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Research Article

Morpho-physiological and fiber quality evaluation of cotton genotypes under normal sowing condition

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is a key fiber crop with significant economic importance, yet Pakistan has recently experienced declining production and yield. There is a need to evaluate cotton germplasm with enhanced yield and quality traits. For this purpose, 79 cotton genotypes were sown at the Cotton Research Station, Vehari under Randomized Complete Block Design (RCBD) to assess morphological, physiological, and fiber quality traits, including plant height, bolls per plant, sympodial branches, boll weight, ginning outturn, seed cotton yield, cell membrane thermostability, pollen viability, fiber length, fiber strength, and fineness. Significant phenotypic variation was observed among genotypes for most traits. Correlation and principal component analysis revealed strong positive associations among yield-related and physiological traits, while fiber quality traits clustered separately. Heat map and mean value analyses identified superior genotypes, with G5, G24, G32, G44, G46, G52, and G60 exhibiting the highest fiber strength and promising performance for seed cotton yield, pollen viability, and cell membrane thermostability. These genotypes constitute an important gene pool for breeding programs focused on improving yield, fiber quality, and heat stress tolerance in cotton. The study provides a foundation for selecting superior parents for future cotton improvement under normal and stress-prone environments.

Keywords: Biplot analysis; fiber quality; *Gossypium hirsutum*; heat map; physiological traits.

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INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is considered an important fibre crop, forming the backbone of the textile industry and contributing significantly to the agricultural economies of many countries (Li et al., 2019). Within this broader context, the gene pool of cotton offers a rich repository of morphological, agronomic, and fibre-related traits that can be harnessed for crop improvement (Kumar et al., 2025; Majeed et al., 2024). The present study seeks to evaluate and document the diversity present in cotton germplasm by assessing key morphological and fibre quality parameters. Such characterization is vital because effective utilization of the gene pool depends on understanding trait variability, heritability, interrelationships among traits, and their responses to environmental stresses (Iqbal et al., 2017; Saleem et al., 2023). A well-characterized germplasm set enables breeders to select parents with complementary traits, devise crossing strategies aimed at improving yield and quality, and incorporate novel traits into elite cultivars (Anwar & Saleem, 2024). In Pakistan, cotton has long been regarded as "white gold" due to its major role in raw-

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material supply to the textile industry, employment generation, and foreign-exchange earnings (Malik et al., 2022). Approximately 70 % of cotton cultivation occurs in the greater Punjab region, 25–30 % in Sindh, and smaller percentages in Khyber Pakhtunkhwa and Balochistan. Recent data show a troubling decline in cotton production and yield in Pakistan: for example, national production decreased to around 5.52 million bales in 2024–25, marking a drop of about one-third compared with 8.39 million bales in the preceding year (Mahmood, 2025). These trends indicate an urgent need for germplasm renewal and trait-based breeding, particularly for stress resilience and fibre quality enhancement (Kumar et al., 2025; Yousaf et al., 2023).

Heat stress represents a major threat to cotton growth, reproduction, and yield, especially in warm cotton-growing regions such as Pakistan (Farooq et al., 2021). High temperature adversely affects morphological development, reproductive stage and fibre development (Sarwar et al., 2023). It is therefore imperative that germplasm characterization also consider performance under elevated temperature regimes, and that breeding efforts incorporate thermotolerance traits (Iqbal Khan et al., 2008).

Two physiological/biochemical parameters central to heat-stress resilience are cell membrane thermostability (CMT) and pollen viability. These serve not only as screening criteria for heat tolerance but also as mechanistic links between high-temperature exposure and yield loss (Abro et al., 2022; Dev et al., 2025). In Pakistan, cotton-growing seasons often experience day-time temperatures of 40–45 °C (and sometimes exceeding 47 °C) combined with elevated humidity during critical reproductive phases. Studies show that elevated temperature at reproductive stages is one of the prime reasons for yield reduction in Pakistan's cotton crop (Majeed et al., 2023). At the morphological and physiological level, heat stress reduces plant height, node number, branch number, boll number, and boll weight; it also adversely affects fibre quality parameters such as staple length, fineness, and strength (Hamidi et al., 2018; Farooq et al., 2023). One major effect of high temperature is disruption of cell membrane integrity and heat causes increased membrane fluidity, protein denaturation, oxidative damage, and increased leakage of electrolytes (Devi, 2023). The parameter of CMT or relative cell injury (RCI) is used to assess the extent of heat induction membrane leakage (Rehman et al., 2021; Zhang et al., 2023; Luqman et al., 2025).

Thus, during characterization of germplasm, CMT and pollen viability offers robust physiological criteria for heat tolerance. In view of this scenario, this study's focus on gene-pool characterization becomes timely: by identifying and germplasm development with improved morphological, fibre, and heat tolerance traits (Shani et al., 2025; Malik et al., 2022). The characterization of cotton germplasm on morphological and fibre-related traits is a foundational step in the breeding pipeline for improved cultivars. The importance of heat stress, particularly via mechanisms of cell membrane thermostability and pollen viability, cannot be overstated in the current climate scenario (Puppala et al., 2023; Arif et al., 2023). Finally, by situating the study within the real-world context of declining cotton production and yield in Pakistan, we underscore the urgency and relevance of this investigation (Mahmood, 2025). There is a dire need to characterize the current cotton germplasm to explore the genetic architecture and quality parameters. The main objectives of the study involved the evaluation of present cotton genepool and incorporate it into breeding programs. The information acquired from germplasm characterization will serve breeders, physiologists, and agronomists alike in designing cross-breeding strategies, selection protocols, and ultimately delivering cotton varieties that can withstand rising temperatures, deliver higher yields, and meet fibre-quality demands.

MATERIALS AND METHODS

The current study was planned at the Cotton Research Station (CRS), Vehari (Longitude 72.3587°E, Latitude 30.03454°N) Punjab, Pakistan, during February to August, 2025 an early cotton growing season. The temperature was around 17-20 °C at sowing time and 35-40 °C at harvesting time. The most rainfall was recorded at the month of July i.e., 45mm. The experimental material consisted of a diverse gene pool of cotton (*Gossypium hirsutum* L.), comprising 79 genotypes including advanced breeding lines, commercial cultivars and germplasm accessions maintained at CRS, Vehari (Table 1.). These genotypes encompass a wide range of gene pool for morphological, physiological, and fiber related parameters.

Experimental design

The experiment was conducted in a Randomized Complete Block Design (RCBD) with triplicates. Each genotype was sown in a plot consisting of three rows, each 5m long, includes 30 cm spacing for plant to plant and 75 cm for row to row. Standard agronomic practices recommended for cotton cultivation in the region were followed throughout the growing season, including fertilizer application, irrigation, and pest management. No growth regulators were applied to avoid confounding effects.

Table 1. List of cotton genotypes used in this study.

Genotype#	Name	Genotype#	Name	Genotype#	Name
G1	326	G27	Dr-Chessi	G53	TIPU-2
G2	1606	G28	FH-142	G54	TMA-1
G3	2085	G29	FH-444	G55	TIPU-1
G4	2307	G30	L-88	G56	US-3116/16
G5	6050	G31	M-3	G57	US-3090
G6	ABC-F-50	G32	M-5	G58	US-58
G7	ABC-Nazeer	G33	M-6	G59	V-3
G8	Acala-1571	G34	MNH-886	G60	VR-33
G9	AC-252	G35	MNH-SG	G61	VH-36
G10	B-64	G36	NEXT	G62	VH-58
G11	B-221	G37	NIAB-1011	G63	VH-189
G12	Bahar	G38	NIAB-545	G64	VH-206
G13	BS-18	G39	NCVT-13/14	G65	VH-259
G14	BTA-1	G40	N-874	G66	VH-285
G15	BT-150	G41	NIBGE-6	G67	VH-289
G16	China-12	G42	NIAB-112	G68	VH-291
G17	China-24	G43	NIAB-444	G69	VH-306
G18	Cyto-313	G44	OKRA-548	G70	VH-319/5
G19	CIM-775	G45	PCCT-16/15	G71	VH-345
G20	CEMB	G46	PRS-72	G72	VH-380
G21	CKC-3	G47	QSR-189	G73	VH-390
G22	CKC-6	G48	Rustam-2	G74	VH-392
G23	CR-276	G49	Roohi	G75	VH-401
G24	CH-38	G50	Super-Shaheen	G76	VH-478
G25	CH-43	G51	Tahafaz-12	G77	VH-446
G26	DH-SA-3	G52	Thakar-808	G78	Wheal-ag-9
G79	ZM-578				

Morphological characterization

Morphological data were recorded on five randomly selected plants per plot for each genotype at the flowering and maturity stages. The following traits were measured according to standard descriptors for cotton like height of the plant (cm), sympodial branches, bolls count for individual plant, yield of seed (g) and weight of the boll (g).

Cell membrane thermostability (CMT)

Cell membrane thermostability was determined following the electrolyte leakage method described by Blum and Ebercon (1981) with slight modifications. Fully expanded young leaves were collected from each genotype. Leaf discs (1 cm diameter) were rinsed with deionized water and kept in test tubes having distilled water of 10 mL.

- Heat treatment: One set of samples was incubated at 50°C for 30 minutes, while a control set remained at 25°C.
- Conductivity measurement: After cooling to room temperature, the initial electrical conductivity (C_1) was measured using a digital conductivity meter. Samples were then autoclaved at 121°C for 15 minutes to obtain total conductivity (C_2).
- CMT (%) was calculated as:

CMT (%)={1-(T1/T2)]/[1-(C1/C2)]} ×100

Where T_1 and T_2 represent the heat-treated readings, and C_1 and C_2 are the control readings.

Pollen viability

Pollen viability was assessed using the 1% acetocarmine solution technique (Rathod et al., 2018). Freshly dehisced pollen grains were collected from three flowers per genotype at anthesis. A small amount of pollen was placed on a glass slide, stained with 1% acetocarmine solution, and covered with a coverslip. Observations were made under a compound microscope at 400× magnification. Pollen grains that stained dark red and retained normal shape were counted as viable, whereas shriveled or unstained grains were considered non-viable. Pollen viability (%) was calculated as:

Pollen viability (%)=Number of viable pollen/Total pollen grains observed×100

Fiber quality analysis

Fiber samples were collected from five randomly selected bolls per genotype at maturity. The samples were ginned using a laboratory roller gin, and fiber traits were analyzed using the High Volume Instrument (HVI) at the Cotton Research Institute, Multan. The following parameters were recorded:

Ginning out turn (GOT, %)
Fiber length (mm)
Fiber strength (g tex^{-1})
Micronaire value (µg inch^{-1})

Statistical analysis

The recorded traits were analyzed using analysis of variance (ANOVA) using RCBD to test the significance of genotypic differences (Steel et al. 1997). Correlation analysis among morphological, physiological, and fiber quality traits was performed using Pearson's correlation coefficients to assess relationships among parameters. To explore patterns of variation and identify key contributing traits, Principal Component Analysis (PCA) was performed using the correlation matrix of standardized data. A biplot analysis based on the first two principal components was generated to visualize the grouping of genotypes and the contribution of different traits to overall variation. All statistical analyses were performed using R so software ware version 3.6.3.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) exhibited significant differences among genotypes for most of the morpho-physiological and fiber quality traits evaluated (Table 2). Mean square values for the sources of variation indicated that genotypes contributed substantially to the total variation observed. Significant genotype effects ($P < 0.01$ or $P < 0.05$) were detected for height of plant (PH), sympodial branches (SM), bolls per plant (BPP), cell membrane thermostability (CMT), pollen viability (PV), weight of the boll (BW), seed yield of cotton (SCY), ginning out turn (GOT), fiber lengths (FL), fiber strengths (FS), and fiber fineness (FF). The significant mean squares for these traits suggest the presence of considerable genetic variability within the tested material, indicating strong potential for selection and genetic improvement.

Table 2. Mean square values of various morpho-physiological and fiber traits.

SOV	DF	PH	SM	BPP	CMT	PV	BW	SCY	GOT	FL	FS	FF
Rep	2	554.74	7.9789	164.06	16.657	32.771	0.0083	1181.5	10.713	12.632	34.732	0.2291
Gen	78	1875.2**	12.256**	365.72**	61.932*	359.19*	0.1239*	2770.4**	57.274*	17.263*	72.092*	0.5213*
Error	156	455.66	2.5174	90.337	24.499	8.9321	0.0117	602.53	11.956	9.623	22.833	0.0233
CV%		9.4	15.23	17.6	19.1	12.78	15.78	11.32	22.17	17.39	16.29	18.28
Total	236											

Where *P < 0.05, **P < 0.01 and ***P < 0.001 SOV= Sources of variation; Gen= Genotype; DF= Degree of freedom; SM= Number of sympodial branches; BPP=Boll per plant; CMT= Cell membrane thermostability; PV= Pollen Viability; BW= Boll weight; SCY= Seed cotton yields; GOT= Ginning out turns; FL= Fiber lengths; FS= Fiber strengths; FF= Fiber fineness.

Correlation analysis of morphological and yield traits under normal conditions

The correlation matrix (Fig. 1) illustrates the interrelationships among the morphological and yield-related traits of the genotypes evaluated under normal conditions. The correlation coefficients range from negative to positive values, depicted by the color intensity and size of the circles. Blue circles indicate positive correlations, while pink shades denote negative associations. The size of each circle represents the strength of the relationship between traits, and asterisks inside the circles indicate statistical significance at $P < 0.05$.

A strong and significant positive correlation was observed among most morphological and yield traits. Plant height (PH) exhibited a highly significant association with the sympodial branches (SM), bolls per plant (BPP), and seed cotton yield (SCY), indicating that taller plants with more sympodial branches tend to produce more bolls and higher yield. Similarly, boll per plant (BPP) and BW showed a positive relationship with SCY, emphasizing their contribution to yield improvement. GOT was positively correlated with SCY, reflecting that plants with higher yield also exhibited better lint percentage.

Among the physiological traits, pollen viability (PV) showed a strong positive association with cell membrane thermostability (CMT), suggesting that both traits contribute jointly to stress tolerance and plant vigor. In terms of fiber quality parameters, fiber length (FL) and fiber strength (FS) were significantly and positively correlated, indicating that improvement in one trait could enhance the other. However, fiber fineness (FF) exhibited weak or negative associations with fiber strength and fiber length, implying a potential trade-off among fiber quality attributes.

Overall, the results indicate that morphological parameters like PH, SM, and BPP are key contributors to seed cotton yield, while physiological traits (PV and CMT) and fiber quality traits (FL and FS) exhibit close internal associations. These findings highlight that simultaneous selection for these positively correlated traits could be an effective strategy in cotton improvement under normal growing conditions.

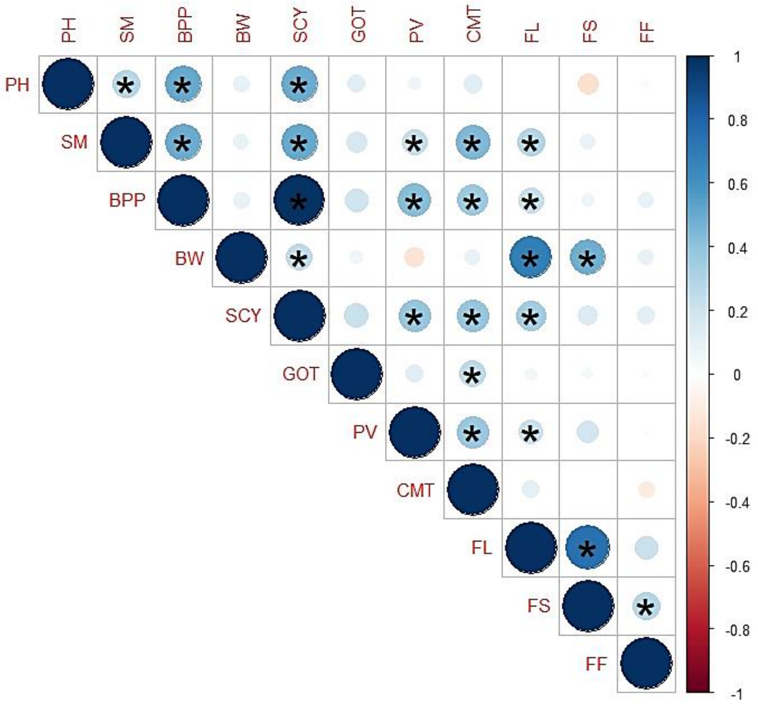


Figure. 1. Correlation plot of morphological and yield traits viz Plant height (PH), Number of sympodial branches (SM), Boll per plant (BPP), Boll weight (BW), Seed cotton yield (SCY), Ginning out turn (GOT), Pollen Viability (PV), Cell membrane thermostability (CMT), Fiber lengths (FL), Fiber strengths (FS) and Fiber fineness (FF) grown under normal condition. Blue shading represents positive correlations, whereas pink shading represents negative correlations. The circle size indicates the degree of association among traits. More size means strong association. The star within the circle indicates the level of statistical significance.

Biplot analysis

The biplot (Figure 2) illustrates the relationships among eleven morphological, physiological, and fiber quality traits in seventy-nine cotton genotypes evaluated under normal field conditions. The first two principal components PC1 and PC2 contributed for 32.1% and 19.6% of overall variation respectively, explained a substantial proportion of the total phenotypic variability among the genotypes. The circle in the plot represents the theoretical maximum extent of the variable vectors, corresponding to a 68% confidence interval, the direction and length of the arrows reflect the contribution of each trait and their correlations with the principal components.

Traits such as SCY, BPP, SM, PH, GOT, PV and cell membrane thermostability (CMT) were closely grouped and oriented in the same direction along PC1, suggesting a strong and positive interrelationship among these traits. This pattern indicates that genotypes with higher values for these morphological and yield attributes are associated with better physiological performance under normal growing conditions.

Conversely, fiber-related traits including fiber strengths (FS), fiber lengths (FL) and fiber fineness (FF) were positioned in a distinct direction, mainly contributing to PC2. This distribution reveals that fiber quality parameters form a separate group of variation, independent of yield-related attributes. The vectors of FL and FS were oriented closely together, suggesting a positive association between these traits, whereas FF showed a somewhat opposite direction.

The distribution of genotypes across the biplot reflects the diversity present within the studied material. Genotypes located near the direction of specific trait vectors exhibited higher expression for those corresponding traits, while those positioned opposite to the vectors showed lower expression. The genotypes G5, G24, G32, G44, G46, G52, and G60 exhibited higher expression for seed cotton yield (SCY), boll weight (BW), plant height (PH), cell membrane thermostability (CMT) and pollen viability (PV). The results emphasize that morphological and yield traits are closely associated with physiological parameters, while fiber quality traits contribute separately to the total genetic diversity under normal environmental conditions.

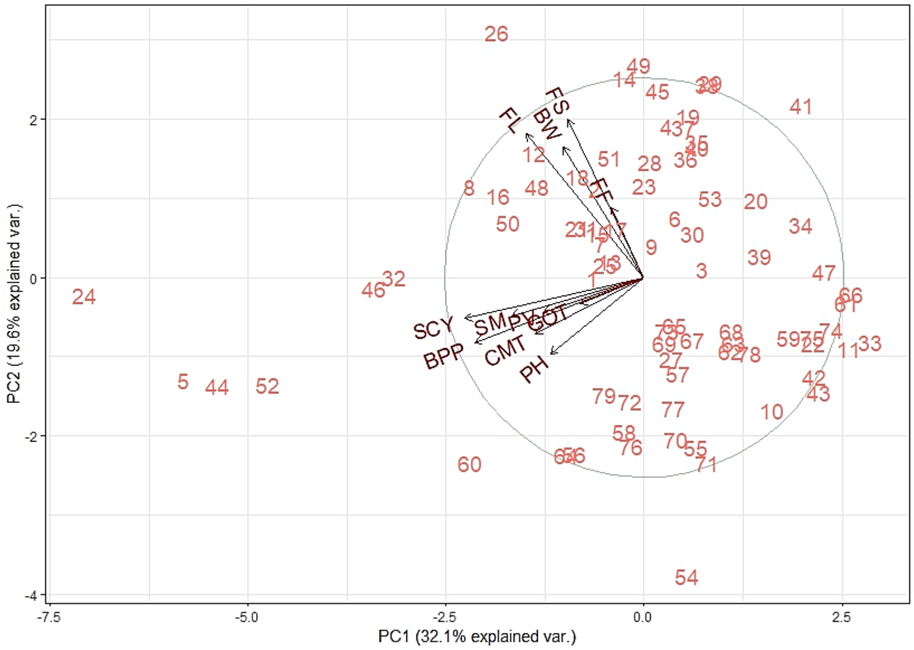


Figure 2. Biplot analysis for morphological and yield traits viz Plant height (PH), Number of sympodial branches (SM), Boll per plant (BPP), Boll weight (BW), Seed cotton yield (SCY), Ginning out turn (GOT), Pollen Viability (PV), Cell membrane thermostability (CMT), Fiber lengths (FL), Fiber strengths (FS) and Fiber fineness (FF) of 79 cotton genotypes under normal field conditions. The circle represents the theoretical maximum length of the arrows, based on a default 68% confidence interval. The arrows indicate the correlations among traits within their respective environments.

Performance heatmap of cotton genotypes

The performance heatmap (Figure 3) presents the mean values of eleven morphological, physiological, and yield-related traits evaluated across 79 cotton genotypes under normal field conditions. The analyzed traits include PH, BW, GOT, SM, FL, BPP, FF, CMT, SCY, FS, and PV.

The color scale represents the relative magnitude of each trait, with purple shades indicating lower mean values and yellow shades denoting higher mean values. Substantial phenotypic variation was observed among genotypes, particularly for PH, SCY, PV, and CMT, suggesting considerable genetic diversity for these traits. In contrast, fiber quality traits such as FL, FS, and FF displayed relatively uniform color intensities, indicating limited variation among genotypes under normal growing conditions. Among the evaluated genotypes, G5, G24, G32, G44, and G46 demonstrated superior performance for key yield-contributing traits, notably PH, SCY, and CMT, as indicated by their higher intensity values on the heatmap. These genotypes can be considered as potential candidates for yield improvement and stress tolerance breeding. Conversely, several genotypes displayed consistently lower mean values across traits, indicating relatively poor performance under normal field conditions.

Overall, the heatmap provides a comprehensive visualization of genotypic performance patterns across multiple traits, facilitating the identification of high-performing and stable genotypes. Such graphical representation is valuable for recognizing key traits contributing to variability and for selecting superior genotypes in cotton improvement programs.

Mean values of studied genotypes

The analysis of 79 cotton genotypes under normal field conditions revealed significant variation in fiber strength (FS). Among the genotypes, G5, G24, G32, G44 and G60 exhibited the highest fiber strength (FS) values, with measurements reaching approximately 27 g tex⁻¹ or higher, indicating superior fiber quality. The genotypes in the purple cluster (left side of the graph) exhibited the highest fiber strength values, consistently ranging between 25 to 27 g tex⁻¹, indicating superior fiber quality. Meanwhile G5, G24, G32 and G63 showed maximum mean values for boll weight (BW), seed cotton yield (SCY), bolls per plant (BPP) and pollen viability (PV) (Figure 4).

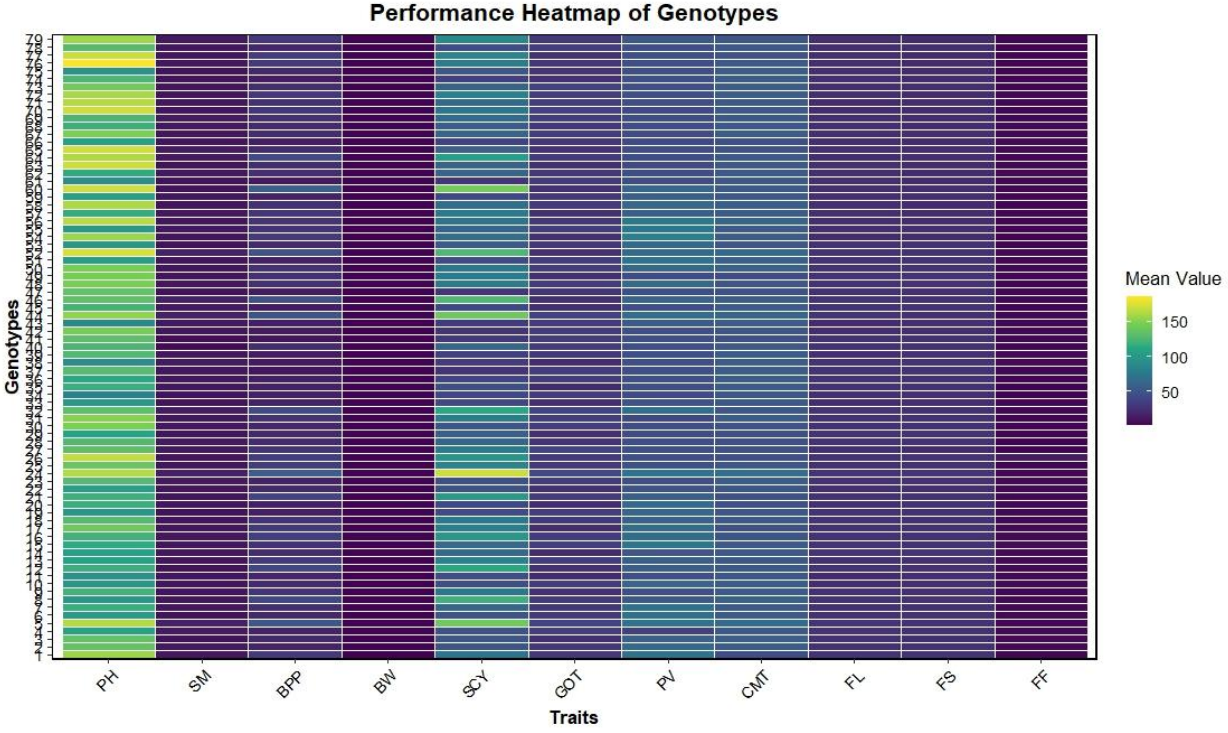
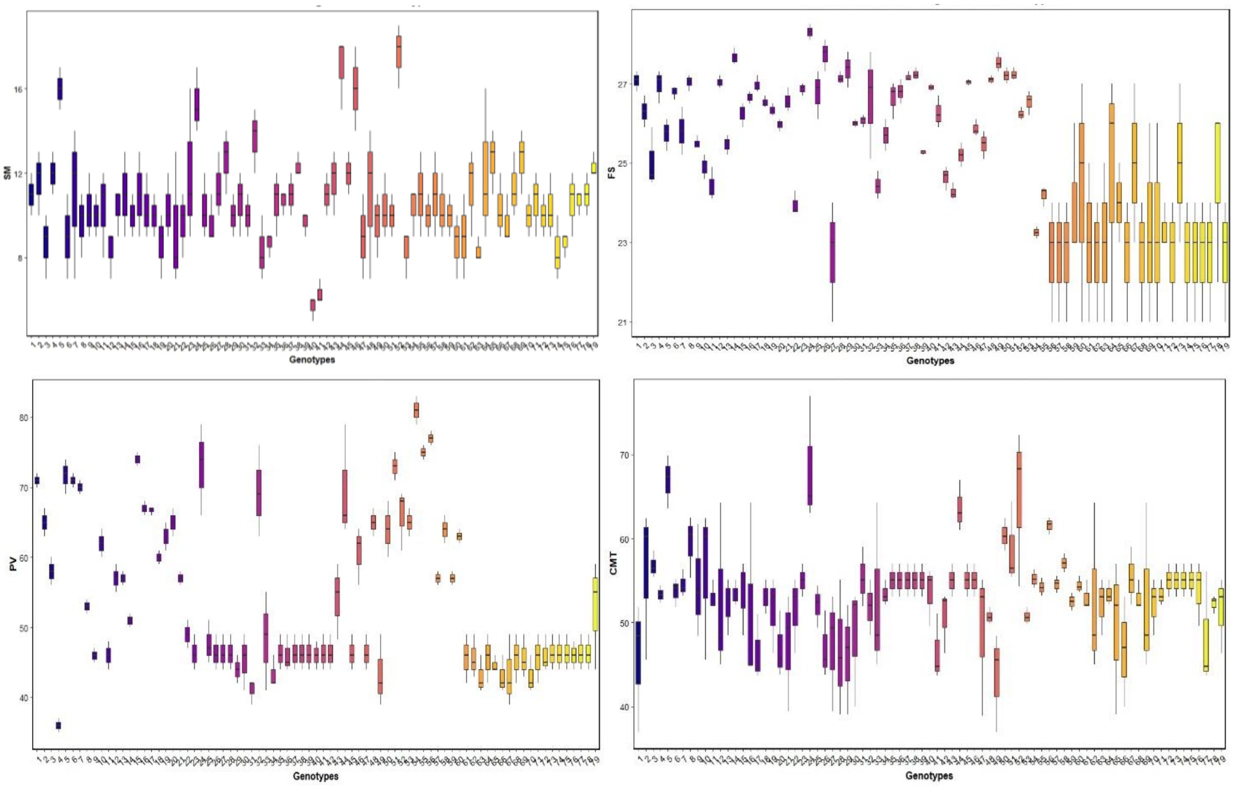


Figure 3. Performance heatmap for morphological and yield traits viz Plant height (PH), Sympodial branches (SM), Boll count per plant (BPP), Bolls weight (BW), Seed cotton yields (SCY), Ginning out turn (GOT), Pollen Viability (PV), Cell membrane thermostability (CMT), Fiber lengths (FL), Fiber strengths (FS) and Fiber fineness (FF) of 79 cotton genotypes under normal field conditions.



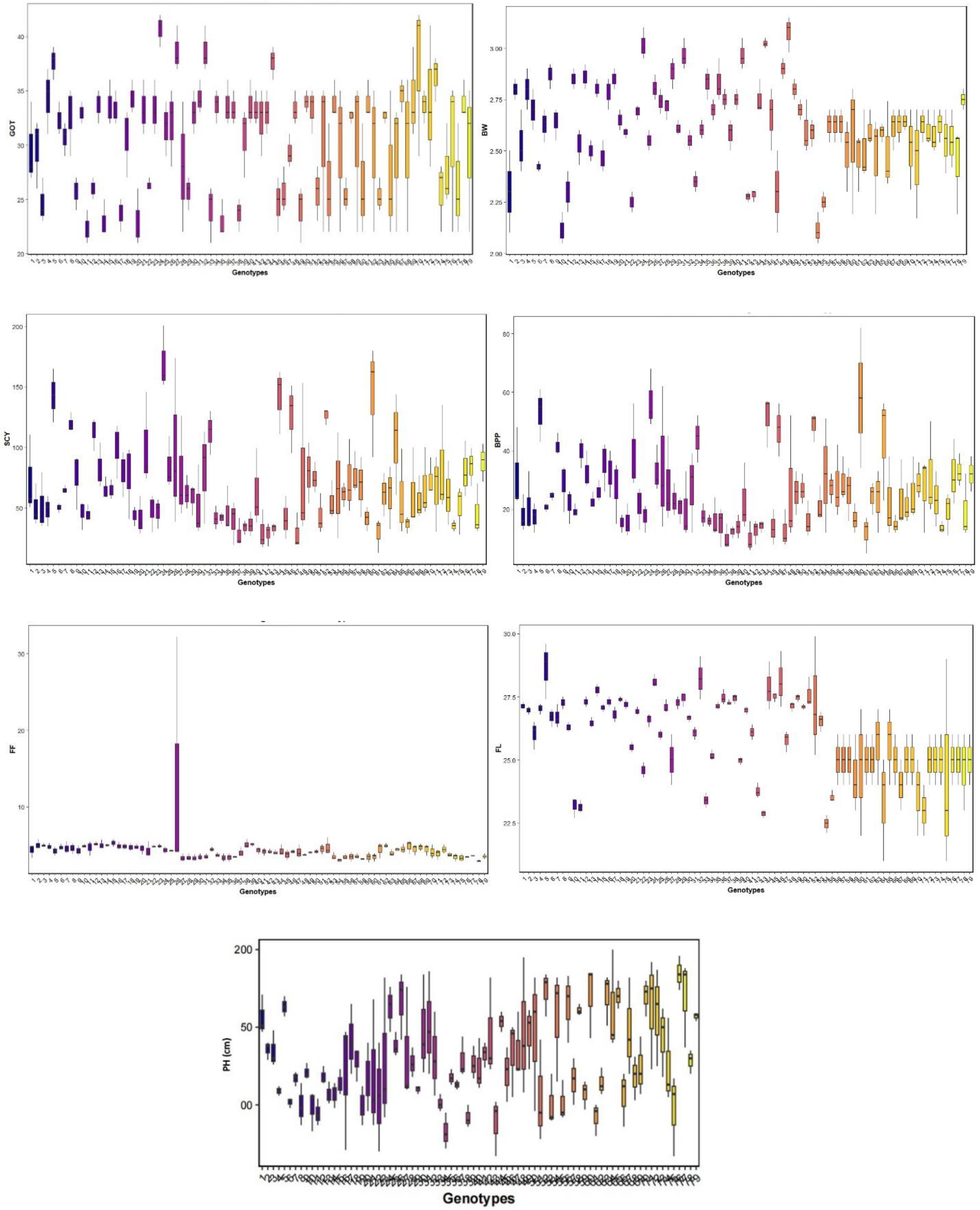


Figure 4. Mean values of studied genotypes regarding morphological and yield traits viz Plant height (PH), Sympodial branches (SM), Bolls per plant (BPP), Boll weight (BW), Seed cotton yields (SCY), Ginning out turns (GOT), Pollen Viability (PV), Cell membrane thermostability (CMT), Fibers lengths (FL), Fibers strengths (FS) and Fibers fineness (FF) of 79 cotton genotypes under normal field conditions.

The present study of 79 genotypes of *Gossypium hirsutum* from the germplasm set maintained at the CRS Vehari has demonstrated substantial variation in morphological, physiological (CMT, pollen viability) and fibre-quality traits under normal growing conditions. This supports the premise that a well-characterized gene pool offers valuable diversity for breeding, as previously emphasized (Li et al., 2019; Kumar et al., 2025). The significant genotypic differences observed for PH, SM, BP and BW reflect the underlying genetic variability that breeders can exploit. These results align with earlier work indicating that morphological trait variation plays vital role for germplasm characterization (Saleem et al., 2023).

The strong positive correlations detected among morphological yield-contributing traits (PH, SM, BP and SCY) underline their joint contribution to yield in upland cotton. This pattern is in accordance with findings of earlier cotton diversity studies, which reported that higher node/branch counts and boll numbers often translate into improved seed cotton yield (Li et al., 2019). Importantly, the positive association between physiological traits (pollen viability and CMT) and morphological/yield traits suggests that heat-stress-resilient mechanisms may be co-optimized with yield potential in cotton germplasm. This supports the idea that selection for thermotolerance need not compromise yield potential when suitable germplasm is selected (Majeed et al., 2024).

With respect to fibre quality, the observed positive correlation between fibre length and fibre strength indicates that improvement in one trait may enhance the other consistent with other upland cotton studies (Side et al., 2025). The weaker or negative relationships of fineness (micronaire) with length and strength reveal a trade-off scenario, as documented in previous fibre-quality research (Wang et al., 2024). This underscores the challenge common to cotton breeding: improving yield while maintaining or enhancing fibre quality without compromising other traits.

One of the study's noteworthy insights is the apparent grouping of the physiological traits (CMT, pollen viability) together with morphological/yield traits in the first principal component, whereas the fibre quality traits contributed separately (PC2) (Majeed et al., 2024). This suggests that the genetic and physiological basis of yield/thermotolerance variation may be somewhat distinct from that of fibre quality. For breeding programmes in Pakistan, this implies that dual improvement of yield/thermotolerance and fibre quality may require selection in parallel rather than assuming strong inherent coupling. It aligns with recent multi-omics and physiological reviews noting that heat-stress tolerance in cotton often involves distinct pathways (photosynthesis, antioxidant systems, HSPs) that may not directly overlap with fibre-quality controlling genes (Ijaz et al., 2024; Luqman et al., 2025).

In the context of Pakistan's major cotton growing areas where day time temperatures often reach 40–45 °C and sometimes exceed 47 °C during reproductive phases. The inclusion of physiological traits like CMT and pollen viability traits are important selection criteria. High temperature at flowering and boll-set stages has been shown to reduce boll retention, seed set, and fibre formation in cotton (Ijaz et al., 2024). The significant positive association between CMT and pollen viability in our germplasm suggests that both parameters may serve as reliable screening indices for thermotolerance in breeding. Given that heat stress can trigger membrane fluidity increase, oxidative damage, protein denaturation and electrolyte leakage, the use of CMT (or relative cell injury) is well supported in literature (Ijaz et , 2024) From a breeding perspective, identifying genotypes that show superior performance for PH, BN, BP, SCY, along with PV and CMT, offers a tangible starting point for parent selection.

Finally, from a broader perspective of gene-pool management and climate-adapted breeding in Pakistan, our study adds valuable data on morphological, physiological and fibre-quality diversity in local germplasm. Given the observed decline in national cotton yield (Mahmood, 2025) and the acknowledged threat of rising temperatures to cotton production, the integration of physiological screening (CMT, pollen viability) into germplasm characterization is timely and strategic (Bista et al., 2025). The adoption of complementary modern tools (high-throughput phenotyping, genomics, multi-omics) will further enhance the utility of such germplasm sets for breeding heat-tolerant, high-yielding and good-quality cotton varieties (Ahmed et al., 2024; Luqman et al., 2025).

CONCLUSION

The observed diversity in traits highlights strong potential for selection and breeding in Pakistan's cotton improvement programmes. Heatmap and mean value analyses identified superior genotypes, with G5, G24, G32, G44, G46, G52, and G60 exhibiting the highest fiber strength and promising performance for seed cotton yield, pollen viability, and cell membrane thermostability. The identified trait relationships and thermotolerance screening provide a strong basis for crossing and selection strategies to combine yield, quality, and resilience. Moving forward, validation under heat-stress environments and use of molecular/omics tools will be essential for the development of new cultivars that meet the demands of the textile industry of Pakistan.

AUTHOR'S CONTRIBUTION

Muhammad Tanees Chaudhary, Shoaib Liaqat and Sabah Merrium, analyzed the data. Muhammad Jamil and Ghulam Sarwar contributed in planning of experiment. Muhammad Tanees Chaudhary and Shoaib Liaqat performed the experiment & wrote the paper. Muhammad Tehseen Azhar, Kamran Javed and Zeenat Javeed did review the manuscript.

FUNDING

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AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed in this study are presented within this article in the form of tables and figures.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current study was checked and approved by the relevant team.

CONSENT FOR PUBLICATION

All authors have reviewed the manuscript and approved it for publication.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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REFERENCES

Abro, S., Rizwan, M., Deho, Z.A., Abro, S.A. and Sial, M.A., (2022). Identification of heat tolerant cotton lines showing genetic variation in cell membrane thermostability, stomata, and trichome size and its effect on yield and fiber quality traits. Frontiers in Plant Science. 12 34-56.

Ahmed, A. I., Iqbal, K. A., Negm, M. A. M., Iqbal, R., Azhar, M. T., Khan, S. H., & Rana, I. A. (2024). Enhancing cotton resilience to challenging climates through genetic modifications. Journal of Cotton Research, (7), 10-19.

Anwar, M., & Saleem, M. A. (2024). Gene pyramiding improved cell membrane stability under heat stress in cotton (Gossypium hirsutum L.). BMC Plant Biology, 24, 886. https://doi.org/10.1186/s12870-024-05610-7

Arif, T., Chaudhary, M.T., Majeed, S., Rana, I.A., Ali, Z., Elansary, H.O., Moussa, I.M., Sun, S. and Azhar, M.T., (2023). Exploitation of various physio-morphological and biochemical traits for the identification of drought tolerant genotypes in cotton. BMC Plant Biology, 23(1), 508-519.

Bista, M.K., Kodadinne Narayana, N., Chakravaram, A., Pieralisi, B., Dhillon, J., Reddy, K.R. and Bheemanahalli, R., (2025). Intensifying heat stress impacts cotton flowering and boll development efficiency. BMC Plant Biology, 25(1), 984-998.

Dev, W., Sultana, F., He, S., Hu, D., Geng, X., Du, X. and Iqbal, B., (2025). Effects of High Temperatures on Pollen Germination and Physio-Morphological Traits in Upland Cotton (*Gossypium hirsutum* L.). Journal of Agronomy and Crop Science, 211(4) 23-35.

Devi, P., Chaudhary, S., Bhardwaj, A., Priya, M., Jha, U., Pratap, A., Kumar, S., Bindumadahva, H., Singh, I., Singh, S. and Vara Prasad, P.V., (2023). Harnessing genetic variation in physiological and molecular traits to improve heat tolerance in food legumes. In Legumes: Physiology and Molecular Biology of Abiotic Stress Tolerance (pp. 27-69). Singapore: Springer Nature Singapore.

Farooq, A., Shakeel, A., Chattha, W.S., Khan, T.M., Azhar, M.T. and Saeed, A., (2021). Genetic variability in cotton germplasm: predicting the agro physiological markers for high-temperature tolerance. The Journal of Agricultural Science, 159(1-2), 11-22.

Farooq, A., Shakeel, A., Saeed, A., Farooq, J., Rizwan, M., Chattha, W. S., & Ramzan, Y. (2023). Genetic variability predicting breeding potential of upland cotton (*Gossypium hirsutum* L.) for high temperature tolerance. Journal of Cotton Research, 6(1), 7.

Hamidi, A., Bazdi, G. and Jafari, Y., (2018). Evaluation of Morphological Characteristics of Cotton (*Gossypium hirsutum* L.) New Genotypes in Golestan Province. Journal of Crop Breeding, 10(27), 66-74.

Ijaz, A., Anwar, Z., Ali, A., Ditta, A., Shani, M. Y., Haidar, S. Khan, M. K. R. (2024). Unraveling the genetic and molecular basis of heat stress in cotton. Frontiers in Genetics, 15, 1296622.

Iqbal, A., Khan, I. A., & Sadaqat, H. A. (2008). Heat tolerance is variable in cotton (*Gossypium hirsutum* L.) and can be exploited for breeding of better yielding cultivars under high temperature regimes. Pakistan Journal of Botany, 40(5), 2053-2058.

Iqbal, M., Ul-Allah, S., Naeem, M., Ijaz, M., Sattar, A. and Sher, A., (2017). Response of cotton genotypes to water and heat stress: from field to genes. Euphytica, 213(6), p.131.

Kumar, S., Jattan, M., Kumar, D., Sharma, A., Malik, K. S., Saini, A. K., & Mandhania, S. (2025). Genetic diversity in Bt-cotton (*Gossypium hirsutum* L.) genotypes for yield and fibre quality traits using multivariate analysis. Journal of Advances in Biology & Biotechnology, 28(6), 1081-1088.

Li, S., et al. (2019). Evaluation of the genetic diversity of fibre quality traits in upland cotton (*Gossypium hirsutum* L.). Journal of Cotton Research, 1(4). 45-52.

Luqman, T., Hussain, M. and Khan, M.K.R. (2025). Harnessing multivariate insights coupled with susceptibility indices to reveal morpho-physiological and biochemical traits in heat tolerance of cotton. BMC Plant Biology, 25(1), 126-139.

Luqman, T., Hussain, M., Ahmed, S. R., Ijaz, I., Maryum, Z., Nadeem, S. Liu, Y. (2025). Cotton under heat stress: a comprehensive review of molecular breeding, genomics, and multi-omics strategies. Frontiers in Genetics. 23: 452-469.

Mahmood, A. (2025, January 4). Cotton production plunges over 33 pc – Pakistan. DAWN.com. https://www.dawn.com/news/1882981

Majeed, S., Chaudhary, M.T., Mubarik, M.S., Rana, I.A., Shaban, M., Tan, D.K., Jia, Y., Du, X., Hinze, L. and Azhar, M.T., (2024). Genetics of biochemical attributes regulating morpho-physiology of upland cotton under high temperature conditions. Journal of Cotton Research, 7(1), 3-15.

Malik, W., Ahmad, M. Q., Baig, M. M. A., Nauman, M., & Arshad, S. F. (2022). Progress and perspective on cotton breeding in Pakistan. Journal of Cotton Research, 5, 29-38.

Nortin J (1966) Testing of plum pollen viability with tetrazolium salts. Proc Am Soc Hortic Sci 89:132–134

Puppala, N., Nayak, S.N., Sanz-Saez, A., Chen, C., Devi, M.J., Nivedita, N., Bao, Y., He, G., Traore, S.M., Wright, D.A. and Pandey, M.K., (2023). Sustaining yield and nutritional quality of peanuts in harsh environments: Physiological and molecular basis of drought and heat stress tolerance. Frontiers in Genetics, 14, 112-146.

Rathod, V., Behera, T.K., Munshi, A.D., Durgesh, K., Jat, G.S., Krishnan, B.G. and Sharma, N., (2018). Pollen viability and in vitro pollen germination studies in Momordica species and their intra and interspecific hybrids. International Journal of Chem. Studies, 6(6). 32-40.

Rehman, A.U., Rana, I.A., Majeed, S., Chaudhary, M.T., Zulfiqar, M., Yang, S.H., Chung, G., Jia, Y., Du, X., Hinze, L. and Azhar, M.T., (2021). Intra-plant variability for heat tolerance related attributes in upland cotton. Agronomy, 11(12), p.2375.

Saleem, M. A., Malik, W., Ahmad, M. Q., Baig, M. M. A., Nauman, M., & Arshad, S. F. (2023). Identification of heat tolerant cotton lines showing genetic variation in cell membrane thermostability, stomata, and trichome size and its effect on yield and fibre quality traits. Plant Cell Reports. 23: 45-49.

Sarwar, M., Saleem, M.F., Ullah, N., Ali, A., Collins, B., Shahid, M., Munir, M.K., Chung, S.M. and Kumar, M., (2023). Superior leaf physiological performance contributes to sustaining the final yield of cotton (*Gossypium hirsutum* L.) genotypes under terminal heat stress. Physiology and Molecular Biology of Plants, 29(5), pp.739-753.

Shani, M.Y., Ditta, A. and Khan, M.K.R., (2025). Characterizing drought-resilient cotton genotypes through morpho-physiological and biochemical traits at flowering stage. BMC Plant Biology, 25(1), pp.1-22.

Side, T.H.R., Yamin, M., Mulyani, S., Qadri, S.N., Ayunawati, L. and Aji, A.P., (2025). Diversity and Selection of Cotton Germplasm Based on Morpho-Agronomic Character Performance. HAYATI Journal of Biosciences, 32(2), pp.341-355.

Steel RGD, JH Torrie, DA Dickey (1997). Principles and Procedures of Statistics - A Biometrical Approach, 3 rd edition, pp:204–207. McGraw Hill Book International Co., Singapore

Wang, H., Cai, X., Umer, M.J., Xu, Y., Hou, Y., Zheng, J., Liu, F., Wang, K., Chen, M., Ma, S. and Yu, J., 2024. Genetic analysis of cotton fiber traits in Gossypium hybrid lines. Physiologia Plantarum, 176(4), p. e14442.

Yousaf, M.I., Hussain, Q., Alwahibi, M.S., Aslam, M.Z., Khalid, M.Z., Hussain, S., Zafar, A., Shah, S.A.S., Abbasi, A.M., Mehboob, A. and Riaz, M.W., 2023. Impact of heat stress on agro-morphological, physio-chemical and fiber related paramters in upland cotton (*Gossypium hirsutum* L.) genotypes. Journal of King Saud University-Science, 35(1), p.102379.

Zhang, J., Cheng, M., Cao, N., Li, Y., Wang, S., Zhou, Z. and Hu, W., (2023). Drought stress and high temperature affect the antioxidant metabolism of cotton (*Gossypium hirsutum* L.) anthers and reduce pollen fertility. Agronomy, 13(10), p.2550.