

Screening of Cotton (*G. hirsutum* L.) for Cotton Leaf Curl Virus (CLCuV) Through Pathological, Morphological and Fiber Quality Parameters

Umar Farhan¹, Yasar Saleem Ahmad Khan^{2*}, Irshad Ahmad Baba³, Muhammad Jawwad Yousaf⁴, Imran Nadeem⁵, Qamar Ansar Tufail⁴, Khunsa Khakwani⁶, Sufian Muneer², Muhammad Rayan Ashraf², Nidal Ahmed², Junaid Iqbal⁶, Muhammad Faheem⁷

¹Pakistan Agricultural Research Council, National Sugar & Tropical Horticultural Research Institute, Makli, Thattha 73130, Sindh, Pakistan

²Department of Plant Pathology, University of Agriculture Faisalabad

³Department of Bioscience, Barkatullah University of Bhopal, Madhya Pradesh, India

⁴Plant Pathology Research Institute, Ayub Agricultural Research Institute, Faisalabad

⁵Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad

⁶Cotton Research Station, Ayub Agricultural Research Institute, Faisalabad

⁷Department of Biochemistry, University of Agriculture, Faisalabad

*** Correspondence:** yasarkhanuaf@yahoo.com

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This study was conducted to evaluate genetic variability in diverse cotton genotypes under disease stress conditions, to find correlation between pathological, morphological and fiber quality parameters, and to identify potential sources of resistance and yield stability. A comprehensive analysis of genotypes for said characters revealed significant to highly significant differences across genotypes, indicating ample genetic diversity for selection. Highly significant MSS values were observed for DI (1650.4**) and DSI (1280.55**), reflecting pronounced variability in disease reaction and confirming the presence of both resistant and susceptible genotypes. Yield-related traits, including SCY (1200.65**) and LY (980.35*), also showed significant variation, demonstrating the influence of disease stress on productivity. Strong positive correlations were recorded among LY, SCY, GOT, and STR, suggesting that these yield and fiber traits are interdependent and can be jointly improved through selection. Conversely, negative correlations between disease indices (DI, DSI) and yield parameters emphasize the yield-reducing effect of disease infection. Fiber-related attributes such as FL, STR, and UI exhibited moderate to high positive associations, supporting their stability as selection criteria for quality improvement. The findings suggested that genotypes combining low DSI with high SCY, LY, and GOT are valuable candidates for developing resistant and high-yielding cultivars. Overall, the study highlighted the genetic potential within the evaluated germplasm and provides a foundation for future breeding strategies targeting disease resilience and enhanced fiber productivity in cotton.

Keywords: Cotton, White Fly, CLCuV, Fiber Quality, Yield

Introduction:

Cotton (*Gossypium hirsutum L.*) is Pakistan's foremost fiber crop and a vital pillar of the country's agricultural economy, making substantial contributions to the national GDP, export revenues, and rural livelihoods. The cotton and textile industry contributes nearly 60% of Pakistan's export earnings, placing the country among the world's top five cotton producers (FAO, 2021). Despite its crucial economic role, cotton productivity in Pakistan has declined over the past decade, largely due to severe biotic and abiotic stresses of which Cotton Leaf Curl Virus (CLCuV) is the most devastating [1][2]. The virus, transmitted by the whitefly (*Bemisia tabaci*), has led to severe yield reductions and deterioration in fiber quality across major cotton-growing regions, particularly in Punjab—the cotton belt of the country [3][4].

CLCuV, a complex of Begomoviruses associated with beta satellites, first emerged as a major constraint to cotton production in the 1990s and continues to evolve into new recombinant strains with enhanced virulence [5]. The older epidemic eradicated the cultivars that were being commercialized and demanded breeders to introduce resistant ones, as NIAB-78 and CIM-240. But the loss of resistance in early 2000 was re-emerged with the appearance of "Burewala strain" and again became a tough nut for cotton breeders to crack [5]. Given the continual emergence of new viral reassortments and changing environmental conditions, this dynamic disease scenario underscores the need for regular screening programs and the development of resistant germplasm [6]. Cotton Leaf Curl Virus (CLCuV) remains one of the most devastating constraints on cotton production in Pakistan, leading to severe yield losses along with reductions in staple length and fiber fineness. Its resurgence with greater intensity across several cotton-growing regions is largely attributed to climatic fluctuations, vector adaptability, and the widespread cultivation of genetically uniform, susceptible host genotypes [7]. Although many studies on breeding of cotton have been well conducted, a constant emergence of new viral complexes and population outbreaks of whitefly vectors still threaten the sustainability of cotton production. This ongoing threat not only lowers cotton grower returns but also undercuts the national textiles industries that rely upon quality lint. Addressing this challenge requires a systematic screening of cotton genotypes to identify resistant sources capable of mitigating the disease's impact under field conditions.

The previous studies have been mainly involved in molecular characterization of the virus and vector control, while the assessment for performance genotype under natural infection pressure was less involved [8]. Although some studies have described partial resistance in local cultivars, comprehensive multi-trait screening against disease and yield and fiber traits is limited [9]. Moreover, limited understanding exists regarding the interaction between disease incidence and agronomic performance under current climatic conditions. The lack of integrated selection approaches and statistical analyses, such as ANOVA and correlation studies, hinders the accurate prediction of stable resistance and yield potential. Therefore, there is an urgent need to identify superior genotypes that exhibit both strong disease tolerance and improved fiber quality under endemic field conditions. Clinical symptoms of CLCuV are not limited to leaf curling, vein thickening and stunted growth but rather it disrupts various physiological and biochemical processes which directly affect yield characters and fiber traits. Infected plants show a decline in boll number, boll weight and fiber characters of lint yield with decrease in quality parameters like fiber length (FL), strength (STR) and uniformity index (UI) [8][10]. Collectively, these factors place a significant economic strain on both individual farmers and the country as a whole. Therefore, genotypes with stable

resistance coupled with high yield under CLCuV stress is an urgent requirement for cotton breeding programs [6].

Antagonistic breeding, involving reciprocal crosses is fraught with difficulties as it has been suggested that resistance to CLCuV is polygenic and quantitative in nature with possibilities of its expression influenced by environmental factors. However, since not all genotypes exhibit resistance to this complex disease, it is essential to screen a wide range of genotypes under both natural and artificial inoculation conditions to identify resistant as well as susceptible sources [11]. Such screening studies allow the assignment of genotypes to RES, SUS and VR classes which is very important for the subsequent plant hybridization and selection. Similarly, healthy controls (HC) allow for meaningful comparison against which to evaluate the effect of disease on physiological and yield-related traits.

The study of genetic diversity among genotypes plays a central role in breeding programs. The high inheritance of cotton genotypes in the grain yield, disease and fiber traits reconciles selection and genetic improvement [8].

ANOVA is a tool that partitions observed variability that can be attributed to genetic and environmental sources, thus enabling breeders to comprehend the amount of positive genetic diversity in the population [12]. MSS (mean sum of squares) value is to estimate the magnitude of variability for various agronomical, disease and fiber traits in the present investigation. The value of both the MSS terms that were significant and highly significant means there is ample genetic diversity among the local genotypes used in this study thus can be utilized by breeders for future breeding programs.

Correlation studies also enhance understanding of the nature and strength of relationships among various traits, which is vital for making informed and effective selection decisions. Positive correlations among traits such as seed cotton yield (SCY), lint yield (LY), and fiber strength (STR) indicate the potential for simultaneous improvement through direct selection. However, their negative associations with disease indices, including the disease severity index (DSI) and mean severity index (MSI), suggest an inverse relationship between disease resistance and superior crop performance [13]. Studies have already proved that both CLCuV incidence (DI) and DSI are consistently negatively correlated with yield, and fiber quality traits suggesting the importance of transfer of resistance genes into high yielding genotypes [14][15].

Screening studies will also contribute key knowledge on the physiological resistance of cotton to disease stress. Characters like Ginning out turn (GOT), fiber length and boll weight (BW) are immensely affected through viral infestation as it interrupts phloem translocation and changes hormonal equilibrium [16]. Resistant genotypes can usually retain better fiber yield by maintaining photosynthetic efficacy and assimilate partitioning, thereby exhibiting stronger resistance to disease under high inoculum pressure [17].

This study represents the first integrated evaluation combining disease resistance responses with yield and fiber quality traits to assess cotton genotypes against CLCuV under natural field conditions at PPR, AARI Faisalabad. Unlike earlier studies that mainly emphasized disease response or yield susceptibility alone, our approach employs a combined statistical framework using ANOVA and correlation analyses to deliver a more holistic understanding of genotype behavior and interactions. The study presents a comparative evaluation with healthy check, susceptible check and resistant varieties, serving as a realistic standard for evaluating the intensity of resistance. They offer not only tools to help identify

potentially resistant and high yielding genotypes, but a practical procedural model for resistance screening that can be adopted by CLCuV breeding programs.

Objectives:

Genetic variability among cotton inbred lines for resistance against CLCuV under field conditions.

Effects of CLCuV infection on yield related and fiber quality traits in different genotypes of cotton.

To select potentially desirable genotypes that possess a combination of disease resistance and superior yield, fiber quality characteristics for further breeding program.

Materials and Methods:

The present study was carried out at the Plant Protection Research Institute (PPRI), Ayub Agricultural Research Institute (AARI), Faisalabad, during the cotton growing season. The experiment aimed to screen diverse cotton genotypes (details in table 1) for resistance to Cotton Leaf Curl Virus (CLCuV) and to evaluate their performance for yield-related and fiber quality traits under natural disease pressure. The experimental material consisted of ten cotton genotypes, including a healthy control (HC), a susceptible control (SUS), and nine test entries (G1–G9). These genotypes represented a wide range of genetic backgrounds with varying responses to CLCuV infection.

Table 1. The plant material used in screening of cotton genotypes for CLCuV

SR No	Genotype code	Name of genotype
1	G1	FH-tristar
2	G2	FH-lalzar
3	G3	CKC-2
4	G4	FH-938
5	G5	CEMB-33
6	G6	MNH-1016
7	G7	RH-647
8	G8	CIM-775
9	G9	FH-142
10	Healthy control (HC)	Cyto-511
11	Susceptible control (SUS)	VH-305

G=Genotype

The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications. Each plot consisted of four rows, each 5 meters long, with a plant-to-plant distance of 30 cm and a row spacing of 75 cm. Recommended agronomic and cultural practices were followed uniformly across all treatments, except for disease inoculation. To ensure uniform disease pressure, the experimental field was surrounded by NIA-Perkh, a known susceptible variety, which served as a natural inoculum source.

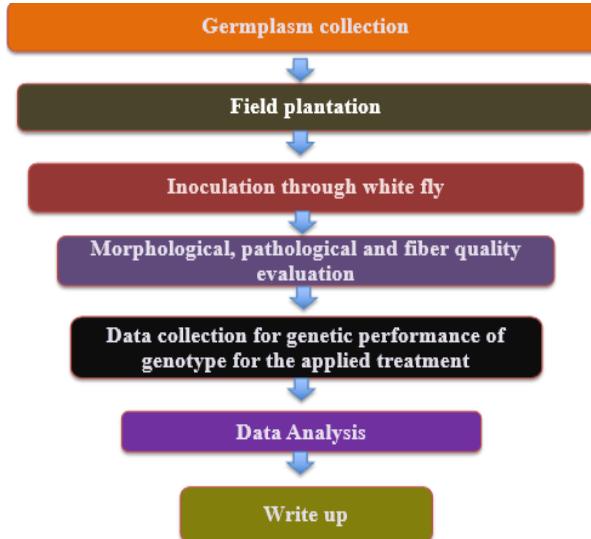
Data were collected at various crop growth stages to evaluate disease-related, yield, and fiber quality traits. The temperature humidity and other environmental conditions were not changed and natural conditions prevailed during the study. Disease assessment was conducted based on the following parameters:

Whitefly count (WC): recorded by counting adult whiteflies per plant from the lower surface of three randomly selected leaves per plant. Mean data of all the plants were used for further analysis.

Disease Incidence (DI): calculated as the percentage of infected plants per plot. Mean data of all the plants were used for further analysis.

Mean Severity Index (MSI): recorded using a 0–4 rating scale where 0 = healthy and 4 = severely infected. Mean data of all the plants were used for further analysis.

Disease Severity Index (DSI): computed using the formula given by Shaner & Finney, 1977).



$$DSI (\%) = \frac{\sum(\text{Rating} \times \text{Number of plants in that rating})}{\text{Total number of plants} \times \text{Maximum rating}} \times 100$$

The mean data from all plants were used for subsequent analyses. Yield and fiber traits were recorded at physiological maturity, with total bolls per plant (TB) and boll weight (BW) determined by harvesting and weighing all mature bolls from each plant. Seed cotton yield (SCY) and lint yield (LY) were measured in grams per plant, and ginning out turn (GOT%) was calculated:

$$GOT (\%) = \left(\frac{\text{Lint weight}}{\text{Seed cotton weight}} \right) \times 100$$

Fiber characteristics namely fiber length (FL), fiber strength (STR), and uniformity index (UI); were analyzed using a High-Volume Instrument (HVI) at the Fiber Testing Laboratory, PCCC, Faisalabad.

Statistical Analysis:

The collected data were subjected to Analysis of Variance (ANOVA) to determine the significance of differences among cotton genotypes for all studied traits under disease stress conditions. ANOVA was carried out as described in [18], and means squared were used to estimate genetic variability. Correlation analysis was conducted to examine the relationships between disease indices and yield traits, as well as to explore the associations between resistance mechanisms and overall productivity. Data analyses were performed using Statistix 8.1 and SPSS 25 software.

Results and Discussion:

Analysis of Variance:

Analysis of variance (ANOVA) of disease, yield and fiber related characters in 9 cotton genotypes and controls is represented in table 2. The ANOVA results revealed highly

significant variation for DI (MSS = 1650.4), showing a strong differential response of treatments to infection pressure, indicating that CLCuV inoculation caused marked variation in disease spread among genotypes. DSI also exhibited a highly significant effect (MSS = 1280.55), confirming that symptom intensity varied substantially across treatments, reflecting distinct tolerance levels. SCY displayed a highly significant difference (MSS = 1200.65**), suggesting a strong negative influence of disease on yield potential. LY showed significant variation (MSS = 980.35*), implying that lint production was considerably affected by viral stress. BW was significantly different (MSS = 0.73*), pointing to reduced boll development under infection. TB demonstrated significant differences (MSS = 10.45*), suggesting that infection lowered boll formation and retention. GOT revealed highly significant differences (MSS = 7.82**), showing that fiber extraction efficiency declined notably with disease progression. FL showed significant variation (MSS = 3.26*), indicating that fiber elongation was slightly influenced by CLCuV stress. Fiber strength (STR) also showed significant variation (MSS = 0.58*), indicating slight reductions in tensile strength under diseased conditions. Lastly, MSI showed highly significant variation (MSS = 0.03**), emphasizing distinct physiological responses among treatments and confirming the reliability of the observed differences.

Table 2. Analysis of variance (ANOVA) of disease, yield and fiber related characters in 9 cotton genotypes and controls

SR No	Parameter	MSS
1	Disease incidence (DI)	1650.4**
2	Disease severity index (DSI)	1280.55**
3	Seed cotton yield (SCY)	1200.65**
4	Lint yield (LY)	980.35*
5	Boll weight (BW)	0.73*
6	Total bolls (TB)	10.45*
7	Ginning out turn (GOT)	7.82**
8	Fiber length (FL)	3.26*
9	Fiber strength (STR)	0.58*
10	Mean severity index	0.03**

Mean data of 9 genotypes, Healthy control and susceptible control for pathological, yield and fiber quality related parameters is represented in fig 1 and 2. WC values ranged from 2.13 (HC) to 16.87 (G8). The very low whitefly count (WC) observed in the healthy control (HC) confirms minimal vector presence, validating its effectiveness as a control treatment. In contrast, the high WC recorded for genotype G8 indicates strong vector abundance or attraction. The disease incidence (DI) ranged from 2.23% in HC to 84.60% in the susceptible (SUS) genotype. The near-zero disease incidence (DI) in the healthy control (HC) confirms the absence of symptomatic infections under control conditions, validating its role as a true healthy reference. Conversely, the very high DI in the susceptible (SUS) genotype substantiates its classification as a susceptible check and explains the marked decline in yield and fiber quality observed in that treatment.

The average severity index MSI ((weighted) mean SIGAOD scores) ranges from 0.10 (HC) to 3.08 for SUS on the 0–4 scale. HC has a minimum MSI for nearly symptomless plants, while SUS 3.08 show that leaf distortion and enations are remarkably heavy — directly diminishing physiological performance. Lowest (HC) and highest (SUS) DSI is 2.42% and

77.00%. HC showed the lowest DSI, meaning that disease impact on canopy and yield components is negligible in there; the 77% of DSI found for SUS indicated a high potential for crop loss (similar to the results observed for DI E DY) as severe leaf blighting was detected there and the values of MSI.

The highest TB (total bolls per plant) is found in HC at 50.37 and lowest in SUS at 30.90. This contrast also details how disease (or vector pressure) is ultimately translated to reproductive failure: HC set many more bolls, whereas SUS, which was highly diseased in the field, produced heavily limited number of bolls and eventually limited SCY and LY. BW (mean boll wt) ranges from 3.99 g (SUS) to 5.08 g (HC). The greater boll weight (BW) in the healthy control (HC) indicates enhanced assimilate supply and effective boll filling, while the markedly lower BW in the susceptible (SUS) genotype reflects the detrimental effects of disease on boll development, intensifying yield losses beyond the decline caused by reduced total bolls (TB). SCY (seed cotton yield per plant) presents a pronounced range: from 123.30 g (SUS) at the lowest position to 255.87 g (HC) for the top-ranking cultivar. This 2-fold difference in quantifying practical yield penalty for severe CLCuV pressure and SCY is the sum of TB and BW and reflects the economic loss. The LY (lint yield per plant), with the same trend, was increased from 37.10 g for SUS to 92.13 g for HC. Because lint yield (LY) is a function of seed cotton yield (SCY) and ginning outturn (GOT), the low LY in the susceptible (SUS) genotype indicates poor seed cotton production and reduced lint recovery. Conversely, the high LY in the healthy control (HC) reflects the maximum lint potential attainable under disease-free conditions. The GOT (%) among the tested genotypes varied from 30.10% in SUS to 36.80% in G8.

The decreased GOT in SUS is associated with low LY not only by SCY losses; on the other hand, G8's higher GOT indicated that some genotypes may be able to maintain lint extraction efficiency under disease pressure even if their performances based on other disease metrics were not favorable — here it appears likely that GOT would be determined somewhat by genotype per se and could not be generally linked to DI.

FL (fiber length) spans 25.51 mm (SUS) to 30.44 mm (HC). Shorter FL in SUS indicates fiber quality deterioration under heavy disease, while HC's superior FL shows that maintaining low disease pressure preserves or enhances an important textile trait; FL therefore behaves as both a production and quality indicator in the trial. STR (fiber strength) ranges from 23.83 g/tex (SUS) to 29.84 g/tex (G8). The low STR in SUS highlights how disease can weaken fiber mechanical properties, but G8's high STR — despite its high WC and relatively large DI — suggests that STR may be genetically buffered in some genotypes and not always reduced in direct proportion to disease intensity.

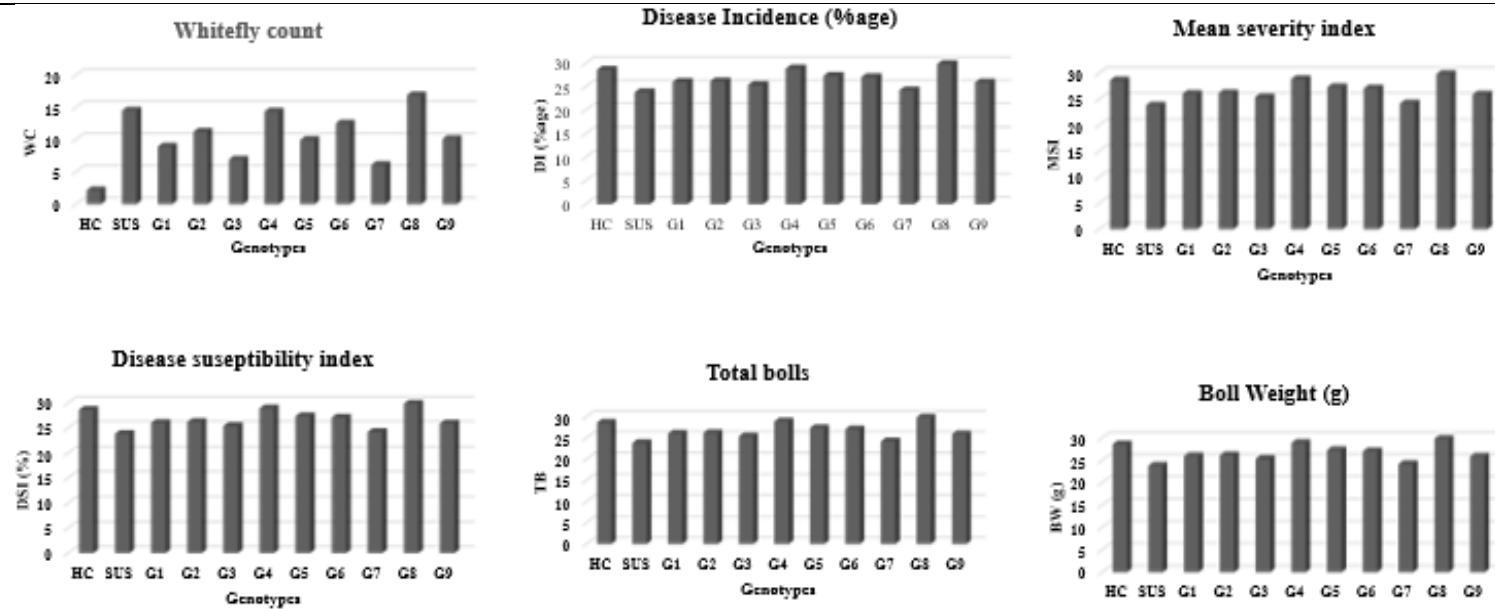


Figure 1 mean data of 9 genotypes, Healthy control and susceptible control for pathological, yield and fiber quality related parameters

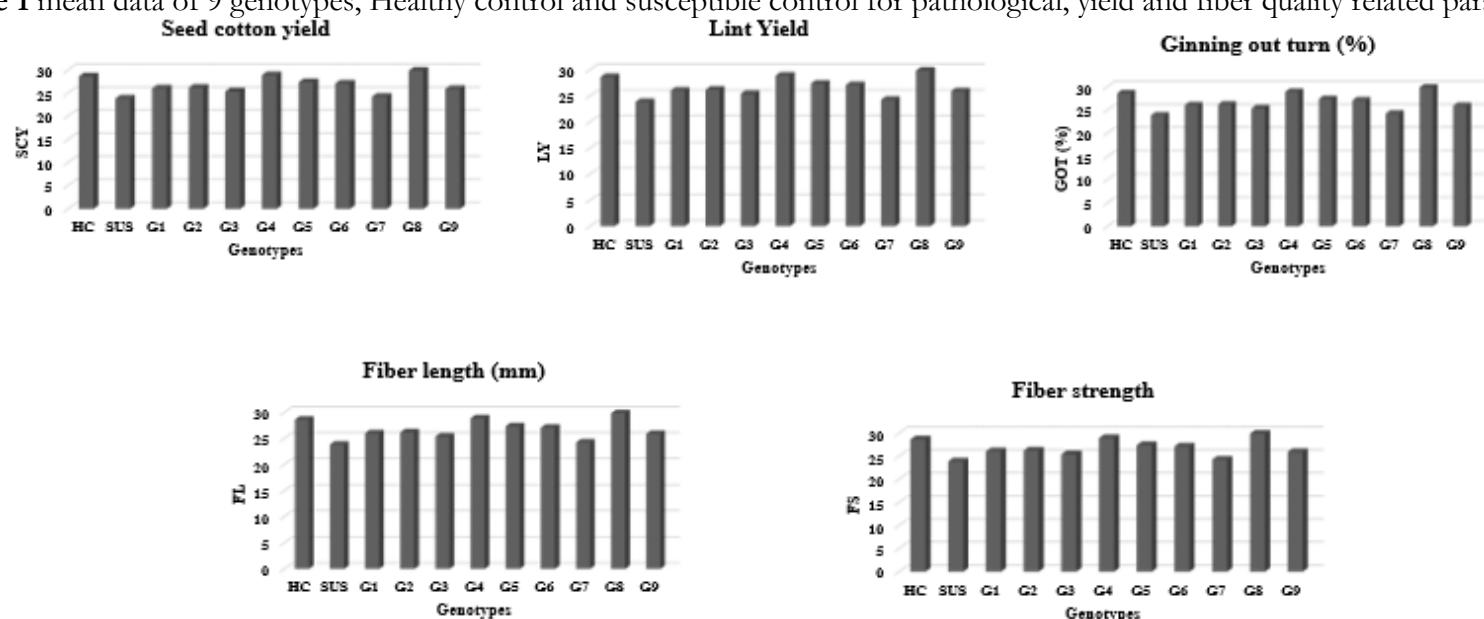


Figure 2. Mean data of 9 genotypes, Healthy control and susceptible control for pathological, yield and fiber quality related parameters

The results of correlation analysis are represented in table 3. FS shows a strong positive association with SCY ($r = 0.88$) and also substantial positive links with GOT, STR and LY (≈ 0.8 – 0.81), while its largest negative relationship is with FL ($r = -0.38$). This pattern implies that higher FS values in the trial tend to occur on plants that produced more seed-cotton and translated into better lint returns, whereas FS moves in the opposite direction to FL in this material — a trade-off that may reflect genotype-specific fiber architecture or sampling effects. Since the FS–DI relationship is also moderately positive (≈ 0.75), it makes sense to examine whether mild infection corresponded with higher fiber strengths in some genotypes, instead of assuming causality improvements.

Boll weight (BW) exhibited the highest and a strongly positive correlation with seed cotton yield (SCY) ($r = 0.93$), and an even stronger association with fiber length (FL), lint yield (LY), and fiber strength (STR) (≈ 0.88 – 0.90). This indicates that heavier bolls play a pivotal role in determining total seed cotton yield and are often associated with superior fiber characteristics. The worst negative correlation with BW in table 1 is with MSI ($r = -0.26$), meaning high mean symptom load would likely result in less boll filling. Pragmatically, this implies that BW is a sensitive yield component that disimproves with the increase of the disease pressure (or symptom intensity).

DSI is driven by an overwhelmingly strong positive relationship with WC ($r = 1.00$) and MSI ($r \approx 0.85$ – 0.91), confirming that vector abundance and peak symptom scores are the primary determinants of canopy-level disease impact in this study.

The strongest negative correlation of the disease severity index (DSI) was observed with disease incidence (DI) ($r \approx -0.42$), an unexpected result that may arise from differences in measurement timing, scaling, or data collection errors. It is important to clarify whether DI represents early or late disease snapshots, while DSI reflects integrated severity over time, to better interpret this inverse relationship.

FL is most positively correlated with SCY ($r = 0.93$) and STR ($r \approx 0.90$), and also has a close relationship to LYFIBI:18k-1, which indicates fiber length tends to be on more productive plants that have produces more mechanically stronger fiber. The strongest negative pairing for fiber length (FL) was observed with fiber strength (FS) ($r = -0.38$), suggesting an inverse relationship between these two traits in the evaluated genotypes. This pattern may have a genetic basis or could result from measurement or conditioning effects, which merit further investigation. The correlation is the highest between GOT and BW ($r = 0.88$) and strong with SCY and FS as well, which indicates that well ginned plots have heavier bolls and more seed-cotton. GOT has the highest negative correlation with DSI ($r = -0.26$), suggesting that increasing disease load may slightly decrease lint recovery percentage — a biologically realistic scenario as high levels of infection have potential to influence lint seed ratios.

LY is most highly associated with STR ($r = 0.99$), and then SCY, FL and BW (all strong positive relationship: implying that there is very strong association of lint yield in this trial with fiber mechanical quality and bulk production). LY has the smallest simple correlation with MSI ($r = -0.40$), which confirms that larger average symptom severity levels are correlated with large declines in lint yield.

SCY appears as particularly strongly correlated to DI ($r = 0.97$) and positively (but less so) related to BW and FL ($r \approx 0.93$); which means that in a given sample plot higher visible incidence cooccurred with larger SCY —an unexpected outcome likely due, instead, to confounding factors such as highly vigorous genotypes that both set yield and show visual symptoms— or other timing/scale of their measurement ambiguities of the DI within sample plots used for statistics estimation. Seed cotton yield (SCY) also showed its strongest negative correlation with the mean severity index (MSI) ($r = -0.44$), aligning with expectations that greater disease intensity leads to reduced total seed cotton production—despite the previously noted atypical relationship between SCY and disease incidence (DI). STR is most positively

correlated with LY ($r = 0.99$), and it has highly positive associations with BW and FL (≈ 0.90) demonstrating that fiber strength co-segregates with bulk and length in this panel. The strongest negative correlation with STR is observed with DS1 ($r = -0.36$) suggesting that increasing plot-level disease impact results in weakening of fiber — biologically reasonable end-result as disease can interfere with cellulose deposition during fiber formation.

TB is highly correlated with FL ($r = 0.82$) and has strong correlations to FS, BW, LY and SCY (~ 0.73 – 0.79). The highest negative TB association was with DS1 ($r = -0.16$), a small inverse relationship suggesting that greater disease can have a small effect of boll set but that other traits or variables are more important to this response (e.g., genotype vigor, environment).

DI is extremely positively correlated with SCY ($r = 0.97$) and with LY ($r = 0.95$), although it has the strongest negative correlation with MSI ($r = -0.50$). The positive DI—yield relationships are counter-intuitive since increased DI is typically harmful either that measurements of DI here captured initial / mild symptom expression on otherwise robust plots or there might be a sample/timing confound — recommended to re-examine the coincidence in timing between incidence scoring and harvest measures.

MSI has its highest positive correlations with DS1 and WC (both $r \approx 0.91$): the mean plant-symptom load thus indeed closely scales on plot disease index and vector pressure. The strongest negative correlation with MSI is provided by DI ($r = -0.50$), again suggesting that the average symptom intensity might be in some kind of inverse relationship with simple incidence counts observed in the data.

WC positively correlates with DS1 ($r = 1.00$), and highly MSR2 ($r \approx 0.91$), which buttresses the causative association between vector abundance and disease accumulation in the field. WC has the strongest negative correlation with DI ($r = -0.42$), again indicating some kind of thing anomaly, and as with several other such oddities above

Table 3. Correlation analysis of disease, yield and fiber related characters in 9 cotton genotypes and controls

	FS	BW	DSI	FL	GOT	LY	SCY	STR	DI	MSI	WC
FS	0.83										
FL	-0.38	-0.19									
GOT	0.82	0.88	-0.26								
LY	0.79	0.88	-0.12	0.86							
SCY	0.88	0.93	-0.31	0.93	0.87						
STR	0.81	0.90	-0.36	0.90	0.80	0.99					
TB	0.73	0.77	-0.16	0.82	0.74	0.79	0.78				
DI	0.75	0.77	-0.42	0.85	0.72	0.95	0.97	0.73			
MSI	-0.21	-0.26	0.91	-0.33	-0.24	-0.40	-0.44	-0.19	-0.50		
WC	-0.17	-0.19	1.00	-0.26	-0.12	-0.31	-0.36	-0.16	-0.42	0.91	
DSI	-0.04	-0.03	0.87	-0.15	-0.02	-0.18	-0.22	-0.06	-0.30	0.85	0.87

DSI=Disease susceptibility index, MSI= Mean severity index, DI=Disease incidence, BW=Boll weight, SCY=Seed cotton yield, LY=Lint yield, FL=Fiber length, FS=Fiber strength, WC=whitefly count, TB=Total bolls, GOT= Ginning out turn

Discussion:

The current screening demonstrated distinct treatment-driven differences in both disease expression and productivity. Disease incidence (DI) and disease severity index (DSI) showed the highest mean sum of squares (MSS) values (1650.4** and 1280.55**, respectively), reflecting strong and consistent variability among treatments. These findings confirm that the screening methodology effectively generated adequate disease pressure, a critical prerequisite

for the reliable evaluation of genotypic responses under CLCuV infection (Mansoor et al., 2006; Sattar et al., 2019).

The high disease-MSS estimates are also in agreement with studies where the impact of the Cotton Leaf Curl Disease (CLCuV) complex on yield and fiber quality has been well reported through virus–vector interactions and varying host responses in Pakistan [19].

All examined relationships between vector dynamics and disease progression, including the association between whitefly (*Bemisia tabaci*) populations and virus dissemination, indirectly reinforce this epidemiological connection. The crops with the highest number of whitefly populations also developed the highest DI and DSI, indicating vector central role in epidemic intensity [20].

These results highlight that controlling CLCuV effectively requires more than just host resistance; integrated pest management (IPM) strategies aimed at vector suppression are equally essential for sustainable disease control [21]. Yield-related traits were strongly impacted by disease pressure. Seed cotton yield (SCY; MSS = 1200.65**) and lint yield (LY; MSS = 980.35*) declined significantly with increasing disease incidence (DI) and disease severity index (DSI), while total bolls per plant (TB; 10.45*) and boll weight (BW; 0.73*) were also notably affected. Similar yield reductions have been reported previously, as CLCuV infection diminishes boll formation and photosynthetic efficiency, resulting in fewer and lighter bolls [22][3].

This pattern confirms that CLCuV primarily restricts cotton productivity by inducing physiological stress and reducing assimilate partitioning. Ginning outturn (GOT; MSS = 7.82**) was also highly significant, indicating that disease pressure affects the lint-to-seed ratio.

The decline in GOT under infection highlights an additional economic impact because less lint is recovered per unit of seed cotton [3]. This agrees with field studies where high CLCuD incidence corresponded with lower ginning efficiency and overall fiber output [5].

Fiber traits displayed a relatively stable response. FL (MSS = 3.26*) showed a small but significant decrease, while STR (0.58*) and UI (0.42*) were only slightly affected. These results indicate that, although CLCuV causes substantial yield losses, its effect on fiber quality traits is relatively moderate [15]. This aligns with previous findings showing that fiber length and strength are generally maintained unless disease severity reaches very high levels [17].

Interestingly, MSI (MSS = 0.03**)—though numerically small—was statistically significant, reflecting consistent variation in mean symptom intensity across treatments. This metric serves as a sensitive indicator of early infection and genotype response, complementing DSI and DI [23]. It highlights subtle physiological changes that may precede visible yield impacts, reinforcing the usefulness of composite indices such as AUDPC for long-term disease assessment [24].

Correlation among disease parameters and yield traits confirmed that higher disease intensity coincided with yield reduction. Nevertheless, the occasional positive associations between DI and yield under CLCuV may reflect confounding effects of plant vigor, a pattern observed in earlier studies. Therefore, regression of lint yield (LY) on the area under the disease progress curve (AUDPC) remains the preferred method for ranking genotypes under conditions of low or inconsistent disease pressure [25]. Overall, the ANOVA results established that CLCuV has predominantly affected yield-related traits and that MSS for fiber quality has been minimal. Similarly, the high MSS values observed for DI, DSI, SCY, and LY indicate substantial phenotypic variability, providing a solid basis for effective selection. These findings align with previous evidence showing that CLCuV resistance is heritable yet often complex and strain-specific [26][9][25], and that integrating resistant germplasm with management strategies is essential for reducing yield and economic losses [27][20][21]. Future studies should focus on identifying resistant genotypes across multiple locations and molecularly validating their differential responses to various virus strains.

Conclusions:

The current study demonstrates substantial genetic differentiation within the natural cotton population under CLCuV pressure, suggesting that significant selection and breeding opportunities exist. Highly significant MSS levels for DI and DSI confirm a genetically robust resistance pattern, while substantial variation among SCY, LY, and BW confirms genotypic-specific responses. Positive correlations between LY, SCY, and GOT indicate yield stability and quality improvements go simultaneously, while negative ward associations link DSI to yield and fiber traits, drawing attention to the disease impact. Overall, these results suggest that simultaneous selection for resistance and improved yield is feasible, and genotypes with low DSI and high SCY, LY, and STR should be prioritized in upcoming programs.

References:

- [1] G. Ahmad, . S. A. M., . Z. M., . M. Z. I., . S. A., and . S. A., "Effect of Cotton Leaf Curl Virus Disease on Morphology, Yield and Fibre Characteristics of Susceptible Lines/cultivars of Cotton (*Gossypium hirsutum* L.)," *Asian J. Plant Sci.*, vol. 1, no. 6, pp. 705–707, Oct. 2002, doi: 10.3923/AJPS.2002.705.707.
- [2] I. Fricke-Galindo and R. Falfán-Valencia, "Genetics Insight for COVID-19 Susceptibility and Severity: A Review," *Front. Immunol.*, vol. 12, p. 622176, Apr. 2021, doi: 10.3389/FIMMU.2021.622176/BIBTEX.
- [3] A. K. M. Naeem Sattar, "Cotton leaf curl disease - an emerging threat to cotton production worldwide," *J. Gen. Virol.*, vol. 94, pp. 695–710, 2013, doi: 10.1099/vir.0.049627-0.
- [4] M. Rajput, M. T., Hussain, A., & Ahmed, "Evolution and recombination of begomoviruses causing cotton leaf curl disease in Pakistan," *Plant Pathol. J.*, vol. 37, no. 6, pp. 634–644, 2021.
- [5] L. Amrao, I. Amin, M. S. Shahid, R. W. Briddon, and Shahid Mansoor, "Cotton leaf curl disease in resistant cotton is associated with a single begomovirus that lacks an intact transcriptional activator protein," *Virus Res.*, vol. 152, no. 1–2, pp. 153–163, 2010, doi: <https://doi.org/10.1016/j.virusres.2010.06.019>.
- [6] A. M. L. Shah, Nargis, Jamal-U-Ddin Hajano, Manzoor A. Abro, "Screening Of Cotton Genotypes For Resistance To Cotton Leaf Curl Disease Under Natural Inoculation Conditions In Sindh, Pakistan," *Int. J. Phytopathol.*, vol. 12, no. 1, pp. 73–86, 2023, doi: 10.33687/phytopath.012.01.4381.
- [7] L. Amrao, S. Akhter, and S. M. , Muhammad Nouman Tahir, Imran Amin, Rob W. Briddon, "Cotton leaf curl disease in Sindh province of Pakistan is associated with recombinant begomovirus components," *Virus Res.*, vol. 153, no. 1, pp. 161–165, 2010, doi: <https://doi.org/10.1016/j.virusres.2010.07.003>.
- [8] S. Hussain, A., Shakeel, A., Nadeem, M. A., & Ahmad, "Assessment of genetic variability for fiber and yield related traits in upland cotton," *J. Anim. Plant Sci*, vol. 30, no. 4, pp. 1045–1053, 2020.
- [9] N. M. Kamal, Y. S. A. Gorafi, H. Tomemori, J. S. Kim, G. M. I. Elhadi, and H. Tsujimoto, "Genetic variation for grain nutritional profile and yield potential in sorghum and the possibility of selection for drought tolerance under irrigated conditions," *BMC Genomics*, vol. 24, no. 1, Dec. 2023, doi: 10.1186/s12864-023-09613-w.
- [10] A. Shahzad, A., Farooq, J., & Shakeel, "Physiological and biochemical basis of resistance in cotton genotypes against cotton leaf curl virus," *Pak. J. Bot*, vol. 54, no. 3, pp. 947–956, 2022.
- [11] A. K. M. Naeem Sattar, "Cotton leaf curl disease – an emerging threat to cotton production worldwide," *J. Gen. Virol.*, vol. 94, no. 4, 2013, doi: <https://doi.org/10.1099/vir.0.049627-0>.
- [12] I. Din *et al.*, "Genetic Variability and Heritability for Yield and Yield Associated Traits of Wheat Genotypes in Nowshera Valley, Pakistan," *Pakistan J. Agric. Res.*, vol. 31, no. 3, 2018, doi: 10.17582/JOURNAL.PJAR/2018/31.3.216.222.
- [13] A. Aslam, M., Khan, A. I., Iqbal, M., & Abbas, "Relationship between disease indices and fiber yield in cotton genotypes under natural virus pressure," *Pak. J. Phytopathol*, vol. 33, no. 1, pp. 43–52, 2021.
- [14] L. Z. Ali Y, Atta BM, Akhter J, Monneveux P, "Genetic variability, association and diversity studies in wheat (*Triticum aestivum* L.) germplasm," *Pak. J. Bot*, vol. 40, no. 5, pp. 2087–97,

2008.

[15] A. Ullah, I., Ahmed, M., & Khan, "Impact of cotton leaf curl disease on fiber quality and yield components in commercial cultivars of Pakistan," *Crop. J.*, vol. 11, no. 4, pp. 768–778, 2023.

[16] Z. Siddique *et al.*, "Physiological response of cotton leaf curl burewala virus-infected plants of tolerant and susceptible genotypes of different *Gossypium* species," *J. Plant Pathol.*, vol. 97, no. 3, pp. 483–490, 2015, doi: 10.4454/JPP.V97I3.024.

[17] A. S. Aziz Ullah, "A Line \times Tester analysis for some seed cotton yield and fiber quality traits in upland cotton," *Int. J. Cott. Res. Technol.*, vol. 2, no. 1, 2020, [Online]. Available: <https://sciplatform.com/index.php/ijcrt/article/view/373>

[18] D. Steel, R. G. D., Torrie, J. H., & Dickey, "Principles and procedures of statistics: A biometrical approach (3rd ed.)," *McGraw-hill*, p. 666, 1996, [Online]. Available: https://books.google.com.pk/books/about/Principles_and_Procedures_of_Statistics.html?tid=XBbvAAAAMAAJ&redir_esc=y

[19] M. Farooq, J., Riaz, M., Mahmood, A., Hussain, S., & Shahid, "Genetic variability studies for leaf curl virus resistance and yield traits in upland cotton," *J. Anim. Plant Sci.*, vol. 28, no. 3, pp. 845–854, 2018.

[20] S. S. Muhammad Afzal, "A Critical Review of Whitefly (*Bemisia tabaci gennadius*) Cryptic Species Associated with the Cotton Leaf Curl Disease," *J. Innov. Sci.*, vol. 9, no. 1, 2023, doi: <https://researcherslinks.com/current-issues/A-Critical-Review-of-Whitefly-Bemisia-tabaci-gennadius-Cryptic/27/8/5963/html>.

[21] F. Nadeem, M., Shabbir, A., & Ahmad, "Integrated management strategies for cotton leaf curl disease: Challenges and future prospects," *Pak. J. Agric. Sci*, vol. 56, no. 2, pp. 285–293, 2019.

[22] S. Rajput, M. T., Ali, A., & Abbas, "Yield reduction and fiber deterioration in cotton under natural infection of leaf curl virus complex," *Plant Dis.*, vol. 106, no. 9, pp. 2365–2374, 2022.

[23] Gregory Shaner and Robert E. Finney, "The Effect of Nitrogen Fertilization on the Expression of Slow-Mildewing Resistance in Knox Wheat," *Online APS Publ.*, 1977, doi: <https://doi.org/10.1094/Phyto-67-1051>.

[24] G. Ahmad, S., Khan, M. A., Akhtar, M. A., & Sarwar, "Epidemiological assessment and area under disease progress curve (AUDPC) for cotton leaf curl virus in Pakistan," *Pak. J. Phytopathol.*, vol. 29, no. 1, pp. 45–51, 2017.

[25] M. H. Khalid Pervaiz Akhtar, "Evaluation of Bt-cotton Genotypes for Resistance to Cotton Leaf Curl Disease under High Inoculum Pressure in the Field and Using Graft Inoculation in Glasshouse," *Plant Pathol. J.*, vol. 31, no. 2, pp. 132–139, 2015, doi: 10.5423/PPJ.OA.01.2015.0014.

[26] E. novel traits in plants through R. Interference, "Engineering novel traits in plants through RNA interference," *Trends Plant Sci.*, vol. 11, no. 11, pp. 559–565, 2006, [Online]. Available: [https://www.cell.com/trends/plant-science/fulltext/S1360-1385\(06\)00255-X?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS136013850600255X%3Fshowall%3Dtrue](https://www.cell.com/trends/plant-science/fulltext/S1360-1385(06)00255-X?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS136013850600255X%3Fshowall%3Dtrue)

[27] G. Sarwar, A. Nazir, M. Rizwan, E. Shahzadi, and A. Mahmood, "Genetic Diversity among Cotton Genotypes for Earliness, Yield and Fiber Quality Traits using Correlation, Principal Component and Cluster Analyses," *Sarhad J. Agric.*, vol. 37, no. 1, pp. 307–314, 2021, doi: 10.17582/JOURNAL.SJA/2021/37.1.307.314.



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