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Testing of Cotton Leaf Curl Virus Resistance of Candidate Varieties/strains Through Different Techniques

Tariq Mahmood, Muhammad Tahir, Hafiz Tariq Mahmood and Sabahat Hussain
Plant Pathology Section, Central Cotton Research Institute, Multan, Punjab, Pakistan

Abstract: Twenty-seven upland cotton (*Gossypium hirsutum* L.) cultivars were screened for their respond to cotton leaf curl virus (CLCV) by artificial inoculation through grafting in controlled condition. The plants of each cultivars/ strains grown in sterilized soil, and were graft inoculated with CLCV infected leaf of S-12 (petiole grafting techniques). Symptoms development was recorded till 60 days after grafting. Presence of CLCV was determined, 60 days after grafting through polymerase chain reaction (PCR). Nine strains/ cultivars demonstrated the typical symptoms after 13-38 days of grafting. One group (41/99, DNH-49, CP-15/2xCRIS-404, AENB-18/10/87, AENB-36/SE/90, BH-125 and SLH-242) showed severe symptoms (13-25 days after grafting), while the other group (MNH-633 and VH-140) developed mild symptoms (35-38 days after grafting). The third group (MNH-536, FH-900, FH-901, NIAB-98, NIAB-94, NIAB-801/F, RH-500, 13/99, CIM-473, CIM-482, VH-141, VH-142, VH-143, LRA-5166xCRIS-404, BH-121 and BH-124) remained asymptomatic. Polymerase chain reaction (PCR) of all the material was also done. The first two groups showed the amplification of virus DNA, while the third group was negative. We concluded that the cultivars /strains belonging to third group are highly resistance to CLCV infection in the Multan region.

Key words: Cultivars, cotton leaf curl virus, *Gossypium hirsutum*, PCR

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important crop of Pakistan, which account for 60% of the total foreign exchange earning through the export of raw and cotton products. It also provides raw material to local domestic cotton industry comprising 503 textile mills, 1135 ginning factories and over 5000 oil-expelling units. It has an 85% share in total vegetable oil produced in the country. Leaf curl disease of cotton is main factor of causing heavy losses to the cotton crop. In Pakistan according to an estimate 7.1 millions bales have been lost during the last decade due to CLCV (Tariq, 1999). Moustafa (1961) stated that cotton leaf curl symptoms appeared in the top leaves of the susceptible cotton 30 days after planting and reached 100 per cent in 15 days. The experiments was carried out at Central Cotton Research Institute (CCRI), Multan indicates that this virus is neither mechanically transmissible nor carried in soil or seed. It is transmitted by the feeding of the whitefly, *Bemisia tabaci* (Genn.), which can complete the entire cycle from acquisition of the virus and infection of the host plant, with in 5-6 hours (Anonymous, 1975). Transmission through grafting and whitefly has been successfully done in Pakistan (Mirza, 1992). Control of CLCV in Pakistan is only resistant varieties. Screening of resistant varieties could be done through different methods but in our previous study it was done through petiole grafting that is the most convenient and easiest method, can be used for screening (Arshad, 2001). PCR is also a highly specific and reliable technique for the detection of gemini viruses.

Aim of this study is awareness about petiole grafting in cotton that is very easy and successful method for the transmission of CLCV into the healthy plants under control condition to check the breeding material before commercialization of new varieties/strains of cotton.

Materials and Methods

The cotton isolate used in this study was collected from naturally infected cotton plant exhibiting characteristic CLCV symptoms from field of CCRI, Multan. This isolate was maintained on cotton variety S-12 through whitefly transmission at Central Cotton Research Institute, Multan. Seeds of twenty-seven upland cotton (*Gossypium hirsutum* L.) cultivars were received from Pakistan Central Cotton Committee, Karachi. All these cultivars/strains were sown in sterilized soil mixture in cages at 30-32 ^{0}C in glass house at CCRI, Multan during the year 2000-2001.

Ten plants of each cultivars/strains were graft inoculated with CLCV infected petiole of S-12. Grafted plants were covered with moist polythene bags and held so until graft was established. Ten healthy S-12 plants were also grafted as control. The observations of graft inoculated plants were taken daily starting from one week after grafting and continued up to 100 days on a scale of 0-6 (Siddiq, 1968) a modification of the system described by Hutchinson and Knight (1950). The scale of symptoms of cotton leaf curl virus given in Table 1.

Table 1: Scale of symptoms of cotton leaf curl virus

Scale	Description	Scale	Description
0	Complete absence of symptoms	4	Large group of vein involved
1	Few small scattered vein thickening	5	All vein involved.
2	Small scattered vein thickening	6	All vein involved and sever curling
3	Vein thickening involving small group of vein	E	Enations

DNA from young leaves of twenty seven upland cotton cultivars/strains which were petiole grafted with CLCV infected leaves of variety S-12, after 60 days were extracted with CTAB method (Gawal and Jarret, 1991). PCR was performed to amplify target DNA with primer PA and PB of Deng *et al*. (1994) having the following sequences.

Primer PA: 5-TAA TAT TAC CGG AGG AGG CCC CC-3
Primer PB: 5-TGG ACC TAA CAA GGG CCT TCA CA-3

Target DNA was amplified in Biometra II system. The amplified product was analyzed by electrophoreses in 1% agarose gel. UV trans-illuminator box was used for examination of DNA amplification.

Results and Discussion

Results showed that out of twenty-seven cultivars/strains, nine demonstrated the typical symptoms of vein thickening after graft inoculation and also expected size of band was seen in PCR amplified product. The typical symptoms of CLCV were observed after 13-38 days of grafting (Table 2).

On the basis of disease severity the tested cultivars can be grouped into three categories:

(a) Sever symptoms in which all vein thickening; sever curling and enation observed under the leaf. In this group the CLCV symptoms appeared 13-25 days after grafting I. e., 41/99, DNH-49, CP-15/2 X CRIS-404, AENB-18/10/87, AENB-36/SE/90, BH-125, and SLH-242.

(b) Mild symptoms in which few small-scattered vein thickenings were observed i.e., MNH-633 and VH-140. In this group CLCV symptom appears 35-38 days after grafting.

Table 2: Screening of NCVT against CLCV through grafting

Name of strain	Source	No. of days taken to appear symptoms	Intensity
41/99	C.R.I, Rahim Yar Khan	12-15	6
DNH-49	C.R.S, D.I.Khan	18-20	6
CP-15/2 x CRIS-404	C.C.R.I, Sakrand	20-25	6
AENB-18/10/87	N.I.A, Tandojam	15-18	6
AENB-36 SE 90	N.I.A, Tandojam	13-15	6
BH-125	C.R.S, Bahawalpur	18-20	5
SLH-242	C.R.S, Sahiwal	18-20	6
MNH-633	C.R.S, Multan	38	1
VH-140	C.R.S, Vehari	35	4
MNH-536	C.R.S, Multan	Disease did not appear	0
FH-900	C.R.I, Faisalabad	Disease did not appear	0
FH-901	C.R.I, Faisalabad	Disease did not appear	0
NIAB-98	NIAB-Faisalabad	Disease did not appear	0
NIAB-94	NIAB-Faisalabad	Disease did not appear	0
NIAB-801/F	NIAB-Faisalabad	Disease did not appear	0
RH-500	C.R.I, Rahim Yar Khan	Disease did not appear	0
13/99	C.R.I, Rahim Yar Khan	Disease did not appear	0
CIM-473	C.C.R.I, Multan	Disease did not appear	0
CIM-482	C.C.R.I, Multan	Disease did not appear	0
VH-141	C.R.S, Vehari	Disease did not appear	0
VH-142	C.R.S, Vehari	Disease did not appear	0
VH-143	C.R.S, Vehari	Disease did not appear	0
LRA-5166XCRIS-278	C.C.R.I, Sakrand	Disease did not appear	0
BH-121	C.R.S, Bahawalpur	Disease did not appear	0
BH-124	C.R.S, Bahawalpur	Disease did not appear	0
FH-930	C.R.I, Faisalabad	Disease did not appear	0
FH-945	C.R.I, Faisalabad	Disease did not appear	0
S-12	Standard	12-15	6

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

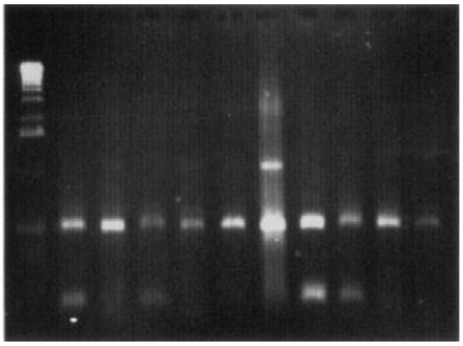

Fig. 1: PCR Reaction of different cultivars. Lane 1, Marker; Lane, 2 S-12; Lane 3, 41/9; Lane 4, DNH-49; Lane 5, CP-15/2 x CRIS-404; Lane 6, AENB-18/10/87; Lane 7, AENB-36/SE/90; Lane 8, BH-125; Lane 9, SLH-242; Lane 10, MNH-633 and Lane 11, VH-140.

(C) Highly resistant/ immune in which complete absence of symptoms till 60 days of petiole grafting i.e., MNH-536, FH-900, FH-901, NIAB-98, NIAB-94, NIAB-801/F, RH-500, 13/99, CIM-473, CIM-482, VH-141, VH-142, VH-143, LRA-5166x CRIS-278, CP x CRIS-404, BH-121, BH-124, FH-930, and FH-945.

Our results are confirmed with previous finding (Hussain *et al*., 1999) that the cultivars developed by CCRI, Multan (CIM-1100, CIM-443, CIM-446 and CIM-448) showed resistance in the field of their experiment. Results that two cultivars/strain (MNH-633 and VH140) gave symptoms of disease very late after grafting. This means these cultivars have tendency to resist against disease and their intensity of disease was also low. Polymerase Chain Reaction (PCR) of all the twenty-seven cultivars was also done to confirm the results. The cultivars/strains i.e., 41/9, DNH-49, CP-15/2 x CRIS-404, AENB-18/10/87, AENB-36/SE/90, BH-125, SLH-242, MNH-633 and VH-140 showed strong bands at 500 bp (Fig.1). No band of expected size was seen in remaining cultivars. Results of this study showed that all these cultivars in third group showed resistance/immune against CLCV in petiole grafting as well as in PCR amplification. It was also confirmed that the cultivars belonging to third group could be released for general cultivation. Some of the cultivars (FH-900, FH-901, and CIM-482) have been already cultivated in the field. The problem should not be considered solved, because four variants of CLCV have been shown to exist in the field (Zhou *et al*., 1998). Therefore, chances of recombination among them or with other whitefly transmitted gemini viruses does exist and may lead into emergence of new more virulent and resistance breaking variants.

Field observations indicated that most of the commercially grown cultivars were susceptible to CLCV infection, but there were different grades of tolerance in different varieties (Mahmood *et al*., 1994), so the use of graft inoculation technique for the screening of CLCV disease and PCR for detection of cotton gemini viruses in alternate host plants should be used carefully and may be confirmed by some other techniques. These methods will be useful in screening of resistance varieties as well as evaluation of breeding lines/strains in relation to gemini viruses species.

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