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Identification of Tomato Leaf Curl Virus (ToLCV) Strain Causing ToLCV in Tomato and Chilli

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Abstract: ***Tomato (Solanum lycopersicum* L.) *and Chilli (Capsicum annuum* L.) *are the most important and widely grown vegetable crop in the world belonging to the family Solanaceae. Tomato is important source of vitamins A and C and Chillies are excellent source of Vitamin A, B, C and E. India is one of the major producers of chilli and tomato. Leaf curl is one of the most important diseases of chilli and tomato causes substantial losses every year. The Tomato Leaf Curl Virus Disease (ToLCVD) is characterized by the upward and downward curling and twisting of leaves followed by marked reduction in leaf size. The diseased plants looks pale and stunted due to shortening of inter nodal length with more lateral branches resulting in a bushy appearance of leaves and shortening of internodes with partial to complete sterility. The DNA extracted by CTAB method from the leaf sample. For identification of ToLCV disease of tomato and chilli primers were designed using NCBI tool by collecting sequences of ToLCV from different regions of India. DNA sample was amplified successively with designed primers using PCR cycles and the band appearance was observed on the gel electrophoresis. From the appearance of the band the disease identification and diagnosis of Tomato leaf curl virus ToLCV was done. In future work ToLCV disease could be confirmed using NESTED PCR.***

Keywords: Chilli, NCBI, NESTED PCR, ToLCV, Tomato.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an important and most widely grown vegetable crop of both tropics and sub tropics of the world, belonging to the family solanaceae and ranks second among vegetables. It is esteemed as an important source of vitamins A and C. Lycopene is a very powerful antioxidant which can help prevent the development of many forms of cancer.

Total area under tomato in the world is 4.81 million ha with production of 163.02 million tons and with productivity of 33.9 tons per ha. In India it occupies an area of about 882000 ha with the production of 18735900 tones *i.e.* 21.2 tons per hectre [1].

The begomoviruses affecting tomato in India are the most devastating. It had been observed that the begomoviruses affecting tomato in Northern India are bipartite and those affecting tomato in Southern India are monopartite [2],[3]. Among the virus diseases, tomato leaf curl disease; *Tomato Leaf Curl Bangalore Virus* (ToLCBV), *Tomato Leaf Curl New Delhi Virus* (ToLCNDV), *Tomato Leaf Curl Karnataka Virus* (ToLCKV) and *Tomato Leaf Curl Gujarat Virus* (ToLCGV) are the important begomoviruses which limit the tomato production.

Chilli (*Capsicum annuum* L.) is an important spice crop in India. The chilli is known for its flavor and pungency due to capsicum and having a medicinal value. It has tonic and carminative action.In India chilli is cultivated in all the states including Andhra Pradesh, Karnataka, Maharashtra, Orissa, Rajasthan, Tamil Nadu and West Bengal. India contributes about 36% to the total world production. Andhra Pradesh is the largest producer of chilli.

Total area under chilli in the world is 1.9 million ha with production of 29.9 million tons and with productivity of 15.8 tons per ha. In India it occupies an area of about 775000 ha with the production of 1492000 tones *i.e.* 1.9 tons per hectare [1].

The *Chilli Leaf Curl Virus* (ChiLCV) disease on chilli was first reported in India by [4]. In India, *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) was recently shown to be associated with chilli leaf curl disease occurring in Lucknow [5]. *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) is a bipartite begomovirus that infects tomato and watermelon crops in the Indian subcontinent [6]. Chilli Leaf Curl Disease (ChiLCD) is an important factor limiting chilli production on the Indian subcontinent and is caused by begomoviruses. The disease was experimentally transmitted from infected to healthy chilli and tomato seedlings by the white fly (*Bemisia tabaci*).

The incidence of ToLCV has become a major limiting factor and challenge to farmers and scientists. Tomato Leaf Curl Virus Diseases (ToLCVD) have seriously hampered the cultivation and production of tomato and chilli. The incidence of ToLCVD in susceptible cultivars caused more than 90 % yield loss [7]. Tomato Leaf Curl Virus Disease (ToLCVD) is characterized by the curling and twisting of leaves followed by marked reduction in leaf size. The diseased plants looks pale and stunted due to shortening of inter nodal length with more lateral branches resulting in a bushy appearance [8].

Indian ToLCV isolates are mostly monopartite (DNA-A) in

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nature with few isolates possessing bipartite (DNA-A and DNA-B) genome organization. Both DNA-A and DNA-B are single-stranded (ss) DNA genomes of approximately 2.7 kb size.

2. Materials and Methods

2.1. Importance of ToLCV Disease

Tomato leaf curl virus was reported to be a serious disease on tomato and chilli throughout India. The disease may cause up to 75 per cent or more reduction in fruit yield and due to its devastating nature it has become a national problem [9], [10]. [10] Reported that ToLCV infected plants produced very few fruits when infected within 20 days after planting and resulting up to 92.3 per cent yield loss. Plants infected 35 and 50 days after transplanting resulted in 74 and 22.9 per cent yield loss respectively.

2.2. Symptomology

The symptoms of *Tomato Leaf Curl Virus* (ToLCV) included vein clearing, reduction in leaf size, stunted growth, deformation of leaf lets, inward and outward curling and puckering of leaf lets. The infected plants produced few fruits in case of late infection and no fruits, if infected at very early stage. Tomato leaf curl symptoms viz., leaf curling, stunting of plants, thickening and greening of the veins of leaves.

2.3. Vector Transmission

Gemini viruses are transmitted from plant to plant by their main insect host well known as white fly (*Bemisia tabaci*). The whiteflies remained infective throughout their life span [11].

2.4. Survey for Occurrence of ToLCV Disease

The natural occurrence of *Tobacco Leaf Curl Virus* (TobLCV) on tomato in India was first observed by [12]. [10] Reported 17.53 per cent incidence of ToLCV in some tomato growing areas of Karnataka during July-November, whereas the incidence was as high as 100 per cent in crops grown in February-May. Similarly, in sequential sowings 90 to 100 per cent of plants were infected in pots sown between the end of January to the end of May.

2.5. Host range of ToLCV

Gemini viruses are transmitted by the white fly, infect dicotyledonous plants, and possess a bipartite genome. In infected plants, the two viral genomic components (designated as DNA-A and DNA-B) were found to be equally represented both in the viral genomic single-stranded DNA (ssDNA) end in its double-stranded DNA (dsDNA) replicative form (RF). The PCR amplification of its DNA with begomovirus specific primers and its strong hybridization with a DNA-A probe of Indian tomato leaf curl virus strongly indicate that it is a begomovirus and confirmation of disease can be done by using NESTED PCR [13].

2.6. Primer Designing

Primers were designed based on the high sequence conservation among tomato leaf curl virus strains from India like New Delhi virus, ToLCV Pune virus, ToLCV Karnataka, ToLCV Bangalore and ToLCV Palampur virus showing significant homology. Blastn analysis was performed to select the homologous sequences for primer designing. The primer designing was done with ClustalW multiple sequence alignment program and Primer 3 software.

Primers were designed to specifically amplify particular viral DNA sequences. DNA fragments were attempted to amplify under moderate stringency conditions. Available complete viral DNA sequences were downloaded from NCBI GenBank and used for primer designing for detection of different viral strains.

Table 1: Primer Designing for Amplification of ToLCV Strains

Sr. No.	Primer code	Base pair	Sequence
1.	PGKF1	20	5'ACACGCCAT TCTCTGCTTGA 3'
2.	PGKR1	21	5'CCATCCRAACATTCAGRGAGC 3'
3.	NDF1	19	5' TGGGATCCATTATTGCACG 3'
4.	NDR1	20	5'TTCAACCACAACCTGCGGAA 3'
5.	NDF2	19	5' TCGTGCAGTTGTCCCCAT T 3'
6.	NDR2	20	5' TGGGGTGTTTTCCAGATCGA 3'

2.7. Maintenance of Source Plants

The *Tomato Leaf Curl Virus* (ToLCV) used during present investigation for the experimental purpose was obtained from different plots of tomato and chilli growing region of Nashik viz. Jopul, Vani, Bhatode & Adgaon showing typical leaf curl symptoms tomato and chilli plant.

2.8. Isolation of Genomic DNA and its purification

DNA was isolated through CTAB protocol [14] with slight modification. Purification of DNA is essential to remove RNA, proteins and polysaccharides which are considered to be the major contaminants in the DNA precipitates. Inclusion of CTAB in DNA extraction buffer helps elimination of polysaccharides. RNA was removed by RNAse treatment and proteins were removed by phenol-chloroform extraction.

2.9. Assessing the quality and quantity of DNA by Bio-Spectrophotometric measurement

The extract of genomic DNA 5 µl was diluted with 445 µl distilled water and concentration was measured using Bio-Spectrophotometer (Eppendorf, USA). The samples were considered pure, if the ratio was between 1.8 and 2.0. If otherwise, the purification steps were repeated to bring the desired ratio. Then the required DNA concentration was prepared for each plant using sterile water. The quality of isolated DNA was evaluated through agarose gel electrophoresis.

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2.10. PCR amplification

The pure DNA obtained by above mentioned procedure was used as template for PCR amplification. Oligonucleotide primers designed were used for amplification purpose. PCR analysis was performed using designed primers (Eurofins, Banglore). 25 µl reaction mixtures containing Deionized nuclease free water 13.2 µl, 10X PCR buffer (Supplied with enzyme) 2.5 µl, 2 mM dNTPs 2.0 µl, 25mM $MgCl_2$ 2.0 µl, forward and reverse primer 2.0 µl each, *Taq* DNA polymerase 0.3 µl, DNA sample template 1.0 µl.
The PCR amplification was carried out in a thermal cycler with initial-denaturation at 94 ºC for two min. followed by 35 cycles each consisting of denaturation at 94 ºC for 45 sec., annealing at 55 ºC for one minute followed by extension at 72 ºC for 1.30 min. and final extension at 72 ºC for 20 min. After the completion of the reaction the products were kept at 4 ºC prior to gel analysis. Amplified DNA fragments were electrophoresed in 1.2 per cent agarose gel.

3. Result

3.1 Genomic DNA Isolation

Infected leaves from both the plant were collected for genomic DNA isolation. CTAB method with certain modifications was found to be the best efficient method. Conformation of DNA was done by 0.8% agarose gel electrophoresis.

3.2. Quantification of DNA

Quantification of DNA was carried out by Bio-spectrophotometric readings and gel electrophoresis. The ratio of absorbance ranged between 1.8-2.0 which indicated that the quality of DNA was good.

3.3. Data Analysis of PCR Amplification

The preliminary screening of ND1, ND2 and PGK primers was done with sample of tomato and chilli. ND1 and ND2 primers gave satisfactory amplification among all 3 primers. Primer PGK was not amplified. ND1 and ND2 are used for further study.

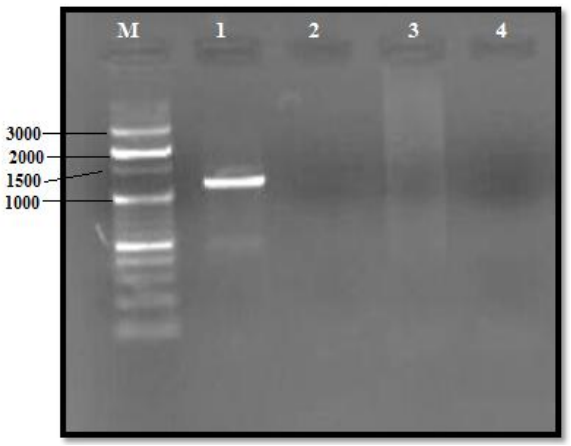

Plate 1: Tomato DNA with ND1 primer. M- Ladder, 1-Jopul, 2-Vani, 3-Bhatode & 4-Adgaon

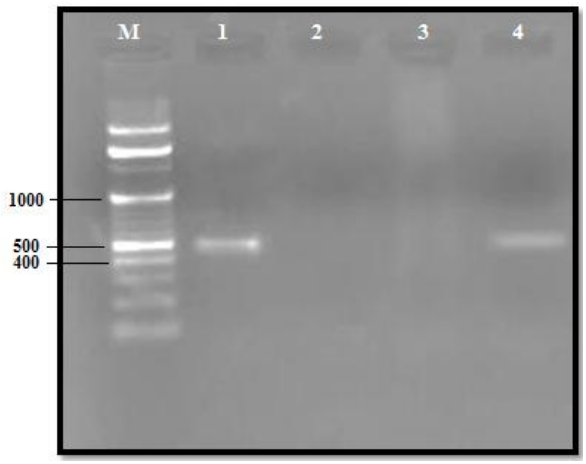

Plate 2: Chilli DNA with ND2 primer. M- Ladder, 1-Jopul, 2-Vani, 3-Bhatode & 4-Adgaon

4. Discussion

Tomato (*Solanum lycopersicum* L.) is an important and most widely grown vegetable crop in India and Chilli (*Capsicum annuum* L.) is an important cash crop in India and is grown for its pungent fruits which are used both green and ripe to impart pungency to the food.

The incidence of ToLCV has become a major limiting factor, Tomato Leaf Curl Virus Diseases (ToLCVD) have seriously hampered the cultivation and production of tomato and chilli. The incidence of ToLCVD in susceptible cultivars caused more than 90% yield loss. DNA which is isolated from curly leaves of tomato and chilli used for amplification with ToLCV specific designed primers helps in diagnosis of ToLCV disease in tomato and chilli.

Three primers were designed from the sequences of ToLCV present in different regions of India. Out of three ND1, ND2 and PGK primers, ND1 and ND2 primers showed satisfactory amplification. ND1 primer showed amplification with tomato from Jopul region. ND1 primer amplified with tomato DNA with 1310 bp distinct band seen in Plate 1 in lane 1. ND2 primer showed amplification with chilli DNA from Jopul and Adgaon region. ND2 primer amplified with chilli DNA with 500 bp band seen in Plate 2 in lane 1 and 4. PGK primer did not show amplification with any tomato or chilli sample. Amplification of designed primer from different regions of India with infected leaf sample of tomato and chilli indicates that the ToLCV is present in tomato and chilli of Nashik region in Maharashtra.

5. Conclusion

The present investigation was proposed and results are summarized here under which, focused on survey for disease incidence and identification of disease. The present study revealed that *Tomato Leaf Curl Virus* (ToLCV) detected from tomato and chilli sample by virus specific designed primers. ToLCV disease in tomato and chilli results in higher yield loss, upto 90-100 percent yield loss occurs due to ToLCV. Hence this study indicates that detection of ToLCV disease is useful to save the tomato and chilli from high range

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of yield loss at early stage.

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