

# Assessment of Cotton Cultivar Resistance to Cotton Leaf Curl Disease in Multan District

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**Abstract:** In Pakistan, cotton enjoys prominent position in agriculture economy. The cotton is known for a best source of fiber along with natural oil and widely known as a white gold. Cotton is cultivated mostly in Punjab and Sindh provinces of Pakistan while Balochistan is an emerging market for organic cotton cultivation. The cotton production is threatened by Cotton leaf curl disease (CLCuD) caused by begomoviruses exclusively transmitted by whitefly, *Bemisia tabaci*. The present study was conducted to ascertain the resistance level in cotton cultivars against CLCuD in Multan district. During cotton seasons 2018-19 and 2019-20, a survey was carried out in Multan district covering all four tehsils viz. Multan city, Multan Saddar, Shujabad and Jalapur Pirwala. The resistance level in cotton cultivars were evaluated using CLCuD rating scale followed by diagnostic PCR amplifying the partial coat protein gene sequence. The two cotton varieties IUB-2018 and MNH-992 were rated susceptible during 2018-19 and 2019-20 with mean disease severity index of 38.49% and 35.94%; 47.91% and 47.87% respectively. The seven cotton varieties IUB-2013, IUB2015, MNH-886, BS-15, BS-18, SS-32 and FH-142 were rated susceptible to highly susceptible during 2018-19 and 2019-2020 with a mean severity index of 46.77% and 61.87%; 48.87% and 63.25%; 61.92% and 61%; 58.39% and 67.55%; 60.68% and 67.50%; 51.75% and 49.25%; 48.37% and 50.80 respectively. During the surveyed seasons, absence of resistant, tolerant and moderately tolerant cotton cultivars were observed. The study reflected an urgent need to release new cotton cultivars resistant to CLCuD for a better cotton production.

**Keywords:** Cotton leaf curl disease, cotton genotypes, CLCuD, whitefly

## Introduction

Agriculture sector have played a significant part in the economy of developing countries like Pakistan. It contributes 18.5% share in GDP of Pakistan and has utilized up to 38.5% labor force of Pakistan. (Pakistan Bureau of Statistics, 2018-19). Cotton (*Gossypium hirsutum*. L) belongs to a family Malvaceae is an important crop for developing countries like Pakistan, and also known as white gold across the globe (Chakravarthy et al. 2014). *Gossypium* genus have 52 species which was consisting of *Gossypium barbadense* as well as *Gossypium hirsutum* have allotetraploid genomes, whereas *Gossypium herbaceum* or *Gossypium arboreum* have diploid genomes (Bakhsh et al. 2015). Cotton is a well-known fiber, and as well as natural food crop which contributes in textiles industries \$600 billion yearly across the globe (Ashraf et al. 2018). Cotton is contributing 80% in the natural production of edible oil (Rehman et al. 2016). Production of cotton in Pakistan during 2018-19 was 9.861million bales, whereas in 2019-2020 cotton production is 9.1 million bales, whereas Punjab and Sindh are main cotton cultivating areas of Pakistan (Pakistan Bureau of Statistics, 2020). IUB-2018, MNH-992, MN-886, BS-15, FH-142, NIAB-878 and IUB-fff2013 are mostly cultivating in Punjab (GOP, 2020). Pakistan was the 4<sup>th</sup> largest producer of cotton and third largest consumer of cotton yarns across the globe. In agriculture GDP it is contributing 4.5% and in national GDP of Pakistan cotton contribution is more than 1% (PCGA, 2019). Cotton production in Pakistan is mostly affected by biotic as well as abiotic factors, these factors involved in decrease of production and eventually low profitability of this crop. (PCGA, 2019). Cotton Leaf Curl Disease (CLCuD) is an important disease of cotton in Pakistan, which causes loss up to 2 to 4 million bales per annum (PCGA, 2019). First time it was reported in Nigeria (Farquarson 1912) and in Pakistan it was first time recorded in 1967 at Tibba Sultanpur which is situated near district Multan in South Punjab, while this disease was also reported from different areas of Sindh in 1997 (Hussain and Ali 1975:

Mansoor et al. 1998). Cotton leaf curl disease is caused by begomoviruses and its associated satellites (Bridson 2003). *Begomovirus* genus belongs to a family Geminiviridae which is comprised of more than 350 species. Mostly dicots plants are infected by the members of these species, and begomoviruses are transmitted by whitefly (*Bemisia tabaci*) (Brown et al. 2015). DNA genome has two circular ssDNA molecules, with size of almost 2.6 kb is known as bipartite and monopartite has size of 2700bp circular ssDNA molecule (Rojas et al. 2005). Alpha and beta satellites have been related with ssDNA satellites (Lozano et al. 2016). CLCuD is transmitted by whitefly (*Bemisia tabaci*) has at least 24 biotypes identified by using molecular as well as biological characteristics (De Barro et al. 2011). The main purpose of our study was to evaluate the cotton genotypes grown in Multan districts and to identify any cultivar exhibiting resistance or tolerance against Cotton leaf curl disease (CLCuD). The study aimed to assess disease incidence and severity under local agro-climatic conditions, thereby identifying resistant varieties that could be recommended for sustainable cotton production and integrated disease management strategies in the region.

## Results and Discussion

During the 2018-19 and 2019-20 seasons, IUB-2013 was rated, on basis of disease rating scale, from susceptible to highly susceptible in district Multan (Multan City, Multan Saddar, Shujabad, Jalalpur Pirwala). The Mean Disease Severity Index of IUB-2013 in 2018-19 was recorded 49.4% in Multan whereas the mean DSI during 2019-20 was 61.87% in Multan. IUB-2015 was rated from susceptible to highly susceptible in district Multan. The cultivar was assessed under field conditions during 2018-19 observed at two different locations and in 2019-20 observed at one location. The MDSI in 2018-19 was 48.87% in Multan, whereas the MDSI during 2019-20 was 63.25%. IUB-2018 was proved to be susceptible to highly susceptible against cotton leaf curl disease in Multan. The variety was examined at 03 and 14 locations during 2018-19 and 2019-20 respectively. The MDSI of IUB-2018 during 2018-19 was 38.58% in Multan, whereas the MDSI in 2019-20 was recorded 41.32% in Multan. MNH-886 was highly susceptible during 2018-19 and 2019-20 in district Multan. The commercial cultivar MNH-886 was assessed at 07 and 04 locations in 2018-19 and 2019-20. MDSI of MNH-886 was 62.92% in Multan during 2018-19, while in 2019-20 the MDSI was 62.81% in Multan. MNH-992 showed susceptible to highly susceptible results against CLCuD during 2018-19 and 2019-20. The variety was observed at 03 and 02 locations in Multan during 2018-19 and 2019-20. The MDSI of MNH-992 was 47.91% in district Multan, respectively the MDSI in 2019-20 was 47.87% in Multan. SS-32 was rated as susceptible to highly susceptible during 2018-19 and 2019-20 in district Multan. SS-32 was evaluated at 07 and 10 locations in Multan during 2018-19 and 2019-20. The MDSI was 58.42% in 2018-19 and in 2019-20 the MDSI was 67.50% in Multan. BS-15 was rated from susceptible to highly susceptible during 2018-19 and 2019-20 in different areas of district Multan. BS-15 was examined at 04 and 03 locations in district Multan during 2018-19 and 2019-20 respectively. The MDSI was 60.37% in 2018-19, while in 2019-20 the MDSI of BS-15 was 70% in Multan. During 2018-19 and 2019-20 BS-18 rated from susceptible to highly susceptible in district Multan. The cultivar assessed at 1 locations in district Multan during 2018-19 and 2019-20. The MDSI was 51.85% and 49.25% respectively during 2018-19 and 2019-20 in Multan. FH-142 was also rated from susceptible to highly susceptible during 2018-19 and 2019-20 respectively in Multan. The commercial cotton cultivars observed at 02 and 05 locations in district Multan during 2018-19 and 2019-20 respectively. The MDSI of FH-142 was 48.37% in 2018-19 while during 2019-20 FH-142 MDSI was 50.50% in Multan district (Figure 1, 2). During 2018-2019 and 2019-2020 MDSI in Multan was 53.17% and 55.58%, whereas in Shujabad and Jalalpur Pirwala MDSI was 54.17%, 54.1% and 55.5%, 54.60% respectively (Figure 3(A, B)).

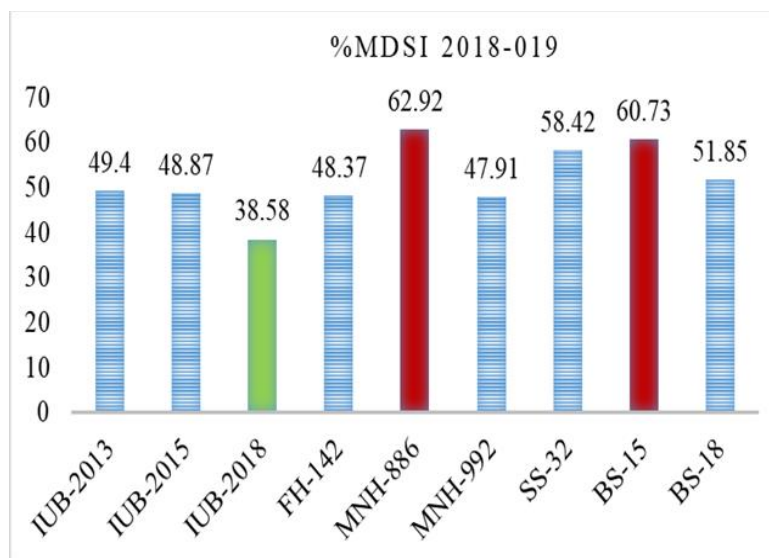


Figure 1. Mean Disease Severity Index During 2018-019.

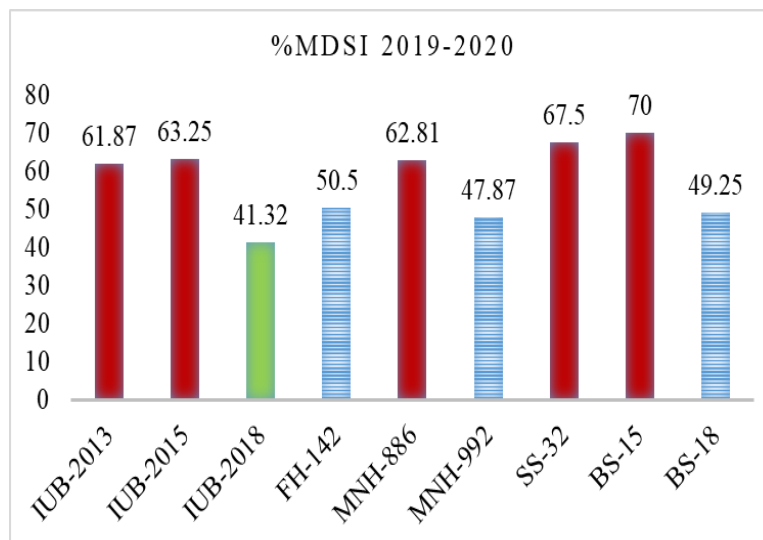


Figure 2 Mean Disease Severity Index during 2019-2020.

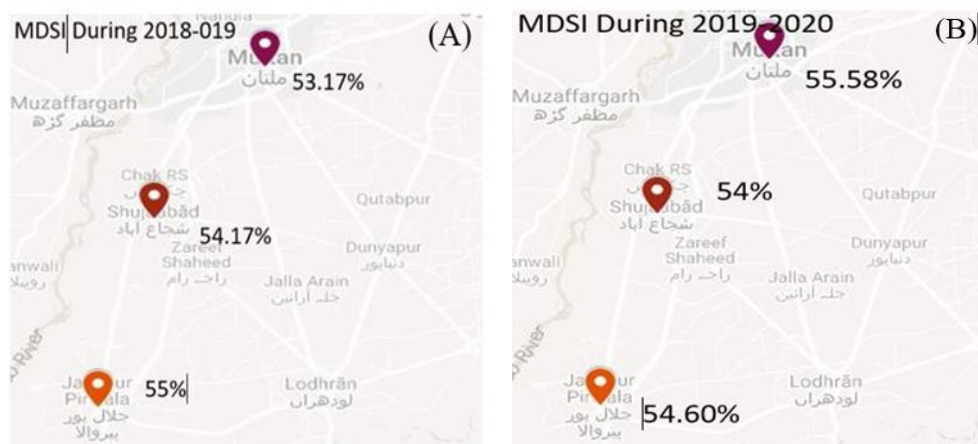


Figure 3 (a). Mean Disease Severity Index 2018-019. (B). Mean Disease Severity Index during 2019-2020.

The molecular detection of begomoviruses infecting cotton cultivars resulted in 180bp partial coat protein gene which proved the presence of begomoviruses in all rated commercial cotton cultivars (Ahmad, 2016) (Table 1) (Figure 4).



Figure 4 Amplification of 180bp partial coat protein gene of begomoviruses

Table 1. Molecular Detection of different cotton cultivars

Sr. No	Cultivars Name	No of Samples Tested	PCR(+/-)	<i>Gossypium hirsutum</i> is the fibrous crop of Pakistan after wheat, belongs to family Malvaceae. Economically cotton can play a pivotal
1	IUB-2013	13	+	
2	IUB-2015	3	+	
3	IUB-2018	15	+	
4	MNH-886	10	+	
5	MNH-992	5	+	
6	SS-32	17	+	
7	BS-15	7	+	
8	BS-18	2	+	
9	FH-142	7	+	

role in the development of Pakistan (Jamshed et al, 2008; Anwar et al, 2009). Cotton cultivated in more than 60 countries with an estimated production of 120 million bales per year. Cotton production was

decreasing day by day by sowing till harvesting, the major reason is more than 75 diseases that's are responsible for 50% yield losses annually, with a net worth of 50 million US dollars all around the globe (Hasan et al, 2019). During the 2018-19 and 2019-20 seasons, IUB-2013 was rated as susceptible to highly susceptible in district Multan (Multan City, Multan Saddar, Shujabad, Jalalpur Pirwala). The mean DSI in 2018-19 was recorded 46.77% with minimum 40.5% and maximum 55.25% in Multan whereas the mean DSI during 2019-20 was 61.87% with minimum 56.25% DSI and maximum 67.50% in Multan. IUB-2015 MDSI in 2018-19 was 48.87% with minimum 47.55% and maximum 50.00% in Multan whereas the MDSI during 2019-20 was 63.25%. IUB-2018 MDSI during 2018-19 was 38.49% with minimum DSI 38.00% and maximum 39.25% in Multan whereas the MDSI in 2019-20 was recorded 35.94 % with minimum DSI 33.75% and maximum 51.75% in Multan. MNH-886 MDSI was 61.92% with minimum DSI was 50% and maximum was 77.25% in Multan during 2018-19 whereas in 2019-20 the MDSI was 61% with DSI ranged from 56.25%-65.25% in Multan. The MDSI of MNH-992 was 47.91% with DSI ranged from 46.50% to 48.75% during 2018-19 in district Multan whereas the MDSI in 2019-20 was 47.87% with minimum DSI was 47.75 and maximum DSI was 48% in Multan. The MDSI of SS-32 was 58.39% with minimum DSI was 52.50% and maximum DSI 61.75 in 2018-19 whereas in 2019-20 the MDSI was 67.55% with minimum DSI was 56.75 and maximum DSI 80% during 2019-20 in Multan. The MDSI was 60.68% with minimum DSI was 54.50 and maximum DSI was 71.25% in 2018-19 while in 2019-20 the MDSI of BS-15 was 67.50% with DSI ranged from 60.50% to 76% in Multan. While previous research about this cultivar showed that DSI of BS-15 in 2016-17 74.13% (CCRI, 2016-17). The MDSI was 51.75% and 49.25% respectively during 2018-19 and 2019-20 in Multan. While the DSI of BS-18 73.02% recorded in 2017-18 (CCRI, 2017-18). The MDSI of FH-142 was 48.37% with minimum DSI was 45.50% and maximum DSI was 53.25% in 2018-19 while during 2019-20 FH-142 MDSI was 50.80% with minimum DSI was 47.25% and maximum was 59% in Multan district. While in previous studies FH-142 was also rated as highly susceptible with DSI 74.75% (CCRI, 2016-17). The latest cultivar CYTO-124 has shown promising resistance against CLCuD (Ashraf, 20023) and along with field screening and molecular identification, qPCR based varietal screening should be made mandatory as demonstrated by Sarwar et al. (2025).

The present study provides a vivid picture of resistance level in cotton varieties with 62.92 percent and 70 percent of disease severity index in 2018-19 and 2019-20 respectively. The presence of begomoviruses were confirmed by molecular diagnostics underlining an emerging threat of new begomoviruses and associated betasatellite recombinants. The new emerging recombinants could have devastating impact of cotton production and reflect dire need for coordination between plant breeders and virologists to combat CLCuD.

## Materials and Methods

### Survey and Sample Collection

District Multan which is situated in the South Punjab, Pakistan have two Tehsils Shujabad and Jalalpur Pirwala, are the major hub of cotton cultivation. Cotton is the most important crop of Pakistan after wheat and maize. Cotton Leaf Curl Disease has been the major threat for cotton production in this area. We surveyed 82 different locations in district Multan for sample collection during 2018-19 and 2019-20. Survey was conducted at reproductive stage, and W Shape method was used for data recording and sample collection. We collected 10 symptomatic samples of every cultivar infected with CLCuD. During survey we observed IUB-2013, IUB-205, IUB-2018, FH-142, MNH-886, MNH-992, BS-15 and BS-18 were mostly cultivated in district Multan. We assessed these cultivars by using disease rating scale (Nazeer et al. 2014) and calculations of disease severity index by using the following formula (CCRI 2016-17).

$$\% \text{ Disease Severity Index} = \frac{0(\text{No of plants}) + 1(\text{No of plants}) + 2(\text{No of plants}) + 3(\text{No of plants}) + 4(\text{No of plants})}{\text{Total no of plants}} \times 100/4$$

No of Symptomatic Plants

$$\text{Disease Incidence} = \frac{\text{No of Symptomatic Plants}}{\text{Total no of tested Plants}} \times 100$$

Table 2. Disease rating scale for the evaluation of cotton cultivars

Disease index (%)	Severity grade	Symptoms	Remarks
0	0	No symptoms	Resistant
1-20	1	Thickening of only secondary and tertiary veins	Highly tolerant
21-30	2	Thickening of secondary and primary (mid rib ) veins	Tolerant
31-50	3	Vein thickening, leaf curling or enation/both	Susceptible
50>	4	Leaf enation and stunting of plant	Highly susceptible

Infected samples with CLCuD was collected on the basis of symptoms such as leaf upward curling, vein thickening, leaf enation and stunting of plants (Figure 5). These samples were brought in laboratory for further procedure.

#### *DNA Extraction and Quantification*

For isolation and extraction of DNA from plant leaf samples CTAB method was used (Doyle and Doyle, 1990). For DNA extraction one composite samples of each cultivar was used. DNA was quantified by Nano Drop or Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA).

#### *Molecular Detection*

Nucleic acid based technique Polymerase Chain Reaction (PCR) was used for the confirmation of samples infected with cotton leaf curl disease with universal primer pair **Forward 5' GAAGCGACCAG-CAGATATAATC 3'/ Reverse 5' CATCCTGTACATCCTGGGCTT 3'** (Ahmad, 2016). PCR program was run with 35 cycles at 94°C for 60sec, annealing at 55°C for 40sec, extension at 72°C for 90sec, and final extension at 72°C for 7 minutes (Rojas et al. 1993). The PCR reactions were carried out in 96 well thermocycler with an individual reaction volume of 50uL.



Figure 5 Symptomatic Pictures of Infected Plants with Cotton Leaf Curl Disease

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**Authors contribution:** MH and HR designed and authored the manuscript. MH executed the research work. HR and MA proof read the manuscript.

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