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Pakistan Journal of Biological Sciences 2 (4): 1494-1496, 1999 **Research Article**

Evaluation of Pepper Lines Against Tomato Leaf Curl Virus

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Abstract

n search of resistance/tolerance source against tomato leaf curl virus (TLCV), a whitefly (*Bemisia tabaci*) transmitted Geminivirus, seven pepper (*Capsicum annuum* L.) lines were inoculated with TLCV through *B. tabaci* in controlled conditions. Symptoms started to appear seven days after inoculation. Downward leaf curling and stunting of plants was he most common symptom observed. Symptom severity matched with ELISA values. All lines were highly susceptible (high ELISA valus), except PBC-491, which showed milder symptoms and low virus titer.

ntroduction

he pepper (Capsicum annuum) is grown all over the Pakistan under diverse ecological, environmental and soil conditions. Area under pepper/chilli cultivation during 996-97 was 87,200 ha with a production of 1,40,100 ones and yield 1,600 kg/ha (Anonymous, 1997). The verage yield of pepper/chilli (1,600 kg ha) is very low as ompared to other countries of the world. Among the actors responsible for low yields in Pakistan, the diseases f viral nature are of great importance.

bout 45 viruses are reported to infect pepper throughout he world (Green & Kim, 1991). However, in many parts of he world, including Pakistan, a whitefly (*Bemisia tabaci* Gennadius) vectored, tomato leaf curl virus (TLCV) is miting factor for good pepper production. TLCV in one of he most devastating viral disease of tomatoes and peppers n Southeast and East Asia, particularly India. Losses upto 0 percent have been reported in many parts of northern ndia (Singh *et al.*, 1979). Although no data is available bout the exact losses caused by TLCV in tomato and epper/chillies, but it is believed that in Pakistan 30-40 ercent losses are caused by TLCV.

ifferent approaches for the management of plant viral iseases can be adopted but use of resistant/tolerant ultivar is still the most preferred choice for combating viral iseases. In pursuance of this strategy pepper germplasm as screened to find out resistance source against TLCV, o that, if found, can be incorporated into a desired variety r be used directly as a commercial cultivar.

laterials and Methods

irus Source: The virus source used in this study was ollected from naturally infected tomato plants from Katha ugral (a highly infected area with TLCV), showing typical isease symptoms. The presence of virus was confirmed rough Triple Antibody Sandwich-Enzyme Linked mmunosorbent Assay (TAS-ELISA). As described by homas *et al.*, 1986; Aiton and Harrison, 1989; Harrison *et l.*, 1997. Nunc Microtiter ELISA plates were coated with Polyclonal antiserum to African cassava mosaic virus (ACMV) as first antibody and Monoclonal Antibodies (MAbs) (SCRI-60) of Indian cassava mosaic virus (ICMV) was used as second antibody.

Purity of the Isolate: Purity of the isolate was checked for ruling out the contamination with other commonly occurring viruses in tomato i.e. tomato mosaic virus (TMV), cucumber mosaic virus (CMV), potato virus X (PVX) and potato virus Y (PVY) using Bioribba ELISA kits in double antibody sandwich (DAS) ELISA as described by Clark and Admas (1977).

Whitefly Culture: Whitefly culture used in this study was originally obtained from the cotton fields, Multan and maintained at Plant Virology Programme, National Agricultural Research Centre (NARC), Islamabad, on cotton plants. To adapt to tomato, whitefly culture was shifted to tomato and kept in transparent perpex cages (36.25 cm x 36.25 cm x 56.25cm). Aeration was provided through muslin cloth sealed holes (diameter 17.5 cm) on both sides of cages. These cages were kept at 28-35 C under artificial light of 14 hours photoperiod. The temperature was maintained by providing heat through blower or heater whenever needed. Tomato plants were periodically replaced with new ones to enhance breeding activities of whiteflies.

Multiplication of Virus Source: Virus source was increased by feeding of non-viruliferous whiteflies on TLCV infected tomato plants for 48-72 hrs in cages. These viruliferous whiteflies were collected with an aspirator and released on cv. Rutger, susceptible to TLCV. After 48-72hrs inoculation feeding, these whiteflies were killed mechanically. As plants developed symptoms, pots were shifted to glasshouse on which non-viruliferous whiteflies were released to increase the population of viruliferous whiteflies.

Plant Material: Seeds of seven pepper lines, i.e., PBC-164, PBC-176, PBC-346, PBC-439, PBC-491, PBC-575 and PBC-576, were provided by Asian Vegetable Research and Development Centre (AVRDC). As a susceptible check cv.

Rutger was included. Seeds were sown in a standardized soil mixture (an equal amount of soil, sand and farmyard manure) in plastic pots (12.50cm.x12.50 cm.) and they were transplanted to individual pots.

Placement in Glass House: At 3-4 leaf stage plants were placed side by side in a square form in the green house. Infectious source (tomato plants infected with TLCV) was placed around healthy plants in such a way that they were completely surrounded by infectious source (Fig. 1). Full strength Hoagland solution (10-15ml/pot) to each pot was added at 15 days interval to meet the nutritional requirements.

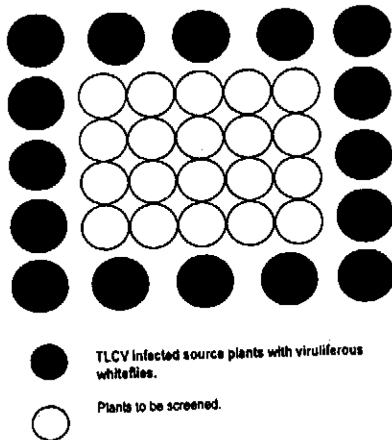

Fig. 1: Placement in glasshouse

Shifting of Whitefly: Viruliferous whiteflies established on surrounding infected tomato plants (Fig. 1), were shifted by shaking the source plants twice a day i.e., once in the morning and then in afternoon.

Symptoms Development: The plants were daily observed for the appearance and development of symptom. Data on first date of symptom appearance, type and severity of symptoms were recorded.

Hybridization Test: Nylon membrane charged with Indian isolate of TLCV (16I-TLCV-Banglore 1), received from AVRDC, were used for hybridization test. Thrity-three (33) samples from randomly selected plants were taken. Small leaf disks were cut from young shoots with corkborer. These disks were placed in the centre of designed squares marked on the membrane with forceps. Disk was covered by a small piece of parafilm, and pressed firmly with a pestle to imprint on membrane. The membrane was sent to AVRDC for hybridization.

Virus Concentration: Three weeks after the inoculation, virus concentration was checked through TAS-ELISA, using polyclonal antibodies to ACMV and MAbs (SCR-60) to ICMV. Five plants of each line were randomly selected for testing through TAS-ELISA. Samples were collected from top, middle and bottom of the plant. These leaves were chopped and pooled and 1gm was used for estimation of virus concentration. A negative and positive control was included and ELISA values were measured in ELISA reader Titertek Multiskan MC.

Results

Symptoms started appearing one week after inoculation. The most prominent symptoms observed were smalling, rolling and severe curling of leaves. Apart from these, stunted growth was also noticed in check (cv. Rutger). The details are given in Table 1. The data revealed that none of the lines were found to be immune or resistant against the used isolate of TLCV. All test lines were highly susceptible and developed severe symptoms. However, the symptoms in line PBC-491 were milder as compared to other lines.

Check (cv. Rutger), PBC-346 and PBC-439 exhibited symptoms within one week with 100, 95 and 90 percent infection, respectively. On the other hand, PBC-164, PBC-176, PBC-575 and PBC-576 showed symptoms two weeks after inoculation with a percentage of 65,75 75.0 and 75 infection, respectively. In case of PBC-491, only four plants exhibited symptoms after two weeks, i.e., percent infection was only 20.0, which increased upto 90.0 after eight weeks as fourteen more plants, became infected. Two plants remained uninfected. All lines showed 100 percent infestation three weeks after inoculation, except PBC-491 (Table-1).

Virus concentration measured through ELISA was on higher side except in PBC-491 (Table 2). Reaction strength in lines PBC-164, PBC-176, PBC-346, PBC-439, PBC-575 & PBC-576 was 3 to 4. In case of PBC-491, two plants were ELISA negatives, in one plant virus was detected in traces and in other two plants reaction strength was 2 (Table 2). In hybridization tests our isolate of TLCV strongly reacted with Indian TLCV probe 16I-TLCV-Banglore 1 (Table 3).

Discussion

Our study showed that all tested lines were susceptible to TLCV infection, but differed in their response to TLCV infection. One group, lines PBC-346 and PBC-439, showed symptoms after one week and second group, lines PBC-164, PBC-176, PBC-575 & PBC-576, within two weeks after inoculation. No significant difference was noticed in ELISA values of these lines (Table 2). Both groups showed severe symptoms and higher virus concentration. However, PBC-491 showed delayed and milder symptoms initially but gradually turned into severe ones as in group one and two. The virus concentration in PBC-491 ranged from zero, traces to 2 (Table-2) indicating that this line has some sort of resistance which not only delayed symptom development but also virus did not multiply well in this compared to other lines.

Although symptoms are good revelation of plant response to viral infection but they are not always indicative of v

Table 1: Symptoms of TLCV on Pepper Lines after 3 to 8 Weeks of Inoculation

S#	Papper Lines	Plant Inoculated	Symptoms	Symptoms after days	% Age Infection after		
					Ist Weeks	2nd Weeks	3rd Weeks
1	PBC-164	20	Severe Curling	11	0	65.0	100
2	PBC-176	20	Severe Curling	09	0	75.0	100
3	PBC-346	20	Severe Curling	07	95.0	100	100
4	PBC-439	20	Severe Curling	07	90.0	100	100
5	*PBC-491	20	Mod. Curling	14	0	0	20.0
6	PBC-575	20	Severe Curling	09	0	75.0	100
7	PBC-576	20	Severe Curling	09	0	75.0	100
8	Check	20	Severe Curling	07	100	100	100

Fourteen plants of PBC-491 showed symptoms eight weeks after inoculation while two plants remained unaffected (might be escaped).

Table 2: ELISA Values of Pepper Lines at 405 nm

#	Lines	Symptoms	Reaction Strength
	PBC-164	Severe Curling	3
	PBC-176	Severe Curling	4
	PBC-346	Severe Curling	4
	PBC-439	Severe Curling	3-4
	PBC-491	Mod. Curling	None-2
	PBC-575	Severe Curling	3-4
	PBC-576	Severe Curling	4
	Check	Severe Curling	4

cale: Trace = 0.15-0.3; 1 = 0.31-0.6; 2 = 0.61-1.2; 3 = 1.21-1.8; 4 = 1.8 -

oncentration in infected plants. Therefore, it is advisable o also measure virus concentration in them so that meaningful conclusion can be drawn. ELISA results of this study enabled us not only to detect virus but also helped for analyzing the extent of virus multiplication/concentration in inoculated lines. A good relationship between symptom severity and virus concentration was noticed. Lines in which severe symptoms were developed also had higher virus concentration and vice versa.

None of the line was found to be free of TLCV infection, but lines showing some level of tolerance (delayed infection and less virus) can be used to reduce the losses caused by TLCV. This might also help in checking disease spread as vector might find difficulties in acquiring the virus from a poorer source and then transmitting it further. Meanwhile, search for high tolerance level or source of immunity against TLCV in pepper should continue.

able 3: Hybridization Results of Pepper Lines

BC 64+	PBC 164+	PBC 176+	PBC 176+	PBC 176-	PBC 176-	PBC 176+	PBC 176+	PBC 346-	PBC 346+
BC 46+	PBC 439+	PBC 439+	PBC 439+	PBC 491+	PBC 491+	PBC 491-	PBC 491+	PBC 491+	PBC 575+
BC 75+	PBC 575+	PBC 575+	PBC 575+	PBC 575+	PBC 575+	PBC 575+	PBC 576-	PBC 576+	PBC 576+
BC 76+	PBC 576+	PBC 576-	PBC 576+	PBC	PBC	PBC	PBC	PBC	PBC

Positive/Diseased DNA imprints
Negative/Healthy DNA imprints

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