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Original Research Article

SCREENING OF COTTON (*GOSSYPIUM HIRSUTUM* L.) GERMPLASM AGAINST COTTON LEAF CURL VIRUS (CLCUV)

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Abstract *Cotton Leaf Curl Virus (CLCuV) is a devastating disease affecting cotton production worldwide, particularly in South Asia and Africa transmitted by the whitefly (Bemisia tabaci). CLCuV infection leads to severe symptoms, including leaf curling, vein thickening, stunted growth, and significant yield losses. Developing resistant cotton varieties is the most suitable strategy for managing CLCuV, given the limitations and environmental concerns associated with chemical controls. This study aimed to evaluate the resistance and susceptibility of different cotton varieties to CLCuV under natural field conditions and artificially infected condition using a disease severity scale ranging from 0 (highly resistant) to 5 (susceptible). A total of five cotton varieties, were assessed at three critical growth stages: vegetative, flowering, and boll formation. Disease severity, and yield components were recorded to determine extent of resistance of each variety. The results indicated that genotypes and treatments had significant variation in control compared to T1. According to DSI, NIAB-852 was scored as resistant with 10% infected leaves. FH-142 and CIM-496 were scored as tolerant according to DSI with 10-25% infection in leaves. NIAB Karishma and CIM-448 were scored as moderately susceptible with 50-75% infection. All genotypes had significant reduction in number of bolls, boll weight and seed cotton yield in T1 than control. These findings provide valuable insights into the effectiveness of existing cotton germplasm against CLCuV and highlight the need for continuous evaluation and development of resistant varieties. The study highlights the importance of generating resistant cultivars, thereby ensuring sustainable cotton production.*

Keywords: *Cotton; Cotton Leaf Curl Virus; Screening; Breeding; Biotic stress*

Introduction

Cotton (Gossypium spp.) is one of the world's most important cash crops, providing a huge amount of natural fiber for the textile industry. Cotton is not only important economically, but also the livelihoods of millions of farmers is attached to this crop in cotton growing regions (Sattar et al., 2013). However, there are several biotic factors that affect cotton production, and one of the most devastating diseases, especially in South Asia and Africa, is Cotton Leaf Curl Virus (CLCuV). The whitefly, *Bemisia tabaci*, is the vector of CLCuV infection. The virus can cause severe symptoms such as upward curling of leaves, thickening of veins, enations, and stunted growth of plants, which can lead to significant losses in production (Farooq et al., 2011).

The first CLCuV epidemic was recorded in Pakistan and India in the 1980s, resulting in a significant decrease in cotton production. The virus is a difficult infection to control because it has been evolved into multiple strains throughout time (Wazeer et al., 2020). The constant introduction of novel virus strains, resistance development in strains of virus and the polyphagous nature and in whitefly populations, has rendered chemical control measures ineffectual and environmentally unsustainable. Therefore, creating resistant cotton types continues to be the most practical and eco-friendly method of controlling CLCuV.

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Plant breeding projects have concentrated on identifying and creating CLCuV-resistant cotton cultivars. However, resistance levels vary significantly among varieties, ranging from very resistant to highly susceptible (Farooq et al 2014). To adequately assess these resistance levels, standardized severity ratings are required. The 0–5 rating system, which was created by Khan et al. (2007) and is commonly used scoring scale, classifies cotton plants into highly resistant (no symptoms) and susceptible (extremely severe symptoms affecting more than 75% of leaves) categories. This scale has been very helpful in identifying CLCuV resistance in cotton germplasm and in directing breeding operations (Ashraf and hanif, 2010).

While various CLCuV resistant cotton genotypes have been created, resistance breakdowns caused by new virus strains and environmental variables. Therefore, continual screening of cotton germplasm for resistance to CLCuV strains is critical step in improving genetic architecture of cotton. Understanding the interactions between various cotton genotypes, whitefly vector dynamics, and environmental circumstances can also aid in the optimization of management techniques for long-term cotton output (Amrao et al., 2010).

This study uses a disease severity scale to assess how various cotton cultivars respond to CLCuV in field conditions. The study examines disease severity, plant growth, and yield factors to discover potential sources of resistance and susceptibility. The findings of this research had provided information on the efficiency of existing cotton types against CLCuV, as well as guideline for developing future breeding strategies aimed at developing more robust, resistant cotton cultivars.

The research was conducted in Plant Pathology Research Institute (PPRI), Ayub Agriculture Research Institute (AARI), Faisalabad. The experiment was carried out in field under factorial CRD with three replications and two treatments i.e. control (Virus free environment) and T1 (Viral inoculated environment). In each replication one row of plants containing 10 plants was planted. Three rows (replications) of each genotype were planted in both control and T1. The control condition was maintained by spraying Diafenthuron @ 200 ml/100 liter of water after observing 6 adults per leaf upper canopy of plants before 10 a.m. The next spray was sprinkeled at same dose after one week. In T1, virus inoculation was carried out by collecting and rearing white flies in the cage on infected leaves for 48 hours for virus acquisition. The viruliferous flies were allowed to feed on plants of T1 which were covered with polyethene for 48 hours to allow infection. The flies were then removed and plants were allowed to grow in natural condition. The symptoms were observed at three critical stages of cotton viz. vegetative (30 days post-inoculation), flowering (60 days), and boll formation (90 days). Data were collected from five representative plants of each row for virus infection at each critical stage according to scale described by Khan et al., (2007) given in table 1. The average score was calculated by taking mean of score of each plant at all critical stages. After harvesting, data of yield related characters were recorded from each representative plant. Average data of each treatment and replication was computed using excel. The data was then analyzed through analysis of variance and correlation analysis by using statistix 8.1. The average data was also represented in the form of graphs.

Material and methods

Table 1 Disease severity scale for evaluation of cotton plants under normal and infected condition

Scoring	Description	Percentage infection	Remarks
0	No visible symptoms of CLCuV infection. Leaves are healthy without any curling, vein thickening, or discoloration. Plants grow normally without any impact from the virus.	0%	Highly Resistant (HR)
1	Very mild symptoms, such as slight vein thickening or mild curling on 1-2 leaves. The symptoms are limited and do not spread to new leaves. There is no significant impact on plant growth or yield.	10% of leaves infected	Resistant (R)
2	Moderate vein thickening and mild curling on some leaves, covering 10-25% of the plant. Plants exhibit minimal growth reduction, with normal flowering and boll formation.	10-25% of leaves	Moderately Resistant (MR)
3	Noticeable leaf curling and moderate vein thickening affecting 25-50% of the plant. Growth is slightly stunted, but the plant still produces a reasonable number of bolls, though yield may be somewhat reduced.	25-50% of leaves	Tolerant (T)
4	Severe curling, thickening of veins, and some leaf enations on the underside of leaves, affecting 50-75% of the plant. The plant is stunted, and flowering and boll formation are significantly affected.	50-75% of leaves	Moderately Susceptible (MS)

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5	Very severe symptoms, including heavy curling, twisting, thickening, and enations on most leaves (over 75%). The plant is highly stunted, with poor or no boll formation, and may eventually die.	on more than 75% of leaves	Susceptible (S)

Table 2 Germplasm of cotton for screening against Cotton Leaf Curl Virus

Sr. No	Genotype	Institutes
1	FH-142	Cotton Research Station (CRS), Faisalabad, Pakistan
2	CIM-496	Central Cotton Research Institute (CCRI), Multan, Pakistan.
3	NIAB-852	Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.
4	NIAB Karishma	Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.
5	CIM-448	Central Cotton Research Institute (CCRI), Multan, Pakistan.

Yield related characters:

1) Boll weight (g)

At boll formation stage, the potential bolls were selected from each five represented plants and weighted by using digital weight balance. Average boll weight of each plant was calculated and then average of all plants of a genotypes for each treatment was calculated.

2) **No of bolls**

After harvesting the picked open bolls were counted from each plant and average of each genotype was calculated.

3) Seed cotton yield

The seed cotton was collected from each plant three times at maturity. Average of each genotype was calculated.

Results and discussions

Average DSI of all five genotypes under observation in control and CLCuV infected environment is represented in fig. 1. In control there was no deliberate infestation of CLCuV and in natural environment the FH-142 showed resistance according to scale. In highly infested environment it was graded as tolerant. CIM-496 showed similar results as it showed resistance in control and tolerance in T1. The NIAB-852 showed resistance in both control and T1. NIAB Karishma and CIM-448 didn't get disease symptoms in control but these two genotypes showed susceptibility in T1.

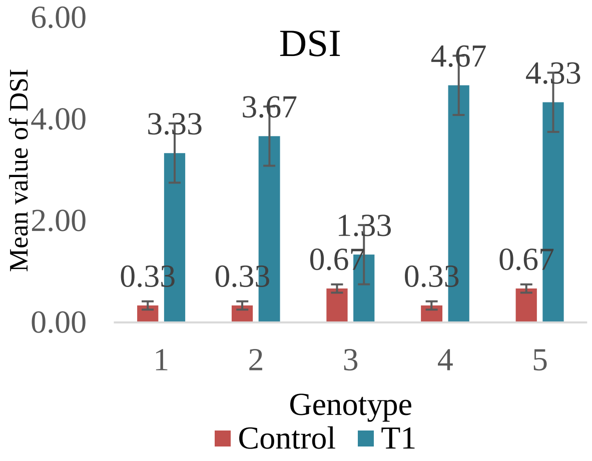

Fig.1 Average DSI of five genotypes under control and CLCuV infected environment

Average Seed Cotton yield of in control and T1 for 5 genotypes is shown in fig.2. FH-142 showed seed cotton yield of 44.91 g, CIM-496 58.02 g, NIAB-852 55.63 g, NIAB-karishma 33.25 g and CIM-448 33.06 g. In T1 the yield reduction was observed and FH-142 showed seed cotton yield of 17.31 g, CIM-496 27.74 g, NIAB-852 28.14 g, NIAB-karishma 6.41 g and CIM-448 7.69 g.

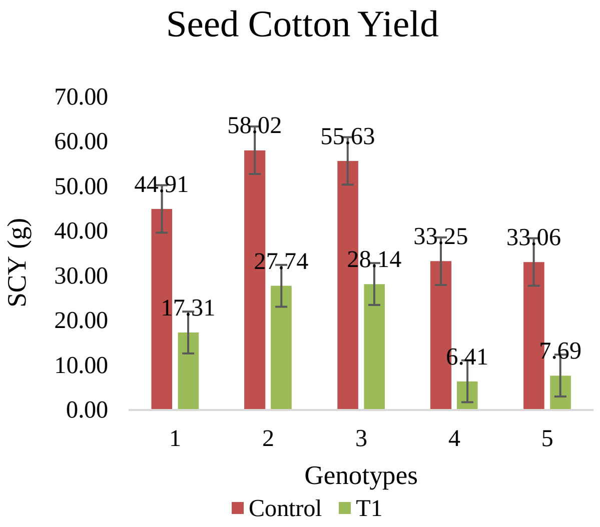

Fig 2. Average Seed cotton yield of five genotypes under control and CLCuV infected environment

Average number of bolls in control and T1 for 5 genotypes is shown in fig.3. FH-142 showed seed cotton yield of 17.67 g, CIM-496 20.33 g, NIAB-852 19.33 g, NIAB-karishma 13.67 g and CIM-448 12.33 g. In T1 the yield reduction was observed and FH-142 showed seed cotton yield of 13.67 g, CIM-496 13.33 g, NIAB-852 4.33 g, NIAB-karishma 4.67 g and CIM-448 8.67 g.

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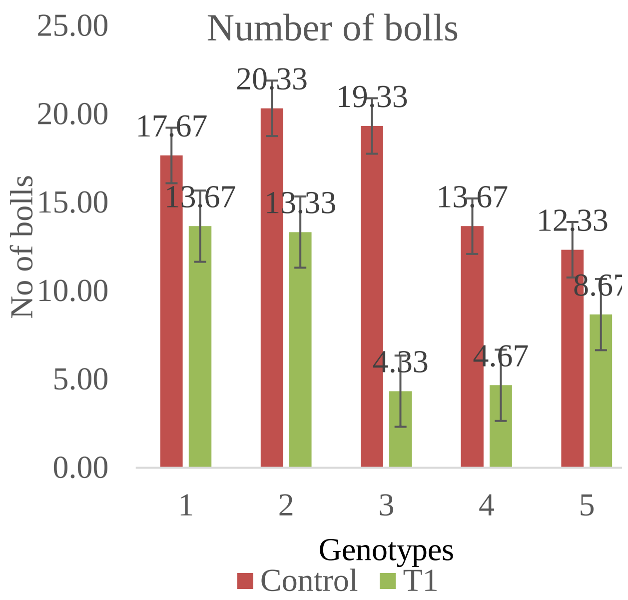

Fig 3. Average number of bolls of five genotypes under control and CLCuV infected environment

Average number of bolls in control and T1 for 5 genotypes is shown in fig.3. FH-142 showed seed cotton yield of 2.54 g, CIM-496 2.85 g, NIAB-852 2.88 g, NIAB-karishma 2.43 g and CIM-448 2.68 g. In T1 the yield reduction was observed and FH-142 showed boll weight of 1.99 g, CIM-496 2.02 g, NIAB-852 2.09 g, NIAB-karishma 1.47 g and CIM-448 1.63 g.

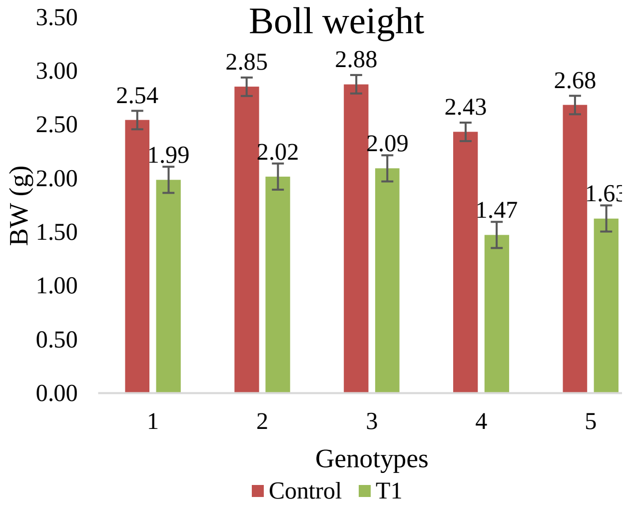

Fig.4 Average boll weight of five genotypes under control and CLCuV infected environment

Table 3 showed the analysis of variance of 5 cotton genotypes under controlled and stressed condition. The genotypes were varied high significantly for SCY, BW and No of bolls. There was found significant variation in genotypes for DSI. The both treatments were highly significant for DSI, SCY, BW and No of bolls.

Table 3 Analysis of variance of 5 cotton genotypes under controlled and CLCuV infected environment

Character	MSS of Genotypes	MSS of Treatments
Disease severiety index	3.58*	27.01**
Seed cotton yield	34.32**	261.42**
Boll weight	15.84**	298.94**
No of bolls per plant	28.04**	132.68**

The table 4 showed correlation matrix of 5 genotypes under controlled and infected environment. Seed cotton yield showed negative correlation with disease susceptibility index and positive correlation with number of bolls and boll weight. There was found negative correlation between boll weigh and DSI.

Yousaf et al., (2015) reported that the NIAB-852 is resistant to CLCuV. The results of research by Siddique et al., (2014) showed that CIM-496 is resistant to cotton leaf curl virus. FH-142 showed resistance against cotton leaf curl virus (Batool et al., 2021). Akhtar et al., (2002) reported susceptibility of NIAB-Karishma and CIM-448 for cotton leaf curl virus. Farooq et al., (2014) studied the effect of CLCuV on Cotton and found that the yield reduction is fate of cotton when there is incidence of CLCuV. Faroo et al 2013 reported positive correlation of number of bolls and boll weight with seed cotton yield in CLCuV infected condition.

Conclusion

The Pakistani germplasm has potential for resistance against CLCuV. The variety named NIAB-852 showed resistance against CLCuV. However, the incidence of virus reduces the seed cotton yield. The extent of yield loss depends upon the genetics of infected genotype.

Table 4 Correlation analysis of 5 cotton genotypes under controlled and CLCuV infected environment

	Bolls	DSI	weight
DSI	-0.76		
Weight	0.88	-0.82	
SCY	0.97	-0.73	0.92

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Declaration

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The study was approved by authors.

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Authors' Contribution

All authors contributed equally.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.