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Screening of Cotton Mutants for the Resistance Against Cotton Leaf Curl Virus (CLCuV)

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Abstract: Cotton germplasm representing twenty two mutants against one positive control S-12 was screened for resistance to CLCuV. BLOT inoculation was found useful in identifying CLCuV resistance. None of the mutant screened through grafting respond immune or highly resistant, while M-811, M-115, PIM-76-8, M-388 and M-564 behaved resistant. Moreover, PIM-76-1 and PIM-76-1a, were as highly susceptible as S-12. In contrast, screening under natural conditions was most useful for identifying insect resistant germplasm. Most of the mutants that were resistant or moderately resistant under BLGT inoculation were found immune or highly resistant under natural conditions. None of the test mutants was found susceptible or highly susceptible under field conditions.

Key words: *Gossypturn* sp., CLCuV, screening, grafting, natural, mutants, cotton

Introduction

Cotton (*Gossypiurn* species) is one of the most important cash crop of Pakistan and is grown over about 12 percent of the total cultivated area of Pakistan (Ahmed, 1999). Raw cotton, its products and by products collectively contribute for about 60 percent of the gross foreign exchange earnings of the country (Hashmi *et al*., 1995). It also provides fiber, food, feed, fodder and fuel for urban and rural population. Cotton seed is a main source of more than half the domestic production of edible oil (Mirza *et al*., 1994).

Cotton plant is naturally susceptible to large number of insect pests and diseases (Anonymous, 1983). Among the later, now cotton leaf curl is a serious viral disease (Muhammad *et al*., 1998). It can cause a reduction of 64.5 percent in yield per plant, 33.8 percent in boll weight and 73.5 percent in number of bolls per plant. CLCuV can also decrease GOT up to 3.93 percent, seed index 17.0 percent, fiber length 3.44 percent, micron are 4.3 percent, maturityratio 7.56 percent, fiber strength 10 percent and elongation percentage 10 percent (Ahmed, 1999).

The best easiest and the cheapest method of controlling plant disease is the development of resistant variety (Agrios, 1978). The main objective of this study was to screen the resistant/tolerant mutants (evolved through the use of radiations) of cotton against CLCuV through grafting and under natural conditions.

Materials and Methods

Cotton germplasm consisting of twenty two mutants developed by cotton group NIAB, Faisalabad through radiation mutation was screened against CLCuV. S-12 a highly susceptible cultivar was included as a positive control for all inoculations. Seeds were dilinted before sowing by using 1 kg of commercial H_2SO_4 for 10 kg of seed. The response of the CLCuV was recorded by using the modified scale (Anonymous, 1996) described in Table 1.

Maintenance of CLCuV culture: Fifteen infected ratoon plants of cotton cultivar S-12 were transplanted from field to earthen pots during 1998. Then these pots were transferred to net house and maintained according to their requirement to develop the CLCuV culture for further studies.

Efficacy of Bottle Leaf Grafting Technique (BLGT): To check the efficacy of bottle leaf grafting technique 15 pots were sown with dilinted seeds of cotton cultivar S-12 (Susceptible to CLCuV). A cut of about 0.5 cm long and 0.1 cm deep was made on the stem of six week healthy S-12 cotton plants. Then a leaf with high incidence of CLCuV was detached from ratoon plants. A similar cut was made on the petiole of that isolated leaf and the corresponding cuts were brought together and tied with para-film to avoid from drying and to stop the entry of air. While tying, care was taken to bring the corresponding cambium surfaces into contact. Here the leaf petiole was placed in a test tube with distilled water until a union is formed within five days. These grafted plants were placed under the insect free cages. Fifteen plants of 5-12 cultivar without grafting were also placed to serve as control. Distilled water were maintained in test tubes by adding for five days. The data on grafted plants were recorded after one week by using the modified scale (Table 1).

Screening of mutants through grafting: Five pots of each test entry were sown (4-5 delinted seeds per pot) during 1998. Then these pots were transferred under insect free cages under net house conditions. Thinning was done having one plant per pot two weeks after germination of seeds. These plants were inoculated at the age of 6-8 weeks following BLGT, using diseased leaf of 5-12 from maintained culture. Plants were maintained through out the experiment according to their requirements and clean tap water was used to irrigate the young seedling through out

Table 1: Modified disease scale for rating of CLCuV

Score	Symptoms	Disease incidence (%)	Disease reaction
0	Complete absence of symptoms	0	Immune
1	Thickening of few small scattered veins	1-5	Highly resistant
2	Thickening of small group of veins	6-10	Resistant
3	Thickening of large group of veins	11-20	Moderately resistant
4	Thickening of all veins	21-30	Moderately susceptible
5	Severe vein thickening, leaf curling developed	31-50	Susceptible
6	Severe curling and severe stunting of plants	51-100	Highly susceptible

Note: Foliar out growths (Enations) will be marked with "E" where they will be observed

the period of study. Data was maintained as success in grafting percentage, infection percentage, average time required for 1st disease symptom appearance after grafting and average disease intensity after 50 days of grafting.

Screening of mutants against CLCuV under natural conditions: A field experiment was laid down during May, 1998 at cotton experimental field NIAB, Faisalabad. Each test entry was planted in three row subplots in complete randomized design with row length of 10 ft, row spacing of 75 cm and plant to plant spacing of 30 cm under normal cultural practices of fertilizer and irrigation. There were three replications in each case, The germplasm was subjected to natural invasion and build up of whitefly (*Bemisia tabacci* Germ), the vector of cotton leaf curl virus and consequently to the infection by CLCuV. The level of resistance/susceptibility to the CLCuV disease was recorded during the first week of September. Data was maintained as percentage of infected plants.

Results and Discussion

The data presented in Table 2 shows that 93.3 percent plants of S-12 were successfully grafted and all successful grafted plants exhibited high intensity of disease within 20-25 days. It is also clear from the data that all successfully grafted plants became infected with CLCuV within 14-17 days after grafting, This data shows that this technique is 100 percent accurate for the transmission of CLCuv. All the S-12 plants placed as check were free from disease. These observation are similar to those of Muhammad *et al*. (1998). Who found best bottle leaf grafting and large cage grafting technique for successful transmission of CLCuV.

None of the mutants tested under BLGT was found to be immune or highly resistant against CLCuV. The mutant M-811, M-115. PIM-801-13, PIM-76-8 and M-388 were resistant while M-224, M-564-, M-588, M-117, PIM-98, PIM-75-4, PIM-78-5 and M-999 were moderately resistant against CLCuv (Table 4) only two mutant PIM-76-1, PIM-76-1a and a commercial cultivar 5-12 which was included as positive control were found highly susceptible while mutant M-884, M-812 and M-118 were susceptible (Table 3). All accessions exhibited that they have not any resistant gene against CLCuV but they showed different degree of reaction against the disease, while transmission/reproduction of virus was found 100 percent successful on all the test plants. Data presented in Table 3 also indicates that six mutant (M-811, M-115, PIM-80-13, PIM-76-5, M-388 and M-564) resists more against CLCuV as they scored average disease intensity 2 to 2+E even after 50 days of grafting and they took more time for first disease symptom

Table 2: Response of cotton cultivar S-12 against CLCuV by bottle leaf grafting technique

Plant No.	Success of grafting	Time taken for lst disease symptom	Disease intensity after (Days)	
	Yes/No	Appearance (Days)	20 d	25 d
1	Yes	15	6	6+E
2	Yes	14	5	6+E
3	Yes	14	5	6+E
4	Yes	16	6	6+E
5	Yes	15	6	6+E
6	Yes	15	6	6+E
7	Yes	17	5	6+E
8	Yes	16	6	6+E
9	Yes	16	6	6+E
10	Yes	15	5	6+E
11	Yes	15	5	6+E
12	Yes	16	6	6+E
13	No	-	-	-
14	Yes	16	6	6+E
15	Yes	17	6	6+E

Control (15 plants): All plants were free from CLCuV

Table 3: Response of cotton mutants against CLCuV

Mutant	Bottle Leaf Grafting Technique (BLGT)		Natural/Field conditions	
	Average time for disease appearance after grafting (Days)	Average disease intensity after 50 days of grafting (0-6+E)	Infection (%)	Decrease in disease incidence Over control (%)
M-118	18	5	8	91.01
M-999	17	3+E	5	94.38
M-224	22	3+E	0	100.00
M-884	19	5	18.28	79.46
M-588	25	3+E	0	100.00
M-388	27	2+E	0	100.00
M-811	27	2	0	100.00
M-812	24	5+E	19.5	78.09
M-112	18	4+E	2.3	97.42
M-825	23	4	5.7	93.60
M-564	28	2+E	0	100.00
M-117	25	3	2.9	91.74
M-115	28	2	0	100.00
PIM-76-1	14	6+E	-	-
PIM-76-1a	16	6+E	-	-
PIM-77-2	26	4	2.4	97.30
PIM-98	25	3	-	-
PIM-75-4	23	3	-	-
PIM-78-5	22	3+E	-	-
PIM-80-13	26	2	-	-
PIM-80-15	15	4+E	-	-
PIM-76-8	23	2	0	100.00
S-12	16	6+E	89	-

Success in grafting and disease infection was 100 percent in case of grafting (BLGT)

Table 4: Mutants exhibiting various levels of resistance/susceptibility based on average percent disease intensity (0-6+E Scale)

Immune	Highly Resistant	resistant	Moderately resistant	Moderately susceptible	susceptible	Moderately susceptible
-	-	M-811	M-588	M-112	M-884	PIM-76-1
-	-	M-115	M-117	M-825	M-812	PIM-76-1a
-	-	PIM-80-13	PIM-98	PIM-77-2	M-118	S-12
-	-	PIM-76-8	PIM-75-4	PIM-80-15	-	-
-	-	M-388	PIM-78-5	-	-	-
-	-	M-564	M-999	-	-	-
-	-	-	M-224	-	-	-

Table 5: Various levels of resistance/susceptibility to CLCuV based on percent disease incidence (percent plant infection)

Immune (0%)	Highly resistant (1-5%)	Resistant (6-10%)	Moderately resistant (11-20%)	Moderately susceptible (21-30%)	Susceptible (31-50%)	Highly susceptible (51-100%)
M-588	M-118	-	M-884	-	-	S-12
M-388	M-999	-	M-812	-	-	-
M-811	M-112	-	-	-	-	-
M-564	M-825	-	-	-	-	-
M-115	M-117	-	-	-	-	-
P1M-76-8	PIM-77-2	-	-	-	-	-
M-224	-	-	-	-	-	-

appearance as 23-28 days as compared with control which exhibited disease within 16 days of grafting (Table 3). Two mutant PIM-76-1 and PIM-76-1 a developed disease after 14-16 days of grafting and they become highly susceptible with 25 days of inoculation to show the high disease intensity as in case of 5-12 (Table 3 and 4).

Screening of mutants under field conditions showed that none of the test mutants were susceptible or highly susceptible against CLCuV (Table 3 and 5). Out of fourteen mutants screened six namely: M-224, M-588, M-388, M-811, M-564, M-1 15 and PIM-76-8 were immune as they show 100 percent decrease over control while M-118, M-999, M-112, M-117 and PIM-77-2 gave highly resistant reaction (Table 3). Two mutant M-884 and M-812 were moderately resistant as they show 79.46 and 78.09 percent decrease over control (Table 3). These results ate similar with results of Tahir *et al*. (1994), Khan and Rashid (1996) and Awan *et al*. (1998). They found that existing varieties/mutants of cotton vary in susceptibility to CLCuV. They also observed S-12 and NIAB-78 as most susceptible cultivars.

The evaluation of cotton mutants under field conditions indicated that sources of resistance are available against whitefly and their resistance could be incorporated into other commercial cultivars against CLCuV. BLGT inoculation studies also showed that accessions receiving low disease severity scores were found immune, highly resistant and resistant under field conditions. It means both techniques are equally important for the evaluation of the resistant strains.

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