

# Screening of Cotton Germplasm Against Cotton Leaf Curl *Begomovirus* (CLCuV)

RASHIDA, P., M.K. SULTAN†, M.A. KHAN† AND NOOR-UL-ISLAM‡

University College of Agriculture, Bahauddin Zakariya University Multan

†Department of Plant Pathology, University of Agriculture, Faisalabad–38040, Pakistan

‡Ayub Agricultural Research institute, Faisalabad–Pakistan

## ABSTRACT

Sixty four cotton varieties/lines were screened against CLCuV disease. Results revealed that two varieties namely Ravi and FDH 170 were highly resistant, 15 were resistant, and 12 were moderately resistant; whereas, nine varieties were found moderately susceptible, 19 were susceptible and seven were found highly susceptible to CLCuV disease.

**Key Words:** Cotton leaf curl disease; *Begomovirus*; Germplasm; Susceptibility; Resistance; CLCuV

## INTRODUCTION

Cotton is the main cash crop of Pakistan as it contributes 60% of total foreign exchange earning of the country. Cotton is grown in 70 countries and over 180 million people are associated with the fibre industry that yields \$20 to 30 billion worth of raw cotton (Anonymous 1996). Cotton belongs to the genus *Gossypium* of the family Malvaceae. The cultivated species are organized in two sections, Herbacea and Hirsuta. Section Herbacea includes two species, *G. herbaceum* L. and *G. arboreum* L. Both are old world diploids, each having a number of cultivars. The vast majority of cotton produced throughout the world is derived from cultivars of two new world species in the section Hirsuta, i.e. *G. hirsutum* (upland cotton) and *G. barbadense* (Sea Island or Egyptian Cotton). Both the new world species are allopolyploids derived from hybridization between two diploid species. *G. barbadense* can be distinguished from *G. hirsutum* in the field as it has a more open canopy and its leaves are palmate and more deeply divided, the flowers tend to be larger, yellow in colour compared with white creamy of *G. hirsutum*. The centre of origin of *G. hirsutum*, is tropical America. Presently, *G. hirsutum* varieties are grown in numerous countries scattered over five continents (Afzal, 1983).

Cotton contributes significantly to the national economy of Pakistan. It accounts for 11.7% of value added in agriculture and 2.9% of GDP. In addition to providing raw material to the local textile industry the lint cotton is a major export item. The area, production and yield of cotton in Pakistan increased from 1.24 m hectare, 1.16 m bales and 477 kg/hectare in 1947-48 to 2.84 m hectares, 12.82 m. bales and 769 kg/hectare in 1991-92 and were 3.116 m. hectares, 10.6 m bales and 579 kg/hectares in 2001-2002 (Anonymous, 2001-2002).

Cotton has the unfortunate characteristics of being vulnerable to many insects, and to maintain yield, insects

are managed with large amounts of insecticides. For the first time, CLCuV was observed in severe forms at the seedling stage on cotton varieties imported from US, such as Delta pine Stoneville and-147-1. The incidence of the disease was as higher as 80% in these varieties (Ali *et al.*, 1995). In 1992, Cotton leaf curl (CLCuV) disease was found well established in the districts of Vehari, Multan, Khanewal, Bahawalpur and Sahiwal, which ranged from 30- 80% with *Bemisia tabaci* Genn. in abundance (Hameed *et al.*, 1994).

By early 1990's, CLCuV disease had become the major malady, which gave serious set back to cotton production in Pakistan and now it has spread into south and west to other provinces of Pakistan. The characteristic symptoms include leaf curling, darkened veins, vein swelling and enations that frequently developed leaf like structures on under side of leaves (Mansoor *et al.*, 1997; Harrison *et al.*, 1997).

The pioneer work was carried out by Central Cotton Research Institute (CCRI), Multan and varieties such as CIM-1100 and CIM-448 were released earlier than FH-634. CCRI, Multan identified CP-15/2 and LRA-5166 as resistance source.

First generation CLCuV resistant variety FH-634 (CLCuV resistant gene Cedix) was released in 1996. Relatively improved variety FVH-53 with CLCuV resistant gene Kivi-1021 followed in 1998. As a result of cultivation of these resistant varieties, spread of CLCuV was successfully curtailed. In 2000, five second generation CLCuV resistant varieties were released for general cultivation, which included; FH-900, MNH-552 and MNH-554 having CLCuV resistant gene LRA 5166, FH-901 with CLCuV resistant gene CP 15/2 and BH-118 with CLCuV resistant gene Cedix. These second-generation CLCuV resistant varieties were instrumental in increasing cotton production substantially (Anonymous, 2000).

Cotton production in Pakistan has declined markedly as a consequence of several factors, including

unprecedented infestations by *cotton leaf curl Begomovirus* and its vector the whitefly. The development of moderate to very strong resistance in *B. tabaci* to all of the commonly used insecticides supported frequent claims that these chemicals are losing their effectiveness under field conditions. These findings reinforce the urgency of identifying and implementing sustainable pest management practices for the cotton cropping system (Cahill *et al.*, 1994). Therefore, in order to attain successful management of the disease, continuous screening of available cotton germplasm for CLCuV is necessary to identify the resistance sources for CLCuV.

## MATERIALS AND METHODS

### Evaluation of cotton germplasm against CLCuV disease.

To catalogue cotton varieties/frontlines/strains, *cotton leaf curl* disease screening nursery was established for one year during cotton growing season of 2000 in the Departmental Research Area of University College of Agriculture (UCA), Bahauddin Zakariya University, Multan. Sixty four commercial cotton varieties/strains collected from Central Cotton Research institute, (CCRI) Multan; Cotton Research Station, (CRS) Multan; Cotton Research Institute, Ayub Agricultural Research Institute, (AARI) Faisalabad; and Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Seed of S-12 was collected from National Institute for Biotechnology and Genetic engineering (NIBGE).

Seeds of these varieties/strains were neither treated with chemicals, nor acid delinted to increase chances of primary infection of disease. Each entry was sown in a row of 3m length with 30 cm plant to plant and 75 cm row-to-row distance. A line of highly susceptible variety i.e. S-12 was sown after every three entries. The field was surrounded by two rows of S-12 as disease spreader. The nursery was sown in three replications.

All conventional agronomic practices were followed to keep the crop in good condition. However, no pesticides were sprayed to allow maximum whitefly population, the insect vector of CLCuV.

*Cotton leaf curl* (CLCuV) disease ratings were taken eight times on biweekly basis according to a scale described by Anonymous (1996) with the initiation of first disease symptoms. Five plants were selected randomly from each row and tagged for further assessment of CLCuV disease severity by visual observation as per rating from scale described below in table I. Difference in disease severity among 64 varieties were determined by Duncan, s Multiple Range Test (DMR) at 5 probability level (Steel & Torrie 1980).

**Detection of CLCuV from different varieties.** After appearance of disease symptoms ten plants showing the typical symptoms of CLCuV were collected randomly from the experimental field. For Polymerase Chain Reaction (PCR), total genomic DNA was extracted from these

samples by CTAB method (Doyle & Doyle, 1990). In order to confirm the presence of CLCuV, three sets of primers were used. One set was specific for DNA-A, second was specific for DNA-1 and the third set was universal primer for DNA  $\beta$  (Bridson *et al.*, 2001).

## RESULTS AND DISCUSSION

**Detection of CLCuV in different varieties.** Three randomly selected samples of different varieties i.e. S-12, CIM-109 and B-622 were collected from experimental field. There were amplification for each three sets of primers indicating the presence of CLCuV in CIM-240, S-12 and BH-95 (Fig. 1a, 1b, 1c).

**Evaluation of cotton germplasm against CLCuV during 2000.** The essence of research endeavour undertaken was to evaluate available popular varieties/strains, bred at different cotton research stations of the country in the Punjab, for CLCuV incidence during 2000.

During 2000, thickening of small and large veins appeared on under surface of top leaves on cotton cultivar S-12 in the first week of July. In mid of July, similar symptoms were recorded on all, varieties/lines except Ravi, FDH-170, MNH-516, BH-118 and RH-500, FDH-170 and Ravi remained asymptomatic, while MNH-516, BH-118, Rli-500, FVH-57, C'M-443, FH-628, FH-900, CIM-435, CIM-473, CIM-482, FH-649, CIM-1100, CIM-448, CIM-446 and FVH-55 showed resistant to tolerant behaviour during 2000.

Twenty six varieties gave susceptible response out of which seven were highly susceptible to CLCuV i.e. CIM-240, FVH-53, S-12, FH-685, SLS-1, AEC/73/3/89 and B-622 (Table II). Only FDH-170 and Ravi both of *G. arboreum* showed highly resistant reaction. Singh *et al.* 1997 found that all entries of *G. arboreum* were free from disease symptoms. Diverse virulence of virus has been reported from different parts of the world.

**Table I. Disease rating scale used to determine the level of resistance or susceptibility of cotton lines to CLCuV**

Grade	Symptoms description	Level of resistance or susceptibility
0	No symptoms	Highly resistant
1	Scattered thickening of small veins	Resistant
2	Thickening of all veins but no curling	Moderately resistant
3	Thickening of veins and curling of leaves at the top leaves(light effect)	Moderately susceptible
4	Thickening of veins and curling of leaves on half of the plant with enation (medium effect)	Susceptible
5	Thickening of veins and curling of leaves (upward/downward) on the entire plant and dwarfing of plant (severe effect)	Highly susceptible

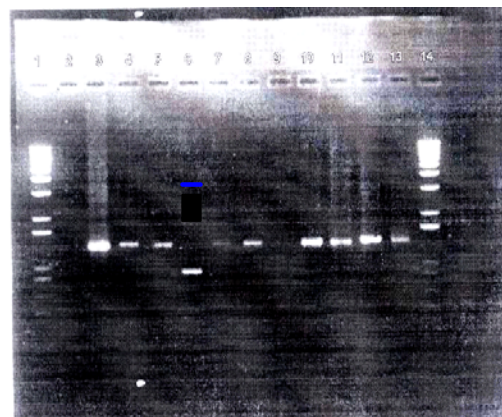
Anonymous (1996)

**Table. II. Reaction of cotton germplasm for source of resistance against CLCuV at the University College of Agriculture, Bahauddin Zakariya, Multan during 2000**

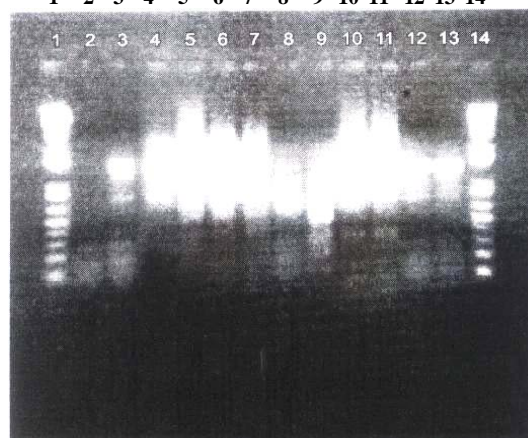
S.No.	Varieties/Strains	Reaction
1	FDH-170	0 HR
2	RAVI	0 HR
3	MNH-516	0.44 R
4	BH-118	0.47 R
5	RH-500	0.48 R
6	FVH-57	0.50 R
7	CIM-443	0.50 R
8	FH-628	0.52 R
9	FH-900	0.53 R
10	CIM-435	0.54 R
11	CIM-4373	0.55 R
12	CIM-482	-0.61R
13	FH-649	0.63 R
14	CIM-1100	0.66 R
15	CIM-448	0.70 R
16	CIM-446	0.77 R
17	FVH-55	0.82 R
18	FVH-49	1.03 MR
19	MNH-554	1.17 MR
20	TSR-23-75	1.19 MR
21	832/97	1.21 MR
22	FH-634	1.41 MR
23	FVH-28	1.49 MR
24	FS-631	1.51 MR
25	MNH-518	1.66 MR
26	VS-135	1.77 MR
27	FH-87	1.86 MR
28	FH-901	1.88 MR
29	MNH-552	1.96 MR
30	CIM-467	2.09 MS
31	840/97	2.25 MS
32	MNH-536	2.31 MS
33	FH-629	2.36 MS
34	493/97	2.38 MS
35	CIM-109	2.50 MS
36	B-850	2.56 MS
37	B-843	2.56 MS
38	B-896	2.82 MS
39	VH-137	3.03 S
40	B-803	3.10 S
41	B-496	3.11 S
42	B-820	3.12 S
43	TCD-3H	3.30 S
44	KARISHMA	3.50 S
45	FH-682	3.54 S
46	MNH-93	3.54 S
47	Bt. Cotton	3.59 S
48	MNH-465	3.66 S
49	FH-657	3.68 S
50	S-14	3.73 S
51	NIAB-78	3.76 S
52	BH-100	3.80 S
53	BH-95	3.86 S-
54	CIM-483	3.93 S
55	DNH-49	3.94 S
56	CIM-465	3.96 S
57	B-821	3.96S
58	CIM-240	4.00S
59	FVH-53	4.01HS
60	SLS-1	4.02HS
61	FH-685	4.09HS.
62	F VH-137	4.21HS
63	AEC/73/3/89	4.21HS
64	B-622	4.22HS

HR= Highly resistant; R= Resistant; MR= Moderately Resistant;  
 MS= Moderately Susceptible; S= Susceptibility; HS = Highly Susceptible  
 Mean values in a column sharing similar letters do not differ significantly as determined by DMR test at p= 0.05

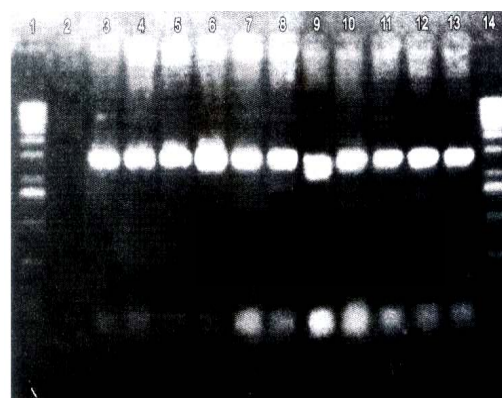
**Fig. 1a. PCR of cotton varieties with DNA 1**



**Fig. 1b. PCR of cotton varieties with DNA β Primer**



**Fig. 1c. PCR of cotton varieties with DNA A Primer**



Lane 1 and 14 1Kb Ladder +  
 Lane 2 -ve (Healthy cotton Plant)  
 Lane 3 +ve (Diseased cotton Plant)  
 Lane 4-6 CIM-240  
 Lane 7-9 BH-95  
 Lane 10-13 S-12

CLCuV disease in Pakistan is caused by several distinct strains like CLCuV-Pak-1, CLCuV-Pak-2, CLCuV-

Pak-3 and CLCuV-Pak-4 (Mansoor *et al.*, 1999). New biotype of *B. tabaci* also reported from Pakistan i.e. *B. argentifolii*, the insect vector of CLCuV (Costa & Brown 1991). Cataloging of cotton germplasm against CLCuV has been reported by several research workers (Alim, 1997; Muhammad *et al.*, 1998; Ashraf *et al.*, 1999; Khan *et al.*, 2000). The commercial cotton varieties responded differently against CLCuV to the changing environmental conditions and presence of whitefly, the insect vector of CLCuV.

CLCuV could be managed by growing arboreum cotton in diseased affected areas. The resistance is controlled by a single gene and can be transferred to any cultivar by a back-cross technique (Alim *et al.*, 1997).

## REFERENCES

- Afzal, M. and M. Ali, 1983. *History of Cotton: Cotton Plant in Pakistan*, pp. 1–9. Aiwani–I–science, Shahrah–I–Romi, Lahore
- Ali, M., Z. Ahmad, M. Tanveer and T. Mahmood, 1995. Identification and characterization of virus in: “*Cotton leaf curl virus* in the Punjab during 1991–92”. CLCuV Project, Asian Development Bank, Ministry of Food, Agriculture and Livestock, Government of Pakistan
- Alim, 1997. Breeding of cotton varieties for resistance to *Cotton leaf curl virus*. *Pakistan J. Phytopath.*, 9: 1, 1–7
- Anonymous, 1996. Minutes of the second meeting on scoring *Cotton leaf curl virus* disease, p. 3. Jointly organized by (i) Ayub Agriculture Research Institute, Faisalabad–Pakistan, (ii) Department of Plant Pathology, University of Agriculture Faisalabad–Pakistan, and (iii) Ministry Food Agriculture and Livestock, Government of Pakistan, Islamabad
- Anonymous, 2000. *Annual Report*. Directorate of Cotton, Ayub Agriculture Research Institute, Faisalabad–Pakistan
- Anonymous, 2001–2002. *Economic Survey: Agriculture*, pp. 3–4. Government of Pakistan. Economic Advisors Wing, Finance Division, Islamabad
- Ashraf, M., Z.U. Zafar, T. McNeilly and C.J. Velthkamp, 1999. Some morpho-anatomical characteristics of cotton (*Gossypium hirsutum* L.) in relation to resistance to cotton leaf curl virus (CLCuV). *Angewandte–Botanik*, 73: 3–14, 76–88
- Bridson, R., S. Mansoor, I. Bedford, M. Pinner, K. Saunders, J. Stanley, Y. Zafar, K. Malik and P. Markham, 2001. Identification of DNA components required for induction of cotton leaf curl disease. *Virology*, 285: 234–43
- Cahill, M., D. Johnson, K. Gorman and I. Denholm, 1994. Insecticide resistance in *Bemisia tabaci* from Pakistan. *Proc. Brighton Crop Prot. Conf. Pest and Diseases.*, 1: 431–6
- Costa, H.S. and J.K. Brown, 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci* and the association of one population with silver leaf symptom induction. *Entomol. Exper. et Appl.*, 61: 211–9
- Doyle, J.J. and L. Doyie, 1990. A rapid DNA isolation procedure for small quantities of fresh tissues. *Phytochem. Bull.*, 9: 11–5
- Economic Survey, 2001–2002. *Agriculture, Government of Pakistan*, pp. 3–4. Economic Advisors Wing, Finance Division, Islamabad
- Hameed, S., S. Khalid, E. Haq and A.A. Hashmi, 1994. *Cotton leaf curl disease* in Pakistan caused by a whitefly transmitted geminivirus. *Pl. Dis.*, 78: 529
- Harrison, B.D., UY.L. Liu, S. Khalid, S. Hameed, G.W. Otinnape and D.J. Robinson, 1997. Detection and relationships of *Cotton leaf curl virus* and allied whitefly transmitted geminiviruses occurring in Pakistan. *Ann. Appl. Biol.*, 130: 61–75
- Khan, N.U., H.K. Abro, G. Hassam, M.B. Kumbhar and A. Razzaq, 2000. *Leaf curl virus* in American upland cotton in NWFP. *Pakistan J. Phytopath.*, 12: 79–86
- Mansoor, S., I. Bedford, M.S. Pinner, J. Stanley and P.G. Markham, 1993. A whitefly–transmitted geminivirus associated with cotton leaf curl disease in Pakistan. *Pakistan J. Bot.*, 25: 105–7
- Mansoor, S., S.H. Khan, A. Bashir, M. Saeed, Y. Zafar, K.A. Malik and P.G. Markham, 1999. Identification of a novel circular single stranded DNA associated with *Cotton leaf curl disease* in Pakistan. *Virolog.*, 259: 190–19
- Muhammad, F., A.H. Tariq, J. Ihsan and A. Saleem, 1998. Evaluation of two *Cotton leaf curl virus* transmission techniques and their response to different cotton cultivars. *Pakistan J. Phytopath.*, 10: 18–22
- Singh, J., A.S. Sohi, H.S. Mann and J.W. Singh, 1997. Screening of cotton germplasm against *Cotton leaf curl viral disease* using its vector *Bemisia tabaci* (Genn.). *J. Res. Punjab. Agric. Univ.*, 34: 3, 294–8
- Steel, R.G.D. and J.H. Torrie, 1986. *Principles and Procedure of Statistics. A Biometrical Approach*, 2<sup>nd</sup> ed., p. 597. McGraw Hill Book Company, New York

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