Genetics of Cotton Leaf Curl Virus in Relation to Morphological Markers in Cotton (*Gossypium hirsutum* L.)

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Cotton is an important fiber crop, but its production is adversely affected by different biotic stresses, especially Cotton Leaf Curl Virus (CLCuV) stress. The objective of this study was to determine the genetic variability and inter-relationship among the morphological, yield-related, and disease-specific traits of eight cotton genotypes under CLCuV infection. The experiment was conducted under screen house conditions in a Completely Randomized Design (CRD) and replicated three times. Ten traits, including plant height, number of leaves, leaf area, leaf curling score, sympodial branches, number of bolls, boll weight, root length, biomass, and disease severity index (DSI), were assessed. Results: Genetic variation was detected for all traits among genotypes. The superior performance of genotypes 1, 2, and 3 was confirmed by the higher values of plant height (98–105 cm), leaf area (179–185 cm²), boll number (23–25), and lower DSI (12–15 %), expressing better resistance against CLCuV. In comparison, reduced growth, bolls, biomass, and higher DSI (55–60%), the genotypes 7 and 8 indicated high susceptibility. Correlation analysis revealed negative significant correlations of DSI and leaf curling score with boll number, biomass, and boll weight, along with positive correlations among growth and yield traits. These results are useful for the strategic selection of genotypes with combined morphological and resistance attributes. Genotypes having low DSI and stable yield components could be proven as important genetic resources in the breeding efforts for the improvement of CLCuV-tolerant cotton varieties.

Keywords: CLCuV, Cotton, Genetic, Morphological Parameters

Introduction:

Cotton (Gossypium hirsutum L.), known as "white gold," is the most important fiber and oilseed crop worldwide. It plays a crucial role in the agrarian economies of many developing countries and ranks as the second most important cash crop after wheat. In Pakistan, cotton has long been a major contributor to foreign exchange earnings, textile production, and employment opportunities. However, these challenges are making the sustainability of cotton production more difficult. Cotton Leaf Curl Virus (CLCuV) is one of the most serious challenges, which individually and together constrain crop yield and fiber quality, and economic crop returns.

Cotton leaf curl virus (CLCuV) belongs to a complex of begomoviruses and is transmitted by the whitefly (Bemisia tabaci), a pest that thrives in hot, dry climates—conditions common in major cotton-growing regions. The virus induces symptoms such as upward or downward curling of leaves, vein thickening, enations, and stunted plant growth. These symptoms reduce photosynthetic area and interfere with hormones and enzyme activity, causing losses in yield which may reach more than 60% in the event of an epidemic[1]. Climate change, high vector populations, and the perpetuation of susceptible genotypes by farmers are worsening the spread and virulence of the virus.[2]. Under the pressure of climate change and viral infection, a more complex and stacked stress situation hampers the resiliency of cotton genotypes.

CCLuV is quite critical for cotton production, particularly in areas such as South Punjab, where both stresses are common. Dual stimuli influence both vegetative development and reproductive efficiency, ultimately affecting yield components. Despite the increasing severity of these problems, few commercially available cotton cultivars have been specifically bred to resist or tolerate both. Thus, it is imperative to examine the morphological and physiological responses of different cotton genotypes to these combined stresses.

Genetic diversity forms the foundation of plant breeding. Identifying and utilizing genotypes with superior performance under adverse conditions is essential for developing resistant or tolerant cultivars. Morphological characteristics such as plant height, leaf number, leaf area, sympodial branch number, boll number, boll weight, and root length are simple but effective parameters for the assessment of plant growth under stress. These characteristics can be easily measured in field conditions with simple equipment and are commonly tested in stress screening experiments. Moreover, they offer an initial understanding of a plant's ability to maintain physiological homeostasis and productivity under stress[3]. Correlation among morphological and disease severity traits can provide a useful relationship that aids in indirect selection for resistance. For instance, a significant negative correlation of leaf curling score with boll load shows that the genotypes with less disease symptom are high in productivity. In the same way, positive relationships between biomass and boll weight or root length with leaf area may also indicate adaptive traits involved in CLCUv tolerance. Knowledge of these interactions can facilitate the identification of elite genotypes based on a combination of several selection criteria[4]. Phenotypic evaluation helps in identifying potential stable performers, and thus it provides valuable information on the extent of heritability of a trait and the ability of the genotype to manage stress. The objectives of this research are as follows:

Objectives:

To evaluate the morphological and yield-related performance of different cotton genotypes under Cotton Leaf Curl Virus and water stress conditions.

To assess the genetic variability among genotypes for disease severity and stress-related traits.

To identify tolerant and susceptible genotypes based on growth, yield, and disease resistance indicators.

To determine the correlation among morphological, yield, and disease traits for effective selection criteria in breeding programs.

Material and Methods:

The experiment was conducted at the Plant Protection Research Institute, Ayub Agricultural Research Institute, and Faisalabad. Eight cotton (Gossypium hirsutum L.) genotypes were selected for the experiment due to their known or presumed variability in response to CLCuV and drought. The selected genotypes included both tolerant and susceptible lines obtained from local breeding programs or previously tested material. For uniformity in data presentation, the genotypes were labeled as G1 through G8.

Table 1: List of germplasm used in the experiment with coding and full names

Code	Name of genotype
G1	FH-lalazar
G2	FH-Tristar
G3	FH-142
G4	FH-942
G5	FH-492
G6	MNH-1016
G7	NIAB-1048
G8	VH-142

The experiment was arranged under a Completely Randomized Design (CRD) with three replications per genotype following control screen house conditions to provide uniform CLCuV exposure. Each replication included five plants per genotype, and all plants were potted in similar earthen pots (40 cm diameter) containing sterilized loamy soil + FYM in the proportion of 3:1. The virus infection was achieved by releasing viruliferous whiteflies (*Bemisia tabaci*) obtained from a naturally infected cotton field on the plants at a 4–5 leaf stage. The whiteflies were confined using insect-proof netting in order to apply consistent infection pressure on all genotypes. The net was removed after 14 days, and the plants were monitored for disease symptom development. Ten morphological, yield-related, and disease traits were recorded 90 days after sowing from five plants randomly selected per replication:

In this study, various morphological, physiological, and pathological parameters of cotton plants were recorded to assess growth and performance under experimental conditions. Plant height (PH) was measured as the distance from the soil surface to the terminal bud using a meter rod (cm), while the number of leaves (NOL) was determined by counting all fully expanded leaves per plant. Leaf area (LA) was recorded using a leaf area meter (cm^2), and the severity of leaf curling was assessed visually through a curling score (CS) on a 0–5 scale, where 0 indicated no symptoms and 5 represented very severe curling. The number of sympodial branches (SB), representing fruit-bearing branches, was counted, while the number of bolls per plant (NOB) was taken as the total number of open bolls per plant at harvest. Boll weight (BW) was measured by weighing five randomly selected bolls on a digital balance (g). Root length (RL) was measured from the crown base to the tip of the longest root (cm) after uprooting the plants, and total biomass was recorded as the fresh weight of the whole plant (g) at harvest. Furthermore, disease severity was quantified using the Disease Severity Index **(DSI%), calculated by the formula:**

$\text{DSI}\ (\%) = \sum (\text{Rating} \times \text{Number of plants at that rating}) \times 100 / \text{Total plants observed}$.

The layout of the study is represented in the form of a flowchart in Figure 1.

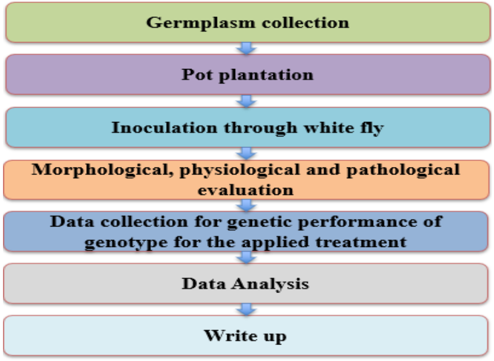

Figure 1. Flow chart of the conducted experiment for genetic evaluation of genotypes for CLCuV

Statistical Analysis:

The value(s) obtained were analyzed using a software Statistix 10 based on ANOVA to determine significant difference(s) among genotypes for each parameter. Correlations between anatomical and disease-related traits were analyzed, and Pearson's correlation coefficients were calculated. Visual data analyses, such as tables and correlation matrices, were done on Microsoft Excel.

Results and Discussion:

Mean Data Representation:

Plant height (PH) of eight cotton genotypes ranged from 75 to 105 cm, which reflects significant differences in genetic variation of the vertical growth under stress. The maximum plant height (105 cm) was observed in genotype G1, followed closely by genotypes G2 and G3, indicating that G2 and G3 also exhibited superior vegetative growth. In contrast, genotypes G7 and G8 exhibited the shortest plants, likely due to the combined detrimental effects of CLCuV and water stress, which restricted cell elongation and internodal growth.

Leaf number per plant varied between 29 and 42; genotype 1 also had the highest number of leaves, followed by genotypes 2 and 3. These genotypes presented a thick canopy, which is usually related to higher photosynthesis rate and biomass formation. Contrarily, genotypes 7 and c-8 produced a lesser number of leaves, probably due to the deleterious effects of viral infection and water stress that restrict the production of leaves or accelerate premature senescence.

Leaf area, an important physiological parameter, was also highly variable among genotypes. The leaf area was largest in genotype 1 (185 cm^2) and smallest in genotype 7 (125 cm^2). The higher leaf area in genotypes 1–3 indicates higher light interception and photosynthetic capacity that might contribute to high yield under stress. On the other hand, susceptible genotypes with smaller leaf areas may reflect virus-induced chlorosis or necrosis, or represent an adaptive trait to reduce transpiration. Leaf curl score (LC), which represents the severity of CLCuV symptoms, was measured, and it varied from 1.0 to 3.4. The lowest ratings (≤ 1.3) were observed for genotypes 1 to 3, showing a lack of visual viral symptoms and suggesting tolerance or resistance of a genetic nature to CLCuV. On the other hand, genotypes G7 and G8 exhibited high curling scores (≥ 3.1), indicating their susceptibility to the disease. Leaf curling disrupts both the structure and function of leaves, with its severity being associated with reduced photosynthesis and stunted growth. Direct fruiting sympodial branches also significantly differed. The highest number of branches (18) was observed in genotypes 1 and 3, indicating that they have positive potential seed yield under stress. Genotypes 7 and 8, showing the least number of branches (11 and 12), seemed to be going through stress-induced recovery and the inhibitory effect of the virus on host plant architecture. The bolls per plant were also affected at the same levels and were between 13 and 25. Genotype 1 produced the maximum number of bolls, followed by genotypes 2 and 3, expressing better boll setting ability than others under stress conditions. Genotypes 7 and 8 showed the least number of bolls, and this might be related to floral abortion, insufficient pollination, or retarded development because of virus injury and water stress.

Boll weight, as an important yield component, varied between 3.6 g and 4.9 g, and genotype 1 bore the heaviest bolls, signifying successful assimilate allocation to boll development. Genotypes 7 and 8 recorded the minimum boll weight, and it could be due to the disruption of photosynthesis and the limited movement of nutrients in the plants subjected to stress. Root length varied between 16 and 23 cm, which corresponded to genotypic differences in below-ground adaptation to water stress. Genotypes 1 and 3 presented long roots that could promote the absorption of water from deeper layers of the soil, reducing the effect of drought. Genotypes with smaller root size, like 7 and 8, could have had restricted access to soil water and thus constrained growth and yield.

Biomass accumulation as an integrated measure of overall plant performance varied from 95g to 145 g, while the highest biomass was obtained in genotype 1, since its plant height, leaf area, boll number, and root length were the best. The biomass of genotype 7 was the minimum, indicating it also vegetatively and reproductively scored lowest under combined stresses. DSI varied from 12% for genotype 1 to 60% for genotype 7. Genotypes 1-3 exhibited the lowest values for DSI, and this supports their resistance or tolerance to CLCuV. In contrast, genotypes G6 to G8 exhibited a high disease severity index (DSI), consistent with their poor performance in other growth and yield parameters. This emphasizes the need for selecting low-DSI genotypes in the breeding program of resistant cotton varieties to the virus.

Table 2. Mean data of all the characters for all the genotypes under study

Genotypes	PH	NOL	LA	CS	SB	NOB	BW	RL	Biomass	DSI (%)
G1	105	42	185	1	18	25	4.9	22	145	12
G2	98	40	179	1.3	17	23	4.7	21	140	15
G3	100	41	181	1.1	18	24	4.8	23	142	13
G4	86	34	145	2.5	14	18	4.1	18	115	35
G5	83	31	140	2.8	13	17	3.9	17	110	42
G6	89	33	150	2.3	15	19	4.2	19	118	30
G7	75	29	125	3.4	11	13	3.6	16	95	60
G8	78	30	130	3.1	12	14	3.7	16.5	98	55

PH=plant height, NOL=no of leaves, LA= leaf area, CS= curling score, SB=sympodial branches, NOB= no of bolls, BW= boll weight, RL=root length, DSI=disease susceptibility index

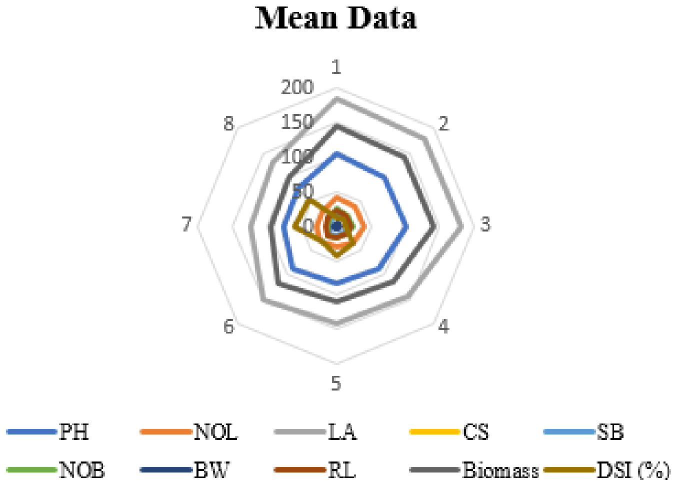

Figure 2. Graphical representation of the mean data of the experiment for evaluating the genetic performance of genotypes for different characters

Analysis of variance:

The mean sum of squares (MSS) for ten traits of CLCuV and water-stressed cotton indicates that there is considerable genetic variability among the genotypes for all the studied traits (Table 1). Disease Severity Index (%) (DSI% reached the highest MSS value (569), indicating strong genetic variability between genotypes to CLCuV infection, and indicating, perhaps, some genotypes harboring resistance/tolerance mechanisms. Leaf area (LA) was also high with an MSS of 301*, indicating large variations in photosynthetic capacity and canopy growth, which are of interest in stress. Leaf curl score (CS) also showed highly significant MSS of 285, indicating a considerable range amongst visual disease symptoms on which this parameter could be used as a criterion for screening of the genotypes for CLCuV resistance.

Biomass (MSS = 225*) and NOB (NOB = 112*) also showed significant differences, indicating that performance under combined stress is discernible from vegetative to reproductive traits. Plant height (PH = 161) and number of leaves (NOL = 82.3*) varied among accessions and reflect plant architecture with consequences in productivity.

Root length (RL = 25.9**) exhibited considerable variation, suggesting that root traits under drought are of major importance, as deep roots enhance stress tolerance.

Table 3. MSS values of Analysis of variance for different Morphological and Disease Parameters of Cotton Genotypes Under CLCuV Stress Conditions

SR no	Characters	MSS
1	PH	161**
2	NOL	82.3*
3	LA	301**
4	CS	285*
5	SB	66.2**
6	NOB	112*
7	BW	77.1**
8	RL	25.9**
9	Biomass	225*
10	DSI (%)	569*

PH=plant height, NOL=no of leaves, LA= leaf area, CS= curling score, SB=sympodial branches, NOB= no of bolls, BW= boll weight, RL=root length, DSI=disease susceptibility index

Correlation Analysis:

Through correlation analysis, multiple significant associations were identified among morphological and disease parameters in cotton under Cotton Leaf Curl Virus (CLCuV) and water stress. Biomass was significantly correlated with the number of bolls (r = 0.36), boll weight (r = 0.65), number of leaves (r = 0.64), and plant height (r = 0.43); the results implied that more vigorous plants are likely to perform better in reproductive growth. Boll weight also had a positive relationship with the number of bolls (r = 0.73), similar to NOB, and both the traits had a significant negative association with leaf curling score (NOB: r = –0.74, BW: r = –0.23), indicating that boll development was lowered by disease symptoms. Cross-correlation of DSI with the boll weight (r = –0.24) and root length (r = –0.36) was found to be significant, as well as with the curling score (r = 0.36), further indicating the effects of viral infection on plant health. Higher curling (r = –0.36) and DSI (r = –0.65) significantly decreased leaf area, which was positively correlated with root length (r = 0.64). A strong negative correlation was observed between plant height and curl score (r = –0.87), indicating that plants exhibiting more severe symptoms tend to have reduced growth. As well, root length and number of leaves (r = 0.32) were positively related, and sympodial branches (r = 0.20) were associated with both biomass and height of plants (r = 0.38*), suggesting that the actual development from them was a combination of structural and yield-related development. These associations highlight the intricate relationship between disease resistance and agronomic traits, which provides important clues in relation to the screening of cotton-tolerant genotypes.

Table 4. Pearson Correlation Matrix Among Morphological and Disease Parameters of Cotton Genotypes Under CLCuV Stress Conditions

	Biomass	**NOB**	**BW**	**CS**	**DSI**	**LA**	**NOL**	**PH**	**RL**
NOB	0.36*								
BW	0.65*	0.73							
CS	-0.26	-0.74*	-0.23*						
DSI	-0.46	-0.37	-0.24*	0.36*					

LA	0.35	0.27	0.46	-0.36*	-0.65*				
NOL	0.64*	0.34*	0.35	-0.32	-0.43	0.35			
PH	0.43*	0.25*	0.68	-0.87*	-0.35	0.45	0.47		
RL	0.42	0.16	0.32	-0.37	-0.36*	0.64*	0.32*	0.55	
SB	0.2*	0.31	0.83	-0.75	-0.32	0.36	0.74	0.38*	0.48

PH=plant height, NOL=no of leaves, LA= leaf area, CS= curling score, SB=sympodial branches, NOB= no of bolls, BW= boll weight, RL=root length, DSI=disease susceptibility index

Discussion:

The ANOVA results showed that there was a significant genetic variation between cotton genotypes for all studied traits under the integrated stress condition (CLCuV-infection and water-deficit, as MSS values confirmed this. The highly significant differences ($p < 0.01$) on traits such as PH, LA, SB, BW, and RL under normal and saline conditions reflect that these traits are strongly controlled by genetic factors even at stress conditions and might be utilized as reliable traits for genotype selection[3]. In particular, the highest MSS value was found for DSI at 569**, indicating that variations in DSI resistance levels exist among genotypes that have previously been shown to be genotype specific and dependent on complex gene interactions[1].

Leaf area (and leaf curling score) present significant variation. Foliar symptoms are variable across genotypes and could be used as phenotypic markers for virus tolerance. These differences are also important when considering the differences in biomass (MSS = 225) and number of bolls (MSS = 112), and reflect that the effects of CLCuV and drought stress on yield-related traits were significant, and the genotypic response in tolerance and compensatory growth, however, it is variable[2]. The differences in plant height (MSS = 161*) and number of leaves (MSS = 82.3) indicate genotypic differentiation in growth responses to the tested genotypes, which may be due to both the direct physiological effect of water stress and the systemic effect of viral infection[5].

Highly significant differences were found for sympodial branches (MSS = 66.2) and boll weight (MSS = 77.1), indicating that these traits also contribute to genetic variability in yield potential under stress. The diversity in root length (MSS = 25.9**) is of particular interest because deep and well-developed roots are important for water uptake in the drought-prone environment and are frequently associated with higher stress tolerance[6]. Statistically significant variation in all the traits studied indicates wide genetic diversity and provides a good opportunity for selection and breeding of stress and disease-resistant genotypes.

These results are in agreement with those reported by[4] and [7], who stated that morphological indicators can also be used to identify cotton genotypes with stable performance under combined biotic and abiotic stresses. The high MSS values reported in the present study indicate that cotton genotypes respond differentially to CLCuV and drought stress, and key morphological traits, including leaf area, boll weight, and root growth, can prove to be valuable selection criteria while breeding for improved stress tolerance.

The association of morphological characteristics and disease response in cotton under Cotton Leaf Curl Virus (CLCuV) and water stress may prove helpful in understanding the interactions among growth, yield, and disease tolerance. There was a significant positive relationship of biomass with the number of bolls ($r = 0.36$) and boll weight ($r = 0.65$), which indicated that greater vegetative growth is in line with better boll production (comparable with the reports of [3], who stated genotypes having strong vegetative growth are the best under both biotic and abiotic stress. Boll weight was also highly associated with the number of bolls ($r = 0.73$*), reflecting the strong correspondence among yield components as previously stressed by[8] while evaluating the productivity of cotton under stress.

Notably, the disease-related traits, such as leaf curling score and DSI, were significantly negatively correlated with the majority of the morphological and yield traits. Leaf curling score was strongly and negatively correlated with the boll number (r = –0.74) and plant height (r = –0.87), suggesting that those that suffered from severe curly leaves produced less growth and fruits. Remarkably, this indicates that the disrupted appearance of the leaf, in addition to limiting photosynthesis, impairs the hormonal balance required for proper development, as previously reported in[5]. Likewise, DSI was negatively and moderately associated with boll weight (r = –0.24) and root length (r = –0.36), indicating that CLCuV had a harmful effect on yield and root development, as was observed on yield parameters and root characteristics by[2].

The leaf area had a significant negative correlation with curling score (r = –0.36) and DSI (r = –0.65), and a positive correlation with the root length (r = 0.64). This means that leaf health and size are at least good indicators of the general plant fitness, and can be used as selection factors in breeding programs, as it has also been reported by Abbas and co-workers. (2016). In fact, the significant positive correlation between the number of leaves and biomass (r = 0.64) and between plant height and biomass (r = 0.43*) suggests that taller plants with a higher number of leaves accumulate more DM, adding higher yield in stress conditions[7].

The sympodial branches, as fruiting points, had significant positive correlation with biomass (r = 0.20) and plant height (r = 0.38), which means the genotypes having higher branching structure will have higher possibility to perform better under combined CLCuV and drought stress which is also corroborated by the findings of[4] who stressed the significance of plant architecture in overcoming stress-induced yield penalties.

Collectively, the findings reveal the intricate and pleiotropic aspects of morphological and disease traits in cotton. The significant associations of boll weight, number of bolls, and plant height with biomass indicate that some genotypes have the genetic capacity to retain growth and productivity under stress, whereas the reverse correlations against disease indices indicate the severity of the effect of CLCuV. These results are useful for breeding programs that wish to breed elite cotton cultivars with resistance to disease and drought, and outstanding morphological characteristics[1].

Conclusions:

The present study demonstrated significant genetic variability among cotton genotypes for morphological, yield-related, and disease-specific traits under CLCuV infection. Superior genotypes such as G1, G2, and G3 expressed greater plant height, leaf area, and boll number, coupled with lower disease severity indices, highlighting their relative tolerance against CLCuV. In contrast, G7 and G8 exhibited reduced growth, fewer bolls, and higher DSI, confirming their susceptibility. The observed negative correlations of DSI and leaf curling score with yield traits, and the positive associations among growth and yield components, emphasize the detrimental impact of CLCuV on cotton productivity. These findings suggest that genotypes with low DSI and stable yield performance can serve as valuable resources for breeding programs aimed at developing CLCuV-tolerant cotton cultivars. The integration of both resistance and agronomic attributes in selection strategies will be crucial for sustainable cotton production under CLCuV pressure.

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