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Response of cotton varieties to cotton leaf curl virus (CLCuV)

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ABSTRACT

The relative resistance /susceptibility of 45 varieties and 40 strains of upland cotton to cotton leaf curl virus (CLCuV) was evaluated under field conditions at Punjab Seed Corporation, Khanewal. Two varieties (CIM-446 & FH-901) and thirteen strains (FH-900S, CIM-473, CM-28, 565/98, 642/98, 726/98, BH-549/99, BH-147, BH-148, VH-137, Z-113, P-6 & P-8) were field immune while twenty two varieties (124F, 199F, 149F, B-557, MNH-93, MS-84, NIAB-78, CIM-70, FH-87, S-12, GOHAR-87, CIM-109, RH-1, NIAB-86, MNH-329, MNH-147, BH-36, FH-682, S-14, SLS-1, Mixture and Karishma) and two strains (MNH-633 & NIAB Karishma) were found to be highly susceptible. Graft inoculation studies of ten varieties and five strains showed that none of the material was immune or highly resistant. Two varieties (NIAB-78 & NIAB-Karishma) showed highly susceptible response and showed disease symptoms after 16 and 15 days of grafting, respectively. Remaining varieties /strains were resistant or moderately resistant. Only two varieties (FH-900 & FH-901) and two strains (NIAB-98 & FH-945) resist more as they delayed disease appearance after grafting till 22, 22, 23 & 22 days respectively. Graft inoculation studies showed difficulty of obtaining highly resistant source, so the preference should be given to the germplasm which resist more against CLCuV and meanwhile search for immune source should be continued. It is also suggested that strains showing "field immune" or "highly resistant" response to virus infection in a field and other desirable agronomic characters may be recommended for high cotton yield.

Key words: Cotton, *Gossypium hirsutum* L., CLCuV, field conditions, grafting, varieties, strains, resistance, susceptibility.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important cash crops in Pakistan. Its raw material and products remain a major source of foreign exchange. More than 60% of the total foreign exchange is earned annually from this crop (Ahmad 1999). The highest ever production of 12.8 million bales was achieved in 1991-92. But, there was an opposite trend in the following two years and production dropped to 8.04 million bales in 1993-94, due to the severe out break of CLCuV disease, which caused heavy damage to the cotton crop. Since then the yield losses have become a regular phenomenon for this crop. Due to CLCuV infection 7.1 million bales have been lost during the last decade (Mahmood 1999).

Cotton leaf curl is a viral disease caused by whitefly (*Bemisia tabaci* Genn.) transmitted Geminivirus, belonging to the genus *Begomovirus* (family *Geminiviridae*). Geminivirus subgroup III (Hameed *et al.* 1994). Cotton leaf curl virus (CLCuV) was first reported in 1912 from Nigeria on *Gossypium barbadense* L. (Farquharson 1912). During 1924 and 1926, this malady was reported from Sudan and Tanzania, respectively (Bailey 1934 and Jones & Mason 1926). CLCuV is characterized by upward and/or downward curling of leaves (Akhtar *et al.* 2000). Veins of leaf become thickened and more pronounced on the underside. Thickening of small veins, which is characterized by small bead-like modifications on the leaves, is a common feature under our conditions. These irregular thickenings gradually extend and coalesce to form a continuous reticulation of the small veins. Under severe attack, frequently one or more cup shaped or leaf laminar outgrowth called "Enation" appears on the underside of the leaf (Fig. 1) (Khalid *et al.* 1999). In Pakistan this disease was first reported during 1967 near Multan (Hussain and Ali 1975) but not much attention was given due to its minor importance. In 1988, the disease appeared in an epidemic form and damaged the crop in about 60 hectares near Multan. Since the disease has been progressively increasing and causing major losses in yield (Mahmood, 1999), development of disease resistant varieties is the only effective or permanent solution to the problem.

The present study was conducted to determine the level of resistance of commercial and promising cotton varieties and strains against cotton leaf curl virus under field conditions and by artificial inoculation through grafting.

Fig. 1. Severe vein thickening, upward and downward leaf curling, leaf enation and stunting of susceptible cotton cultivar due to CLCuV.

MATERIALS AND METHODS

Field survey

In order to measure the response of varieties and strains, of cotton against CLCuV a survey was conducted during August, 2000 (when plants were 12 weeks old), at Punjab Seed Corporation Farm, Khanewal, where the disease has been reported regularly and supposed to be a hot-spot for CLCuV. Ten to eighty four un-sprayed plants of 45 test varieties and 40 strains (Table 2 & Table 3) were observed and percentage of disease index and reaction of varieties were recorded (Table 1).

The percent disease index was calculated as follows:

$$\% \text{ Disease index} = \frac{\text{Sum of all disease ratings}}{\text{Total No. of plants assessed}} \times \frac{100}{6}$$

Artificial inoculation

Source of viral inoculum and maintenance of culture

The viral isolate used for grafting was collected from naturally infected cotton plants exhibiting characteristic symptoms of CLCuV. The virus was maintained in a net house through grafting of infected plant collected from field on to the S -12

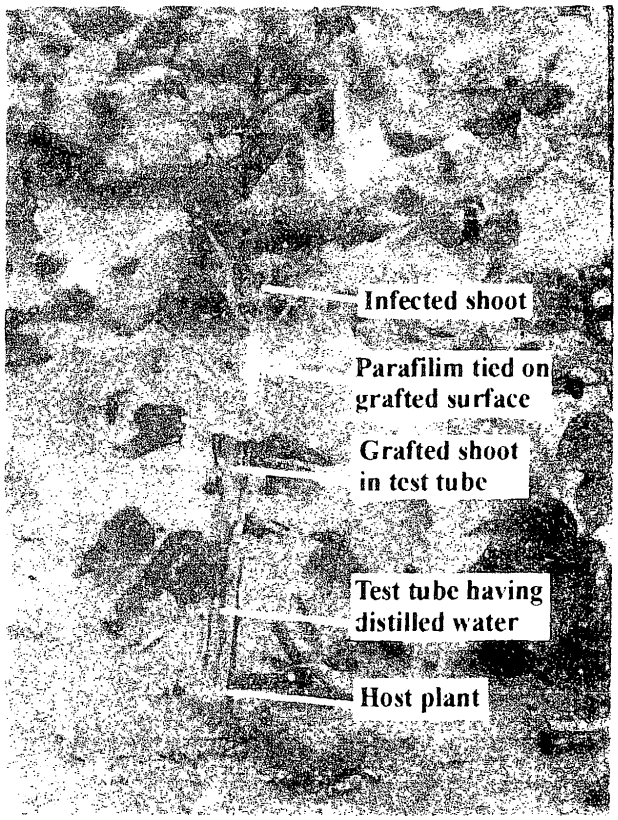

Fig. 2. Bottle shoot grafting method for artificial inoculation of CLCuV

Table 1. Modified disease scale for rating of cotton leaf curl virus (CLCuV)

Rating	Symptoms	% disease index	Disease reaction
0	Complete absence of symptoms	0	Field immune*
1	Thickening of few small scattered veins after careful observations	0.1-5	Highly resistant
2	Thickening of small group of veins	5.1-10	Resistant
3	Thickening of all veins but no curling of leaves	10.1-20	Moderately resistant
4	Severe vein thickening and leaf curling developed at the top of the plant (on one third of the plant)	20.1-30	Moderately susceptible
5	Severe vein thickening and leaf curling developed on half of the plant	30.1-50	Susceptible
6	Severe vein thickening, leaf curling and stunting of the plant with low yield or less fruit bearing	50.1-100	Highly susceptible

*Cooper and Jones, 1983.

plants. Grafting was performed by the bottle leaf grafting method described by Mirza (1992) with necessary modifications.

Graft inoculation

Four to six seeds of ten varieties (CIM-1100, CIM-443, CIM-446, CIM-448, FH-634, FH-900, FH-901, NIAB-78 CIM-482 & N-Karshma) and five strains (CIM-473, NIAB-98, NIAB-94, NIAB-801, FH-945) were sown in ten earthenware pots, having 12 inch diameter, under insect free cages in a net house during May, 2000. These pots were filled with soil taken from cotton experimental area of NIAB. Thinning was done keeping one plant per pot, two weeks after germination of seeds. These plants were grafted following improved grafting method. Six-week-old plants were selected for grafting and one sliced cut of 1 to 2 cm long and 0.1 to 0.2 cm deep was made on the stem near 2nd last internode of test

Table 2. Response of cotton varieties against CLCuV at PSC Farm, Khanewal

Sr. No	Varieties	Total plant Observed.	Diseased Plants	Infection type range*	% disease index
1	4F	22	3	1-6	9.85
2	289F	18	11	5-6E	32.41
3	LSS	18	6	5-6E	32.41
4	269/F43	20	10	5-6E	45.00
5	124F	22	17	5-6E	68.18
6	199F	22	17	5-6E	68.94
7	216F	22	4	1-3	14.39
8	238F	22	7	2-6	28.03
9	268F	22	20	2-6E	70.45
10	L-11	22	8	1-6	20.45
11	AC-134	22	7	4-6	25
12	BS-1	22	1	5	3.79
13	MS-39	22	14	5-6	59.09
14	MS-40	22	2	2	3.03
15	149F	22	17	6	77.27
16	B-557	22	21	5-6E	93.94
17	MNH-93	22	22	5-6E	95.45
18	MS-84	22	21	5E	79.55
19	NIAB-78	22	18	5-6E	69.70
20	CIM-70	22	18	5-6E	69.70
21	FH-87	22	22	4-6E	95.45
22	S-12	22	17	6E	77.27
23	GOHAR-87	22	20	6E	90.19
24	CIM-109	22	20	6E	90.91
25	RH-1	22	19	5-6E	72.73
26	NIAB-86	22	21	5-6E	93.18
27	MNH-329	22	22	1-6E	96.21
28	MNH-147	22	22	5E	83.33
29	CIM-240	22	9	6E	40.91
30	BH-36	22	21	4-6E	77.27
31	BH-682	22	20	3-6E	71.97
32	S-14	22	22	6E	100
33	SLS-1	22	22	6E	100
34	CIM-448	22	2	1-2E	2.27
35	CIM-1100	22	1	1E	0.76
36	FH-634	22	6	2-5E	13.64
37	CIM-443	40	1	2	0.833
38	CIM-446	40	0	0	0
39	FVH-53	45	1	1E	0.37
40	CIM-482	50	2	1-6E	2.33
41	Mixture	42	30	5-6	66.27
42	FH-900	55	2	1-2E	0.91
43	FH-901	55	0	0	0
44	BH-118	55	1	1E	0.37
45	N-Karishma	44	41	5-6e	88.64

Foliar outgrowths (Enation) will be marked with "E" where they will be observed.

* Infection type range represents the lowest and highest values for the ratings of the CLCuV infected plants of a particular cultivar at the time of observations.

Table 3. Response of cotton strains against CLCuV at PSC Farm, Khanewal

Sr. No	Strains	Total plant Observed	Diseased Plants	Infection type range	% disease index
1	FH-900 S	84	0	0	0
2	FH-945	50	1	1E	0.3
3	NIAB-98	70	4	1-6	2.62
4	NF-801	56	6	1-6	5.06
5	NIAB-94	62	11	1-6	10.21
6	CIM-473	56	0	0	0
7	CM-19	56	1	2	0.59
8	CM-28	56	0	0	0
9	CM-29	56	3	1-2E	2.38
10	CM-39	56	1	1E	0.3
11	MNH-633	10	10	6E	100
12	565/98	10	0	0	0
13	642/98	13	0	0	0
14	675/98	13	1	1	1.28
15	713/98	13	1	1	1.28
16	726/98	13	0	0	0
17	MNH-552	70	2	2	1.05
18	BH-121	70	2	1	0.48
19	BH-124	56	0	0	0
20	BH-125	70	3	6	2.86
21	BH-146	56	1	6	1.79
22	BH-549/99	56	0	0	0
23	BH-147	56	1	4	0.59
24	BH-148	56	0	0	0
25	RH-500	56	1	1-2	1.2
26	VH-59	56	3	2	1.49
27	VH-137	56	0	0	0
28	S-1	56	8	4	7.14
29	S-81	56	2	3	2.38
30	S-109	56	1	3	0.89
31	Z-113	56	0	0	0
32	P-4	28	3	3-6	6.55
33	P-5	40	5	4	8.33
34	P-6	56	0	0	0
35	P-7	56	9	3	13.09
36	P-8	84	1	1-2	0.59
37	P-9	39	2	2-6	1.11
38	K-1	56	4	2-5	5.36
39	4-2	56	4	2.5	5.06
40	N-Karishma	56	50	5-6E	77.38

Foliar outgrowths (Enation) will be marked with "E" where they will be observed.

Infection type range represents the lowest and highest values for the ratings of the CLCuV infected plants of a particular cultivar at the time of observations

plant. A CLCuV infected branch with 20 cm long growing tip was detached from diseased plant (maintained culture). A similar cut (as in test plant) was made on this branch and corresponding cut surfaces were brought together and tied with parafilm to avoid drying and to stop the entry of air. Care was taken to bring the corresponding cambium surfaces into contact. This stem was then placed in a test tube having 2 cm diameter with 16 cm length, filled with distilled water, which was changed daily (Fig. 2). After five days, these tubes were removed and plants were observed daily. Experimental unit was fertilized with urea (9 g /plant) once a month and clean tap water was applied to young seedlings according to the requirements throughout the period of study. Data were collected on % success in grafting, infection percentage, mean latent period (average time taken for appearance of first symptoms after grafting) and average disease severity after 90 days of grafting. Disease reaction and severity were recorded according to the Table 1.

RESULTS AND DISCUSSION

The CLCuV infection caused severe yield losses in the cotton crop (Mahmood, 1999). Undoubtedly, the best way to reduce CLCuV-induced damage is by breeding cotton for resistant to the virus. Here we report results obtained with the commercial cotton varieties and new cotton strains. Out of 45 varieties and 40 strains, only two varieties (CIM-446 & FH-901) and 13 strains (FH-900S, CIM-473, CM-28, 565/98, 642/98, 726/98, BH-549/99, BH-147, BH-148, VH-137, Z-113, P-6 and P-8) were found to be

Table 4. Summary statement of cotton varieties evaluated against cotton leaf curl virus (CLCuV) under field condition.

Sr. No.	Field immune	Highly resistant	Resistant	Moderately resistant	Moderately Susceptible	Susceptible	Highly susceptible
1	CIM-446	BS-1	4F	216F	238-F	289-F	124F
2	FH-901	MS-40	-	FH-634	268-F	LSS	199F
3	-	CIM-448	-	-	L-11	269/F43	149F
4	-	CIM-1100	-	-	AC-134	CIM-240	B-557
5	-	CIM-443			MS-39	-	MNH-93
6	-	FVH-53	-	-	-	-	MS-84
7	-	CIM-482-	-	-	-	-	NIAB-78
8	-	FH-900	-	-	-	-	CIM-70
9	-	BH-118	-	-	-	-	FH-87
10-	-	-	-	-	-	-	S-12
11	-	-	-	-	-	-	GOHAR-87
12	-	-	-	-	-	-	CIM-109
13	-	-	-	-	-	-	RH-1
14	-	-	-	-	-	-	NIAB-86
15	-	-	-	-	-	-	MNH-329
16	-	-	-	-	-	-	MNH-147
17	-	-	-	-	-	-	BH-36
18	-	-	-	-	-	-	FH-682
19	-	-	-	-	-	-	S-14
20	-	-	-	-	-	-	SLS-1
21	-	-	-	-	-	-	Mixture
22	-	-	-	-	-	-	N-karishma

Table 5. Summary statement of cotton strains evaluated against cotton leaf curl virus (CLCuV) under field condition.

Sr. No.	Field immune	Highly resistant	Resistant	Moderately resistant	Moderately Susceptible	Susceptible	Highly susceptible
1	FH-900 S	FH-945	NF-801	NIAB-94	-	-	MNH-633
2	CIM-473	NIAB-98	S-1	P-7	-	-	N-Karishma
3	CM-28	CM-19	P-4	-	-	-	-
4	565 98	CM-29	P-5	-	-	-	-
5	642 98	CM-39	K-1	-	-	-	-
6	726.98	675.98	K2	-	-	-	-
7	BH-124	713.98	-	-	-	-	-
8	BH-549 99	MNH-552	-	-	-	-	-
9	BH-148	BH-121	-	-	-	-	-
10	VH-137	BH-125	-	-	-	-	-
11	Z-113	BH-146	-	-	-	-	-
12	P-6	VH-69	-	-	-	-	-
13	P-8	VH-59	-	-	-	-	-
14	-	S-81	-	-	-	-	-
15	-	S-109	-	-	-	-	-
16	-	BH-147	-	-	-	-	-
17	-	P-9	-	-	-	-	-

Table 6. Response of commercial cotton varieties and promising strains to CLCuV through grafting

Variety/ Strains	success of grafting (%)	Infection percentage	Mean latent period** (days)	Disease severity after 90 days of grafting (0-6E)	Disease reaction
			Varieties		
CIM-1100	100	100	17	3E	Moderately resistant
CIM-443	100	100	18	2E	Resistant
CIM-446	100	100	18	2E	Resistant
CIM-448	100	100	17	2E	Resistant
FH-634	100	100	17	4E	Moderately susceptible
FH-900	100	100	22	2	Resistant
FH-901	100	100	22	2	Resistant
NIAB-78	100	100	16	6E	Highly susceptible
CIM-482	100	100	17	2E	Resistant
			Strains		
CIM-473	100	100	18	2E	Resistant
NIAB-98	100	100	23	2	Resistant
NIAB-94	100	100	19	3	Moderately resistant
NIAB-801	100	100	19	3	Moderately resistant
FH-945	100	100	22	2	Resistant
N-Karishma*	100	100	15	6E	Highly susceptible

Foliar outgrowths (Enation) will be marked with "E" where they will be observed; * - Control; **- Average time taken for first disease symptom appearance after grafting.

field immune as none of the plants were found to be infected, while twenty-two varieties (124F, 199F, 149F, B-557, MNH-93, MS-84, NIAB-78, CIM-70, FH-87, S-12, GOHAR-87, CIM-109, RH-1, NIAB-86, MNH-329, MNH-147, BH-36, FH-682, S-14, SLS-1, Mixture and N-Karishma) and two strains (MNH-633 & Karishma) were found to be highly susceptible as they showed severe vein thickening, curling of leaves and stunting of plants. Nine varieties (BS-1, MS-40, CIM-448, CIM-1100, CIM-443, FVH-53, CIM-482, FH-900 & BH-118) and seventeen strains (FH-945, NIAB-98, CM-19, CM-29, CM-39, 675/98, 713/98, MNH-552, BH-121, BH-125, BH-146, RH-500, VH-59, S-81, BH-124, S-109 & P-9) were found to be highly resistant (Tables 2, 3, 4, 5).

Graft inoculation studies revealed that, none of the single plants of cotton cultivars / strains appeared to be immune or highly resistant to CLCuV. Reaction of cotton germplasm under graft-inoculated conditions varied greatly. Six commercial varieties (CIM-446, CIM-448, CIM-443 FH-900, FH-901 and CIM-482) and three strains (CIM-473, NIAB-98 and FH-945) were found to be resistant. One variety CIM-1100 and two strains (NIAB-94 & NIAB-801/F) were moderately resistant as they produced thickening of all veins with various leaf enations. FH-634 was moderately susceptible showing severe vein thickening and curling of leaves on top of the plant with different shapes and sizes of enations. NIAB-78 and NIAB-Karishma were found to be highly susceptible as it produced both upward and downward leaf curling, vein thickening and stunting of plants with enations of varying sizes. Symptoms started after 15 days of inoculation on NIAB-78 & N-Karishma and inoculated plants became completely infected after 20 days. The remaining cultivars exhibited minor infection as they showed thickening of few small scattered veins within 18-23 days of inoculation, while NIAB-98 showed high level of tolerance by delayed infection, as it took more time (23 days) to produce mild symptoms, as compared with other varieties and strains. Our findings do not agree with those of Ali *et al.* (1995) and Shah *et al.* (1999) who observed that CIM-1100, CIM-436, CIM-446, CIM-443 and CIM-448 remained symptomless till 90 days after grafting. On the contrary, our findings are in agreement with that of Akhtar *et al.* (2001) who found for the first time that CIM-443, CIM-446, CIM-448, CIM-1100, FH-634 and LRA-5166 are prone to CLCuV infection under graft inoculation.

This screening revealed scarcity of resistance in varieties, while encouraging results were observed in case of new strains. These resistant strains, if found with desirable for other agronomic traits, can be released for general cultivation. They can also serve as resistant sources for breeding to incorporate their resistance into susceptible commercial cultivation possessing desirable agronomic traits other than resistance. It is extremely essential to determine whether their resistance is mono or polygenic and whether their resistance is controlled by the dominant or recessive allels of gene/genes controlling their resistance. It was also observed that most of the varieties /strains showed different

reactions to CLCuV under both screening methods. Most of the entries showing resistant response through artificial inoculation were field immune or highly resistant under natural infestation of whiteflies, which indicated that this material may have some structural defensive mechanisms, which can restrict the vector from proper transmission of virus at proper place in host. So, one should consider the results obtained by both methods in evaluating the cotton germplasm for screening against CLCuV. These studies also suggest that the preference should be given to only those varieties / strains, which exhibit resistant response after artificial inoculation.

The problem should not be considered resolved, because four variants of CLCuV have been shown to exist in the fields (Zhou *et al.* 1998). Multiple infection of CLCuV and other whitefly transmitted Geminiviruses (WTGs) in cotton and other cotton growing areas is prevalent. Therefore, chances of recombination among them and other WTGs does exist which may lead into the emergence of new more virulent and resistant breaking variants (Shah *et al.* 1999).

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