Research Article
Agrobiological Records
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

GENETIC ASSESSMENT OF ELITE COTTON GENOTYPES FOR COTTON LEAF CURL VIRUS DISEASE (CLCuD) THROUGH MULTIVARIATE ANALYSIS

Hina Firdous[1,2], Amer Habib[1], Huma Abbas[1,]* and Muhammad Asim Jamil[2]

[1]State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China
[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

**Corresponding author*: huma.abbas@uaf.edu.pk

ABSTRACT

This study aimed to investigate the genetic variability of six elite cotton genotypes for agronomic and fiber quality traits under cotton leaf curl virus disease (CLCuD) pressure using multivariate approaches. The selected genotypes were developed by different research institutes across Pakistan and evaluated in a randomized complete block design with three replications. For each genotype, the cotton leaf curl virus disease index (CLCVDI%) was estimated and observations were recorded on plant height, monopodial and sympodial branches, number of bolls, boll weight, seed cotton yield, lint percentage, lint index, seeds per boll, seed index, fiber length, fiber strength and fiber fineness. Correlation analysis indicated that CLCVDI% was negatively associated with seed cotton yield, number of bolls, boll weight, lint index, seed index, and fiber quality attributes. In contrast, seed cotton yield exhibited strong positive associations with sympodial branches, lint percentage, number of bolls, boll weight, lint index, seeds per boll, seed index, and fiber fineness, but a significant negative relationship with CLCVDI%, fiber length, and monopodial branches. Hierarchical clustering and principal component analysis (PCA) further identified MNH88 and FH2015 as superior genotypes, demonstrating enhanced yield potential and fiber quality under CLCuD stress, thereby suggesting greater tolerance to disease pressure.

Keywords: Cotton, Cotton leaf curl virus disease (CLCuD), Multivariate analysis

Article History (ABR-25-048) || Received: 07 May 2025 || Revised: 25 Jun 2025 || Accepted: 15 Jul 2025 || Published Online: 11 Aug 2025

1. INTRODUCTION

Cotton is the major source of fiber being cultivated worldwide (Zhang et al., 2015). Cotton is chief agro-industrial crop of Pakistan. The cultivation of cotton had 0.8 % contribution in GDP of Pakistan. In 2023-24, the cotton production was decreased by 22.8%, from 9.148 million bales to 7.024% than previous year (Anonymous 2023-24). The demand of cotton is increasing day by day due to increased population in Pakistan (Zafar et al., 2024). Different biotic and abiotic stresses are responsible for lower production of cotton (Zafar et al., 2025), but the CLCuV is an important biotic factor in reducing the cotton production in Pakistan. Firstly, it was reported in Nigeria in 1912 and then from their it spread worldwide (Rahman et al., 2017). In Pakistan, in 1988 this disease dropped 0.3 billion bales and from 1988 to 2002 it causes the losses of 7.7 million bales (Akram et al., 2025). The CLCuV is characterized by vein thickening and curling of upward or downward of leaves. As compare to healthy leaves, the most infected leaves become highly thick and brittle (Siddique et al., 2014). The severe infection of this disease cause stunted growth and lower the number of bolls and size and ultimately lower the yield (Monga and Sain 2021). The extent of yield losses can be 15 t0 70% (Brown 2001; Yousaf et al., 2013). It also deteriorate fiber quality (Akhtar et al., 2009). A complex of gemini viruses is responsible for CLCuV and is disseminated by whitefly (Rahman et al., 2017).

In control strategies of CLCuV, the management of whitefly is important. The intense use of pesticides to control whitefly caused resistance in whitefly (Nauen et al., 2015). Along with pesticide resistance the use of chemicals also polluted the environment as well as toxic to human health. So, the development of resistance genotypes is eco-friendly and economic way for the management of CLCuV (Akhtar et al., 2009). To sort out this problem, in different countries as well as Pakistan, various Bt cotton varieties was commercially adopted. But the status of these Bt varieties against CLCuV is not cleared. There is uttermost need to screen the exotic as well as indigenous cotton germplasm against CLCuV. The seed cotton yield is a complex character and affected by both environmental and genotypic factors. For the improvement of cotton genotypes for yield and fiber

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quality traits, the knowledge regarding relationship of these characters among themselves is prerequisite (Desalegn et al., 2009). Different studies suggested the relationship of seed cotton yield with number of bolls (Zafar et al., 2022), boll weight (Javed et al., 2017) and fiber quality traits (Zafar et al., 2023) while it also revealed negative association with CLCuV (Saeed et al., 2014). In this study we evaluated the different elite cotton genotypes for yield, ionic and fiber quality characters as well as the association of studied characters among themselves under CLCuV infestation.

2. MATERIALS AND METHODS

The proposed research was conducted in the experimental area of PBG (Department of Plant Breeding & Genetics), UAF. In this experiment six genotypes (FH113, MNH886, MNH888, IR4, VH61 & FH2015) was used. These genotypes were grown under normal field conditions to assess their genetic variability for agronomic, yield, and fiber quality traits under CLCuV infestation. The experiment was carried out under RCBD with three replications. All agronomic practices were kept constant. Throughout the experiment, no pesticides were applied against whitefly to ensure maximum inoculum pressure. The disease index was calculated according to the method of (Akhtar et al., 2010). At maturity five plants were selected for every genotype per replication and data was recorded for plant height (cm), number monopodial and sympodial branches, CLCVDI%, number of bolls, boll weight (g), seed cotton yield (g), lint (%), lint index (g), seed per boll, seed index (g), lint (%), fiber length (mm), fiber strength (g/tex) and fiber fineness (µg/inch).

1.1. Statistical Analysis

The multivariate analysis, including correlation matrix, PCA, and cluster analysis, was performed using statistical software packages: SAS-JMP Pro 15 (SAS Institute Inc., Cary, NC, USA, 1989–2019).

3. RESULTS

Mean square values of genotypes depicted significant differences for all studied traits except fiber length in six elite genotypes of cotton (Table 1). Significant differences among studied genotypes suggest that variability can be further utilized in cotton breeding program. The univariate simple statistics revealed significant variation in genotypes for studied characters under disease infection (Table 2).

Table 1: Mean square values for the influence of Heat stress on different traits of cotton genotypes

Source	DF	P.H	NB	BW	SCY	GOT%	CLCVDI%	H2O2	CAT	POD	SOD	TSP	FL	FS	FF
Replications	2	43.198	0.222	0.017	5.34	139.96	1.55	0.00	29.86	26.74	0.42	0.00	96.80	64.40	2.26
Genotypes	5	227.40**	1.822**	0.32**	72.07**	10.23**	14.85*	29.950**	222.77**	11.18**	12.58**	10.05*	0.13NS	0.28*	0.45*
Error	10	19.888	2.622	0.031	13.35	4.34	7.62	0.00	7.05	0.80	1.23	0.00	0.44	0.14	0.009
Total	17														

Table 2: Univariate Simple Statistics

Column	N	DF	Mean	Std Dev	Sum	Minimum	Maximum
PH	6	5.00	87.2679	11.5071	523.608	66.0620	99.1451
MB	6	5.00	0.9567	0.3889	5.7400	0.6000	1.6000
SB	6	5.00	21.0600	6.6380	126.360	11.5000	27.1400
CLCVDI %	6	5.00	17.8333	8.1452	107.000	10.0000	30.3333
NB	6	5.00	13.0042	5.9968	78.0255	5.1895	20.6297
BW	6	5.00	4.3073	1.2873	25.8439	2.9022	6.1052
SCY	6	5.00	25.4512	9.7704	152.707	11.4704	35.8723
Lint%	6	5.00	39.2820	3.1765	235.692	34.5229	42.9631
LI	6	5.00	4.8767	0.9533	29.2600	3.5000	5.8000
SPB	6	5.00	21.4083	2.5519	128.450	18.7000	25.6000
SI	6	5.00	6.5967	0.6217	39.5800	5.9000	7.4000
FF	6	5.00	5.0038	0.6889	30.0227	3.9400	5.6700
FS	6	5.00	26.2592	1.7144	157.555	24.2770	28.5645
FL	6	5.00	28.4277	2.8205	170.566	25.0112	32.3165

1.2. Parallel Coordinate Plots

The parallel coordinate plots are used for interpretation of multi-dimensional data set. It is an effective technique to explain multivariate data. The parallel coordinate plots showed that the increased in CLCVDI% reduced the seed cotton yield, lint%, boll weight, number of bolls, lint index, seed per bolls, seed index, fiber

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fineness and fiber strength. The disease index is negatively correlated with all yield and fiber quality traits. Under CLCVDI% the yield of all cotton genotypes was decreased (Fig. 1).

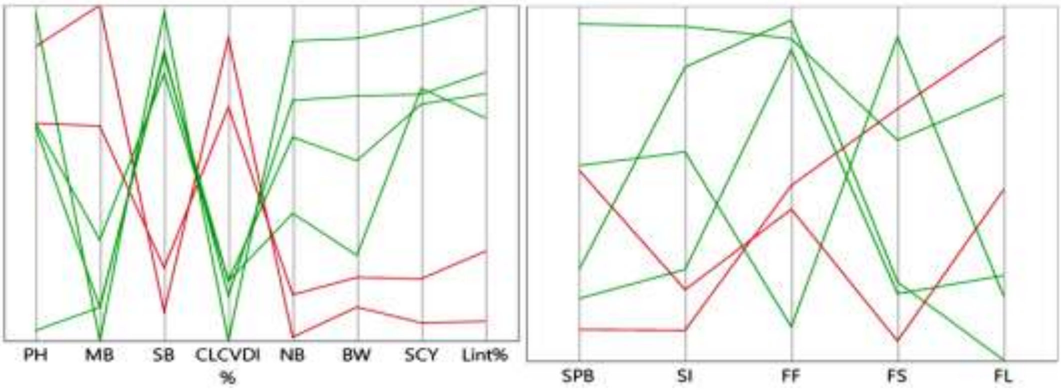


Fig. 1: Parallel coordinate plots (PCPs) for yield and fiber related traits of six cotton genotypes under disease infection. P.H. = Plant Height (cm), MB= number of monopodial branches, SB= number of sympodial branches NB= number of bolls, BW= Boll weight (g), SCY= Seed cotton yield (g), lint%, LI= lint index (g), SI= seed index (g), SPB= seed per bolls, FS= Fiber strength (g/tex), FF= fiber fineness (µg/inch), FL=fiber length (mm).

1.3. Mean Performance of Genotypes for Yield and Fiber-related Traits under Disease Infection

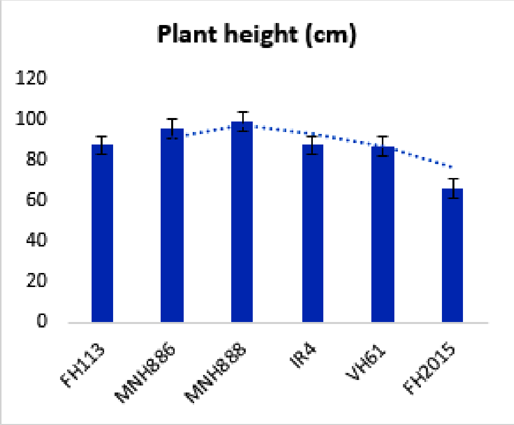
The mean performance of elite genotypes revealed that the MNH888 showed maximum plant height followed by MNH886, FH113, IR4, VH61 and FH2015. The FH113 exhibited higher number of monopodial branches and MNH888 showed least number of monopodial branches. The MNH888 showed higher number of sympodial branches from rest of all genotypes and MNH886 contains lower number of sympodial branches (Fig. 2). The MNH886 revealed highest disease index and MNH888 showed least disease index from rest of all genotypes. The genotypes MNH888 and FH2015 performed superior for number of bolls, boll weight, seed cotton yield, lint%, lint index seed per boll, seed index, fiber strength, and fiber fineness whilst MNH886 performed inferior for above mentioned traits from rest of all genotypes (Fig. 2). Interestingly the genotype MNH886 showed good fiber length under high disease index, so this genotype can be used in future breeding programs for improvement of fiber length under disease infestation.

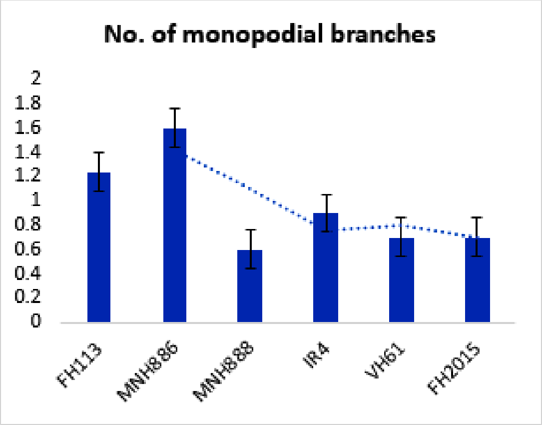
1.4. Correlation Analysis

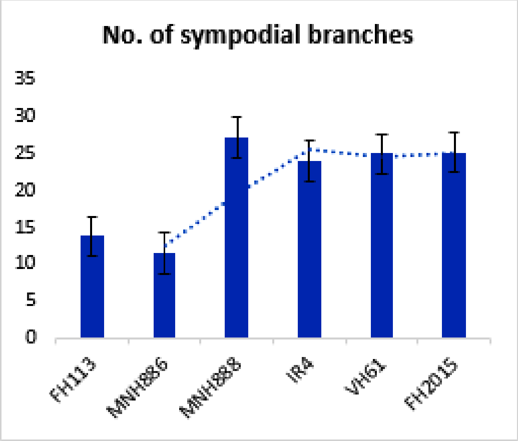
Plant height showed highly positive association with FL and lower positive relationship with FS. Plant height had negative association with SB, NB, BW, lint%, SCY, SI, LI and FF. SCY revealed highly significant and positive relationship with SB, lint%, NB, BW, LI, SPB, SI and FF whilst it exhibited significant negative correlation with CLCVDI%, FL and MB (Fig. 3). The SB was positively correlated with NB, SCY, BW, lint%, LI, SPB, SI and FF. The NB were significantly and positively correlated with lint%, SCY, SB, SPB and SI as well as negatively related with CLCVDI% (Fig. 3). BW showed positive association with SCY, lint%, NB, SB, LI, SI and FF while it revealed negative association with MB, CLCVDI%, P.H and FL. The character lint% was negatively correlated with MB, CLCVDI%, P.H and FL. Cotton leaf curl virus disease index was negatively correlated with SCY, GOT%, NB, BW, LI, SI, SPB, FF and FS. FF showed positive relationship with SI, LI, SCY, lint%, NB and BW while it showed significant negative association with CLCVDI%, FS and FL (Fig. 3). The FL revealed negative relationship with FF, SI, SCY, lint%, NB, BW, FF, SI and SB. The character FS exhibited negative correlation with FF and CLCVDI%. The traits LI and SI were positively related with NB, BW, SCY, lint% and FF. The number of bolls, boll weight, seed cotton yield and GOT% revealed highly significant positive relationship among themselves (Fig. 3).

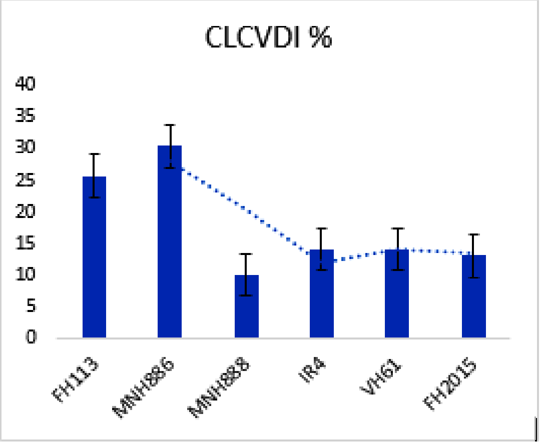
1.5. Hierarchical Clustering

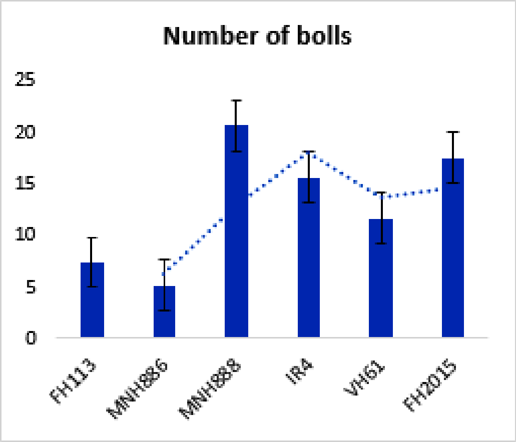
The cluster analysis was performed for cotton genotypes on the basis of agronomics, ionic and fiber quality related traits. The cluster analysis classified the cotton genotypes into two groups. The first group consists of FH113 and MNH886. The second group contains MNH888, IR4, VH61 and FH2015 (Fig. 4). The cluster analysis revealed that the genotype MNH88 and FH2015 are superior for yield and fiber quality traits under disease stress and exhibits tolerance against disease (Fig. 4). The genotypes in first and second group are represented by red and green color respectively.

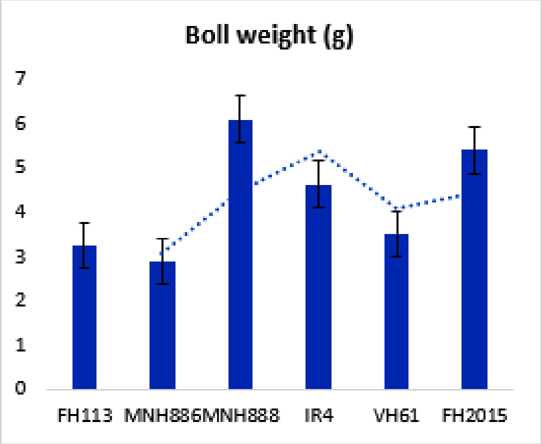


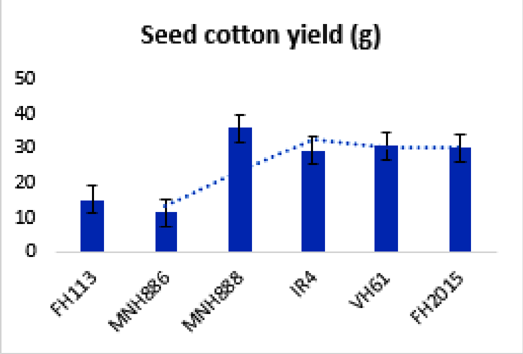


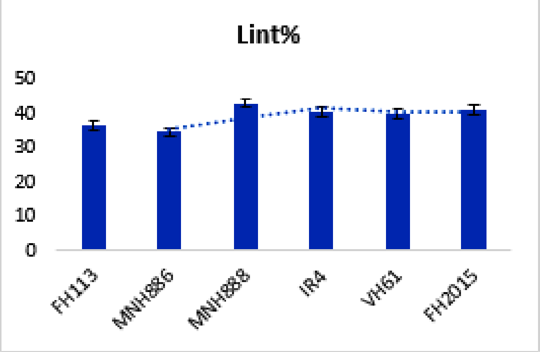












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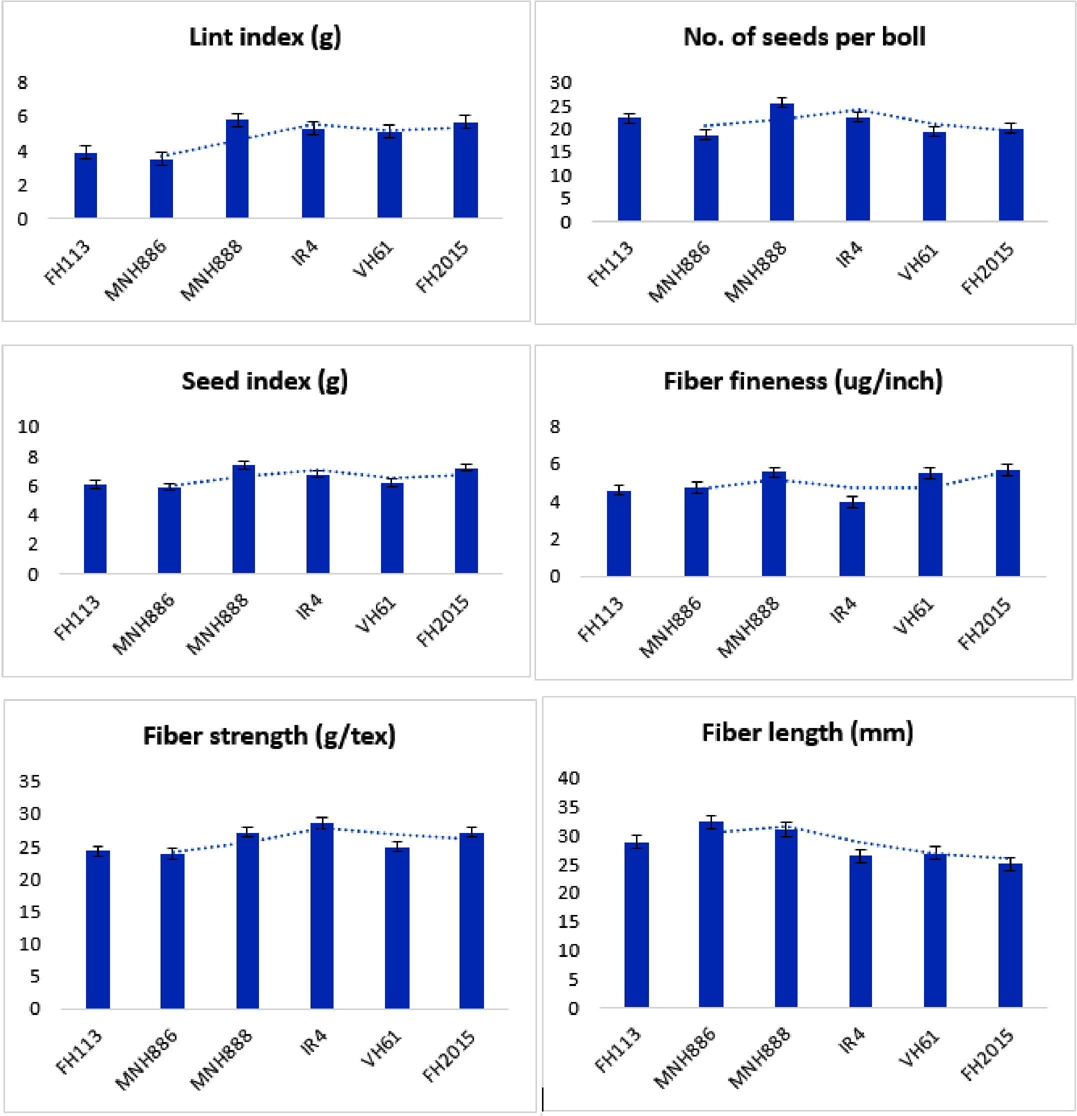

Fig. 2: Mean performance graphs for yield and fiber-related traits of six cotton genotypes under disease infection.

1.6. Principal Component Analysis

Principal component analysis was used to find out the relationship between different variables that lead to variability in the studied data. Principle component analysis that has Eigen values >1 was taken under consideration. The first three PCs had more than one eigen value. These three PCs contributed 91.48% variation towards total variability (Fig. 5). The PC1 revealed 65.83% followed by PC2 (17.32%) and PC3 (8.32%) variability of the total variability (Table 2). The first two principal shared 83.15% variability of the total variation. In PC1, among all studied traits in cotton genotypes, contribution of LI (0.995), lint% (0.994), NB (0.978), SCY (0.972), SB (0.970), SI (0.916), BW (0.914), SPB (0.529), and FF (0.471) were maximum to the total observed variability. On second principal component, P.H (0.838), FS (0.747), FL (0.697), and SPB (0.644) were the major loading factors. In PC3 the variation was explained by FF (0.743) and FL (0.483) (Table 3). The SPB (0.404) and FS (0.345) were the major loading factors in PC4 and PC5 respectively.

Table 2: Eigenvalues for studied traits under diseases infection

Number	Eigenvalue	Percent	Cum Percent
1	9.2161	65.830	65.830
2	2.4256	17.326	83.155
3	1.1653	8.323	91.479
4	0.6217	4.441	95.920
5	0.5713	4.080	100.000

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Table 3: Formatted Loading Matrix

	Prin1	Prin2	Prin3	Prin4	Prin5
LI	0.994752	-0.029001	-0.067579	-0.067218	0.023284
Lint%	0.994473	0.068078	-0.007645	-0.051743	-0.060439
NB	0.977725	0.157776	-0.033616	0.092745	0.097101
SCY	0.971850	0.032133	0.004781	-0.219170	-0.080100
SB	0.970023	-0.043896	-0.039025	-0.228446	-0.058463
SI	0.916074	0.170476	-0.008380	0.280027	0.230782
BW	0.914952	0.222844	0.030349	0.253708	0.218895
PH	-0.291077	0.837843	0.279619	-0.291923	-0.223357
FS	0.051325	0.746753	-0.512569	-0.240691	0.345061
FL	-0.495584	0.697146	0.483066	-0.033554	0.184137
SPB	0.529481	0.644097	0.139932	0.404688	-0.348476
FF	0.471515	-0.361898	0.743006	-0.124808	0.281193
CLCVDI %	-0.981349	0.020231	0.047471	0.153623	0.103398
MB	-0.959809	0.147239	-0.093836	0.125369	0.180458

Loading plot developed for all variables revealed that PC1 was plotted on X-axis while PC2 was plotted on Y-axis. Variables having large vector length on PC1 at X-axis contributed maximum variability towards PC1. The first two PCs contributed 83.15% variability towards total variation (Table 2). The LI, lint%, NB, SCY, SB, and SI have long vectors and lied towards the direction of PC1 and showed higher correlation among themselves. The character PH, FS and FL lied in the direction of PC2 and revealed positive correlation among themselves (Fig. 5A). The MNH888 and FH2015 are promising genotypes for yield and fiber quality traits under CLCuD. The genotypes IR4 and VH61 showed superior performance for most of studied traits after MNH888 and FH2015. The genotype MNH886 and FH113 revealed higher disease index. The score plot revealed that the MNH888 and FH2015 are promising genotypes for studied traits.

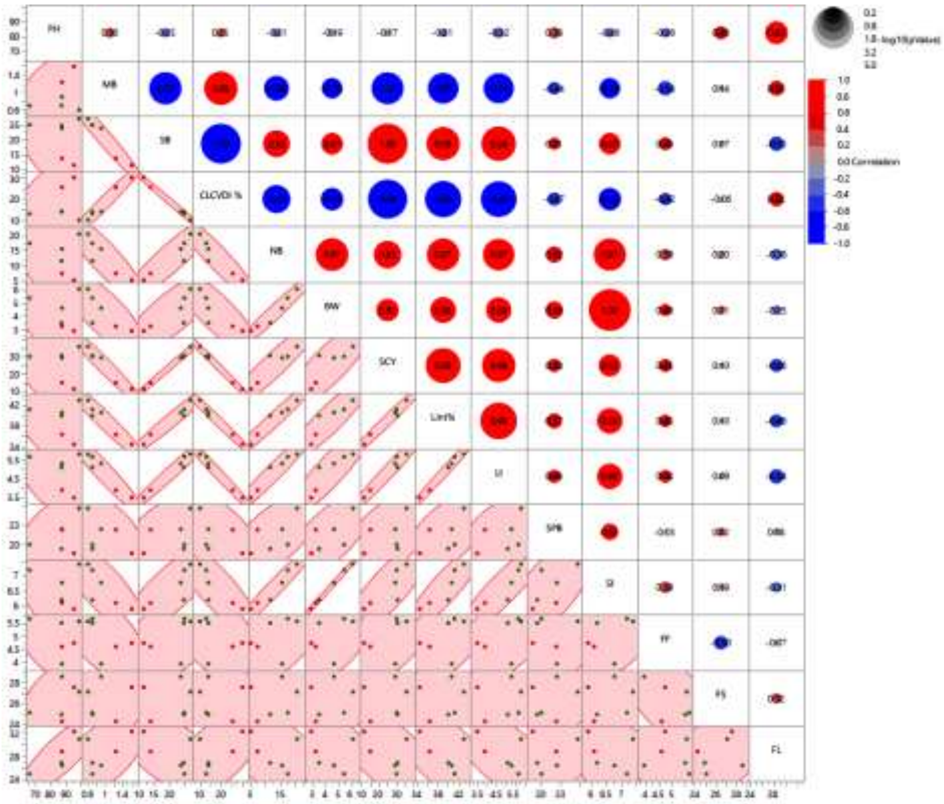

Fig. 3: Scatterplot correlation matrix for yield and fiber related traits of six cotton genotypes under disease infection. In the upper panel, red and blue boxes indicate positive and negative correlations, respectively, with increasing color intensity reflects a higher coefficient. The lower panel indicates the bivariate density distribution with ellipses between each pair of traits and trendline of the correlated traits.

The first three components explained 91.47% variability of the total variation. The agronomic, and fiber quality characters are illustrated by factor map squared cosines (Fig. 5B). The PC-I covered all MB, SB, NB, CLCVDI%, BW, SCY, lint%, and SI. The PC2 covered PH, SPB, FS and FL. Only FF fell in PC3. The genotypes FH113, FH2015, MNH86 and MNH888 are covered by PC1. The genotype VH61 fell in PC2. The genotype IR4 was occupied by PC3 (Fig. 5B).

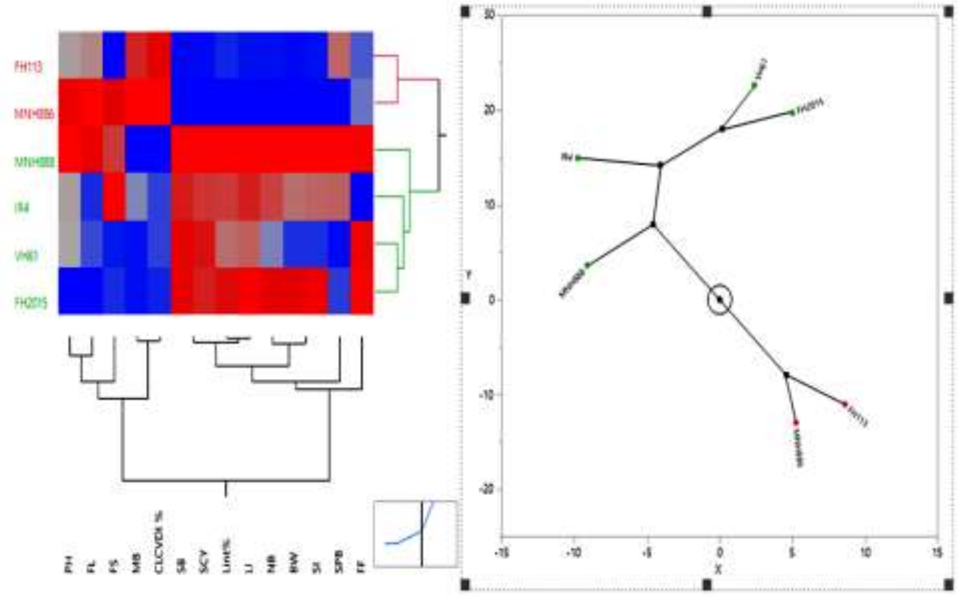

Fig. 4: Hierarchical cluster (left) and constellation plot (right) of 6 cotton genotypes for yield and fiber-related traits under disease infection.

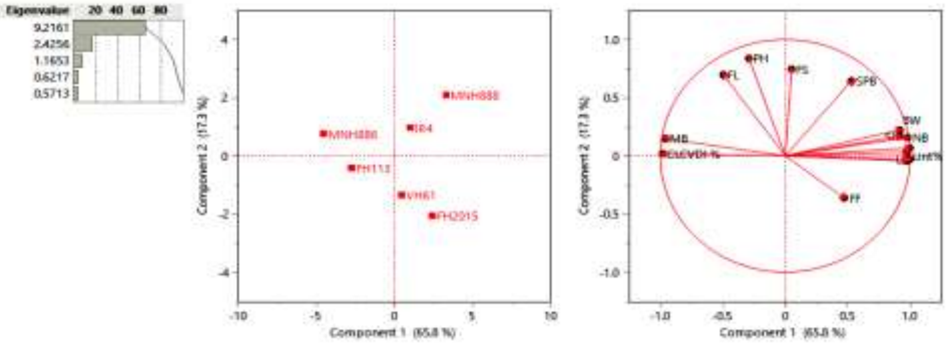

Fig. 5: Summary plots with (**left**) biplot between PC1 and PC2 displaying the distribution of 6 cotton genotypes under disease infection; (**right**) contribution of various traits in variation for genotypes under disease infection.

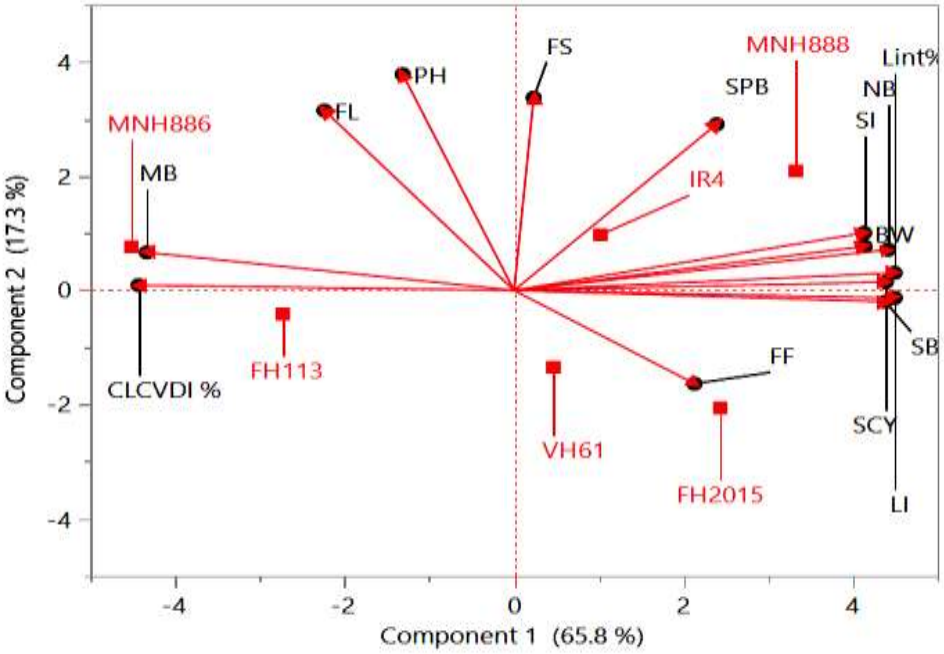

Fig. 5A: Principal component biplot for the contribution of traits under disease infection.

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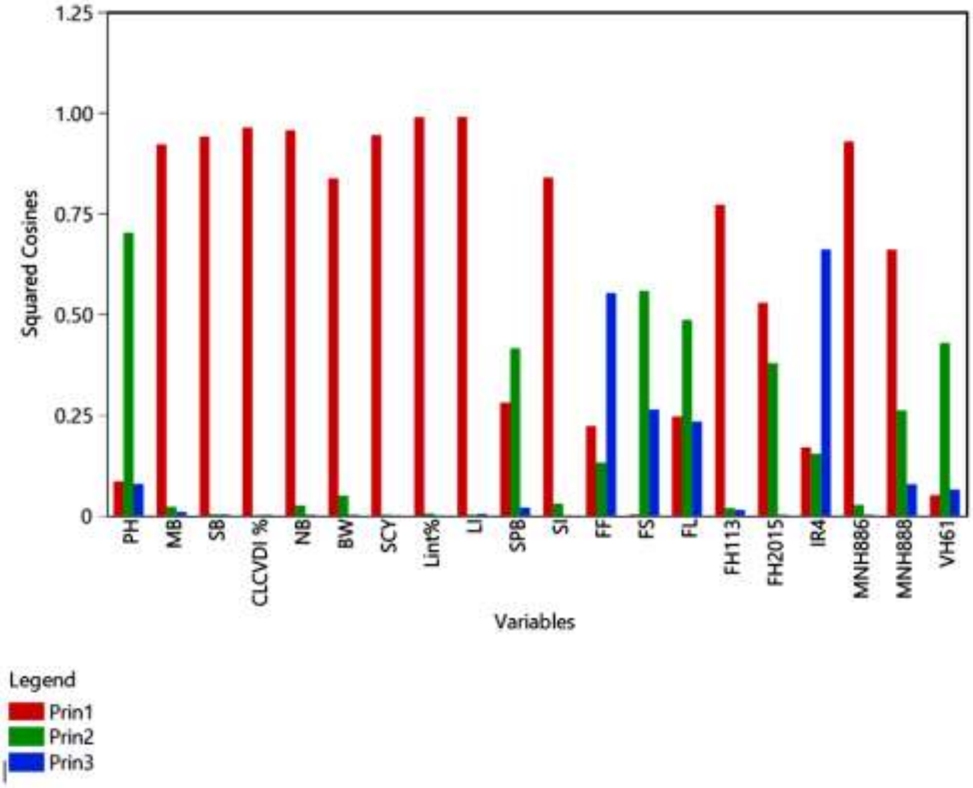

Fig. 5B: Squared cosines associated with the principal components for the studied traits under disease infection.

4. DISCUSSION

Improving yield potential and fiber quality remains the foremost objective in cotton breeding programs, particularly under the combined challenges of biotic and abiotic stresses (Zafar et al., 2025). The present study assessed the tolerance of elite cotton genotypes against cotton leaf curl virus disease (CLCuD), a devastating constraint to cotton production in South Asia. Evaluations were carried out under natural field conditions, which provided a realistic disease pressure scenario for the identification of tolerant and high-performing genotypes. Significant mean square values across all studied traits highlighted the presence of considerable genetic variability, confirming the potential of these genotypes as valuable sources for breeding interventions. The presence of such variability is a prerequisite for genetic improvement and has been widely reported in cotton germplasm under different stress conditions (Salahuddin et al., 2010; Chapepa et al., 2020; Zafar et al., 2020). In any breeding effort, the simultaneous improvement of yield-contributing traits and fiber quality attributes requires a detailed understanding of the correlation structure among traits. In this study, correlation analysis revealed that CLCuD severity was strongly and negatively associated with key agronomic traits such as PH, SB, BW, and SCY. Similar associations have been reported in earlier studies, where virus infection resulted in suppressed plant growth, reduced boll retention, and impaired fiber development (Sarwar et al., 2021; Manan et al., 2021). Fiber-related parameters such as lint percentage and fiber quality indices were also negatively influenced by CLCuD, corroborating earlier findings that viral stress compromises lint allocation and reduces overall fiber quality (Akhtar et al., 2009; Sahar et al., 2021). Conversely, SCY exhibited significant positive associations with BW, NB, lint%, LI, and SI. These interrelationships imply that selection for one yield component could indirectly enhance other complementary traits, thereby accelerating the breeding process. This synergistic effect among traits has also been observed by previous researchers, who demonstrated that simultaneous improvement of BW and lint% often leads to higher SCY (Méndez-Natera et al., 2012; Javed et al., 2017). Therefore, breeders can exploit these associations to design effective selection indices that enhance both yield and fiber quality under disease stress conditions. Interestingly, lint percentage showed a negative relationship with FL, a trade-off that has also been noted by Khan et al., (2017). Such antagonistic associations emphasize the importance of balancing yield and fiber quality in breeding strategies (Jamil et al., 2020; Kamal et al., 2024).

Multivariate techniques such as principal component analysis (PCA) and cluster analysis provided further insights into the genetic diversity among the studied genotypes. PCA confirmed substantial variation for yield and fiber-related traits, with the first two principal components capturing the largest proportion of total variation. High eigenvalues for lint index, lint percentage, and number of bolls, boll weight, seed index, and seed cotton yield in

PC1 indicated that these traits contributed most to the observed variability. This pattern is consistent with previous studies, where PCA was effectively employed to partition variability and identify key discriminating traits in cotton germplasm (Amna et al., 2013; Isong et al., 2017; Sahar et al., 2021). Such multivariate approaches are valuable not only for understanding trait interrelationships but also for designing more efficient breeding programs by targeting the most informative traits.

Hierarchical clustering supported the PCA results and grouped genotypes according to their performance under CLCuD stress. Notably, MNH888 and FH2015 emerged as superior genotypes, displaying a combination of higher yield potential and acceptable fiber quality despite heavy disease pressure. These findings suggest their potential role as parental lines in breeding programs aimed at developing CLCuD-tolerant cultivars. The identification of such genotypes is of immense practical importance, as sustainable cotton production in Pakistan and other affected regions depends on the deployment of cultivars that combine stress resilience with desirable agronomic and fiber traits. Overall, the integration of correlation analysis, PCA, and cluster analysis in this study provides robust evidence of exploitable genetic variability in cotton germplasm under CLCuD stress (Kamal et al., 2024). These results reinforce the importance of employing multivariate statistical tools to guide the selection of superior genotypes (Zafar et al., 2025). Future research should expand the genetic base by incorporating diverse germplasm, including wild relatives and exotic lines, while also integrating molecular markers linked to CLCuD resistance. Such approaches will accelerate the development of resilient cotton cultivars capable of sustaining yield and fiber quality under the dual threats of viral disease and climate-induced stresses.

5. CONCLUSION

The findings of this study demonstrated that the genotypes MNH888 and FH2015 exhibited superior performance for key agronomic and fiber quality traits under cotton leaf curl virus (CLCuV) pressure. Their consistent yield potential and relatively better fiber attributes, despite high disease stress, suggest an inherent tolerance to the virus. These genotypes can serve as valuable genetic resources for incorporation into future cotton improvement programs. By utilizing them as parental lines, breeders may accelerate the development of new cultivars that combine disease resistance, yield stability, and fiber quality, thereby contributing to sustainable cotton production in virus-affected regions.

DECLARATIONS

Funding: This research received no external funding.

Acknowledgement: We are thankful to the Department of Plant Breeding and Genetics (UAF), Pakistan for providing us with the facilities and materials to experiment.

Conflicts of Interest: The authors declare that they have no conflicts of interest in the publication of the manuscript.

Consent for Publication: Not applicable.

Data Availability: Not applicable.

Ethics Statement: Not applicable.

Author's Contributions: HF wrote the initial draft of the manuscript. AH and HA made all necessary corrections and carried out final editing of the manuscript. HA and MAJ proofread the manuscript. All authors have reviewed and approved the final version of the manuscript for publication.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Citation: Firdous H, Habib A, Abbas H, Jamil MA, 2025. Genetic assessment of elite cotton genotypes for Cotton Leaf Curl Virus Disease (CLCuD) through multivariate analysis. Agrobiological Records 21: 67-77. https://doi.org/10.47278/journal.abr/2025.034

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