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Comparison of Resistance Level to *Cotton leaf curl virus* (CLCuV) Among Newly Developed Cotton Mutants and Commercial Cultivars

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Four newly developed cotton mutants (M-111, M-7662, M-358 and M-218) were compared for their resistance against *Cotton leaf curl virus* (CLCuV) together with commercial resistant (CIM-443, CIM-482, CIM-473, FH-900 and FH-901) and susceptible (S-12) varieties by artificial inoculation through grafting and under natural field conditions. Infectivity and success of grafting were 100% in all cases. None of the grafted plants were found immune or asymptomatic. All the grafted mutants and most of their single plant progeny rows (SPPRs) showed highly resistant responses as the symptoms displayed by these mutants were milder than the commercial cultivars. Grafted mutants also had delayed disease reactions as they took more time (25-30 days) to produce disease symptoms, as compared with resistant commercial varieties that produced disease 18-22 days after inoculation. Growth of the grafted SPPRs of tested mutants was normal, which is an indication that there will be no production losses. Observations under natural infestation of whitefly showed that two SPPRs of M-11/CE and M-7662-1/2 and one resistant variety CIM-443 exhibited slight incidence of disease, while one SPPR of M-11/59 and S-12 were moderately susceptible and highly susceptible with 21% and 97.1% disease incidence, respectively. This study also showed that plants displaying more disease symptoms through grafting were easily infected under natural conditions. These results suggest that preference should be given to those plants that exhibited highly resistant responses after artificial inoculation.

Keywords : cotton, *Cotton leaf curl virus*, *Geminivirus*, *Gossypium hirsutum* L., grafting, mutants and commercial varieties, natural infestation, resistance.

Cotton is one of the leading crops in Pakistan, accounting for 60% of the total foreign exchange earning through the export of raw cotton and cotton products. It also provides raw material to the local domestic cotton industry comprised of 503 textile mills, 1,135 ginning factories, and over 5,000 oil expelling units. It has an 85% share in the total vegetable oil produced in the country. Cottonseed cake, an important by-product, is a valuable source of protein for ruminant cattle. Forty percent of the country's labor force is employed in cotton fields and cotton processing mills (Riaz, 1997). Due to its wide production and diverse uses, cotton is rightly considered as the backbone of Pakistan's economy. Pakistan ranks fourth in area and production of cotton in the world (Mahmood, 1999).

Cotton plants are naturally susceptible to a large number of diseases. The actual number is still not known, but nearly 75 of cotton diseases have been described as pathogenic (Watkins, 1981). At present *Cotton leaf curl virus* (CLCuV) is a serious threat to successful cotton production in Pakistan. This disease is reported to be caused by a whitefly (*Bemisia tabaci* Gann.) transmitted *Geminivirus*, having single stranded circular DNA (ssDNA) with twinned geminate particles. This virus belongs to the family *Geminiviridae*, genus *Begomovirus*, and *Geminivirus* subgroup III (Hameed et al., 1994). CLCuV disease is characterized by upward and/or downward curling of leaves. The veins of the leaf thicken, which become more pronounced on the underside. In extreme but frequent cases, one or more cup-shaped outgrowths called "enation" appear on the underside of the leaf blade. Under severe attack, infected plant remains stunted (Hussain et al., 1991).

In Pakistan, the disease was first observed near Multan on a few cotton plants in 1967. Since the disease was of minor importance, it did not attract serious attention although it has been noted consequently. The disease persisted in the same area until 1987. In 1988, it damaged 60 ha of cotton plantation in the Multan district. Since then, the disease has been spreading every year. In 1991-1992, Pakistan achieved a record production of 12.8 million bales, which declined to 7.9 million bales in 1994-1995 (Anonymous, 1997). The yield losses have become a constant phenomenon every year due to CLCuV. More than 7 million bales have been lost during the last decade (Mahmood, 1999a).

Resistant varieties are the only permanent solution to the problem. During the last decade, considerable efforts have been made by different research organizations to develop CLCuV resistant cultivars. For a successful breeding pro-

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gram, a reliable screening technique is necessary to identify genetic resources. Current screening procedure relies upon natural field infection and/or inoculation through grafting. Field observation showed that most commercially grown cultivars were susceptible to CLCuV but there were different levels of tolerance in different varieties. Varieties initially found to have some tolerance eventually become susceptible. Artificial inoculation techniques were not available, therefore, the only way to study the response of cotton germplasm is to expose them to high inoculum pressure by planting in natural hot spots. However, different responses were noticed from the same variety in different locations, e.g., CIM-109 and CIM-240 showed susceptibility in Mian-Channu area, while they appeared to be resistant in Lodhran (Ali, 1997). In the absence of a uniform technique, no meaningful results could be drawn.

The current study was carried out to compare the level of resistance of newly developed mutants with commercially grown resistant varieties against CLCuV through grafting and under natural field conditions.

Materials and Methods

Source and maintenance of viral inoculum. The viral isolate used in this study was collected from naturally infected cotton plants exhibiting characteristic symptoms of CLCuV (Fig. 1). This isolate was maintained in a net house through grafting of infected plant (selected from the field) onto the S-12 plants grown in the net house. Improved grafting method as discussed below was followed. The virus was deposited in the Plant Virus GenBank (http://www.virusbank.org), Seoul, Korea with accession number PV-0231 as dried leaf samples.

Inoculation method. For all artificial inoculations, improved grafting method was followed. For this purpose, 6-week-old plants were selected for grafting and a cut of 1-2 cm long and 0.1-0.2 cm deep was made on the stem near the second last internode of the test plant. A CLCuV infected branch with a growing tip of about 20 cm long was detached from the diseased plant (maintained culture). A similar cut (as in the 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. Distilled water was changed daily at 12-13 h for 5 days. After 5 days, these tubes were removed and the plants were observed daily after grafting to see their success and disease transmission.

Disease evaluation. For the assessment of CLCuV disease, 0 to 6E modified disease scale was followed as shown in Table 1.

The percent disease incidence was calculated as follows:

$$\text{Sum of all disease ratings} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of plants assessed}} \times \frac{100}{6}$$

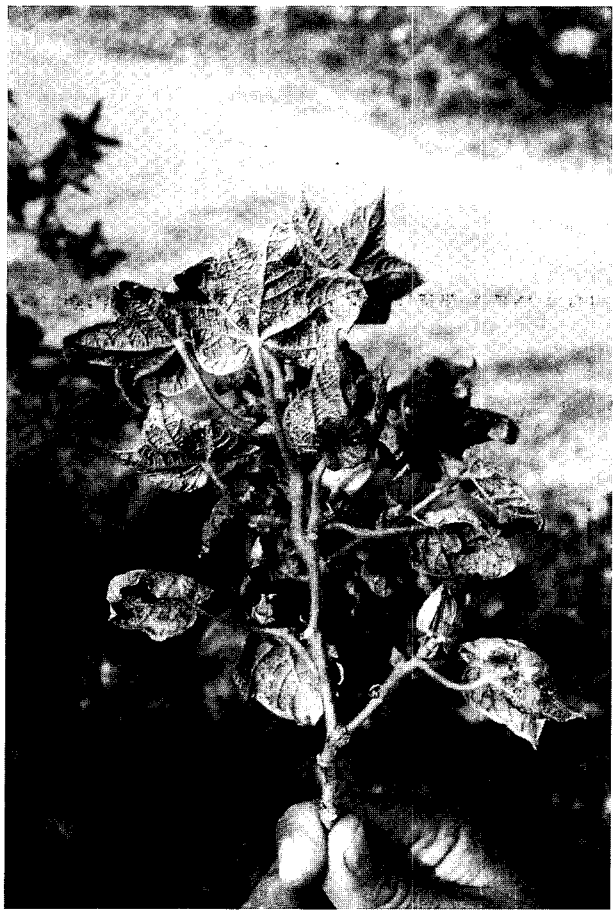

Fig. 1. Severe vein thickening, leaf curling (upward and downward), leaf enation, and stunting of the plant of susceptible cotton cultivar S-12 due to *Cotton leaf curl virus* (CLCuV), used as a source for graft inoculation in this study.

Plant materials. Four new mutants (NIAB-111, NIAB-358, NIAB-7662 and NIAB-218) were compared with five CLCuV resistant commercial varieties (CIM-443, CIM-482, CIM-473, FH-900 and FH-901). A highly susceptible cultivar S-12 was included as positive control (Table 2).

Grafting under net house conditions. Twenty pots of each test entry were sown (4-5 delinted seeds per pot) in the net house. Then, these pots were transferred inside insect free cages. Thinning was done by maintaining one plant per pot 2 weeks after germination of seeds. These plants were grafted following the improved bottle shoot grafting method described above, using diseased stem of S-12 from maintained culture. The following data were then collected: success in grafting, infectivity, average time taken for first symptom appearance after grafting, infection type range, and average disease severity after 70 days of grafting and disease reaction.

Grafting under field conditions. Seeds of SPPRs namely, M-7662-1/1, M-7662-1/8, M-7662-1/2, M-358/2, M-358/12, M-358/26, M-11/2, M-11/CE, M-11/59 and M-218 were sown in the field. One resistant commercial variety CIM-473 and susceptible

Table 1. Modified disease scale for the rating of CLCuV disease

Rating[a]	Symptoms	Disease incidence (%)	Disease reaction
0	Complete absence of symptoms	0	Immune
1	Thickening of few small scattered veins	0.1-5.0	Highly resistant
2	Thickening of small group of veins	5.1-10.0	Resistant
3	Thickening of all veins	10.1-20.0	Moderately resistant
4	Severe vein thickening and leaf curling developed at the top of the plant	20.1-30.0	Moderately susceptible
5	Severe vein thickening and leaf curling developed on the half of the plant	30.1-50.0	Susceptible
6	Severe vein thickening, leaf curling and stunting of the plant	50.1-100	Highly susceptible

[a] Foliar outgrowths (enation) were marked with "E" where observed.

Table 2. Cotton mutants/varieties, origin, present status, and their methods of development

Mutant/variety	Origin[a]	Present status	Method of development
M-111	NIAB, Faisalabad, Pakistan	Mutant	Through radiation
M-218	NIAB, Faisalabad, Pakistan	Mutant	Through radiation
M-7662	NIAB, Faisalabad, Pakistan	Mutant	Through radiation
M-358	NIAB, Faisalabad, Pakistan	Mutant	Through radiation
FH-900	AARI, Faisalabad, Pakistan	Approved	Conventional breeding
FH-901	AARI, Faisalabad, Pakistan	Approved	Conventional breeding
CIM-443	CCRI, Multan, Pakistan	Approved	Conventional breeding
CIM-482	CCRI, Multan, Pakistan	Approved	Conventional breeding
CIM-473	CCRI, Multan, Pakistan	Candidate	Conventional breeding
S-12	CRS, Multan, Pakistan	Approved	Conventional breeding

[a] NIAB = Nuclear Institute for Agriculture and Biology; AARI = Ayub Agricultural Research Institute; CCRI = Central Cotton Research Institute; CRS = Cotton Research Station.

S-12 were included as controls. Each mutant/cultivar was planted in three replicates under normal cultural practices of fertilizer, plant protection, and irrigation. After thinning, 10 selected plants were covered with cages having very fine meshed cloth for insect protection. At the age of 6 weeks, one plant under each cage was grafted following improved bottle shoot grafting method. Data collected were success in grafting, disease transmission, days taken for first symptom appearance after grafting, average disease severity after 70 days of grafting, disease reaction, and growth of the grafted plants.

Field observations. Response of 10 SPPRs namely, M-7662-1/1, M-7662-1/8, M-7662-1/2, M-358/2, M-358/12, M-358/26, M-11/2, M-11/CE, M-11/59 and M-218 was recorded against CLCuV during the month of September under natural infestation of whitefly. Observations were also made against resistant commercial variety CIM-473 and susceptible S-12. The following data were collected: total plants inspected, infected plants, infection type range, % disease incidence, and reaction of the variety/mutants.

Results

Plants of various entries were grafted for symptom development and disease severity during their growth period under net house conditions. The test was carried out during the summer season with an average temperature of about 39°C. Infectivity and success of grafting was 100% in all cases. Plants of the susceptible variety S-12 were the first to produce symptoms after 15 days of grafting. Symptoms started with small vein thickening, progressed to severe vein thickening, upward and/or downward curling of leaves with various types and sizes of enations, and stunting of the plants after 20 days of inoculation. Plants of the resistant commercial cultivars CIM-443, CIM-482, and CIM-473 (candidate variety) were the next to produce disease after 18 days of grafting (Table 3). Diseased plants produced thickening of small group of veins and enation until 70 days of grafting. Two approved resistant varieties FH-900 and FH-901 took 22 days for the development of disease. Only two plants of FH-900 and three plants of FH-901 showed highly resistant responses as they produced thickening of few small, scattered veins while the remaining plants of these cultivars showed thickening of small group of veins. In contrast, plants of the new mutants namely, M-111, M-358, M-7662 and M-218 initiated disease after 25-28 days. All the plants of these mutants showed highly resistant response as they produced thickening of few small, scattered veins. Symptoms displayed by these mutants were much milder than the susceptible control S-12 (Table 3).

Grafting of SPPRs of mutant M-7662 (M-7662-1/1, M-

Table 3. Response of mutants and commercial cotton varieties to CLCuV through grafting under net house conditions

Mutant/ Variety	Grafting success (%)	Infectivity	Average no. of days of 1st symptom appearance after grafting	Infection type range[a]	Average disease severity after 70 days of grafting	Disease reaction
M-111	100	100	28	1	1	Highly resistant
M-358	100	100	28	1	1	Highly resistant
M-218	100	100	27	1	1	Highly resistant
M-7662	100	100	25	1	1	Highly resistant
FH-900	100	100	22	1-2	2	Resistant
FH-901	100	100	22	1-2	2	Resistant
CIM-473	100	100	18	2E[b]	2E	Resistant
CIM-482	100	100	17	2E	2E	Resistant
CIM-443	100	100	17	2E	2E	Resistant
S-12	100	100	15	6E	6E	Highly susceptible

[a] Infection type range is based upon 0-6E scale as described in Table 1.
[b] Foliar outgrowths (enation) were marked with "E" where observed.

Table 4. Response of SPPRs of mutant M-7662 against CLCuV through grafting under field conditions

SPPR/variety	Plant No.	Grafting success	Disease transmission	Days to 1st symptom	Disease severity after 70 days	Disease reaction	Growth of grafted plant[a]
M-7662-1/8	1	+	+	22	2	Resistant	Good
	2	+	+	23	1	Highly resistant	Good
	3	+	+	22	1	Highly resistant	Good
	4	+	+	22	2	Resistant	Good
	5	+	+	23	1	Highly resistant	Good
	6	+	+	23	1	Highly resistant	Good
	7	+	+	24	1	Highly resistant	Good
	8	+	+	23	1	Highly resistant	Good
	9	+	+	25	1	Highly resistant	Good
M-7662-1/1	1	+	+	26	1	Highly resistant	Good
	2	+	+	27	1	Highly resistant	Good
	3	+	+	27	1	Highly resistant	Good
	4	+	+	26	1	Highly resistant	Good
	5	+	+	25	1	Highly resistant	Good
	6	+	+	25	1	Highly resistant	Good
	7	+	+	23	1	Highly resistant	Good
	8	+	+	25	1	Highly resistant	Good
	9	+	+	25	1	Highly resistant	Good
	10	+	+	27	Traces	Highly resistant	Good
M-7662-1/2	1	+	+	18	3E	Moderately resistant	V. poor
	2	+	+	20	3E	Moderately resistant	V. poor
	3	+	+	18	4E	Moderately susceptible	V. poor
	4	+	+	19	3E	Moderately resistant	V. poor
	5	+	+	19	3E	Moderately resistant	V. poor
	6	+	+	19	5E	Susceptible	V. poor
	7	+	+	19	3E	Moderately resistant	V. poor
	8	+	+	18	3E	Moderately resistant	V. poor
	9	+	+	18	5E	Susceptible	V. poor
CIM-473	10 plants	+	+	18	2E	Resistant	Poor
S-12	10 plants	+	+	15	6E	Highly susceptible	V. poor

[a] Good = growth comparable with non-inoculated plants; Very poor = plants showing stunted growth; and Poor = visually shows 25% reduction in growth in comparison with healthy plants.

7662-1/8 and M-7662-1/2). Twenty-eight selected plants from M-7662-1/1, M-7662-1/8, and M-7662-1/2 were inoculated through improved bottle shoot grafting method. The whole experimental unit was successfully grafted which showed more or less resistant reaction to CLCuV. All plants of SPPR M-7662-1/1 and seven plants of M-7662-1/8 exhibited highly resistant response. The remaining two plants of M-7662-1/8 showed resistant response with thickening of small group of veins. Nine grafted plants of M-7662-1/2 developed disease after 18-20 days of grafting. Six plants showed moderately resistant reaction as they all produced thickening of large group of veins while one was moderately susceptible and two were susceptible. Growth of this SPPR was very poor as in the case of S-12. Among these SPPRs, M-7662-1/1 was excellent because disease was minor and appearance of symptom was delayed (23-27 days) as compared with that of CIM-473, S-12, and other SPPRs of this mutant. Plant No. 10 of this SPPR was found to have excellent results, producing minor thickening of veins. After careful search, 2-3 minute spots of small vein thickening were found on three leaves (Table 4).

Grafting of SPPRs of mutant M-111 (M-11/2, M-11/CE and M-11/59). Eighteen plants of three different SPPRs of M-111 were grafted following improved bottle shoot grafting method under field conditions. Disease transmission and success of grafting was 100% in all cases. None of the grafted plants were without symptoms. Different reactions to CLCuV were observed in the tested materials. All the tested plants of M-11/2 were highly resistant as they produced mild disease and took 27-29 days for disease development. Four plants of M-11/CE showed highly resistant response and two showed resistant response within 26-27 and 25 days, respectively. M-11/59 was moderately resistant and took 19-21 days for disease development. Disease development was earlier in the resistant control CIM-473, which took 18 days as compared to that of SPPRs of M-111. Visual observation showed that the growth of the grafted plants of the progenies M-11/2 and M-11/CE was normal as in the case of non-inoculated plants, while poor growth was recorded after grafting in the case of M-11/59 and resistant control CIM-473. S-12 was highly susceptible and the disease developed after 15 days with very poor growth (Table 5).

Grafting of SPPRs of mutant M-358 (M-358/2, M-358/12 and M-358/26). Twenty-eight plants of three different SPPRs of M-358 were inoculated under field conditions. None of the inoculated plants were found immune. This material took 27-30 days for symptom appearance and disease rating was observed between 1 and 2. M-358/26 was excellent because five plants produced two to three minor spots of vein thickening on three to five upper leaves. These spots were found after careful search. The remaining

Table 5. Response of SPPRs of mutant M-111 against CLCuV through grafting under field conditions

SPPR/variety	Plant No.	Grafting success	Disease transmission	Days to 1st symptom	Disease severity after 70 days	Disease reaction	Growth of grafted plant[a]
M-11/2	1	+	+	29	1	Highly resistant	Good
	2	+	+	27	1	Highly resistant	Good
	3	+	+	29	1	Highly resistant	Good
	4	+	+	29	1	Highly resistant	Good
	5	+	+	29	1	Highly resistant	Good
	6	+	+	28	1	Highly resistant	Good
	7	+	+	28	1	Highly resistant	Good
M-11/CE	1	+	+	26	1	Highly resistant	Good
	2	+	+	26	1	Highly resistant	Good
	3	+	+	27	2	Resistant	Good
	4	+	+	25	1	Highly resistant	Good
	5	+	+	26	1	Highly resistant	Good
	6	+	+	25	2	Resistant	Good
M-11/59	1	+	+	21	2	Resistant	Poor
	2	+	+	20	2	Resistant	Poor
	3	+	+	19	2	Resistant	Poor
	4	+	+	19	3	Moderately resistant	Poor
	5	+	+	19	2	Resistant	Poor
CIM-473	10 plants	+	+	18	2E	Resistant	Poor
S-12	10 plants	+	+	15	6E	Highly susceptible	V. poor

[a] Good = growth comparable with non-inoculated plants; Very poor = plants showing stunted growth; and Poor = visually shows 25% reduction in growth in comparison with healthy plants.

Table 6. Response of SPPRs of mutant M-358 against CLCuV through grafting under field conditions

SPPR/variety	Plant No.	Grafting success	Disease transmission	Days to 1st symptom	Disease severity after 70 days	Disease reaction	Growth of grafted plant[a]
M-358/2	1	+	+	27	2	Resistant	Good
	2	+	+	27	1	Highly resistant	Good
	3	+	+	24	1	Highly resistant	Good
	4	+	+	28	2	Resistant	Good
	5	+	+	28	1	Highly resistant	Good
	6	+	+	28	2	Resistant	Good
	7	+	+	24	2	Resistant	Good
	8	+	+	24	1	Highly resistant	Good
	9	+	+	25	1	Highly resistant	Good
M-358/12	1	+	+	23	1	Highly resistant	Good
	2	+	+	23	1	Highly resistant	Good
	3	+	+	22	2	Resistant	Good
	4	+	+	23	1	Highly resistant	Good
	5	+	+	23	1	Highly resistant	Good
	6	+	+	25	1	Highly resistant	Good
	7	+	+	23	2	Resistant	Good
	8	+	+	25	1	Highly resistant	Good
	9	+	+	22	1	Highly resistant	Good
	10	+	+	23	2	Resistant	Good
M-358/26	1	+	+	28	1	Highly resistant	Good
	2	+	+	29	1	Highly resistant	Good
	3	+	+	28	1	Highly resistant	Good
	4	+	+	29	Traces	Highly resistant	Good
	5	+	+	29	Traces	Highly resistant	Good
	6	+	+	29	Traces	Highly resistant	Good
	7	+	+	29	1	Highly resistant	Good
	8	+	+	28	Traces	Highly resistant	Good
	9	+	+	28	Traces	Highly resistant	Good
CIM-473	10 plants	+	+	18	2E	Resistant	Poor
S-12	10 plants	+	+	15	6E	Highly susceptible	V. poor

[a] Good = growth comparable with non-inoculated plants; Very poor = plants showing stunted growth; and Poor = visually shows 25% reduction in growth in comparison with healthy plants.

four plants produced thickening of few small, scattered veins. SPPRs M-358/2 and M-358/26 showed highly resistant to resistant response, with a disease rating of 1 or 2. This material exhibited highly resistant response as compared with CIM-473 and S-12. Growth of the grafted plants of all SPPRs of M-358 was normal while poor and very poor growth were observed for CIM-473 and S-12, respectively (Table 6).

Grafting of SPPR of mutant M-218. Fifteen selected plants from this mutant were grafted. None of the inoculated plants were disease free. All the tested plants produced thickening of small group of veins within 25-30 days of grafting and were highly resistant to resistant. Plants No. 2, 7, and 10 showed better results. Only three to four minute spots of small vein thickening were found on three to five upper leaves of these plants. Size of these leaves was normal and curling was absent. Growth of the plants was also normal, an indication that it will give normal yield. This material showed high level of resistance as compared with the resistant control CIM-473 in terms of disease severity and delayed disease development (Table 7).

Response of the tested material under natural conditions. Advance cotton germplasm representing 10 SPPR (M-7662-1/1, M-7662-1/8, M-7662-1/2, M-358/2, M-358/12, M-358/26, M-11/2, M-11/CE, M-11/59 and M-218) along with CIM-443, CIM-473, CIM-482, FH-900, FH-901 (resistant control) and S-12 (susceptible control) were screened for their resistance to CLCuV under natural conditions of whitefly transmission. Two SPPRs M-11/CE, M-7662-1/2 and one resistant control CIM-443 showed

Table 7. Response of SPPRs of mutant M-218 against CLCuV through grafting under field conditions

SPPR/variety	Plant No.	Grafting success	Disease transmission	Days to 1st symptom	Disease severity after 70 days	Disease reaction	Growth of grafted plant[a]
M-218	1	+	+	26	2	Resistant	Good
	2	+	+	27	Traces	Highly resistant	Good
	3	+	+	27	1	Highly resistant	Good
	4	+	+	25	2	Resistant	Good
	5	+	+	27	1	Highly resistant	Good
	6	+	+	28	1	Highly resistant	Good
	7	+	+	28	Traces	Highly resistant	Good
	8	+	+	25	1	Highly resistant	Good
	9	+	+	26	Traces	Highly resistant	Good
	10	+	+	24	1	Highly resistant	Good
	11	+	+	26	2	Resistant	Good
	12	+	+	25	1	Highly resistant	Good
	13	+	+	26	1	Highly resistant	Good
	14	+	+	25	2	Resistant	Good
	15	+	+	25	1	Highly resistant	Good
CIM-473	10 plants	+	+	18	2E	Resistant	Poor
S-12	10 plants	+	+	15	6E	Highly susceptible	V. poor

[a] Good = growth comparable with non-inoculated plants; Very poor = plants showing stunted growth; and Poor = visually shows 25% reduction in growth in comparison with healthy plants.

Table 8. Response of SPPRs of different mutants and commercial cotton cultivars to CLCuV under natural infection of whitefly

SPPR/variety	No. of plants inspected	Infected plants	Infection type range[a]	Disease incidence (%)	Disease reaction
M-11/2	267	0	–	0	Immune
M-11/CE	265	2	1	0.1	Highly resistant
M-11/59	28	9	2-5	21.0	Moderately susceptible
M-358/2	39	0	–	0	Immune
M-358/12	39	0	–	0	Immune
M-358/26	39	0	–	0	Immune
M-7662-1/8	60	0	–	0	Immune
M-7662-1/1	61	0	–	0	Immune
M-7662-1/2	58	2	5-6E	3.4	Highly resistant
M-218	70	0	–	0	Immune
FH-900	28	0	–	0	Immune
FH-901	26	0	–	0	Immune
CIM-482	26	0	–	0	Immune
CIM-473	56	0	–	0	Immune
CIM-443	296	9	1-3E	1.3	Highly resistant
S-12	70	68	6E	97.1	Highly susceptible

[a] Infection type range is based upon 0-6E scale as described in Table 1.

highly resistant response with percent disease incidence of 0.1%, 3.4%, and 1.3% respectively. One SPPR M-11/59 was moderately susceptible. Out of 28 plants, 9 had disease symptoms with 2-5 infection type range and disease incidence of 21%. Maximum percent disease incidence of 97.1% was recorded in S-12. Seventy plants of this variety were observed and 68 were infected with a disease rating of 6E. The remaining materials were found to be immune (Table 8).

Discussion

CLCuV infection cause severe losses in cotton production. The best way to reduce CLCuV induced damage is by

breeding high yielding cotton resistant to the virus. Results of this study showed that four mutants exhibit a high level of resistance to CLCuV. Their resistance level compared with that of commercial cultivars (known to be resistant and susceptible against CLCuV) were evaluated through grafting and in natural conditions under high inoculum pressure. None of the materials tested through grafting were immune. These results are consistent with the observations of Akhtar et al. (2001). They observed for the first time that all the resistant materials present in the field were susceptible to CLCuV under graft inoculation. The susceptible control showed high intensity of disease through grafting and under natural field conditions and produced no yield at all. However, the grafted resistant entries and plants infected under natural conditions were capable of growing well to give the normal yield. Consequently, the plants were capable of resisting the adverse effects of the virus under extreme conditions.

The present study showed that all the tested materials are prone to CLCuV infection, but they differ in their responses and time of disease development. Plants of the new mutants M-111, M-358, M-218, and M-7662 exhibited the highest level of resistance to CLCuV through grafting under net house conditions. Compared with that of resistant and susceptible control, they produced milder infection and took more time (25-28 days) for the development of disease, as against FH-900, FH-901, CIM-473, CIM-482, CIM-443 and S-12, which developed the disease after 15-22 days. The same results were obtained in the case of most SPPRs of the new mutants when grafted under field conditions.

Results of the present study also showed that the growth of the inoculated plants of the SPPRs namely, M-358/2, M-358/12, M-358/26, M-11/2, M-11/CE, M-7662-1/8, M-7662-1/1 and M-218 was good even under high inoculum pressure, while poor and very poor growth were recorded in the case of the other SPPRs namely, M-11/59, M-7662-1/2, CIM-443 (resistant control) and S-12 susceptible control. It is also clear from the present study that materials showing highly resistant reaction under graft inoculation were immune under field conditions. Materials resistant under graft inoculation had varied responses under field conditions. Some entries like M-11/CE, M-7662-1/2, and CIM-443 showed 0.1%, 3.4%, and 1.3% disease incidence, respectively, under natural conditions and were all from the highly resistant group, while M-11/59 showed 21% disease incidence and become moderately resistant under natural conditions. On the basis of these observations, it is suggested that SPPRs M-11/59 and M-7662-1/2 should not be used in the future, while the others are recommended for further multiplication.

This study also showed that all cotton materials are susceptible to CLCuV infection. However, their level of susceptibility varies. Some genotypes are easily infected while others are harder to infect and has slow disease development, especially those materials developed through radiation. The problem should not be considered solved, because four variants of CLCuV have been shown to exist in the field (Zhou et al., 1998). Multiple infection of CLCuV and other whitefly-transmitted *Geminiviruses* (WTGs) in cotton and other cotton growing areas are prevalent, therefore, chances of recombination among them and other WTGs do exist, which may lead to the emergence of new, more virulent and resistant breaking variants (Shah et al., 1999). Results of this study suggest that preference should be given to those plants that exhibited highly resistant responses after artificial inoculation. Meanwhile, the search for immune materials (through artificial inoculation) should continue.

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