Assesment of cotton germplasm of Pakistan against Cotton leaf curl disease (CLCuD)

By

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Abstract

Twenty three cotton lines included in National Coordinated Varietal Trial (NCVT) of Pakistan were assessed against Cotton leaf curl disease (CLCuD) through whitefly transmission and petiole graft technique. All the strains found susceptible against CLCuV. Out of twenty three lines only CIM-608 appeared as moderately resistant with minimum disease rating of 0-3 , where as all other lines were moderately susceptible against CLCuD. Petiole graft technique (PGT) found more reliable and convenient for effective screening breeding material.

Introduction

Cotton (Gossypium hirsutum L.) is one of the most important cash crops of Pakistan. cotton crop accounts for 60% of the total foreign exchange earning by export of raw products. It provides raw material to domestic cotton industry. Seed cotton yield of Pakistan 653 Kg/hectare is lower as compared to other countries of the world like America 871, Syria 10206, China 1271, Turkey 1365, Brazil 1453 and Australia 1844 ha -1 (Cotistics 2010). Cotton accounts for 8.6% of the value added in agriculture and about 1.8% to the GDP of Pakistan(Anonymus,2007). Pakistan is facing lot of hurdles, challenges contributing in low yield of cotton. CLCuD is the major disease reducing cotton yield since last ten years. In Pakistan CLCuD first time observed in 1967 near Multan district. At initial disease was ignored due to its low intensity, later on disease appeared as epidemic cotton disease (Saif *et al;* 1997) and 80% losses were recorded by Hussain & Mahmood (1988). CLCuD can decrease fiber length 3.44%, fiber strength 10% and elongation percentage up to 10% (Akhtar et al; 2009). Gradually disease spread in other parts of the country in cotton growing regions. Gemini virus (begomo virus) commonly spread by white flies, contains two circular single-strand DNA molecules. These viruses damages a lot of crop plants such as cotton, tomato beans, squash, cassava and potato. Spreading may be directly linked to the inadvertent world wide dissemination of the b-type; or silver leaf biotype of the whitefly *Bemisia tabaci*. This vector is an indiscriminate feeder, encouraging rapid and efficient spread of viruses from indigenous plants species to neighboring crops. Zhou *et al,* (1998) suggested that four variants of CLCuV exist in the field . The chances of recombination among the Gemini virus does exist and may led to the emerging of more virulent and resistance breaking verities (Shah *et al;* 1999). CLCuV disease starts appearing from the marginal

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areas of the leaves at the top of the plant. In most susceptible strains with few small scattered veins thickening. (Mahmood *et. al.* 2002). Gradually it affects whole plant with upward curling symptoms. Veins become thick and more pronounced at lower surface of the leaf and all thick veins link together with swear curling showing cup shape leaves (Watkins 1981). At present none of the commercial cultivars has resistant gene against CLCuD disease. The purpose of this study to locate genetic potential, tolerance level against CLCuD and virus transmission ways from National Coordinated Varietals Trials of Pakistan.

Material & Methods

Experiment was performed at green house of Central Cotton Research Institute (CCRI) Multan during 2011.Twenty three advanced strains were received from Pakistan central Cotton committee, (The source of these material is given in Table-1). All the strains were sown in three sets of earthen pots, filled with sterilized soil mixture.

CLCuV Transmission Methodology

Two methods were practiced for virus transmission i.e. a) Whitefly transmission and b) Petiole graft technique.

a) Whitefly Transmission

The culture of healthy whitefly was reared on healthy cotton plants in glass cages kept in seprate green house in control conditions. All precautionary measures were adopted by using yellow sticky trape, spraying by imidacloprid and keeping the material in glass cages inside green house to avoid natural infection. The healthy whitefly culture was shifted to affected cotton plants of CIM-496 with CLCuV by manual aspirator. After acquiring virus, viruliferous whitefly was shifted to test material as well on control (CIM-496) healthy plants. Whitefly completes acquisition of virus in 4hours called acquiring time period (ATP) and it completes inoculation access period from diseased to healthy plant in 8 hours. The actual virus transmission efficiency (ATE) was recorded in 24 hours (Gupta V.K.2010). After 48 hours test material was sprayed with imidacloprid 20 SL,@ 2.5ml/liter of water to kill whiteflies and stop the continues feeding and oviposining.Insectide spirotetramat applied in mixture with imidacloprid associated or not with methyled soy oil was efficient on the control of whitefly being viable as a part of the cotton pest management (Papa *et al.*,2009)The appearance of CLCuV symptoms recorded daily and classified according to the scale (0--6) developed by Hutchins and Knight (1950) and Siddiq (1968) which is given in Table-2.

b) Graft Transmission

Cotton isolates of variety CIM-496 were collected from naturally infected fields exhibiting characteristics of CLCuV symptoms and maintained in green house under control

condition at temperature 30—35 °C. Test material (NCVT's strains) comprising 23 strains along with control (CIM-496) grown in earthen pots with 4 plants of each strain kept in cages to avoid natural infection of virus. Grafting was started at the age of 50 days. In petiole graft technique a single compatible leaf is selected from diseased plant. The petiole of this leaf is prepared in to cleft shape representing as scion attached to test plant that represents as stock. The top portion of branch (3 cm) of test plant is removed, a wedge is prepared in test plant at cut portion and diseased leaf petiole is inserted inside and Para film tape is wrapped at junction shown in figure-1.CLCuV symptoms observed on daily basis in newly emerged fresh leaves (Mahmood, 2002).

Results and Discussion

Results of petiole graft given in Table-3, found more accurate than whitefly transmission. The strain CIM-608 behave well as compared to all other strains included in (NCVT) against CLCuV with mild symptoms (0-3) according to disease rating scale, Table-2, took maximum time period (26-35) in its defense. Seven strains CIM-608, SLH-334, NIAB-9811, NIAB-2010, NIAB-112, PB-38, and CIM-591 showed mild virus symptoms. Ten strains,V H-300, BH-176, BH-175, NIBGE-314, NIAB-2009, IUB-11, FH-2015, NIA-80, CRIS-494 and MNH-786 showed medium symptoms, while six strains CRIS-510, GH-114, GS-378, MPS-1, JS-212 and GS-212 showed severe symptoms in minimum (6-7) days.

Results of virus transmission through petiole graft method proved better as compared to virus transmission by means of whitefly. Petiole graft method is more reliable, convenient and cheep. A little difference observed in latent period (number of days taken to appear symptoms). In whitefly transmission symptoms appeared within (10—15) days given in Table-4, earlier than graft method. Transmission studies showed that CLCuV could be acquired by whitefly within 4 hr and transmitted to test plant within 1 hr of feeding (Khan. J.A& Ahmed .J 2005). Out of twenty three strains screened only one strain CIM-608 showed remarkable performance according to report of all breeders of research stations described in Table-4.

Some time viruliferous whitefly failed to spread/transmit disease in first attempt and process repeated again. In this method whitefly can be released over test plant in age of (10-15) days and petiole grafting is done in age of fifty (50) days of plant. In whitefly virus transmission escape of disease chances prevails, some time whitefly acquires less feeding from affected plants and some time whitefly stays on affected plant for short period. In both cases whitefly fails to express symptoms. In both of the procedures results were differed a little when data compared with disease rating scale (0-6) developed by knight

(1950), and (Siddiq,1968). Appearance of CLCuV symptoms on strains through graft transmission showed significance difference in their tolerance against CLCuV strain (Mahmood.2003). Grafting may be successful in transmission of viruses where other methods fail (Mathews R.E.F.1970)

In virus transmission through grafting it was observed that it is laborious and it does not work at high temperature because of quick evaporation from the bottle/test tube (Akhter K.P.2002), but in my study petiole graft performed very well even at high temperature. At present according to situation of CLCuD, it is great achievement for the cotton breeder of Central Cotton Research Institute. This study suggest that Cotton leaf curl virus has mutated with complex combination of old and new strains, responsible for breaking resistance of newly evolved cotton verities.

Table- 1 Strain names, sources and their description.

Name of strain	Source of strain	Name of strain	Source of strain
NIAB-9811	NIAB, Faisalabad	GS-444	Gohar Seed Corporation Multan
PB-38	University of Agriculture, Faisalabad	BH-175	CRS, Bahawalpur
NIAB-2010	NIAB, Faisalabad	BH-176	CRS, Bahawalpur
SLH-334	CRS, Sahiwal	FH-2015	CRI, Faisalabad
NIAB-2009	NIAB, Faisalabad	CRIS-494	CCRI, Sakrand
GH-114	CRS, Ghotki	CRIS-510	CCRI, Sakrand
NIAB-112	NIAB, Faisalabad	MNH-786	CRS, Multan
NIA-80	NIA, Tandojam	CIM-608	CCRI, Multan
JS-212	Jullundur Seed Corporation, Rahim Yar Khan	CIM-591	CCRI, Multan
IUB-11	Islamia University, Bahawalpur	VH-300	CRS, Vehari
MPS-II	CRS, Mirpur Khas	NIBGE-314	NIBGE, Faisalabad
GS-378	Gohar Seed Corporation, Multan	----------	----------

NIAB = Nuclear Institute for Agriculture and Biology
NIA = Nuclear Institute of Agriculture
CCRI = Central Cotton Research Institute
CRS = Cotton Research Station
CRI = Cotton Research Institute

Table-2. Disease rating scale

Disease grade	Description	Disease grade	Description
0	Complete absence of symptoms	3	Vein thickening involve in to small group of veins
1	Few small scattered vein thickening	4	Large group of veins involved
2	Small scattered vein thickening	5	All veins involved and curling of leaves
6	All veins involved with sewer curling and stunted plant		

Table-3. Reaction of (NCVT) strains against CLCuV with Petiole graft

NCVT Strains	Latent Period	Severity Scale (0-- 6)	Disease reaction	NCVT Strains	Latent Period	Severity Scale (0-- 6)	Disease reaction
CIM-608	22-27	0-3	+	CIM-591	12-16	4-6	++
SLH-334	15-18	3-5	+	FH-2015	10-13	5-6	+++
NIAB-9811	16-20	3-6	+	NIA-80	10-12	5-6	+++
VH-300	14-16	4-6	++	CRIS-494	10-12	5-6	+++
BH-176	12-14	4-6	++	MNH-786	8-12	4-6	++
BH-175	12-15	4-6	++	CRIS-510	7-10	5-6	+++
NIAB-2010	13-17	4-5	++	GH-114	7-10	5-6	+++
NIAB-112	13-17	4-5	++	GS-378	7-10	5-6	+++
NIBGE-314	10-14	4-6	++	MPS-1	8-10	5-6	+++
PB-38	12-14	4-5	++	JS-212	7-10	5-6	+++
NIAB-2009	12-14	4-6	++	GS-444	7-9	5-6	+++
IUB-11	12-14	4-6	++	CIM-496--	--6--8	--5-6	-+++

Mild + Medium + + Severe + + +

Table-4. Reaction of (NCVT) strains against CLCuV with whitefly transmission.

NCVT Strains	Latent Period	Severity Scale (0-- 6)	Disease reaction	NCVT Strains	Latent Period	Severity Scale (0-- 6)	Disease reaction
CIM-608	12-18	0-2	+	CIM-591	7-12	4-5	+
SLH-334	8-15	2-4	+	FH-2015	6-11	5-6	++
NIAB-9811	18-14	2-5	+	NIA-80	6-10	5-6	++
VH-300	7-14	4-5	+	CRIS-494	6-10	5-6	++
BH-176	6-13	4-5	+	MNH-786	6-11	4-5	++
BH-175	6-13	4-5	+	CRIS-510	6-10	4-6	+++
NIAB-2010	17-14	4-5	+	GH-114	6-9	5-6	+++
NIAB-112	7-13	4-6	+	GS-378	6-9	5-6	+++
NIBGE-314	6-10	4-6	+	MPS-1	7-9	5-6	+++
PB-38	7-13	4-5	+	JS-212	6-9	5-6	+++
NIAB-2009	7-13	4-5	+	GS-444	6-8	5-6	+++
IUB-11	7-13	4-6	+	CIM-496--	-6--8-	5--6--	+++-

Mild + Medium + + Severe + + +

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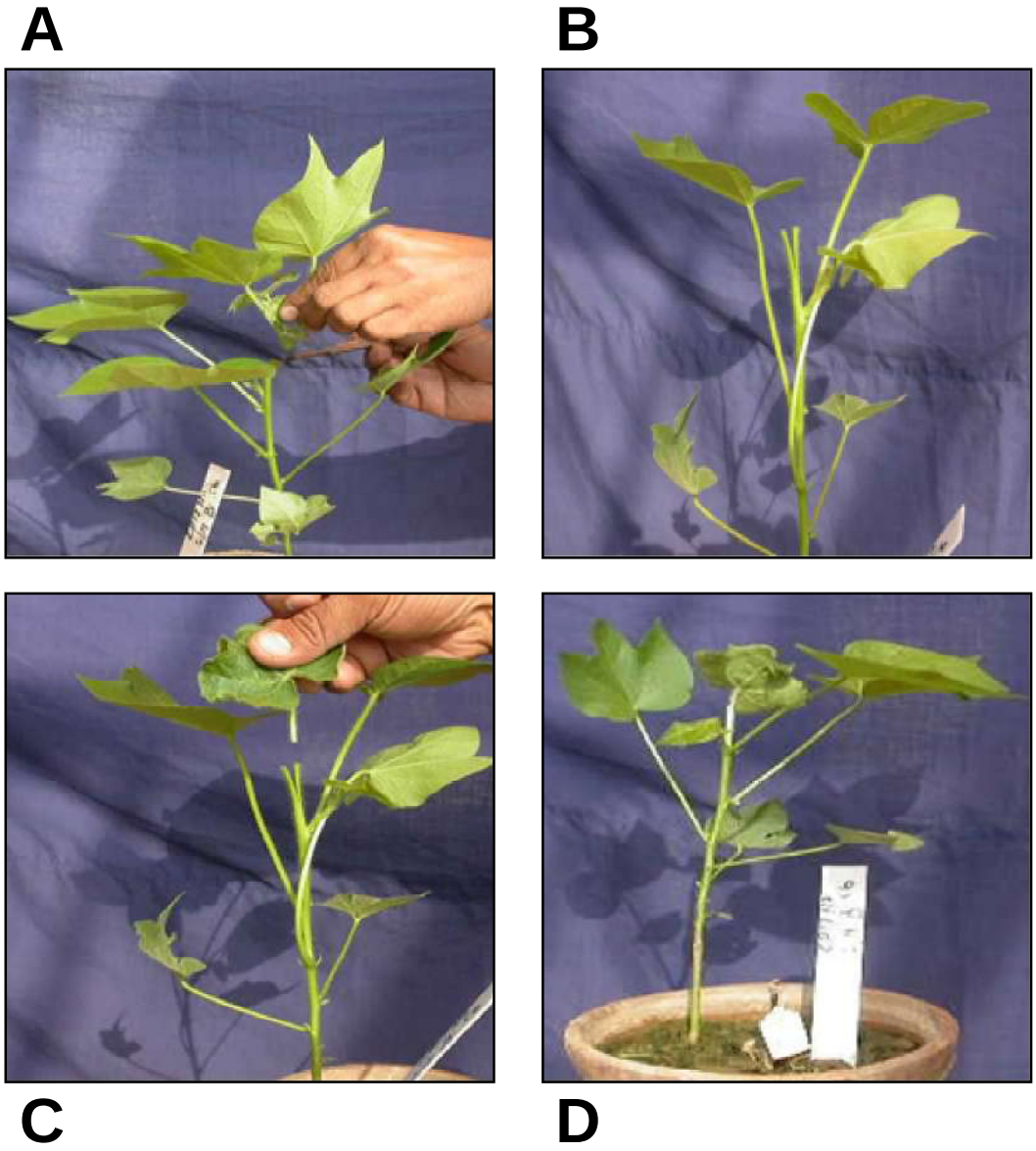

Fig.1: Various Steps of Petiole Graft Technique (a) Top of the test plant removed with scalpel (b) Vertical cut was made in the stem of test plant (c) CLCuV effected petiole inserted in the stem of test plant (d) Para film tape wrapped at the junction