Pak. J. Bot., 42(5): 3327-3342, 2010.

SCREENING OF COTTON GERMPLASM AGAINST COTTON LEAF CURL VIRUS

SAGHIR AHMAD[*], NOOR-UL-ISLAM[1], ABID MAHMOOD[1], FARZANA ASHRAF, KHEZIR HAYAT AND MAMOONA HANIF

Cotton Research Station, Multan, [1]Cotton Research Institute, Faisalabad
**Corresponding author's E-mail: saghirahmad_1@yahoo.com*

Abstract

Cotton leaf curl virus poses a major threat to cotton productivity in Pakistan. Screening of germplasm to explore resistant source is a basic step towards the solution of this hazardous problem. With this objective genetic material comprising 11060 genotypes of cotton of different research centers was tested at Cotton Research Station, Vehari during 2002 to 2007. During 2002-03, 3694 genotypes were tested on the basis of cotton leaf curl virus disease incidence and 30 (Code No./varieties 124, 141, 170, 185, 218, 244, 252, 338, FVH-153, VH-176, 609, 721, 723, 724, 752, 834, 891, 918, 1018, 106, 111, 114, 115, 141, 156, 160, 175, 178, 183, 253) lines showed no symptoms of this disease. Out of 2792 genotypes screened during 2003-04, 7 (Code No./varieties 124, 170, 244, 252, VH-176, VH-209, 609) were found virus free. During next three years i.e., 2004 to 2006, all the genotypes (4364) showed susceptibility to cotton leaf curl virus disease. Two genotypes (China-1 and China-2) out of 210 tested during 2007 exhibited resistance to cotton leaf curl virus (CLCuV). 119 genotypes were screened on the basis of cotton leaf curl virus intensity during 2002-07, out of which 37 (Code No./varieties 654, 1050, 1411, 1448, 124, 141, 170, 185, 218, 244, 252, 338; 609, 721, 723, 724, 752, 834, 891, 918, 1018; NIBGE-106, NIBGE 111, NIBGE 114, NIBGE- 115, NIBGE 141, NIBGE156, NIBGE160, NIBGE 175, NIBGE 178, NIBGE 183, NIBGE 253, FVH-153, VH-176, VH-209, China-1, China-2) lines were found virus free. Twenty four lines were tested against Cotton Leaf Curl Virus on the basis of sick plot technique during 2002-07. Among these lines 609 from Cotton Research Station Multan, 3232 from Central Cotton Research Institute Multan, VH-156 from Cotton Research Station Vehari, China-14 from Nanjing Agri.University China showed minimum disease incidence 0.8%, 7.6%, 14.75% and 23.5%, respectively. The results of recent research demonstrated that it is possible to explore resistant material from germplasm through screening on the basis of incidence and intensity. The same can be utilized in the breeding programme for evolving CLCuV tolerant/resistance verities of cotton.

Introduction

Cotton (*Gossypium hirsutum* L.) is the world's leading food and fiber crop. Cotton recognized as white gold of Indo-Pak plays a pivotal role in the economy of Pakistan contributing a lion's share of 57% in the foreign exchange earning. Since 1947 Pakistan has made a significant increase of 11.2 and 4.4 times in lint production and seed cotton yield per acre, respectively. But still there is a wide gap between yield of our cottons and that of the advanced cotton growing countries of the world.

One of the major reasons constituting a primary limit is Cotton Leaf Curl Virus (Watkins, 1981; Briddon *et al*., 2000). This disease was first reported in 1967 on upland

cotton (*Gossypium hirsutum* L.) at Khokhran near Multan on few individual plants (Hussain & Ali, 1975). It became a serious problem after 1991-92 and continued till the development of Cotton Leaf Curl resistant variety CIM-1100 in 1996. But this problem arose again during 2001 in Burewala breaking the resistance of all the available cotton cultivars/lines (Mansoor *et al*., 2003). Now cotton leaf curl virus is one of the most destructive disease of cotton in the Punjab area of Pakistan. This virus is 8% different from the previous virus on molecular basis (Mansoor *et al*., 2003), but symptoms are almost the same to the previous CLCuV.

This disease is characterized by upward curling of leaves, the veins of the affected leaves become thickened which are most pronounced on the underside. Two types of veins thickenings are seen, small vein thickening and main vein thickening. Initial symptoms consisted for transient vein clearing on young leaves (Mansoor *et al*., 1993; Nateshan *et al.*, 1996). Infected plants develop both upward and downward curling of leaves accompanied by thickening and sometime development of leaf enations (oval or cuplike foliar worth) on the underside of leaf. In severe conditions, plants became stunted and give fewer yields. Highest reduction in seed cotton yield due to cotton leaf curl virus disease has been reported by many workers (Harrison *et al*., 1997; Brown, 2001; Ahmad *et al*., 2002; Idris 1990). Mahmood *et al*., (1996) reported that in cotton cultivars the average reduction in plant height 40.6%, boll weight 33.8%, number of bolls per plant 72.5%, ginning outturn 3.9%, fibre length 3.4% and fibre strength 0.7% due to cotton leaf curl virus disease. Russel (1982) found that boll weight was negatively affected by CLCuV.

The long-term approach to cope with this problem and to save this crop from the ravages of CLCuV is the development of Cotton Leaf Curl resistant varieties (Akhtar *et al*., 2002), as previously practiced in Sudan and Egypt (Kirkpatrick, 1931; Khan *et al*., 2001). The first step is to explore resistant genetic sources through massive screening in the environment highly favorable for the incidence/intensity of Cotton Leaf Curl Virus. Cotton Research Station, Vehari being located in the epidemiological zone of new Cotton leaf Curl Virus (Burewala strain) was selected by the provincial as well as federal governments for the screening of germplasm of all the cotton centers of Pakistan. The main objective of the present study was to find out resistant material/genetic source, which if possesses desirable characteristics, can directly be used for commercial cultivation, or it can be used in hybridization programme for the development new resistant varieties.

Material and Methods

Experiment 1

Screening on the basis of cotton leaf curl virus incidence: The genetic material/ germplasm comprising 11060 genotypes (exotic and local) of different Cotton Research Centers of Pakistan were pooled at Cotton Research Station, Vehari during 2002-03 to 2007-08 in normal growing season for screening against cotton leaf curl virus (Table 2). Each genotype was planted with plant to plant and row to row distance of 30 cm and 75 cm, respectively. Sowing was done from May to June in simple lay out (non-replicated). Recommended agronomic practices were carried out from sowing to harvesting. All the plants of a genotype were thoroughly observed for incidence/appearance of cotton leaf curl virus symptoms such as vein reticulation, vein thickening, leaf curling along and stunting of plant etc. Any plant showing these symptoms was considered as "diseased/susceptible". Data were recorded on fortnightly basis. Only cotton leaf curl virus free (no symptoms of CLCuV) lines were accounted for results.

Table 1. Cotton leaf curl virus intensity and grading of cotton genotypes according to modified 0–6 disease scale.

	Symptoms	Rating/Disease severity	Disease index (%)	Disease reaction
1.	Complete absence of symptoms	0	0	Immune
2.	Thickening of few small-scattered veins or only presence of leaf enations on ten or less than ten leaves of a plant found after careful observations.	1	0.1-1	Highly tolerant
3.	Thickening of small group of veins.	2	1.1-5	Tolerant
	Thickening of all veins but no curling of leaves.	3	5.1-10	Moderately tolerant
4.	Severe vein thickening and leaf curling developed at the top of the plant (on one third of the plant).	4	10.1-15	Moderately susceptible
5.	Severe vein thickening and leaf curling developed on half of the plant.	5	15.1-20	Susceptible
6.	Severe vein thickening, leaf curling and stunting of the plant with no or les fruit bearing.	6	>20	Highly susceptible

Table 2. Germplasm of different research centers of Pakistan tested for cotton leaf curl virus incidence at Cotton Research Station, Vehari during 2002-03-2006-07.

Research centers	Years/genotypes						
	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	Total
Cotton Research Institute, Faisalabad	412	800	175	174	287	52	1900
Cotton Research Station, Vehari	85	335	515	45	54	60	1094
Cotton Research Station, Multan	800	570	12	10	-	-	1392
Cotton Research Station, Bahawalpur	-	142	13	3	-	-	158
Cotton Research Station, Sahiwal	110	7	5	-	-	-	122
Cotton Research Station, Rahim Yar Khan	118	6	-	-	-	-	124
Central Cotton Research institute, Multan	1464	206	87	53	-	-	1810
Cotton Research Station, Tandojam	25	540	78	110	19	19	791
NIBGE, Faisalabad	262	170	1300	12	1100	-	2844
NIAB, Faisalabad	-	-	11	35	14		60
NIA, Tandojam	-	-	10	19	-	-	29
Cotton Research Institute, Sakrand	375	-	115	4	46	34	574
Plant Virology, Ayub Agri. Institute, Faisalabad	17	-	-	-	-	25	42
National Coordinated Varietal Trial Res. PCCC, Karachi	26	16	18	20	20	20	120
Total	**3694**	**2792**	**2339**	**485**	**1540**	**210**	**11060**

Table 3. List of promising genotypes of different research centers of Pakistan tested for cotton leaf curl virus intensity at Cotton Research Station, Vehari during 2002-03-2006-07.

Research centers	Total entries	Promising genotypes
Cotton Research Institute, Faisalabad	16	Code No. 124, 141, 170, 185, 218, 244, 252, 338, 768, FH-133, FH-115, FH-127, FH-925, FH-945, FH-1000, FH-207
Cotton Research Station, Vehari	12	FVH-153, VH-142, VH-144, VH-148, VH-156, VH-176, VH-209, VH-231., VH-255, VH-259, VH-280, VH-289
Cotton Research Station, Multan	18	Code No. 609, 721, 723, 724, 752, 834, 891, 918, 1018, MNH-635, MNH-700, MNH-786, MNH-789, CRSM-38, CRSM-70, MNH-6070, S-12, S-14
Cotton Research Station, Bahawalpur	4	BH-147, BH-160, BH-162, BH-167
Cotton Research Station, Sahiwal	4	SLH-224, SLH-227, SLH-279
Cotton Research Station, Rahim Yar Khan	3	RH-112, RH-510, RH-610.
Central Cotton Research Institute, Multan	21	Code No. 654, 1050, 1411, 1448, 3232, CIM-70, CIM-240, CIM-443, CIM-473, CIM-506, CIM-511, CIM-707, CIM-496, CIM-497, CIM-499, CIM-538, CIM-541, CIM-554, CIM-557, CP-15/2, LRA-5166
Cotton Research Station, Tandojam	2	TH-35-99, TH-41-83
NIBGE, Faisalabad	13	NIBGE-2, NIBGE-4, NIBGE-106, NIBGE-111, NIBGE-114, NIBGE-115, NIBGE-141, NIBGE-156, NIBGE-160, NIBGE-175, NIBGE-178, NIBGE-183, NIBGE-253
NIAB, Faisalabad.	7	NIAB-111/S, NIAB-98, NIAB-777, NIAB-824, NIAB-846, NIAB-884, NIAB-999
Cotton Research Station, Ghotki	2	GH-99, GH-102
Cotton Research Institute, Sakrand	8	CRIS-129, CRIS-342, CRIS-460, CRIS-461, CRIS-168, CRIS-466, CRIS-467, CRIS-468
Private Sector	6	Alseemi-151, ASR-1, MJ-6, MJ-7, NEELUM-111, GS-1
Agricultural University, Faisalabad	1	PB-899
China	2	China-1, China-2
Total	**119**	

Table 4. List of promising genotypes of different research centers of Pakistan tested for cotton leaf curl virus on the basis of sick plot technique at Cotton Research Station, Vehari during 2002-03-2006-07.

Research centers	Total entries	Promising genotypes
Cotton Research Institute, Faisalabad	4	Code No. 252, 768, FH-207, FH-113.
Cotton Research Station, Vehari	6	VH-156, VH-231, VH-209, VH-255, VH-259, VH-257.
Cotton Research Station, Multan	6	Code No. 609, 723, 724, MNH-786, MNH-789, S-12.
Central Cotton Research Institute, Multan	5	Code No. 654, 3232, CIM-473, CIM-496, CIM-557,
NIBGE, Faisalabad	3	NIBGE-114,NIBGE-115,NIBGE-253,
Total	**24**	

Experiment 2

Screening on the basis of cotton leaf curl virus intensity: The experimental material consisted of 119 promising strains of different research centers of Pakistan along with 4 highly susceptible lines (Table 3). Depending upon the response of different genotypes to cotton leaf curl virus intensity, number of entries varied in each year. Genetic material was sown during May in field in non-replicated fashion with plot size of 10m × 3m. Recommended agronomic practices were carried out through out the crop season. Data for cotton leaf curl virus intensity were recorded on fortnightly basis. At the time of maturity seed cotton yield was calculated and the genotypes were classified on the basis of disease intensity (Akhtar *et al*., 2002) and seed cotton yield (kg/ha).

Experiment 3

Screening on the basis of sick plot technique: In this technique two rows of resistant/tolerant genotype along with one row of a highly susceptible cultivar were planted in field. Sowing was done in May with row length of 20 m, plant-to-plant and row-to-row distance was 30 and 75 cm, respectively. The objective was to verify resistance/tolerance of a genotype by providing full inoculum of cotton leaf curl virus. Employing this technique a total of twenty-four highly promising genotypes (Table 4) were tested in the field during the year 2003 to 2007. Inoculum of whitefly (carrier of virus) was also created for spread of cotton leaf curl virus disease. All recommended agronomic practices were kept same for all genotypes. Fortnightly data were recorded for incidence of CLCuV. At the time of crop maturity, data for plant height (cm) and seed cotton yield per plant (g) was recorded.

Results and Discussion

a. Screening of cotton germplasm on the basis of cotton leaf curl virus incidence: A total of 11060 genotypes both local as well as exotic (Table 2) of cotton were screened for incidence of Cotton Leaf Curl Virus disease at Cotton Research Station, Vehari, Pakistan during 2002-2007 under natural field conditions, where virus source (diseased plants) and vector (whitefly) were abundantly present. The results showed that during 2002, only 30 genotypes out of 3694 were found free from CLCuV symptoms (Table 5), 8 entries (124, 141, 170, 185, 218, 244, 252, 338) from Cotton Research Institute (CRI), Faisalabad, 2 strains (FVH-153, VH-176) from Cotton Research Station (CRS), Vehari, 9 lines (609, 721, 723, 724, 752, 834, 891, 918, 1018) belonged to Cotton Research Station (CRS), Multan and 11 entries (106, 111, 114, 115, 141, 156, 160, 175, 178, 183, 253) from Nuclear Institute for Biology and Genetic Engineering (NIBGE), Faisalabad. During 2003 only 7 lines (124, 170, 244 and 252 of CRI, Faisalabad, VH-176 and VH-209 from CRS, Vehari and 609 from CRS, Multan, did not show symptoms of this disease (Table 5). Later on from 2004 - 2007 we could find only two CLCuV free entries (China source). Cataloging of cotton Germplasm against CLCuV has been reported by several research workers (Alim, 1997; Muhammad *et al*., 1998).

b. Screening of cotton germplasm on the basis of cotton leaf curl virus intensity: It has been observed that the cotton germplasm varied greatly in its reaction/intensity to cotton leaf curl virus, which was based on symptom expression. Genotypes showing

100% incidence of cotton leaf curl virus differed in seed cotton yield. For assessing the yield potential and tolerance of genotype to cotton leaf curl virus, it is advisable to screen the genetic material on the basis of cotton leaf curl virus intensity rather incidence. A total of 119 genotypes (Table 6) of cotton were screened during 2002- 2007 in the field for determining their response to cotton leaf curl virus disease. Field observation indicated that most of the lines were susceptible to CLCuV infestation, but there were different grade of tolerance in different varieties (Muhammad *et al*., 1998) During 2000-2003 crop season, out of 67 entries 34 entries (Code No. 654, 1050, 1411, 1448, 124, 141, 170, 185, 218, 244, 252, 338; 609**, 721, 723, 724, 752, 834, 891, 918, 1018; NIBGE-106, NIBGE 111, NIBGE 114, NIBGE- 115*, NIBGE 141, NIBGE156, NIBGE160, NIBGE 175, NIBGE 178, NIBGE 183, NIBGE 253, FVH-253 & VH-176) were found virus free (having no symptoms of CLCV), with yield range of 3035-3455 kg/ha. Five entries i.e. (BH-147, NIAB-111/S, SLH-224**, SLH-227 & VH-142) showed high tolerance while the yield range was 2500-2656 kg/ha, 15 entries (CIM-496, CIM-497, CIM-506**, CIM-511, CIM-707, CRIS-168, FH-925, FH-945, FH-1000*, MNH-635, NIBGE 1, NIAB-98, RH-112, CP-15/2, LRA-5166) and 5 (BH-160*, CIM-499, CRIS-467, FH-925**, MNH-636) exhibited tolerance and moderately tolerance having yield range of 2005-2259 and 1518-1913 kg/ha respectively, 3 entries (CRIS-468**, NIAB-999, CIM-473) were moderately susceptible exhibiting yield range 1278-1474 kg/ha and 5 were highly susceptible (CIM-70, CIM-240**, CIM-443, S-12, S-14*) showing yield range of 211-471 kg/ha (Table 6).

During 2003-2004, out of 41 entries, consisting of virus free lines of previous year and some promising lines of different research centers, 8 entries (Code No. 609, 124*, 170, 244, NIBGE-114, NIBGE-115, VH-176, VH-209**.) found CLCuV free and yield remain in the range of 3311-3745 kg/ha, 3 (Code No. 654*, 723, 252**) having high tolerance were exhibiting yield range of 2611-2678 kg/ha, ten genotypes i.e. CP-15/2, CIM-506*, Code NO. 218, 338, 752, 1018, FH-768, FVH-153, LRA-5166, VH- 176** found moderately tolerant with yield range of 1602-1790 kg/ha, three entries (CRIS-168*, CRIS-468**, RH-510) moderately susceptible having yield range of 1225-1434 kg/ha, thirteen genotypes i.e. CP-15/2, CIM-473*, CIM-496, CIM-497, FH-925**, NIAB-111/S, NIBGE-2, SLH-279, VH-144, TH-41-83, MNH-700, BH-160, LRA-5166 were susceptible possessing yield range of 549-914 kg/ha and four (CIM-70, CIM-473, NIAB-999**, S-12*) were observed highly susceptible with yield range of 250-406 kg/ha.

During 2004-05 twenty strains were studied for CLCuV tolerance and among these there was no CLCuV free varieties but fifteen highly tolerant genotypes were found (Code No. 609, Alseemi-151, BH-162*, CIM-534, CRIS-460, CRIS-468, FH-115, NIAB-98, NIAB-884, PB-899, NEELUM-111, NIBGE-2, MNH-700, SLH-279**, VH-209) having yield range of 3026-3900 kg/ha, two lines (CIM-496**, MJ-7*) were tolerant with yield range of 2355-2421 kg/ha, one entry (CIM-499) moderately tolerant with yield figure of 1890 kg/ha and two entries were (S-12*, CIM-70**) moderately susceptible possessing yield range of 1222-1485 kg/ha.

In 2005-06 total no. of strains screened for CLCuV tolerance were 28, among these 23 strains showed high tolerance to CLCuV which included Alseemi-151, BH-162, CIM-499, CIM-534, CIM-538, CRIS-461, CRIS-466, FH-113**, FH-115, MJ-7, FH-207, GH-99, NIAB-824, MNH-786, MNH-789, MNH-6070*, NIAB-884, NIBGE-4, PB-899, VH-231, VH-148, TH-35/99, TH-84/99 with yield range of 2555-3698 kg/ha, one entry (VH-156) moderately tolerant was exhibiting yield range of 2100-2500 kg/ha, three entries (CIM-70**, S-12, S-14*) were susceptible having yield range of 600-1000 kg/ha.

Table 5. Screening of germplasm of cotton (11060 genotypes) based on leaf curl virus incidence during 2002-2007.

Research centre	2002-2003		2003-2004		2004-2005		2005-2006		2006-2007		2007-2008	
	T.L.	CLCV.F.L.	T.L.	CLCV.F.L.	T.L.	CLCV F.L.	T.L.	CLCV F.L.	T.L.	CLCV F.L.	T.L.	CLCV F.L.
Cotton Research Institute, Faisalabad	412	8 (124, 141, 170, 185, 218, 244, 252, 338)	800	4 (124, 170, 244, 252)	175	-	174	-	287	-	52	-
Cotton Research Station, Vehari	85	2 (FVH-153, VH-176)	335	2 (VH-176, VH-209)	515	-	45	-	54	-	60	2
Cotton Research Station, Multan	800	9 (609, 721, 723, 724, 752, 834, 891, 918, 1018)	570	1 (609)	12	-	10	-	-	-	-	-
Cotton Research Station, Bahawalpur	-	-	142	-	13	-	3	-	-	-	-	-
Cotton Research Station, Sahiwal	110	-	7	-	5	-	-	-	-	-	-	-
Cotton Research Station, Rahim Yar Khan	118	-	6	-	-	-	-	-	-	-	-	-
Central Cotton Research Institute,Multan	1464	-	206	-	87	-	53	-	-	-	-	-
Cotton Research Station, Tandojam	25	-	540	-	78	-	110	-	19	-	19	-
NIBGE, Faisalabad	262	11 (106, 111, 114, 115, 141, 156, 160, 175, 178, 183, 253)	170	-	1300	-	12	-	1100	-	-	-
NIAB, Faisalabad	-	-	-	-	11	-	35	-	14	-		-
NIA, Tandojam	-	-	-	-	10	-	19	-	-	-	-	-
Cotton Research Institute, Sakrand	375	-	-	-	115	-	4	-	46	-	34	-
Plant Virology, Ayub Agri. Institute, F. bad	17	-	-	-	-	-	-	-	-	-	25	-
National Coordinated Varietal Trial Res. PCCC, Karachi	26	-	16	-	18	-	20	-	20	-	20	-
Total	**3694**	**34**	**2792**	**9**	**2339**	**-**	**485**	**-**	**1540**	**-**	**210**	**2**

Summary

Total lines	CLCV Free entries	Percentage of CLCV entries
11060	2	0.02

T = Total, CLCuV = Cotton leaf curl virus, F = Free, L = Line - = No entry/line

Table 6. Screening of promising genotypes (119) of different research centers of Pakistan based on cotton leaf curl virus intensity during 2002-2007.

Year	Infection capacity	No. of genotypes	Disease severity	Disease index (%)	Seed cotton yield (kg/ha) and range	Promising genotypes
2002-03	Virus free	34	0	0	>3000 (3035*-3455**)	Code No. 654, 1050, 1411, 1448; 124, 141, 170, 185, 218, 244, 252, 338; 609**, 721, 723, 724, 752, 834, 891, 918, 1018; NIBGE 106, NIBGE 111, NIBGE 114, NIBGE 115*, NIBGE 141, NIBGE 156, NIBGE 160, NIBGE 175, NIBGE 178, NIBGE 183, NIBGE 253 and FVH-53, VH-176
	Highly tolerant	5	1	0.1-1	2600- ≥3000 (2500*-2656**)	BH-147, NIAB-111/S, SLH-224**, SLH-227, VH-142*
	Tolerant	15	2	1.1 to 5.0	2100-2500 (2005*-2259**)	CIM-496, CIM-497, CIM-506**, CIM-511, CIM-707, CRIS-168, FH-925, FH-945, FH-1000*, MNH-635, NIBGE-1, NIAB-98, RH-112, CP-15/2, LRA-5166
	Moderately tolerant	5	3	5.1 to 10	1600-2000 (1518*-1913**)	BH-160*, CIM-499, CRIS-467, FH-925**, MNH-636
	Moderately susceptible	3	4	10.1 to 15	1100-1500 (1278*-1474**)	CRIS-468**, NIAB-999*, CIM-473.
	Susceptible	-	5	15.1 to 20	600-1000	-
	Highly susceptible	5	6	>20	≤ 500 (211*-471**)	CIM-70, CIM-240**, CIM-443, S-12, S-14*
	Total	67				
2003-04	Virus free	8	0	0	>3000 (3311*-3745**)	Code No. 609, 124*, 170, 244; NIBGE-114, NIBGE-115, VH-176, VH-209**
	Highly tolerant	3	1	0.1-1	2600-≥3000 (2611*- 2678**)	Code No. 654*, 723, 252**
	Tolerant	-	2	1.1 to 5.0	2100-2500	-
	Moderately tolerant	10	3	5.1 to 10	1600-2000 (1602*-1790**)	CP-15/2, CIM-506*, Code No. 218, 338, 752, 1018, FH-768, FVH-153, LRA-5166, VH-176**
	Moderately susceptible	3	4	10.1 to 15	1100-1500 (1225*- 1434**)	CRIS-168*, CRIS-468**, RH-510
	Susceptible	13	5	15.1 to 20	600-1000 (549*914**)	CP-15/2, CIM-473*, CIM-496, CIM-497, FH-925**, NIAB-111/S, NIBGE-2, SLH-279, VH-144, TH-41-83, MNH-700, BH-160, LRA-5166
	Highly susceptible	4	6	720	≤ 500 (250*-406**)	CIM-70, CIM-473, NIAB-999**, S-12*
	Total	**41**				

Table 6. (Cont'd.).

Year	Infection capacity	No. of genotypes	Disease severity	Disease index (%)	Seed cotton yield (kg/ha) and range	Promising genotypes
2004-05	Virus free	0	0	0	>3000	-
	Highly tolerant	15	1	0.1 to 1	2600- ≥ 3000) (3026*-3900**)	Code No. 609, Alseemi-151, BH-162*, CIM-534, CRIS-460, CRIS-468, FH-115, NIAB-98, NIAB-884, PB-899, NEELUM-111, NIBGE-2, MNH-700, SLH-279**, VH-209
	Tolerant	2	2	1.1 to 5.0	2100-2500 (2355*-2421**)	CIM-496**, MJ-7*
	Moderately tolerant	1	3	5.1 to 10	1600-2000 (1890)	CIM-499
	Moderately susceptible	2	4	10.1 to 15	1100-1500 (1222*-1485**)	S-12*, CIM-70**
	Susceptible	0	5	15.1 to 20	600-1000	-
	Highly susceptible	0	6	>20	≤ 500	-
	Total	20				
2005-	Virus free	0	0	0	>3000	-
	Highly tolerant	24	1	0.1 to 1	2600-≥3000 (2555*-3698**)	Alseemi-151, BH-162**, CIM-499, CIM-534, CIM-538, CRIS-461, CRIS-466, FH-113**, FH-115, MJ-7, FH-207, GH-99 NIAB-824, MNH-786, MNH-789, MNH-6070*, NIAB-884, NIBGE-4, PB-899, VH-231, VH-148. TH-35/99, TH-84/99
	Tolerant	1	2	1.1 to 5.0	2100-2500	VH-156
	Moderately tolerant	0	3	5.1 to 10	1600-2000	-
	Moderately susceptible	0	-	10.1 to 15	1100-1500	-
	Susceptible	3	4	15.1 to 20	600-1000	CIM-70**, S-12, S-14*
	Highly susceptible	0	5	>20	≤ 500	-
	Total	**28**	**6**			-

Table 6. (Cont'd.).

Year	Infection capacity	No. of genotypes	Disease severity	Disease index (%)	Seed cotton yield (kg/ha) and range	Promising genotypes
2006-07	Virus free	0	0	0	>3000	-
	Highly tolerant	1	1	0.1 to 1	2600 - ≥3000 (2611)	Code No. 609
	Tolerant	4	2	1.1 to 5.0	2100-2500 (2150*-2193**)	FH-113*, Code No. 3232, MNH-786**, VH209
	Moderately tolerant	4	3	5.1 to 10	1600-2000 (1547*-1849**)	CRIS-342**, CRIS-466*, NIAB-846, NIBGE-115
	Moderately susceptible	6	4	10.1 to 15	1100-1500 (1190*-1359**)	CIM-538, NIBGE-4*, MNH-789, NIAB-824, SLH-284**, VH-148
	Susceptible	8	5	15.1 to 20	600-1000 (639*-988**)	Alseemi-151, ASR-1**, BH-167*, CIM-541, CIM-496, FH-127, GH-99*, MJ-6
	Highly susceptible	3	6	>20	≤ 500 (150*-235**)	TH-84/99**, S-12*, CIM-70
	Total	26				
2007-08	Virus free	2	0	0	>3000	China-1, China-2. (These sources are being utilized in hybridization programme)
	Highly tolerant	2	1	0.1 to 1	2600-≥3000 (3550*-5544**)	280**, VH-289*
	Tolerant	1	2	1.1 to 5.0	2100-2500 (2100)	VH-255*
	Moderately tolerant	1	3	5.1 to 10	1600-2000 (1508)	CRIS-342
	Moderately susceptible	11	4	10.1 to 15	1100-1500 (1033*-1440**)	CIM-554, CRIS-129, CRSM-38, CRSM-70, FH-113, GH-102, NIAB-777, NIAB-846**, NIBGE-115, RH-610*, SLH-284
	Susceptible	3	5	15.1 to 20	600-1000 (771*-973**)	GS-1, TH-86/02*, TH-198/94**
	Highly susceptible	7	6	>20	≤ 500 (360*-461**)	ASR-1*, CIM-496, BH-167**, CIM-541, S-12, CIM-70, NIBGE-115
	Total	**27**				

* = Lowest yield kg/ha, ** = Highest yield kg/ha

During 2006-07, 26 entries were observed for CLCuV comparison and out of these only one strain showed highest tolerance to CLCuV i.e. Code No. 609 with yield value of 2611 kg/ha, four strains (FH-113, Code No. 3232, MNH-786**, VH-209) showing tolerance were giving yield range of 2150-2193 kg/ha, four genotypes (CRIS-342**, CRIS-466*, NIAB-846, NIBGE-115) having moderate tolerance were exhibiting yield range of 1547-1849 kg/ha, six strains (CIM-538, NIBGE-4*, MNH-789, NIAB-824, SLH-284**, VH-148) were found moderately susceptible possessing yield range of 1190-1359 kg/ha, eight were susceptible (Alseemi-151, ASR-1**, BH-167, CIM-541, CIM-496, FH-127, GH-99*, MJ-6) with yield range of 639-988 kg/ha and three strains (TH-84/99**, S-12*, CIM-70) were highly susceptible to CLCuV having yield range of 150-235 kg/ha (Table 6).

For CLCuV screening in 2007-08, 27 genotypes were studied among them 2 lines (China-1, China-2) were CLCuV free, two lines (VH-280, VH-289) having high tolerance were showing the yield range of 3550-5544 kg/ha , one (VH-255*) showing tolerance exhibit yield figure of 2100 kg/ha, one (CRIS-342) showing moderate tolerance gave the yield value of 1508 kg/ha, , eleven lines, i.e. CIM-554, CRIS-129, CRSM-38, CRSM-70, FH-113, GH-102, NIAB-777, NIAB-846**, NIBGE-115, RH-610*, SLH-284 were moderately susceptible with yield range of 1033-1440 kg/ha, three strains (GS-1, TH-86/02*, TH-198/94**) were susceptible possessing yield range of 771-973 kg/ha and seven entries (ASR-1*, CIM-496, BH-167**, CIM-541, S-12, CIM-70, NIBGE-115) were highly susceptible having yield range of 360-461 kg/ha (Table 6). These may or may not produce infection started appearing 20 days after sowing and continued up to maturity of the crop is in close conformity to previous work reported by Shah and Khalid (1998) who found an incubation period of 2-3 weeks. For the first time such a large number of genotypes were put together in field for testing against cotton leaf curl virus. Normally a set of 10-20 varieties had been evaluated for assessing disease resistance (Hameed *et al.*, 1994; Mirza *et al.*, 1994; Tanveer *et al.*, 1995; Ali, 1997; Shah and Khalid, 1997).

The data are consistent with hypothesis that the resistance against Gemini virus could be governed by many factors (Thomas *et al*; 1988). As stated by Mansoor *et al*; 2003) Gemni virus diseases are rapidly spreading in terms of their geographical distributions host range. Threatening nature of their virus and their world wide spread is attributed to the new biotype *Bemisia tabaci*, now regarded as new species *Bemisia argentifolii* which is reported to have much wider host range preference for feeding (Polston *et al.*, 2002). It will be desirable to monitor the population of new biotype of white fly and establish it as a potential vector of CLCuV. From breeding point of view, it should be determined whether dominating or recessive genes for which all types of genotypes on available govern resistance in cotton against CLCuV.

c. Screening of cotton germplasm on the basis of sick plot technique: Increase in seed cotton yield is one of the major objectives in cotton breeding programmes. Genetic variability in cotton genotype with respect to seed cotton yield has been reported in various studies (Murtaza *et al.*, 1992a; Azhar and Rana, 1993; Sayal *et al.*, 1996; Ahmad and Azhar, 1999).For assessing the yield potential and tolerance of genotypes against CLCuV, it was necessary to screen the genetic material in sick plot technique. Twenty four varieties/strains obtained from different cotton research centers of Pakistan were screened against CLCuV using sick plot technique at Cotton Research Station Vehari during 2003-2007.

Table 7. Cotton leaf curl incidence (%) and seed cotton yield of some promising genotypes (24) screened against Cotton Leaf Curl Virus in Sick plot technique disease during 2003-2006.

Year	Lines	Research center	Incidence (%)	Plant height (cm)	Av. yield/ plant (g)
2003	654	Central Cotton Research Institute (CCRI), Multan	12.5	115	100
	252	Cotton Research Institute, Faisalabad	10.0	110	48
	768	Cotton Research Institute, Faisalabad	8.0	130	105
	609	Cotton Research Station, Multan	0.8	118	125
	723	Cotton Research Station, Multan	3.5	140	85
	724	Cotton Research Station, Multan	5.7	105	65
	NIBGE-114	Nuclear Institute for Biology and Genetic Engineering (NIBGE), Faisalabad	12.5	90	71
	NIBGE-115	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	11.4	100	103
	NIBGE-253	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	10.5	108	82
	CIM-473 (Standard)	Central Cotton Research Institute, Multan	21.3	120	55
	S-12 (Spreader)	Cotton Research Station, Multan	100	45	7
2004	654	Central Cotton Research Institute, Multan	100	70	46
	3232	Central Cotton Research Institute, Multan	7.6	130	140
	252	Cotton Research Institute, Faisalabad	13.5	140	110
	768	Cotton Research Institute, Faisalabad	40	125	65
	609	Cotton Research Station, Multan	8	145	90
	723	Cotton Research Station, Multan	35	110	45
	724	Cotton Research Station, Multan	60	85	30
	NIBGE-114	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	35	135	100
	NIBGE-115	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	25	140	115
	NIBGE-253	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	55	90	65
	VH-156	Cotton Research Station, Vehari	11.5	125	120
	VH-231	Cotton Research Station, Vehari	15.4	120	150
	CIM-496 (Standard)	Cotton Research Station, Multan	27.5	95	60
	S-12 (Spreader)	Cotton Research Station, Multan	60	60	25

Table 7. (Cont'd.).

Year	Lines	Research center	Incidence (%)	Plant height (cm)	Av. yield/ plant (g)
2005	654	Central Cotton Research Institute, Multan	100	50	30
	3232	Central Cotton Research Institute, Multan	100	60	38
	252	Cotton Research Institute, Faisalabad	60	110	70
	768	Cotton Research Institute, Faisalabad	70	100	55
	609	Cotton Research Station, Multan	25	100	90
	723	Cotton Research Station, Multan	90	60	35
	724	Cotton Research Station, Multan	100	50	25
	NIBGE-114	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	55	110	60
	NIBGE-115	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	35	130	90
	NIBGE-253	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	90	60	40
	VH-156	Cotton Research Station, Vehari	11.5	125	120
	VH-231	Cotton Research Station, Vehari	15.4	120	150
	CIM-496 (Standard)	Central Cotton Research Institute, Multan	90	60	35
	S-12 (Spreader)	Cotton Research Station, Multan	100	35	12
2006	CIM-557	Central Cotton Research Institute, Multan	45	120	85
	FH-207	Cotton Research Institute, Faisalabad	22	130	120
	FH-113	Cotton Research Institute, Faisalabad	28	120	145
	609	Cotton Research Station, Multan	35	90	75
	MNH-786	Cotton Research Station, Multan	30	145	135
	NIBGE-115	Nuclear Institute for Biology and Genetic Engineering, Faisalabad	60	110	60
	VH-156	Cotton Research Station, Vehari	18	136	136
	VH-209	Cotton Research Station, Vehari	20	132	145
	CIM-496 (Standard)	Central Cotton Research Institute, Multan			
	S-12 (Spreader)	Cotton Research Station, Multan	100	35	20
2007	China 14	Nanjing Agri. University, China	23.5	120	130
	VH-255	Cotton Research Station, Vehari	25.5	145	100
	VH-257	Cotton Research Station, Vehari	25.0	75	85
	NIBGE-115	NIBGE, Faisalabad	29.5	142	78
	609	Cotton Research Station, Multan	25.5	150	60
	CIM-496 (Standard)	Central Cotton Research Institute, Multan	75.5	115	30
	S-12 (Spreader)	Cotton Research Station, Multan	100	60	18

During 2003, the results revealed (Table 7) that out of 10 entries maximum yield (125 g/plant) was produced by entry 609 belonging to Cotton Research Station Multan with minimum incidence of CLCuV (0.8%) while plant height was 118 cm followed by entry coded as 723 which show the incidence of 3.5% with yield figure of 85 g/plant and plant height was 140cm, the spreader S-12 showed stunted growth with plant height of 45cm at 100% incidence. This indicated that plant height was reduced due to CLCuV infestation. These findings are in accordance with (Brown, 2001) who reported decrease in plant height due to CLCuV. In 2004 minimum incidence (7.6) was showed by entry 3232 belonging to Cotton Research Station Multan, with average yield of 140 g/plant and plant height was 130cm followed by entry 609 showing incidence of 8% with average yield of 90 g/plant and plant height was 145cm.Minimum yield(25 g/plant) was given by spreader S-12 at 60% incidence. It indicated that seed cotton yield decreases significantly under higher CLCuV infestation. Similar findings were earlier reported in different studies (Tahir and Mehmod 2005).

In 2005 out of 14 entries minimum incidence 11.5, 15.4% of CLCuV was observed in entries VH-156 and VH-231 belonging to Cotton Research Station Vehari, with yield figure of 120,150 g/plant while plant height was 125 and 120 cm respectively. In 2006 two entries VH-156 and VH-209 from Cotton Research Station Vehari were at lowest incidence level (18, 20%), yield of seed cotton/ plant was 136 and 145 g with plant height of 136 and 132 cm, respectively.

In 2007 minimum rate of incidence (25.0, 25.5) was exhibited by VH-257 and VH-255 from Cotton Research Station Vehari, these entries gave yield figure of 85, 100 g/plant while plant height was 75, 145 cm respectively. Minimum yield (18 g/plant) was given by S-12 which was used as spreader in the field. The consequences of this research validates the method of screening to exploit resistant material for CLCuV on the basis of intensity, sick plot technique and incidence and to use the resistant material against CLCuV in further breeding programme.

References

Ahmad, G., S.A. Malik, Z. Mahmood, M.Z. Iqbal, and S. Ahmad. 2002. Effect of cotton leaf curl disease severity on morphology, yield and fibre characteristics of susceptible lines/cultivars of cotton (*G. hirsutum* L.). *Asian J. Plant Sci*., 1: 705-707.

Ahmad. M. and F.M. Azhar, 1999, Genetic mechanism controlling seed cotton yield and its components in F_1 and F_2 generations of *G. hirsutum* L. *Int. J. Biol*., 4: 227-233.

Akhtar, K.P., M.A. Haq, M. Hussain and A.I. Khan. 2002a.Whitefly transmitted geminiviruses and associated disorder in cotton, a: review. *Pak. J. Phytopath*., 14: 140-150.

Ali, M. 1997. Breeding of cotton varieties for resistance of cotton leaf curl virus. *Pak. J. Phytopath*., 9(1): 1-7.

Alim, 1997. Breeding of cotton varieties for resistance to cotton leaf curl virus. *Pak. J. Phytopath*.,, 9: 1-7.

Anonymous. 2003. Cotisties (Cotton Statistical Bulletin) Pak. Central Cotton Committee. Ministry of Food, Agriculture and Livestock, January Vol. 32.

Ashraf, M., Z.U. Zafar, T. McNeilly and C.J. Veltkhamp. 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.

Azhar, F.M. and A.H. Rana. 1993. Genetic analysis of lint percentage, staple length and fibre fineness of upland cotton. *Pak. J. Agric. Sci*., 30(3): 289-302.

Briddon, R.W., S. Mansoor, I.D. Bedford, M.S. Pinner, K. Saurdess, J. Stanley, Y. Zafar, K.A. Malik and P.G. Markham. 2000. Clones of cotton leaf curl Gemini virus induce symptoms atypical of cotton leaf curl disease. *Virus Genus*, 20(1): 19-26.

Brown, J.K. 2001. Viral and phytoplasma disease: Cotton leaf curl disease. p. 52-54. In: *Compendium of cotton disease.* (Eds.): T.L Kirkpatrick and C.K. Rothrock. 2nd ed. American psychopathological Society, St.Paul, MN.

Gusain, T., M. Tahir and T. Mahmood. 1991. Cotton Leaf Curl Disease. A Review, *Pak. J. Phytopath*., 3(1-2): 57-61.

Hameed, S., S. Khalid, E. Haq and A.A. Hashmi. 1994. Cotton leaf curl disease in Pakistan caused by whitefly transmitted Gemini virus. *Plant Dis*., 78(9): 529.

Harrison, B.D., Y.L. Liv, S. Khalid, S, Hameed, G.W. Otim Nape and D.J. Robinson. 1997. Detection and relationship of cotton leaf curl virus and allied white fly transmitted gemini viruses accruing in Pakistan. *Ann. Appl. Biol*., 130: 61-75.

Hussain, T. and M. Ali. 1975. A review of cotton diseases of Pakistan. *The Pak. Cottons*, 19(2): 71-86.

Idris, A.M. 1990. Cotton leaf curl disease in Sudan. Med. Fac. Landbow, Rijksunir. *Genet*., pp. 55.

Khan, T.A., I.A. Khan, M.A. Khan and N. Murtaza and A.M. Khan. 1992. Genetic analysis of upland cotton under Faisalabad conditions, yield of seed cotton and its components. *Pak. J. Agri. Sci*., 29(2): 170-171.

Kirkpatrick. T.W. 1931. Further studies on leaf curl of cotton in the Sudan. *Bull. Entomol. Res*., XIII (3): 323-363.

Mahoomad T., M. Tahir, M. Tanveer and M.B. Mirza, 1996. Effect of cotton leaf curl virus on yield components and fibre properties of four commercial varieties. *Pak. J. Phytopath*., pp. 68-70.

Mansoor, S., I. Amin, S. Iram, M. Hussain, Y. Zafar, K.A. Malik and R.W. Briddon. 2003. The breakdown of resistance in cotton to cotton leaf curl disease in Pakistan. New Disease Reports 7: 10-2.

Mansoor, S., I. Staniey, K.A. Malik and P.G. Markham. 1993. Molecular characterization of a Geminivirus associated with cotton leaf curl disease in Pakistan. Proc. Int. Symp. On Biotech. Sustainable Dev. NIBGE, Faisalabad.

Mirza, J.H., W. Ahmad, M.A. Ayyub, O. Khan and S. Ahmed. 1994. Studies on the identification, transmission and host range of cotton leaf curl disease in Punjab with special reference to its control. Final Res. Report, Dept. Path., Univ. Agric., Faisalabad.

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 cultivars. *Pak. J. Phytopath*., 10: 18-22.

Murtaza, N., M.A. Khan, I.A. Khan, T.M. Khan and A.M. Khan. 1992a. Inheritances of quantitive traits in cotton (G. Hirsutum L.) yield and yield components. *Pak. J. Agri. Sci*., 29(4): 420-405.

Nateshan, H.M., V. Muniyappa, M.M. Swanson and B.D. Harrison. 1996. Host range, vector relations and serological relationships of cotton leaf curl virus from Southern India. *Ann. Appl. Biol*., 128(2): 233-244.

Polston, J.E., T. Shervood, R.C. Rosell and A. Nava. 2002. Transmission of begomovirus, by whitefly (B. Tabact)- Do begomoviruses replicate in their vector. Gulf Coast Research and Irrigation Centre, Hoston, TX. pp. 2: 45.

Russel, T.E. 1982. Effect of cotton leaf curl crumple disease (CLC) on stab and planted cotton. Cotton. Coll. Agric. Rep. Ser., pp: 56. Cop. Ext. Ser. / Agric. Exp. Stn. Uni. Ariz./ Us. Dep. Agric., pp. 170.

Shah, H. and S. Khalid. 1997. Response of cotton germplasm to cotton leaf curl virus. Int. Conf. On integrated Plant disease management for sustainable agriculture. Nov. 10-15 New Delhi, India. (Abst), pp. 224.

Syal, O.U. Salam and M.Z. Sulemani. 1996. Comparison of gene action controlling the qualitative traits in some early maturing cultivars of American Cotton (*Gossypium hirsutum* L.). *Sarhad J. Agric*., 12(2): 137-145.

Tahir, M. and T. Mahmood. 2005. Occurrence of cotton leaf curl virus in the main cotton belt of Punjab and its impact on yield losses during 2004-05. *The Pak Cottons*, 50(3 & 4): 65-73.

Tanveer, M., T. Mehmood and Z. Ahmad. 1995. Study on cotton leaf curl virus in the Punjab, 5th Nat. Conf. Pl. Scientists, NARC, Islamabad (Abst.) p. 119.

Thomas, J.E., D.M. Parsley, D., J. Megrath and A.M. Hibberd. 1988. Virus diseases of tomato and pepper in Queensland and some aspects of their control. Queensland Dept. of primary Industries, 28: pp. 249-259.

Watkins, G.M. 1981. Compendium of cotton diseases. The American Phytopathological Society. 3340. Pilot Knob Road, Minnesota, U.S.A.

Watkins. G.M. 1981. Compedium of cotton diseases. The Amercan Phytopathological Society. 3340. Pilot Road, Minnesota, USA.

(Received for publication 2 February 2009)