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Classification and Characterization of Elite Cotton Lines for their Agronomic Performance through Multivariate Statistical Approaches

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Abstract

Cotton (*Gossypium hirsutum* L.) productivity in semi-arid regions is constrained by climatic stress and genetic limitations, necessitating the identification of high-performing and adaptable genotypes. The present study was conducted to evaluate the genetic variability, trait associations, and yield potential of thirty-two cotton genotypes under semi-arid conditions at the Cotton Research Station, Bahawalpur, Pakistan. The experiment was laid out in a randomized complete block design with three replications. Data were recorded on key agronomic, physiological, and fiber quality traits, including plant height, nodes per plant, monopodial and sympodial branches, green bolls, open bolls, seed cotton yield, fiber length, net photosynthetic rate, and ginning out turn. Analysis of variance revealed highly significant differences among genotypes for all studied traits, indicating substantial genetic diversity. Correlation analysis showed that seed cotton yield was positively and significantly associated with open bolls ($r = 0.470^*$), green bolls ($r = 0.455^*$), sympodial branches ($r = 0.443^*$), monopodial branches ($r = 0.424^*$), nodes per plant ($r = 0.369^*$), and plant height ($r = 0.446^*$), while a significant negative correlation was observed between yield and fiber length (r = -0.769**). Cluster analysis grouped the genotypes into two distinct clusters, with Cluster-1 exhibiting superior yield, higher boll number, and enhanced photosynthetic activity. Principal component analysis explained 78.41% of the total variability through the first three principal components, highlighting yield and yield-contributing traits as major sources of genetic divergence. Cotton genotypes Emp-1, SI-3, and SI-22 demonstrated superior performance and can be exploited in future breeding programs aimed at improving cotton productivity under semi-arid environments.

Keywords: Upland Cotton, multivariate analysis, principal component analysis, correlation

Introduction

Cotton (*Gossypium hirsutum* L.), widely referred as Upland cotton, is a cornerstone of global agriculture, recognized as the foremost natural fiber crop and an essential component of the world economy (Monicashree et al., 2017). Globally, it covers nearly 33–35 million hectares, representing around 2.5% of cultivable land, and generates over 70 million tons of seed cotton each year, with an average lint productivity of about 800 kg/ha under varying agroecological zones (ruti et al., 2020). Major cotton producing nations including China, India, the United States, and Pakistan account for more than 70% of total global output, with China attaining yields close to 1,992 kg/ha through sophisticated irrigation and management systems (Shabbir & Yaqoob, 2019). In Pakistan, cotton remains a fundamental component of the agricultural sector, cultivated on 2.143 million hectares primarily across Punjab and Sindh and produces nearly 9.86 million bales annually (Younas et al., 2025).

Cotton is one of the most important fiber crops worldwide; however, Pakistan ranks fifth in global scenario (about 4% of the world cotton area). The average per yield of cotton in Pakistan is (699 kg per ha) when compared to the world average (730 kg per ha) and some of the leading producers of lint are namely, Australia (1781 kg per ha), China (1761 kg per ha), Brazil (1522 kg per ha), USA (974 kg per ha), and India (560 kg per ha) (ruti et al., 2020). In 2024/25 Pakistan's cotton output is forecast at approximately 5.2 million bales, reflecting a substantial reduction compared to past levels (USDA, 2024). Pakistan's cotton production for 2024/25 is estimated at 4.8 million bales, marking a 4 percent decline from the previous year 2024/25. This reduction is primarily due to lower yields in Punjab, where adverse weather conditions and monsoon season flooding negatively affected crop performance. In several affected areas, growers have already harvested their cotton crops to enable the timely sowing of winter wheat (USDA, 2025). As compared to leading countries such as India (20.1%) and China (25.6%), Pakistan accounts for 4.4% global output (Zhang et al., 2025). The relatively lower cotton yield in Pakistan can be scientifically attributed to two principal factors: intense insect-pest pressure (whitefly and pink bollworm), which significantly damage cotton bolls and reduce lint yield; and suboptimal irrigation conditions, especially in flood-prone or poorly drained areas, which compromise crop development and fiber quality (Hussain et al., 2025). Therefore, despite cotton's global significance as a natural fiber crop, Pakistan's cotton production remains constrained not due to lack of demand, but because of agronomic and environmental challenges that negatively affect productivity and yield stability.

Several approaches exist to enhance cotton production; among these, the evaluation of cultivated germplasm is considered the most effective strategy (Stiller & Wilson, 2014). Following systematic evaluation, high-performing varieties and lines can be identified. These superior genotypes may subsequently be advanced either through a direct variety release program or incorporated into hybrid breeding programs, with the objective of developing new varieties that combine high yield potential with superior fiber quality. The present experiment was therefore designed to assess the performance of existing cotton germplasm at the Cotton Research Station, Bahawalpur. At the conclusion of the study, the best-performing lines and varieties will be selected for further utilization in breeding and variety development programs.

Material and Method

Site and Research Material

The research was conducted at the Cotton Research Station, Bahawalpur, located in Punjab, Pakistan. The site is characterized by a semi-arid climate. Thirty-two genotypes of cotton were used in this research (Table1); the research was laid out in a Randomized Complete Block Design (RCBD) with three replications. Sowing is done with the help of a dibbler, @ 3 seeds/hill. At the early seedling stage, out of the three plants, the healthy plantlet was left standing, and the other plants were removed. All practices, including agronomic and plant protection, were carried out for all the treatments.

Data were collected on different agronomic traits, including plant height (PH), number of Node (Node), monopodial branches per plant (MNP) and sympodial branches per plant (SYP), number of green bolls per plant (GB), number of opened bolls per plant (OB) and Seed cotton yield (Yield). Plant height was measured with the help of a meter rod. These traits were assessed at physiological maturity. Fiber length (FL) was measured with the help of High-Volume Instrument (Uster HVI 1000). Net photosynthetic rate (Pn) was measured using Infrared gas analyzer (IRGA) equipment, CI-340 Handheld Photosynthesis System.

Data recording and analysis

The collected data were subjected to analysis of variance (ANOVA) using RCBD design to determine significant differences among genotypes, multiple comparisons, correlation coefficient, cluster analysis, and biplot analysis (Steel et al., 1997). Correlation analysis was conducted to explore the relationship between agronomic traits. Statistix 8.1, R/R Studio, as well as Microsoft Excel, were used to analyze the data and illustrate the graphs. Data was also recorded for the soil properties, on which experiment was conducted (Table 2).

Table 1: Genotype to be used in the study

Sr No.	Genotypes	Sr No.	Genotypes
1	Emp-1	17	SI-12
2	Emp-2	18	Emp-12
3	Amp-1	19	Emp-14
4	SI-21	20	Emp-16
5	Emp-3	21	Emp-17
6	SI-3	22	Emp-18
7	FMP-4	23	SI-20
8	Emp-5	24	SI-21
9	Emp-6	25	Emp-23
10	SI-7	26	SI-23
11	HB-1	27	SI-24
12	HB-2	28	Emp-25
13	HB-HSP-1	29	SI-25
14	SI-9	30	HB-HSP-2

15	SS-10	31	Emp-27
16	Emp-11	32	SI-28

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

Sr. No.	Soil Property	Value/Reading
1	Soil Texture	Clay Loamy
2	pH	7.9
3	EC	4.9 dSms
4	Organic Matter	0.74
5	Available P	7.6 ppm
6	Available K	84 ppm
7	Zinc	1.36 ppm

Results and Discussion

Analysis of Variance (ANOVA)

The data were subjected to analysis of variance (ANOVA) unveiled highly significant differences among studied plant. Highly significant differences were found for the studied plant traits i.e., Plant height (PH; 1154.32**), nodes per plant (NODES; 173.09**), monopodia (MNP; 2.00**), sympodia (SYMP; 220.06**), green bolls (GB; 32.26**), open bolls (OB; 445.22**), seed cotton yield (Yield; 4908156**), fiber length (FL; 1.51**), net photosynthetic rate (Pn; 9.72**), ginning out turn (GOT; 10.56**) under cotton genotypes (Table 3).

The highly significant ANOVA results for all studied traits confirm the presence of substantial genetic variability among the evaluated cotton genotypes, indicating strong potential for selection and crop improvement. Significant differences in plant height, nodes per plant, monopodia, and sympodia suggest variation in plant architecture and growth patterns, which directly influence reproductive development and boll formation. In cotton, sympodial branches are closely associated with fruiting sites and yield potential, highlighting the importance of architectural traits in productivity (Younas et al., 2025). Likewise, the highly significant variation observed for green bolls, open bolls, and seed cotton yield demonstrates that yield performance is strongly genotype-dependent, as yield is a complex trait governed by boll number, retention capacity, and assimilate partitioning (Hussain et al., 2024a). The significant differences in fiber length and ginning out turn further indicate the presence of genetic diversity for fiber quality traits, which are critical for spinning performance and economic value (Hussain et al., 2023a; Hussain et al., 2024b). Moreover, variation in net photosynthetic rate suggests physiological diversity among genotypes, and enhanced photosynthetic efficiency is known to contribute to greater biomass accumulation and yield formation (Javed et al., 2024). These findings confirm broad genetic variability within the germplasm, providing a solid foundation for breeding programs aimed at simultaneous improvement of yield, fiber quality, and physiological efficiency in cotton.

Table 3: Analysis of Variance for studied plant traits in thirty-two cotton genotypes

Source of Variation	Replication	Varieties	Error
Degre of Freedom (df)	*2*	*31*	*62*
Plant Height	295.7	1154.32^{**}	159.31
Monopodial Branches per Plant	0.78	2.00^{**}	0.42
Sympodial Branches per Plant	88.32	220.06^{**}	20.85

Nodes per Plant	58.15	173.09**	23.5
Open Bolls per Plant	159.87	445.22**	41.39
Ginning Out Turn	4.73E-28	10.56**	7.64E-31
Green Bolls per Plant	19.76	32.26**	5.84
Net Photosynthetic rate	0.34	9.72**	0.0003
Fiber Length	0.32	1.51**	1.64E-30
Seed Cotton Yield	5571	4908156**	11783

*Note: ** = significant at 1%; * = Significant at 5%, ns = non-significant*

Correlation Coefficient Analysis

The correlation coefficient analysis revealed the relationship among plant height, nodes per plant, monopodia, sympodia, green bolls, open bolls, yield, fiber length, net photosynthetic rate and ginning out turn for elite cotton genotypes evaluated under semi-arid conditions. Highly significant differences of yield were found with open bolls (r = 0.470*), green bolls (r = 0.455*), sympodial branches (r = 0.443*), monopodial branches (r = 0.424*), nodes per plant (r = 0.369*) and plant heigh (r = 0.446*) (Table 3). However, highly significant but negative correlation was also found between seed cotton yield and fiber length (r = -0.769*). Similarly, the relationship of fiber length was also negative correlated with sympodial branches (r = -0.398*) and green bolls (r = -0.369*). Highest correlation coefficient was also found between sympodial branches and nodes per plant (r = 0.976**) followed by open bolls and plant height (r = 0.890**), sympodial branches and plant height (0.875**).

Table 3: Correlation Matrix of studied plant traits in thirty-two cotton genotypes

Variables	PH	NODE	MNP	SYMP	GB	OB	Yield	Pn	GOT
NODE	**0.865**								
MNP	**0.533**	0.304							
SYMP	**0.875**	**0.976**	**0.359**						
GB	**0.643**	**0.592**	**0.521**	**0.601**					
OB	**0.890**	**0.862**	**0.670**	**0.873**	**0.683**				
Yield	**0.446**	**0.369**	**0.424**	**0.443**	**0.455**	**0.470**			
Pn	-0.067	-0.145	0.335	-0.089	0.214	0.109	0.183		
GOT	0.159	0.150	0.130	0.200	0.138	0.192	0.076	0.260	
FL	-0.341	-0.328	-0.314	**-0.398**	**-0.369**	-0.320	**-0.769**	-0.202	-0.071

The correlation analysis demonstrated strong and meaningful associations among morphological, yield, and physiological traits in the evaluated cotton genotypes under semi-arid conditions. Seed cotton yield exhibited significant and positive correlations with open bolls, green bolls, sympodial branches, monopodial branches, nodes per plant, and plant height, indicating that yield improvement is closely linked with enhanced vegetative growth and reproductive development. These findings suggest that traits contributing to greater fruiting sites and boll retention directly enhance productivity, which aligns with previous reports that boll number and fruiting branches are primary determinants of yield in cotton (Yousaf et al., 2024). The exceptionally high positive correlation between sympodial branches and nodes per plant further indicates that node development strongly influences fruiting branch formation, while strong associations between open bolls and plant height and between sympodial branches and plant height highlight the importance of vigorous plant architecture in supporting reproductive success (Yousaf et al., 2022).

Conversely, fiber length showed a highly significant and strong negative correlation with seed cotton yield, indicating a potential trade-off between yield and fiber quality traits. Negative associations of fiber length with sympodial branches and green bolls further suggest that increased reproductive load may reduce assimilate allocation to fiber elongation. Such inverse relationships between yield and fiber quality have been reported in cotton breeding programs, where selection for high yield may sometimes compromise fiber characteristics due to resource partitioning constraints (Bradow & Davidonis, 2000). The generally weak correlations of net photosynthetic rate (Pn) and ginning out turn (GOT) with yield indicate that while these traits contribute to overall performance, they may not serve as primary selection criteria under semi-arid conditions. The correlation matrix underscores the importance of fruiting and architectural traits for yield improvement while highlighting the need for balanced selection strategies to avoid compromising fiber quality.

Cluster Analysis

The cluster analysis categorized thirty-two cotton genotypes into two distinct clusters based on their performance across plant height, nodes per plant, monopodial branches, sympodial branches, green bolls, open bolls, fiber length, net photosynthetic rate, ginning out turn and seed cotton yield (Table 4). The dendrogram, using Euclidean distance and Ward's method, divided the genotypes into two clusters, with cluster-1 comprised of 13 genotypes and cluster-2 comprised of 19 genotypes shown in Figure 1 and Table 5. Cluster-1 gave the highest seed cotton yield (3238.714 kg/ha), greater number of open bolls (32.205), and highest net photosynthetic rate (27.715 µmol m^{-2} s^{-1}) (Table 4). Cluster-2 comprised of genotypes having lowest seed cotton yield (967.632 kg/ha), number of open bolls (22.649) and sympodial branches (25.561).

Table 4: Cluster means for studied plant train in cotton genotypes

Cluster	PH	NODE	MNP	SYMP	GB	OB	Yield	Pn	GOT	FL
1	132.6	36.15	0.87	32.1	9.23	32.2	3238.7	27.7	37.6	27.7
2	116.2	31.32	0.25	25.5	6.09	22.6	967.6	27.2	37.6	28.8

Table 5: Genotypes falling under their respective clusters

Cluster	No. of Genotypes	Genotypes under Respective Clusters
Cluster-1	13	Emp-1, Emp-2, Amp-1, SI-22, Emp-3, SI-3, FMP-4, Emp-5, HB-1, HB-2, HB-HSP-1, SI-20, HB-HSP-2
Cluster-2	19	Emp-6, SI-7, SI-9, SS-10, Emp-11, SI-12, Emp-12, Emp-14, Emp-16, Emp-16, Emp-17, Emp-18, SI-21, Emp-23, SI-23, SI-24, Emp-25, SI-25, Emp-27, SI-28

The cluster analysis effectively grouped the thirty-two cotton genotypes into two distinct clusters, indicating substantial genetic divergence among the evaluated material. Using Euclidean distance and Ward's method, the dendrogram clearly separated the genotypes into Cluster-1 (13 genotypes) and Cluster-2 (19 genotypes), reflecting differences in agronomic, physiological, and yield-related traits. Cluster-1 exhibited superior performance, characterized by higher seed cotton yield (3238.7 kg ha^{-1}), greater number of open bolls (32.2), higher sympodial branches (32.1), and comparatively higher net photosynthetic rate (27.7 µmol m^{-2} s^{-1}). These results suggest that genotypes in Cluster-1 possess favorable plant architecture and reproductive efficiency, contributing to enhanced productivity under semi-arid conditions. The association of higher yield with increased open bolls and sympodial branches aligns with established findings that fruiting structures and assimilate partitioning are primary determinants of yield performance in cotton (Pettigrew, 2004).

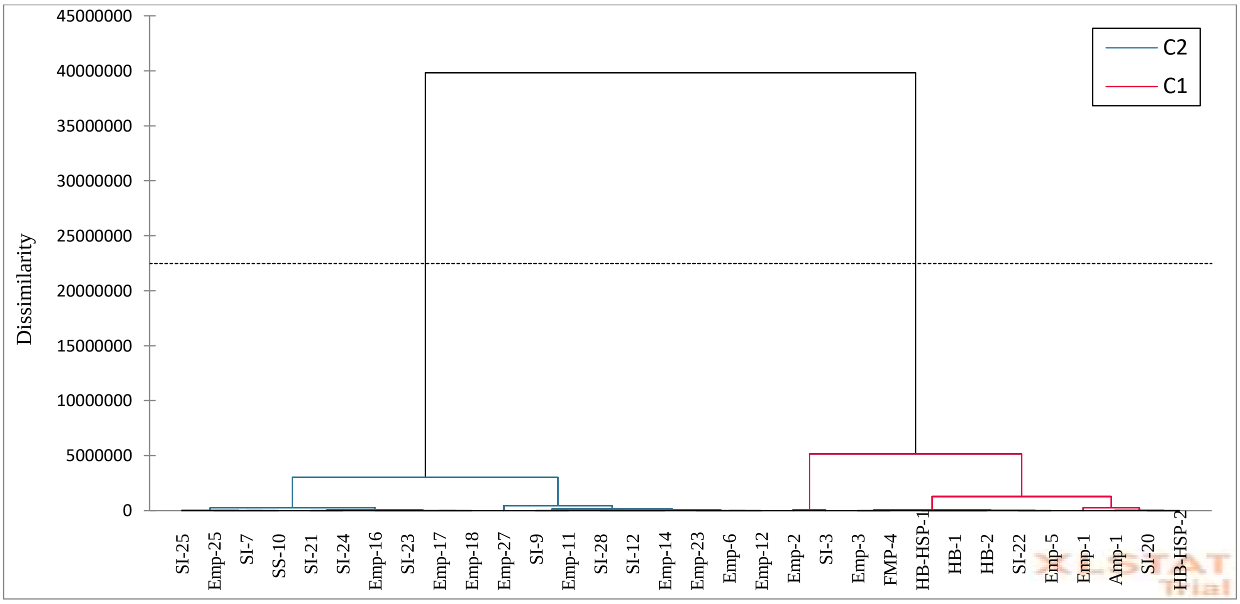

Figure 1: *Dendrogram based on cluster analysis in thirty-two cotton genotypes*

Moreover, the relatively higher plant height and node number in Cluster-1 further indicate vigorous growth, which may support improved canopy development and reproductive success.

In contrast, Cluster-2 comprised genotypes with comparatively lower seed cotton yield (967.6 kg ha^{-1}), fewer open bolls (22.6), and reduced sympodial branches (25.5), indicating comparatively weaker reproductive potential. Although fiber length was slightly higher in Cluster-2 (28.8 mm) compared to Cluster-1 (27.7 mm), this pattern supports the previously observed inverse relationship between yield and fiber length, suggesting a possible trade-off between productivity and fiber quality traits (Bradow & Davidonis, 2000). The minimal variation observed in ginning out turn and net photosynthetic rate between clusters indicates that yield differences were primarily driven by structural and reproductive traits rather than lint percentage or photosynthetic capacity alone. The clustering pattern highlights the presence of considerable genetic diversity among the genotypes and suggests that crosses between contrasting clusters may enhance heterosis and broaden the genetic base for future cotton breeding programs.

Principal Component Analysis (PCA)

The principal component analysis (PCA) provided an exhaustive understanding of the variation among thirty-two cotton genotypes under semi-arid climate, as demonstrated through eigenvalues, scree plot, correlation circle, variable contributions to PC-1 and PC-2, combined PCA biplot, individual observation plot. The scree plot explained that PC-1, PC-2 and PC-3 together explained a significant portion of divergence, with PC1 accounting for 50.996%, PC2 for 15.908% and PC3 for 11.505%, totaling 78.409% of the divergence, suggesting these components represent the primary sources of trait differentiation among the cotton genotypes. All other seven PCs collectively explain 21.591% of variation that is non-significant (Figure 2).

The correlation circle show that PC1 was strongly influenced by yield, open bolls, plant height, nodes per plant and sympodial branches, all of which were positively aligned along the positive PC-1 axis, while fiber length was negatively associated with PC-1. PC-2 was driven by net photosynthetic rate which is positively aligned, contrasting with fiber length on the negative PC-2 axis (Figure 3). The variable contribution to PC-1 and PC-2 confirmed that PH, Nodes, SYMP, OB contribute the most to PC-1 (>10%), while Node, Yield, Pn, FL contribute the most to PC-2 (>10%) (Table 7).

The combined PCA biplot and individual observations plot positioned genotypes like Emp-1, SI-3, SI-22 along the positive PC-1 axis, close to Yield, Monopodial branches and green boll, open boll, indicating their high yielding potential, while SI-22, SI-24 and SS-10 were exhibited superior fiber length and had lower seed cotton yield (Figure 4&5). The PCA results reveal the genetic diversity among the genotypes like Emp-1, SI-3, SI-22 for high productivity.

Table: 6 Eigenvalues, Individual and Cumulative Variability in ten Principal Components

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigenvalue	5.10	1.59	1.15	0.88	0.50	0.39	0.22	0.12	0.04	0.02
Variability (%)	51.00	15.91	11.51	8.75	4.97	3.92	2.22	1.18	0.38	0.17
Cumulative %	51.00	66.90	78.41	87.16	92.13	96.05	98.27	99.45	99.83	100.00

Table: 7 Contribution of variables in five Principal Components

Plant Parameters	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Plant Height	16.221	4.268	0.332	0.321	0.945
Nodes per Plant	14.877	10.732	0.069	1.519	4.576
Monopodial Branches per Plant	8.067	8.482	2.537	25.022	37.604
Sympodial Branches per Plant	16.030	6.673	0.033	2.544	2.412
Green Bolls per Plant	11.793	0.783	0.572	4.626	17.245
Open Bolls per Plant	17.297	0.922	2.417	2.740	0.524
Seed Cotton Yield	8.273	11.263	20.600	3.564	2.839
Net Photosynthetic rate	0.300	40.222	10.551	2.147	26.700
Ginning Out Turn	1.050	3.712	36.850	47.113	7.131
Fiber Length	6.091	12.942	26.039	10.401	0.022

The principal component analysis (PCA) effectively summarized the multivariate variation among the thirty-two cotton genotypes under semi-arid conditions, revealing that the first three principal components (PC1, PC2, and PC3) accounted for 78.41% of the total variability, with PC1 alone explaining 50.99% of the divergence. This high proportion of variance captured by the first component indicates that a substantial part of genetic differentiation among genotypes is governed by yield-related and architectural traits. The correlation circle and variable contribution analysis demonstrated that PC1 was primarily influenced by plant height, nodes per plant, sympodial branches, open bolls, and yield, all positively aligned, suggesting that this component represents a productivity axis.

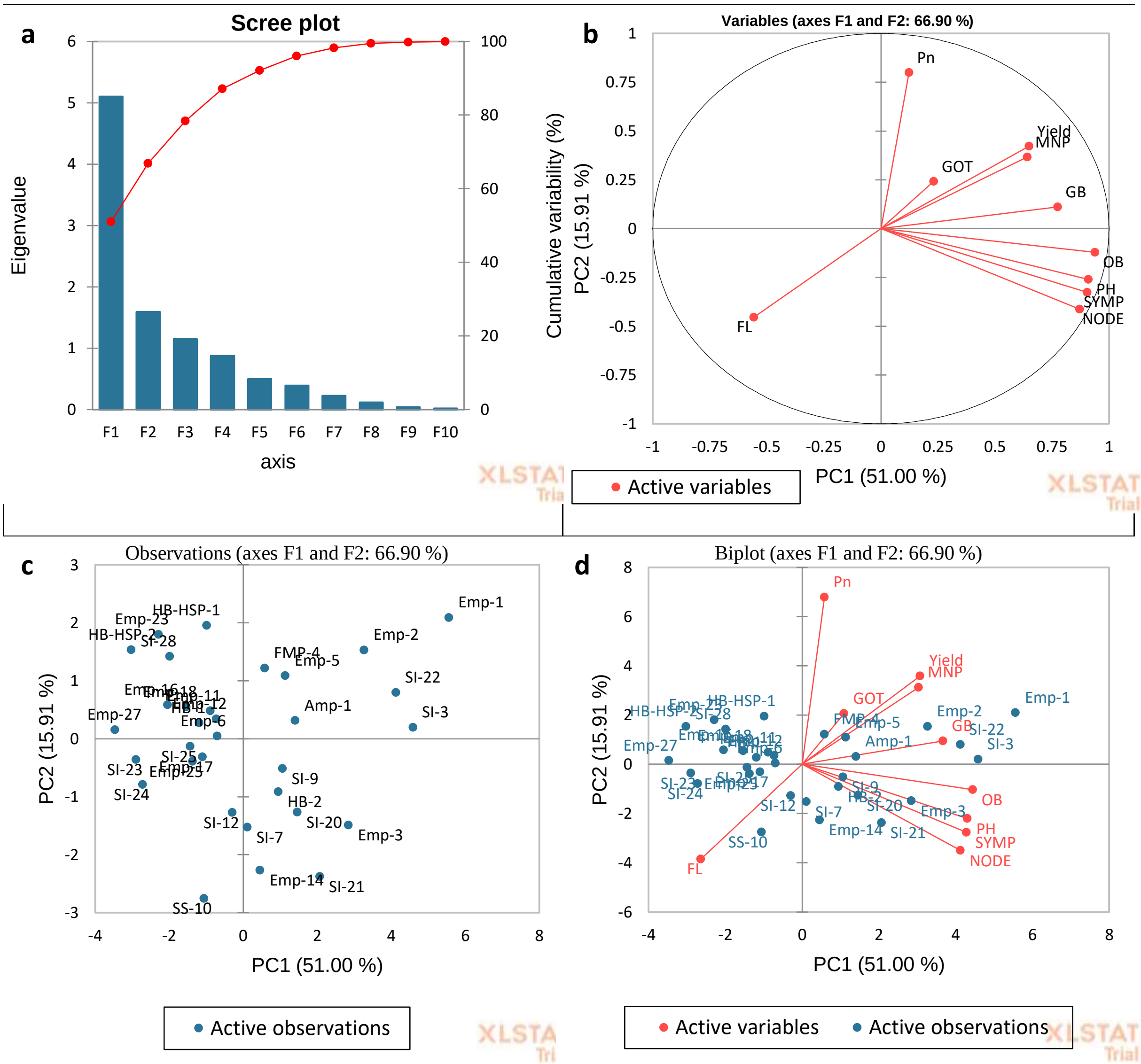

Figure 2: Principal Component Analysis in thirty-two cotton genotypes **(a)** Screeplot of Principal Component Analysis **(b)** Variable biplot **(c)** Observation biplot **(d)** Combined PCA Biplot

In contrast, fiber length showed a negative association with PC1, reinforcing the previously observed trade-off between yield and fiber quality. PC2, explaining 15.91% of the variation, was largely driven by net photosynthetic rate (40.22% contribution), along with notable contributions from fiber length and yield, indicating a physiological–quality dimension of variability. The substantial contribution of ginning out turn and fiber length to PC3 further highlights the role of fiber-related attributes in differentiating genotypes beyond yield components. Such patterns are consistent with earlier studies reporting that yield and fiber traits often load differently across principal components due to their complex genetic and physiological control (Iqbal and Khan, 2008; Khan et al., 2009).

The combined PCA biplot and observation plots provided clear visualization of genotype grouping and trait associations. Genotypes such as Emp-1, SI-3, and SI-22 were positioned along the positive PC1 axis in close proximity to yield, open bolls, and monopodial and sympodial branches, indicating their superior yield potential and favorable plant architecture. Conversely, genotypes like SI-22, SI-24, and SS-10 were associated with higher fiber length but comparatively lower yield, further confirming the inverse relationship between productivity and fiber quality traits. The spatial separation of genotypes across principal components reflects considerable genetic diversity within the studied material, suggesting opportunities for selecting contrasting parents for hybridization to exploit heterosis and broaden the genetic base. The PCA proved to be an effective tool for identifying key traits contributing to variability and for distinguishing high-yielding genotypes from fiber-quality-oriented lines, thereby facilitating strategic selection in cotton breeding programs.

Conclusion

The present study demonstrated substantial genetic variability among the evaluated cotton genotypes under semi-arid climatic conditions, providing valuable opportunities for selection and genetic improvement. Yield performance was primarily governed by yield-contributing traits such as open bolls, green bolls, sympodial branches, nodes per plant, and plant height, emphasizing their importance as key selection criteria in breeding programs. The observed negative association between seed cotton yield and fiber length suggests a trade-off between productivity and fiber quality, which should be carefully managed in future varietal development. Multivariate analyses, including cluster analysis and principal component analysis, effectively differentiated the genotypes and identified superior performers. Genotypes grouped in Cluster-1, particularly Emp-1, SI-3, and SI-22, exhibited higher yield potential, improved boll retention, and favorable physiological performance, making them promising candidates for varietal release or use as parental material in hybridization programs. Overall, the findings provide a robust scientific basis for selecting high-yielding and adaptable cotton genotypes and contribute to the development of improved cultivars suited for semi-arid regions.

Author's Contribution

MIY; *Conceptualization and Execution,* **MJ**; *Supervision, Planning,* **MIY, AR, MH, NK, SH**: *Data retrieval, Writing – original draft;* **IA, SWHS, SS**; *Writing – review & editing,* **MS, GM**; *Data Visualization and Analysis,* **MI**; *Soil Data Analysis*

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