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## Burewala Strain of Cotton Leaf Curl Virus: A Threat to CLCuV Cotton Resistant Varieties

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**Abstract:** Cotton Leaf Curl Virus (CLCuV) is one of the disease of cotton, which is transmitted by whitefly (*Bemisia tabaci* Gem). In a survey conducted during the year 2001-2002, it was observed that a new variant of Multan CLCuV was appeared on all resistant cotton varieties in culture at various locations in Vehari district especially in Burewala territory. The appearance of symptoms was experimentally confirmed through graft and whitefly transmission techniques under controlled environments at Central Cotton Research Institute, (CCRI) Multan, Pakistan. Results showed that all commercial varieties in culture, which were resistant to Multan CLCuV disease, fell prey to new variant to Burewala-CLCuV. Not with standing that even parental genetic stock from which these varieties were bred had shown their susceptibility to this new variant of Multan CLCuV (commonly known as Burewala-CLCuV).

**Key words:** *Gossypium hirsutum*, cotton leaf curl virus disease (CLCuV), geminivirus, graft transmission, whitefly transmission, Burewala-CLCuV: Punjab: Pakistan

### Introduction

Cotton Leaf Curl Virus (CLCuV) disease was first reported during 1967 near Multan (Hussain and Ali, 1975). It is a viral disease transmitted by whitefly (*Bemisia tabaci* Gem) and belongs to the genus Begomovirus (family geminiviridae), Gemini virus subgroup III (Hameed *et al.*, 1994). During the year 1991-1992, the highest cotton production was 12.82 million bales in Pakistan. During the year 1992-1993, CLCuV disease appeared in epidemic form, which caused decrease in cotton, yield down to 9.05 million bales and further was decreased to 8.04 million bales during 1993-94. CLCuV disease caused reduction of 7.1 million bales (during the last decade); which has accrued a loss of Rs.70 billion to national economy (Mahmood, 1999).

After the development and introduction of resistant varieties in the cotton belt, yield losses were recovered gradually and production regained to 11.17 million bales during 1999-2000 (Anonymous, 2001).

A team of cotton breeders and pathologist of Central Cotton Research Institute, (CCRI) Multan conducted a survey of Vehari district during the year 2001-2002. They recorded mild symptoms of CLCuV disease on terminal leaves of the plants of the varieties resistant to CLCuV. The infection of the disease was more prevalent at various locations of cotton crop in district Vehari in general and Burewala area in particular.

Therefore, an experiment was conducted at CCRI, Multan to confirm the susceptibility of Multan-CLCuV resistant cotton varieties against this new variant of CLCuV. The out-come of this effort will be a data base containing information about this new strain.

### Materials and Methods

A green house study was undertaken to determine tolerance level of new variant of CLCuV to commercial cotton cultivars, which were resistant to CLCuV disease. Nine cultivars viz., CIM-446, CIM-443, FH-901, BH-118, RH-500, CIM-473, CIM-478, CIM-482 and FH-900 resistant to CLCuV and cv. S-12 (a highly susceptible to CLCuV) were selected for this study. Experiment consisted of grafting and whitefly transmission. The appearance of symptoms of the disease was recorded by using the modified scale described in (Siddig, 1968) and is given in Table 1.

### Source of viral inoculum and maintenance of cultivars:

The viral inoculum used in this study was consisted of Burewala and Multan infected cotton plants and was maintained in glass house at CCRI, Multan.

**Transmission through grafting:** Six-week-old plants were selected for graft transmission. In one set, nine resistant varieties and one susceptible to CLCuV were grafted with Burewala infected plants. Graft transmission was performed through leaf-petiole graft technique as described by (Ali *et al.*, 1995). In the other set, varieties were grafted with Multan-CLCuV source. The grafted plants were covered with moist polyethylene bags and held so until grafts were established. Data on graft-inoculated plants were taken daily i.e. starting from one week after graft transmission and continued up to 90 days. The symptoms of CLCuV were graded according to the technique described by Siddig, (1968).

Table 1: Scale of symptoms of cotton leaf curl virus (CLCuV)

Scale	Description	Scale	Description
0	Complete absence of symptoms	4	Large group of veins involved
1	Few small scattered vein thickening	5	All veins involved
2	Small scattered vein thickening	6	All veins involved and sever curling
3	Vein thickening involving small groups of veins	E	Foliar outgrowth (i.e. Enations) present

**Transmission through whitefly:** In this study three weeks old cotton plants were selected. The cultivars were inoculated with viruliferous whiteflies collected from Burewala and Multan viral inoculums and were kept in separate cages. After 48 h, the plants were sprayed by a recommended insecticide to kill the whiteflies and to stop the continuous supply of viral inoculums. The appearance of the disease symptoms were recorded daily after insecticidal spray.

**Evaluation of parent genetic stock:** The parent genetic stock (LRA-5166 and CP-15/2) used for breed hybridization programmed was also included to check the response of this new virulent. Cultivar CIM-240 was used a test plant to determine the susceptibility of Multan-CLCuV strain.

**Results and Discussion**

Data for appearance of symptoms of CLCV disease in commercial cultivars through graft transmission showed significant difference in their tolerance to two sources of CLCuV disease. Cultivars except cv S-12 showed their complete resistance to Multan-CLCuV disease. In contrast, CLCuV symptoms appeared within 16-30 days after graft transmission with Burewala-CLCuV strain. The intensity of disease according to scale of CLCuV symptoms ranged from 2 to 5 (Table 2).

The data clearly depict that cultivars showed their complete resistance to Multan-CLCuV disease, whereas, the lot fell prey to Burewala-CLCuV disease. Data demonstrate that Burewala-CLCuV is new variant and more virulent than that of earlier Multan strain present in the vicinity. Cultivars differed at little in their response to Burewala-CLCuV. Cultivar FH-901, BH-118, RH-500 showed higher intensity of disease with concurrent less number of days for showing symptoms compared to other ones.

All the commercial varieties except cv. S-12 showed their complete resistance to Multan-CLCuV source. On the other hand, CLCuV disease symptoms appeared within 7-15 days after whitefly inoculation with Burewala-CLCuV strain (Table 3). The intensity of the disease ranged from 2-5.

Data clearly depict that cultivars showed complete differential behavior in tolerance and / or susceptibility to CLCuV disease. Simulation of CLCuV infection through

Table 2: Comparison of commercial resistant varieties against CLCuV disease through graft transmission

Variety	Multan CLCuV Source		Burewala CLCuV Source	
	Disease appeared (days)	Intensity	Disease appeared (days)	Intensity
CIM-446	Not appeared	0	22-28	2
CIM-443	-do-	0	20-26	2
CIM-473	-do-	0	22-29	2
CIM-448	-do-	0	25-60	2
CIM-482	-do-	0	20-26	2
FH-900	-do-	0	20-23	2
FH-901	-do-	0	18-25	3
BH-118	-do-	0	17-22	3
RH-500	-do-	0	17-20	3
S-12	20-23	5	16-20	5

Table 3: Comparison of commercial resistant varieties against CLCuV-disease through Whitefly transmission

Variety	Multan CLCuV Source		Burewala CLCuV Source	
	Disease appeared (days)	Intensity	Disease appeared (days)	Intensity
CIM-446	Not appeared	0	11-13	2
CIM-443	-do-	0	10-14	2
CIM-473	-do-	0	12-14	2
CIM-448	-do-	0	10-15	2
CIM-482	-do-	0	10-12	2
FH-900	-do-	0	7-10	2
FH-901	-do-	0	11-15	3
BH-118	-do-	0	11-14	3
RH-500	-do-	0	12-14	3
S-12	13-15	5	6-10	5

Table 4: Comparison of Parents Material against CLCuV disease through Graft and whitefly transmission

Variety	Multan CLCuV Source		Burewala CLCuV Source	
	Disease appeared (days)	Intensity	Disease appeared (days)	Intensity
<b>Graft transmission</b>				
LRA-5166	Not appeared	0	20-25	2
CP-15/2	-do-	0	17-22	2
CIM-240	18-25	5	17-20	5
<b>Whitefly transmission</b>				
LRA-5166	Not appeared	0	10-13	2
CP-15/2	-do-	0	7-10	3
CIM-240	10-12	6	7-9	6

whitefly transmission took significantly less number of days compared to that of grafting transmission technique. There was 50% reduction in period for simulation of disease through whitefly transmission (Table 3). The pattern of appearance of CLCuV disease intensity was similar as that of through graft transmission technique. The parental genetic stock (LRA-5166 and CP-15/2) under study showed susceptibility to Burewala disease (Table 4). The grades of intensity of disease were similar to that of hybrids of these parentage (Table 2 and 3).

Similar results have been reported by Singh *et al.* (1999). Thus the present scenario leads to two assumptions: (i) whether the existing commercial cultivars have lost their resistance to CLCuV ? and or (ii) mutation has occurred in the existing CLCuV strain. If we suspect that present commercial cultivars have lost their resistance to CLCV, then it would have shown their complete susceptibility to Multan-CLCV with concurrent to Burewala-CLCV. Therefore, this assumption negated by data presented in Table 2 and Table 3. So, these results indicating the emergence of a resistance breaking strain of virus (Burewal-CLCuV) in the Punjab.

Recognizing the great current threat to this new variant of Multan-CLCuV disease needs intensive and concerted efforts by researchers.

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