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Detection and Identification of Tomato Leaf Curl Virus (ToLCV) using Molecular Technique - PCR Method

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A B S T R A C T

The cultivated tomato (*Solanum lycopersicum* L.) is economically one of the most important and widely grown plants of the Solanaceae family. Epidemics of Tomato leaf curl virus associated with upsurge of whiteflies (*Bemasia tabacci*) on tomato crop has been frequently reported which up to 100% yield losses (Shelat, *et.al*, 2014). In all twenty six samples were collected to see the presence of tomato leaf curl virus (ToLCV). Polymerase chain reaction (PCR) in the moist sensitive approach to detect a minute amount of viral nucleic acid it is the most method to amplified geminvirus as they replicate via-double-stranded, circular DNA from. In this study, geminivirus specific detergents primer was employed to detect ToLCV occurring in it vector whitefly *Bemisia tabaci* by PCR based approach. The presence of virus was observed through PCR method with specific primer CPAV1 forward and reveres on different annealing temperature range from 52ºC to 56ºC. The presence of virus was observed at 54ºC annealing temperature in sample no. 4, 11, 15, 22, 24, 25, 26) and confirm the presence of ToLCV.

Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is economically one of the most important and widely grown plants of the Solanaceae family. It has been one of the first crop plants for which a genetic linkage map was constructed (Rick, 1975). Tomato leaf curl disease (ToLcD) is a serious and threatening disease of tomato causing losses of up to 100% various part in the world. The characteristically have ssDNA as genome of some bengomo virus is mono partite (DNA a alone), while a majority have bipartite genome DNA A&B (Shiva Lingam, *et al.,* 2007) However, there were substantial differences between genotypes in disease incidence, spread, symptom severity and crop yield (Castililo *et al.,* 2000). Tomato leaf curl disease (ToLCD) is associated with *begomovirus, with* their generic name as tomato leaf curl virus ToLCVs. Exist both with mono/bipartite genome, whereas ToLCVs carry mono partite genome. For

management of these viruses, three resistance locii Ty 1 to Ty 3 are being deployed, with provides different degree of resistance. In the plant, the virus develops within the phloem and induces cytological changes (Channarayappa *et al.,* 1992). Symptoms appear only 15day after inoculation. Tomato plants infected at an early stage are severely stunted; their terminal and auxiliary shoots are erect, and their leaflets are reduced in size and abnormally shaped. Leaves that develop soon after infection are cupped downward, whereas leaves developing later are prominently chlorotic and deformed, with leaf margins rolled upwards and curling between the veins. No flower symptoms are observed but dropping of flowers is common (Abou-jawdah *et al.,* 1995).

The life cycle of whitefly is 18 to 28 days from egg to adult in warm weather and 30 to 48 days in winter. At 221c° the greenhouse whitefly completes its life cycle in about 28 days (Abdullah and Singh, 2004). Reference is for analyzing the internal anatomy of whitefly species. Several publications have focussed on the anatomy of *B. tabaci* mouthparts (Rosell *et al.,* 1995), anterior alimentary canal (Hunter *et al.,* 1996), and digestive tract, filter chamber and salivary glands (Cicero *et al.,* 1995; Harris *et al.,* 1995, 1996; Ghanim *et al.,* 2001). One of the important damage cause by the whitefly is vectoring various plant viruses transmitted by whiteflies cause over 114 diseases of vegetables and fibers crops worldwide and 111 of those virus species are vectored by the species *B. tabaci h*as a circulative mode of transmission, requiring an average of 6-12hr prior to be transmission event.

The present study aims to detection and identification of Tomato leaf curl virus using molecular technique with two objectives. 1. To isolate DNA from tomato sample.2. To detect tomato leaf curl virus (ToLCV) by PCR method.

Materials and Methods

The present study "Tomato leaf curl virus detection by PCR Method" carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001, during Jan-2016 to March-2016, with objects to detection and identification of tomato leaf curl virus (ToLCV) by using molecular technique. Chemicals used for present study were of good quality (AR-grade) from various agencies.

PCR amplification

Good quality genome DNA isolated from different Tomato sample. The ToLCV specific primer CPAV1.was subjected to PCR reaction to detection of ToLCV in different sample. The composition of PCR reaction is presented in table 1.

Procedure

Sterile micro centrifuge tubes were numbered and placed on PCR tube stand.

0.5µl of DNA was added to each PCR tube followed by 637 µl master mix.

1µl of each forward and reverse primer was added in each PCR tube respectively. Separately transfer 24.5µl PCR master mix in PCR tube and add 0.5 µl DNA sample added.

PCR was run on the programme thermal cycler with the following by programme:

Results and Discussion

Source of tomato leaf sample

Twenty six tomato leaf samples were collected from Nidhona village farm in Jalna (Fig. 1).

Fig.1 Tomato leaf sample used for detection of tomato leaf curl virus (TLCV)

Fig.2 DNA band of different tomato leaf sample

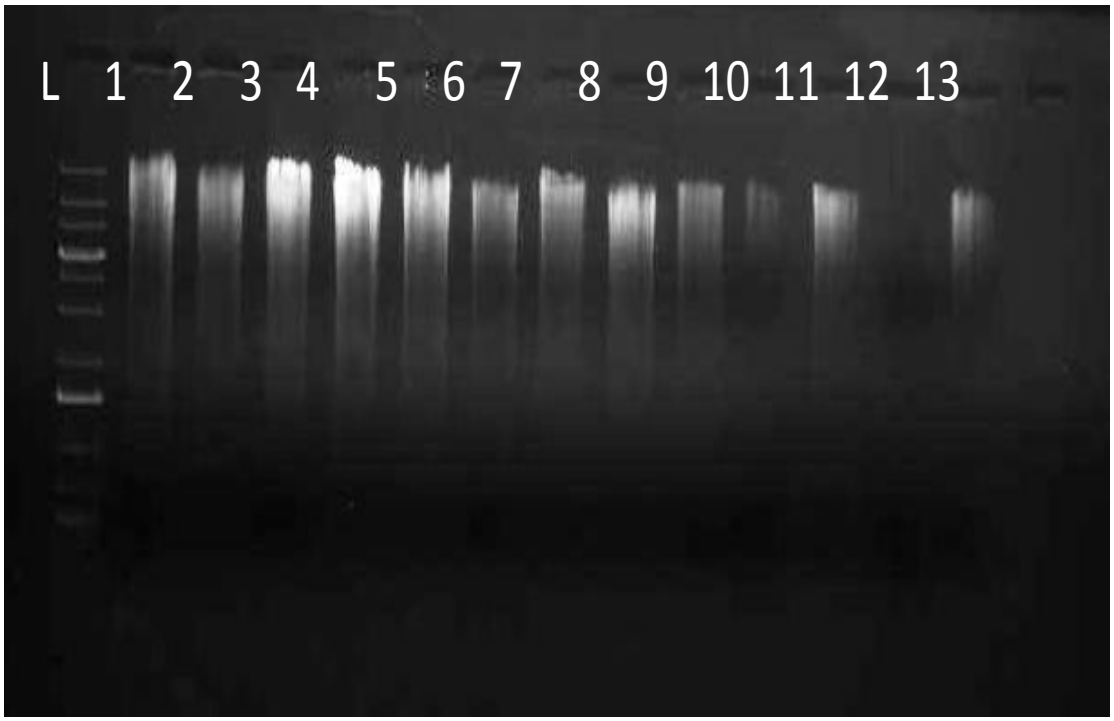

Fig.3 Amplification of DNA band

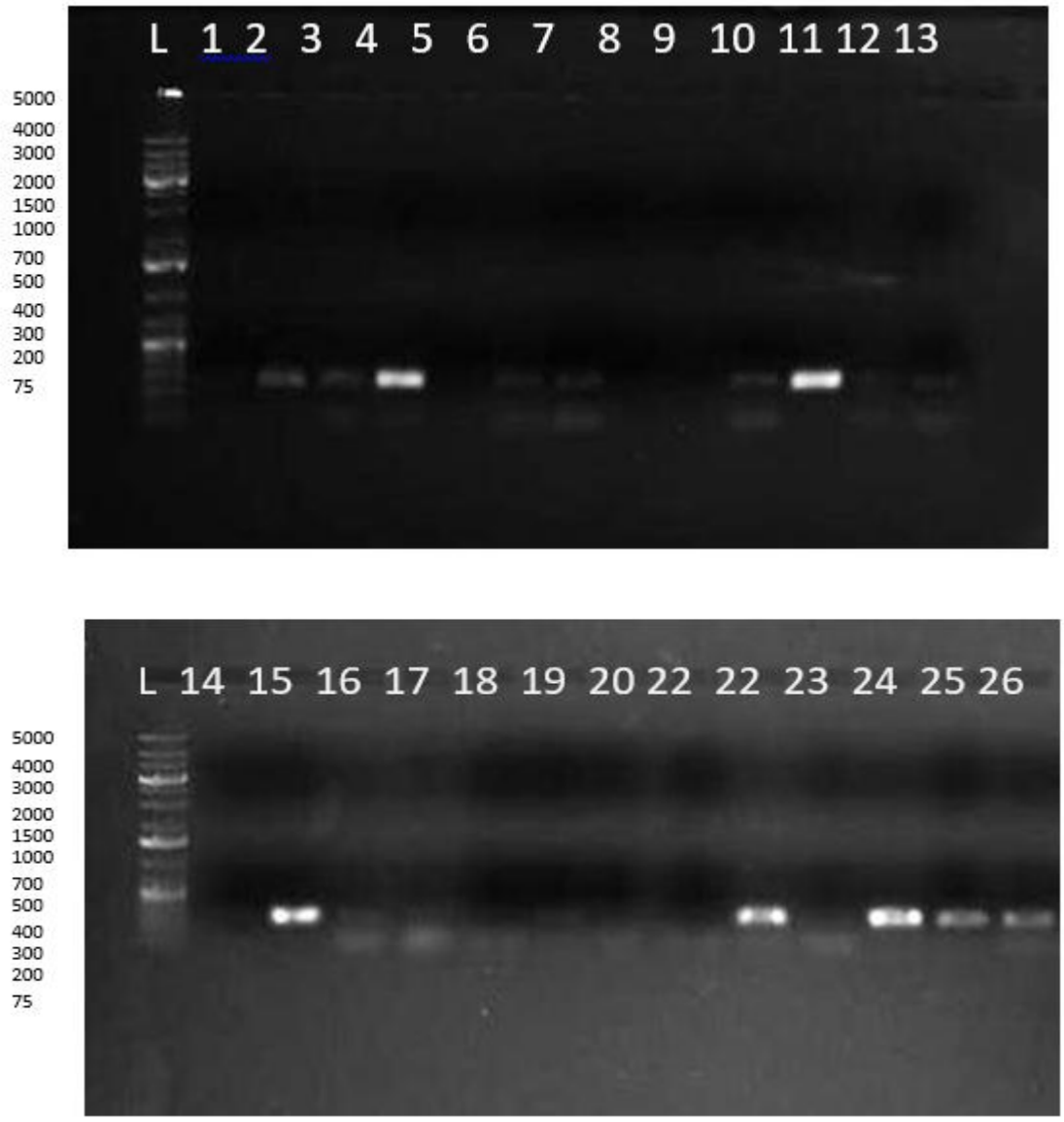

Table.1 Composition of PCR reaction mixture

Sr. No.	Components	Quantity in 1 sample in µl	Quantity in 26 sample in µl
1	10x buffer	2.5	65
2	dNTP	1	26
3	Forward Primer	1	26
4	Reverse Primer	1	26
5	*Taq Polymerase*	0.2	5.2
6	Template DNA	0.5	13
7	SDW	18.8 (To make up 25 µl)	488.8
8	TOTAL	25	650

We screened the 26 samples for Tomato leaf curl virus (ToLCV). The collected samples were subjected to PCR amplification with primer CPAV1 (Monoprimer)

Isolation of genomic DNA

Genomic DNA isolated from tender leaves of selected 26 different Tomato sample. A good quality genomic DNA isolation by using CTAB method.

DNA confirmation

Isolated genomic DNA from each Tomato leaf sample was loaded on 1% agarose gel electrophoresis to determine DNA quality. DNA sample as shown in figure 2.

PCR amplification

The collected tomato leaf sample (possible symptoms of ToLCV) was subjected to PCR amplification for detection of ToLCV with specific primer, CPAV1. After completion of the cycles keep the samples at 4°C till electrophoresis. Samples were loaded on 1% gel electrophoresis (Fig. 3).

These banding pasterns seen under gel doc. Out of 26 sample PCR amplification with ToLCV specific primer, sample 4, 11, 15, 22, 25 and 26 were shown presence 6 bands. It means these samples shown negative means not infected to ToLCV. 1stband is of 400bp, 2ndband is of 430, 3rd band is of 400, 4thband is of 500, 5thband is of 530bp, 6thband is of 480bp, 7^{th} band is of 490bp.

In present study we were isolated DNA from infected tomato leaf sample and after PCR by primer (CPAV1). We got positive result for Tomato leaf curl virus in some sample. We selected or collected 26 samples from different region and after screening for virus we analyzed that out of 26 samples we found TLCV in following lanes 4, 11, 15, 22, 24, 25, 26. The underlying reasons for some plants of Fiona not showing symptoms in the glasshouse are unknown but could be due to several reasons including the multiple infections with ToLCVs. Viral DNA from weed and whitefly-mediated transmitted. Degenrate DNA B-specific PCR primer has not allowed to amplify from plant infected by known bipartite begomovirus. The full length 2759 nucleotide DNA-A-like viral genome was sequenced, similarly to other monopartite ToLCV. Isolates from Bangalore constitute of a group of virus separate from this northern India (Muniyappa *et al.,* 2000; Kirthi *et al.,* 2002). The importance of this disease has promted a great need for a rapid identification of ToLCV in it host and vector One primer pair amplified ToLCV DNA fragment about 1.1kb representing partly replicated gene, intergenic reagion and partly coat protein amplified fragment of about 0.5kb was obtain. This approach is highly useful for and early detection of ToLCV occurring very small amount in the vector *B. tabaci*, its amplification in geminivirus mangment strategies and their differentiation and being discussed (Khan, 1999).

Unfortunately the tomato crop is highly susceptible to fungal and viral disease viruses are playing the major role and put the farmer in to the huge losses. Amongst the virus tomato leaf curl virus play major role considering the importance of the virus disease, the present project has been undertaken. In all twenty six samples were collected to see the presence of tomato leaf curl virus (ToLCV). The presence of virus was observed through PCR method with specific primer CPAV1 forward and reveres on different annealing temperature range from 520C to 560C. The presence of virus was observed at 540C annealing temperature in sample no. 4, 11, 15, 22, 24, 25, 26) and confirm the presence of ToLCV.

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