigation every 10 days using canal water, fertilizer inputs of 220 kg nitrogen per hectare, 100 kg phosphorus per hectare, and 98 kg potassium per hectare supplied as urea, diammonium phosphate, and sulfate of potash, respectively, and pest and disease management through integrated pest management (IPM) protocols involving manual weeding and selective pesticide applications.

Data Collection

Data were recorded on key physiological, agronomic, and fiber quality parameters, including plant height, number of monopodial and sympodial branches, photosynthetic rate, stomatal conductance, transpiration rate, fiber length, fiber fineness, fiber strength, ginning out-turn, boll weight, and lint yield per hectare. Data regarding agronomic traits, i.e., plant height, number of monopodial and sympodial branches, ginning out-turn, boll weight, and lint yield per hectare, were recorded at physiological maturity/harvest. Data on physiological parameters (net photosynthetic rate, stomatal conductance, transpiration rate) were measured during the boll-filling stage using a portable, handheld photosynthesis system (CI-340, LI-COR Biosciences, USA). Fiber quality parameters, i.e., fiber length, fiber fineness, and fiber strength, were analyzed post-harvest using a High-Volume Instrument (HVI-1000, Uster Technologies, Switzerland).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the RCBD model to determine significant differences among cotton genotypes (11). Statistical analyses were performed with the software package Statistix 8.1 (Analytical Software, USA) and R/RStudio (version 4.3.1). Correlation coefficient analysis was conducted to explore relationships between seed cotton yield and different agro-physiological plant traits.

Results & Discussion

Analysis of Variance (ANOVA)

The ANOVA results confirmed significant genotypic variation among the ten cotton genotypes for most physiological and yield-related traits evaluated under semi-arid conditions, highlighting their potential for genetic improvement (Table 1). Genotypes exhibited highly significant differences ($p \leq 0.01$) for plant height, number of nodes, sympodial branches, photosynthetic rate, stomatal conductance, transpiration rate, fiber length, fiber strength, ginning out-turn, boll weight, and seed cotton yield, indicating strong genetic control over these traits, which can be targeted for selection to enhance performance in water-limited environments (12). Monopodial branches and fiber fineness showed non-significant genotypic variation, suggesting limited genetic diversity for these traits, possibly due to environmental constraints in semi-arid conditions (13).

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

Plant Traits/Source of Variations	Replications	Genotypes	Error
df	2	9	18
Plant Height	37.43	1067.27^{**}	305.51
Nodes per Plant	7.433	35.411^{**}	5.878
Monopodial Branches	0.1333	0.35556^{NS}	0.8
Sympodial Branches	12.4	7.4963^{*}	3.4741
Net Photosynthetic Rate (Pr)	21.43	15.793^{**}	6.026
Stomatal Conductance (Ci)	220.3	4620.7^{**}	1136.1

Transpiration Rate (Tr)	0.072463	0.144015**	0.0512
Fiber Length (FL)	0.15433	0.89189**	0.2987
Fiber Strength (FS)	8.5637	22.731**	8.337
Fiber Fineness (FF)	0.025963	0.073852^{NS}	0.1511
Ginning Out Turn (GOT)	0.3543	4.5372**	1.6677
Boll Weight (BW)	0.005108	0.197075**	0.05522
Seed Cotton Yield (Yield)	0.14158	2.89245**	0.18556

Correlation Coefficient Analysis

The correlation coefficient analysis revealed significant relationships between physiological parameters—photosynthetic rate, stomatal conductance, transpiration rate—and yield-related traits—boll weight, number of bolls, sympodial branches, and seed cotton yield (Yield)—among the ten cotton genotypes, alongside other traits like plant height, number of nodes, monopodial branches, fiber length, fiber strength, fiber fineness, and ginning out-turn (Figure 1). Seed cotton yield showed a strong positive correlation with sympodial branches per plant ($r = 0.75^{*}$, $p \leq 0.05$) and a moderate positive but non-significant correlation with boll weightaq1 ($r = 0.25$), indicating that genotypes with more fruiting branches and heavier bolls, such as BH-226 and BH-410, are likely to achieve higher seed cotton yields, aligning with findings by Hussain et al. (11). Net photosynthetic rate exhibited a strong positive correlation with stomatal conductance ($r = 0.962^{**}$, $p \leq 0.01$) and transpiration rate ($r = 0.929^{**}$, $p \leq 0.01$), reflecting their interdependence in driving photosynthetic efficiency, but its negative correlation with Yield ($r = -0.18$) suggests that excessive gas exchange may not always translate to yield gains under semi-arid conditions, possibly due to water loss (14) Notably, fiber length and fiber strength were negatively correlated with Yield ($r = -0.33$ and -0.24, respectively), highlighting a yield-quality trade-off, while GOT showed a negative correlation with net photosynthetic rate ($r = -0.65^{*}$), indicating that high photosynthetic rates may reduce lint recovery (Sabagh et al., 2022). Plant height and number of nodes were positively correlated ($r = 0.68^{*}$), supporting their role in enhancing fruiting positions, indirectly boosting yield through sympodial branches ($r = 0.53$ with PH).

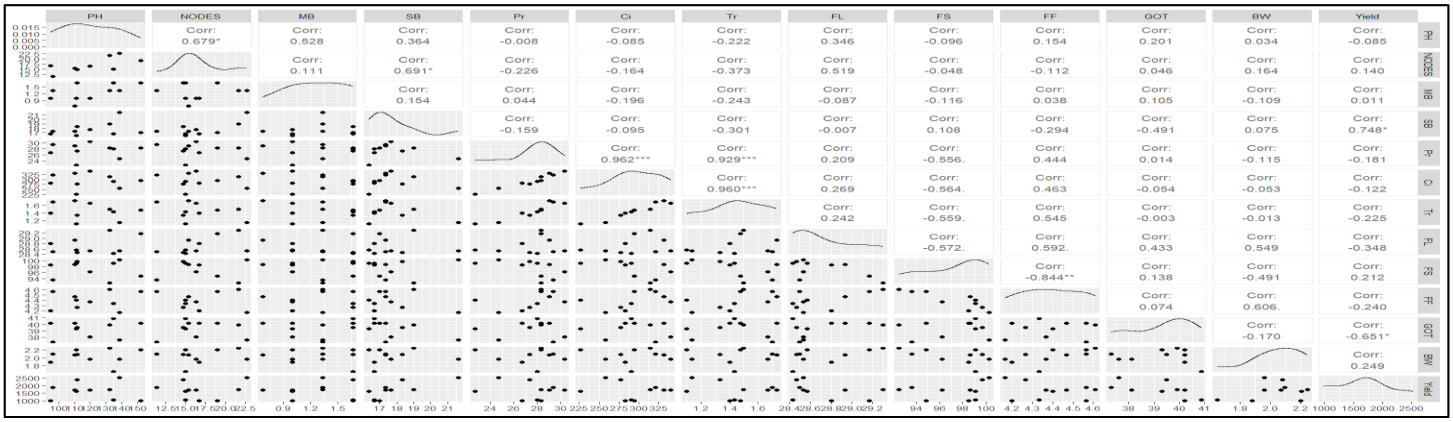
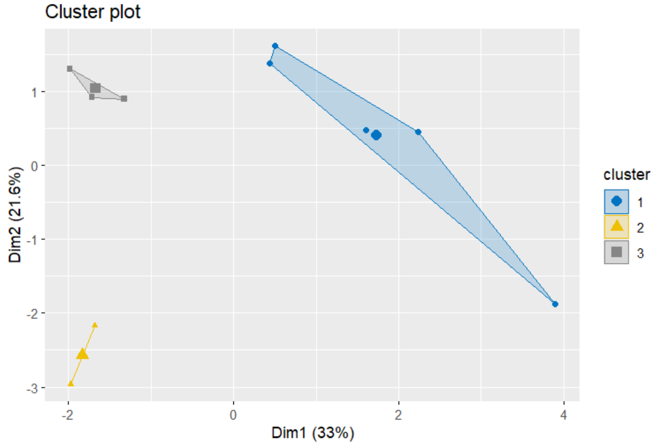


Figure 1: Correlation Matrix of studied plant traits in ten cotton genotypes

Cluster Analysis

Cluster analysis grouped the genotypes into three clusters based on their physiological and yield-related traits, as shown in the cluster means table (Figure 2). Cluster-1 (BH-188, BH-226, BH-410) exhibited the highest yield (4.56 t/ha), driven by superior net photosynthetic rate (30.33 µmol m^{-2} s^{-1}), Ci (340.3 mmol m^{-2} s^{-1}), BW (2.08 g), and SB (21.66), reflecting their adaptation to semi-arid conditions through efficient photosynthesis and robust boll production, a trait desirable for yield-focused breeding (Hussain et al., 2024b). Cluster-2 (BH-291, BH-403, BH-407, BH-423) showed moderate yield (1.90 t/ha) but excelled in Tr (5.63 mmol m^{-2} s^{-1}) and Bolls (55.3), indicating a balance between water use and reproductive output, which could be leveraged for resilience under variable moisture regimes (15).

Cluster-3 (BH-563, BH-184, CIM-600) had the lowest yield (1.76 t/ha), with a lower net photosynthetic rate (24.64 µmol m^{-2} s^{-1}) and sympodial branches (11.0). This suggests limited physiological and agronomic performance, possibly due to environmental constraints or inherent genetic limitations (16). This clustering underscores the genetic diversity for selecting high-yielding, physiologically efficient genotypes.



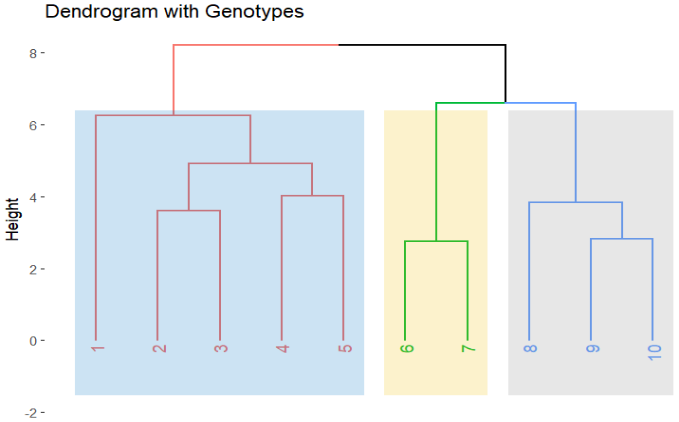


Figure 2: Cluster Plot

Principal Component Analysis (PCA)

The PCA results provided a comprehensive understanding of trait variation among the ten cotton genotypes, as illustrated by the scree plot (Figure 4a), correlation circle (Figure 4b), individual variable plot (Figure 4c), variable contribution to PC1 and PC2 (Figure 4d), and individual observations plot (Figure 4e) and combined PC1/PC2 biplot (Figure 4f). The scree plot indicated that PC1 and PC2 together explained 54.6% of the total variance, with PC1 accounting for 33% and PC2 for 21.6%, suggesting that these components capture the primary sources of trait differentiation among the genotypes, a finding consistent with PCA applications in cotton studies (17) (Figure 4a). This level of explained variance highlights the effectiveness of PCA in reducing dimensionality while retaining key information about genotypic performance under semi-arid conditions.

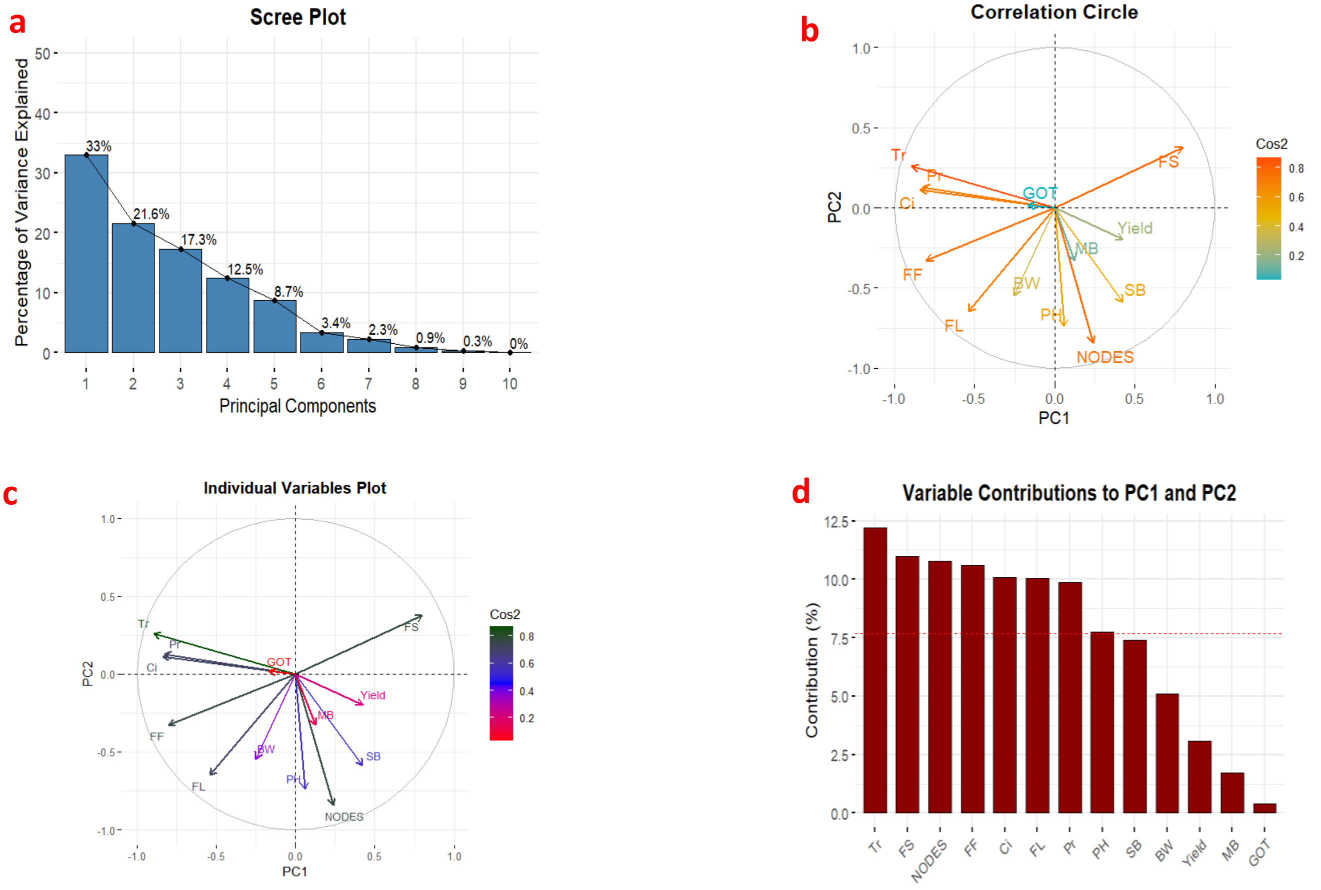
The correlation circle and individual variable plot revealed the trait associations driving PC1 and PC2 (Figure 4b and 4c). PC1 was strongly influenced by Yield, SB, and MB (all positively aligned), indicating that yield and fruiting structures are major contributors to genotypic variation. At the same time, FL, FF, and BW were negatively associated with PC1, reflecting a trade-off between yield and fiber quality traits (18) PC2 was dominated by FS (positive) and GOT (near the origin, indicating low contribution to variance), with Pr, Ci, and Tr also contributing positively, contrasting with PH and Nodes on the negative PC2 axis, suggesting that physiological traits and fiber strength play a significant role in separating genotypes based on quality and stress adaptation (24, 25) (Figure 4d & 4e). The variable contribution plot confirmed that Tr, FS, Nodes, FF, and Ci were the top contributors to PC1 and PC2 (each >7.5%), underscoring their importance in genotypic differentiation (Figure 4e).

The individual observations plot illustrated the distribution of genotypes across PC1 and PC2, aligning with the cluster analysis results (Figure 4e).

Figure 3: Dendrogram of Ten Cotton Genotypes

Cluster-1 genotypes (BH-188, BH-226, BH-410, BH-403, BH-407) were positioned along the positive PC-1 axis, close to Yield and SB, confirming their high-yield potential, with BH-226 and BH-410 particularly excelling in productivity traits. Cluster-2 genotypes (BH-291, BH-423) were located near the origin of PC1 but varied along PC2, indicating a balance between yield and quality traits like FS. In contrast, Cluster-3 genotypes (BH-563, BH-184, CIM-600) were positioned negatively on PC1, reflecting lower yield performance and closer association with FL and FF, consistent with their role as checks with limited adaptability to semi-arid conditions (19). These PCA findings highlight the genetic diversity among the genotypes, enabling the selection of high-yielding strains like BH-226 for productivity and quality-focused strains like BH-563 for breeding programs in semi-arid environments.

The combined PCA biplot illustrated the relationships between physiological and yield-related traits i.e., photosynthetic rate, stomatal conductance, transpiration rate, boll weight, number of bolls, sympodial branches, seed cotton yield, plant height, number of nodes, monopodial branches, fiber length, fiber strength, fiber fineness, and ginning out-turn—and the distribution of the ten cotton genotypes across PC1 (33%) and PC2 (21.6%), which together explained 54.6% of the total variance (Figure 4f). Genotypes BH-226, BH-410, and BH-423, positioned on the positive PC1 axis, were closely associated with Yield, SB, and MB, indicating their superior yield potential driven by robust fruiting structures, aligning with their Cluster 1 grouping and high yield performance (4.56 t/ha) from the cluster analysis (20). In contrast, BH-563, BH-184, and CIM-600, located on the negative PC1 axis near FL, FF, and BW, reflected a focus on fiber quality over yield, consistent with their Cluster 3 classification and lower yield (1.76 t/ha), highlighting a yield-quality trade-off (16).



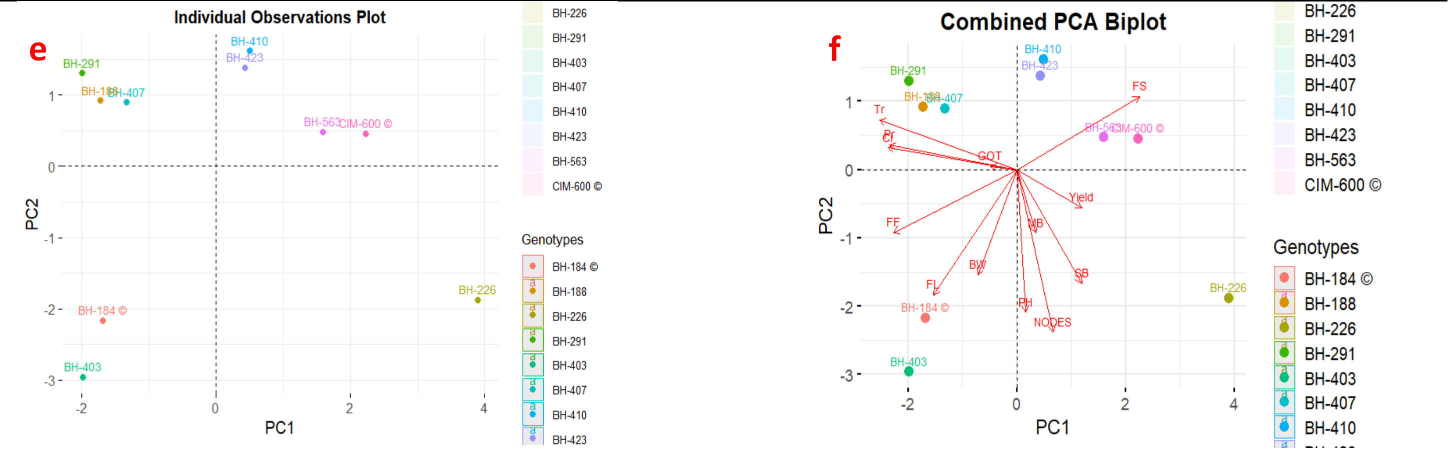


Figure 4: Principal Component Analysis in ten cotton genotypes (a) Screeplot of Principal Component Analysis (b) Correlation circle (c) Individual Variable Plot (d) Variable contribution to PC1 and PC2 (e) Individual Observation Plot (f) Combined PCA Biplot

BH-291 and BH-403, positioned near the origin of PC1 but varying along PC2, were associated with FS, Pr, Ci, and Tr, suggesting a balance between physiological efficiency and fiber strength, as seen in Cluster 2. The biplot also showed that physiological traits (Pr, Ci, Tr) were positively aligned with PC2, contrasting with PH and Nodes on the negative PC2 axis, indicating their role in separating genotypes based on stress adaptation and quality traits (2124). This biplot underscores the genetic diversity among the genotypes, supporting the selection of BH-226 and BH-410 for yield-focused breeding and BH-563 for quality improvement in semi-arid environments.

Conclusion

This study elucidated the genetic associations between physiological parameters and yield-related traits in *Gossypium hirsutum L.*, providing valuable insights for breeding programs in semi-arid regions like Bahawalpur, Pakistan. Genotypes in Cluster 1, particularly BH-226 and BH-410, demonstrated superior yield potential (4.56 t/ha), driven by high photosynthetic rates (30.33 µmol m^{-2} s^{-1}), sympodial branches (21.66), and boll weight (2.08 g), making them prime candidates for enhancing productivity under water-limited conditions. The significant genotypic variation confirmed by ANOVA, coupled with strong correlations between yield and sympodial branches (r = 0.75*) and boll weight (r = 0.25), underscores the importance of these traits in selection programs. PCA further highlighted the role of physiological traits in yield variation, explaining 54.6% of the variance. In contrast, the negative correlation between photosynthetic rate and yield (r = -0.18) suggests optimizing water-use efficiency to mitigate trade-offs in semi-arid environments. By leveraging the genetic diversity identified through cluster and PCA analyses, breeders can develop high-yielding, resilient cotton varieties through targeted hybridization, ultimately improving productivity and supporting sustainable cotton production in challenging agroecological zones.

Declarations

Data Availability statement
All data generated or analysed during the study are included in the manuscript.

Ethics approval and consent to participate
Approved by the department concerned. (IRBEC-24)

Consent for publication
Approved

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Not applicable

Conflict of interest

The authors declared the absence of a conflict of interest.

Author Contribution

MY, MZ, MKM, BHB
Coordination of collaborative efforts.
Study Design, Review of Literature.
Conception of Study, Development of Research, Methodology Design, Study Design, manuscript Review, Conception of Study.
AHM, WS, GMS, JI
Sampling, Study Design, Manuscript revisions, and critical input.
Data acquisition and Data analysis, Coordination of collaborative efforts.
MT, AGK, MS, MAS
Statistical Analysis.
Data acquisition, analysis.
Manuscript drafting.
II, SWHS, GM, IA, MRF
Study Design, Review of Literature.
Data entry, data analysis, and drafting the article.
Data acquisition, analysis.
SK, JQ, NK, SH, MIY
Coordination of collaborative efforts.
Methodology Design, Study Design, Review of manuscript, Conception of Study.

All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.

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