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Molecular Detection and Identification of Begomovirus Isolate on Tomato from Central Region of India

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Abstract

The natural occurrence of leaf curl and blisters disease on tomato was observed at Bhopal, India in the January, 2015. The begomovirus was amplified on symptomatic tomato by polymerase chain reaction (PCR) using coat protein gene specific primers. The purified PCR product (~800 bp) was sequenced and submitted in GenBank database (KU760803) and identified by their sequence analyses. The isolate under study (KU760803) showed 97% to 99% sequence identities and closest phylogenetic relationships with various isolates of *Tomato leaf curl New Delhi virus* (ToLCNDV), therefore, the isolates under study were identified as isolates of ToLCNDV associated with leaf curl and blister disease on tomato in the first time from central region of India.

Keywords: Begomovirus; Polymerase chain reaction; Sequence analyses; Phylogenetic relationship

Introduction

The horticultural crops in India cover approximately 13.6 million hectares of arable land (7% of the gross cropped area) and contribute 18% to 20% of the gross value of India's agricultural output (FAO, 2004). India is the second largest producer of fruits and vegetables in the world.

Madhya Pradesh is a central region of India and agriculture is one of the main sectors of the state's economy. About 73% population of the state is rural, which directly or indirectly depends on the agriculture. In Madhya Pradesh soyabean, mongbean, pea, linseed, tomato, brinjal, potato, okra, chili, cucumber, cotton, and cucurbits vegetable crops are grown commercially throughout the years by the farmers. Commercially cultivated plants/crops of Madhya Pradesh have been affected by several diseases caused by bacteria, fungus, phytoplasma and viruses. Pathogens causing these diseases are still unidentified at species level

The Madhya Pradesh has plant diversity and flexible temperatures, which are favorable to virus vectors (aphids, whiteflies, leafhoppers, and planthoppers). Now a days there are no reports on virus infection on any crops or weeds on Madhya Pradesh state except one begomovirus on *J. gossypifolia* [1].

Begomoviruses of the family *Geminiviridae* that are whitefly transmitted cause diseases of important crops in the tropics and subtropics [2].

Their genome consists of one or two circular single-stranded DNA components, referred as DNA-A and DNA-B, each about 2.6 kb to 2.8 kb in size [2,3]. Monopartite Begomovirus (have DNA-A genome only) are predominantly found in the old world and are often associated with satellite DNAs (alpha- and betasatellites), which may or may not contribute to pathogenicity [4]. Some nanovirus-like DNA components known as alphasatellites (DNA-1) have also been reported with many begomovirus disease complexes [5].

The tomato (*Solanum lycopersicum* L., family *Solanaceae*) is one of the most important vegetable crops grown commercially worldwide for its edible fruits that have achieved tremendous popularity over the last two century. India is the fourth producer of tomato with an annual production of 7.6 million tons. Within India, major tomato producing states are Uttar Pradesh, Karnataka, Punjab, West Bengal and Assam (FAO, 2003).

Tomato crop is susceptible to infection by a variety of causal agents generally showing symptoms of any array of diseases mainly caused by insects, nematodes, fungus, bacteria and viruses. Of which, viruses are known to cause huge loss to productivity of the crop. Tomato cultivars are susceptible host for a wide range of DNA and RNA viruses, which cause significant economic losses. Major viruses infecting tomato includes: *Cucumber mosaic virus* (CMV); *Tomato spotted wilt virus* (TSWV), *Tomato aspermy virus* (TAV); *Tobacco mosaic virus* (TMV); *Tomato bushy stunt virus* (TBSV); *Potato Y virus* (PVY) and *Tomato leaf curl virus* (TLCV) [6-10]. Moreover, these viruses can frequently occur in mixed infections [11].

During a survey in January 2015, begomovirus-like symptoms such as severe leaf curl and blisters on tomato accompanied with reduction of leaf size and stunting of whole plant were observed in the agriculture field near at Hoshangabad road, Bhopal, Madhya Pradesh of India (Figure 1). The causal pathogen was suspected to be a begomovirus due to the large population of whitefly (*Bemisia tabaci*) observed in the area. Three symptomatic plant samples were collected for detection and identification of associated begomovirus on these plant species.

To detect begomovirus species on these symptomatic vegetable plant associated with the severe leaf curl and blisters, the total DNA was isolated from 100 mg from newly emerging symptomatic leaves by the Dellaporta method [12] method and polymerase chain reaction (PCR) was performed using a pair of begomovirus coat protein (CP) gene specific primers CPIT-T/CPIT-T [13]. PCRs was set up in a 50 µL reaction mixture containing: template DNA (100 ng), dNTPs (10 mM each), primers (each 25 pmol), *Taq* DNA polymerase (1.5

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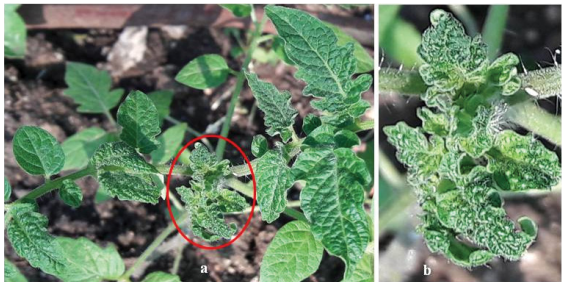

Figure 1: Naturally infected *Solanum lycopersicum* (tomato) plants exhibiting severe leaf curl, blistering reduction of leaf size and stunting of whole plant (a) and its close view (b).

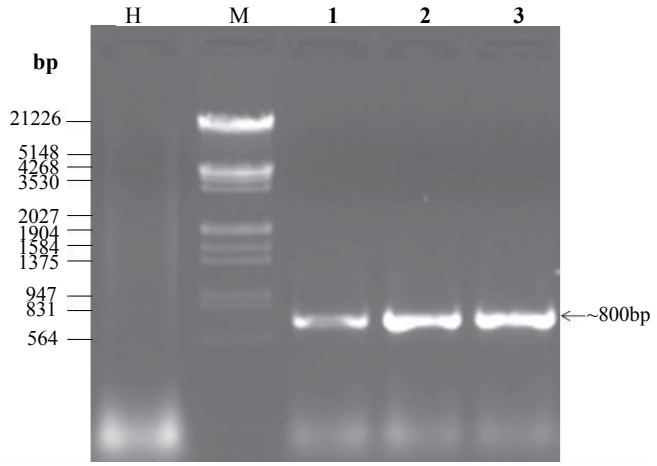

Figure 2: 1% Agarose gel electrophoresis of PCR products (~800 bp) amplified from all symptomatic tomato plants (lanes 1-3) but not in healthy one (lane H) by Begomovirus coat protein gene specific primers: CPIT-I /CPIT-T (Singh, 2005). Lane M: Lambda DNA digested with *Eco*RI/*Hin*dIII (Genei Pvt. Ltd, Bangalore, India) as marker.

U), assay buffer (5 mL 10 Meark Pvt. Ltd) and were cycled 30 times (denaturation: 94°C for 5 min; 47°C; annealing temperatures: for 1 min; extension: 72°C for 1.5 min; and final extension was 7 min.

The PCR products were analysed by 1% agarose gel electrophoresis. The PCR products showed the expected size ~800 bp amplicons in symptomatic plant samples but no such amplicons were obtained in healthy samples (Figure 2). The PCR product was purified by using PCR Clean-up System (Promega, USA) Kit and sequenced and the data obtained was submitted to NCBI GenBank database under Accession number (KU760803).

The analyses of the sequence data revealed presence of 771 nt of DNA-A genome which contained AV1 (coat protein gene) complete ORFs using the BLASTn (http://www.ncbi.nlm.nih.gov/BLAST). The nucleotide sequence data of tomato isolate under study (KU760803) shared highest 99% identity in The BLASTn with several isolates of *Tomato leaf curl New Delhi virus* (KP235542, KP235540, HM007120, HQ141673, HM3459790, FJ468356) on chilli, tomato from Pune, Plampur, Tumkur, India.

The sequence similarities of tomato isolate (KU760803) were also obtained using Genomatix DiAlign program based on its complete coat protein gene at nt and aa level and showed highest 97% to 98% sequence similarities at nt level and 97% to 99% at aa level with the several isolates of ToLCNDV (HQ141673, KP235542, KP235540, HM007120, HQ141673, HM3459790, FJ468356). However, 96% sequence similarities at nt level and 98% at aa level with the several isolates of ToLCV (AJ810365, AY691900) and 73% to 94% sequence similarities at nt level and 78% to 98% at aa level with the other Begomovirus isolates (Table 1).

During phylogenetic analysis of tomato isolate under study (KU760803) with some selected begomovirus isolates reported from India, the isolate showed closest relationships with isolates of *Tomato leaf curl New Delhi virus* (KP235542, KP235540, HM007120, HQ141673, KF515616, FJ468356, HM345979, HQ141673, JN129254, DQ272540, KU760804) on tomato, chilli and pumpkin from India and showed distinct relationships with *Tomato leaf curl virus* (ToLCV: AY691900), *Squash leaf curl China virus* (SqLCChV: AY396151, JN587811), *Papaya leaf curl virus* (PLCV: EU126822), *Tomato leaf curl Palampur virus* (ToLCPaV: KC456161, HG934859), *Tomato leaf curl Karnataka virus* (ToLCKaV: KP178731, HM803118), *Tomato leaf curl Rajasthan virus* (ToLCRajV: DQ339117), *Tomato leaf curl Bangalore virus* (ToLCBaV: GU474418, KP164859), *Chilli leaf curl virus* (ChLCV: KP868762, KM921669) and *Tomato leaf curl Gujarat virus* (ToLCGujV: KP178726, NC_004558) reported on tomato, pumpkin and papaya from India (Figure 3). Based on highest sequence similarities and close

Accession number	Abbreviated virus name	Host plant	Location in India	%[A] Nucleotide identity	%[A] Amino Acid identity
HQ141673	ToLCNDV	Tomato	Pune	98 (0.956)	99 (0.995)
KP235542	ToLCNDV	Chilli	Pune	97 (0.955)	99 (0.991)
KP235540	ToLCNDV	Chilli	Palampur	97 (0.955)	99 (0.991)
HM007120	ToLCNDV	Chilli	Tumkur	97 (0.955)	99 (0.991)
HM345979	ToLCNDV	Tomato	Pune	97 (0.951)	99 (0.992)
FJ468356	ToLCNDV	Tomato	Pune	97 (0.952)	99 (0.992)
KF515616	ToLCNDV	Tomato	Bhavnagar	97 (0.932)	98 (0.982)
JN129254	ToLCNDV	Pumpkin	New Delhi	97 (0.928)	98 (0.984)
DQ272540	ToLCNDV	Bottle gourd	New Delhi	97 (0.930)	98 (0.986)
KU760804	ToLCNDV	Chilli	Bhopal	97 (0.902)	97 (0.961)
AJ810365	ToLCV	Tomato	Rohtak	96 (0.936)	98 (0.982)
AY691900	ToLCV	Tomato	Jabalpur	96 (0.914)	98 (0.983)
AY396151	SqLCChV	Pumpkin	Lucknow	94 (0.845)	98 (0.988)
JN587811	SqLCChV	Pumpkin	New Delhi	94 (0.846)	97 (0.968)
EU126822	PLCV	Papaya	New Delhi	92 (0.801)	94 (0.931)
KC456161	ToLCPaV	Tomato	Punjab	82 (0.533)	91 (0.904)
HG934859	ToLCPaV	Tomato	Palampur	82 (0.524)	91 (0.912)
DQ339117	ToLCRajV	Tomato	Rajasthan	81 (0.558)	94 (0.935)
KP178731	ToLCKaV	Tomato	Maharahtra	79 (0.481)	93 (0.927)
HM803118	ToLCKaV	Tomato	New Delhi	79 (0.477)	93 (0.926)
KP868762	ChLCV	Tomato	Varanasi	77 (0.446)	94 (0.940)
KM921669	ChLCV	Tomato	Sonipat	75 (0.425)	92 (0.921)
KP178726	ToLCGujV	Tomato	Varanasi	76 (0.400)	89 (0.878)
NC_004558	ToLCGujV	Tomato	Varanasi	73 (0.322)	78 (0.751)
GU474418	ToLCBaV	Tomato	Bangalore	74 (0.380)	87 (0.847)
KP164859	ToLCBaV	Tomato	Tamilnandu	73 (0.372)	87 (0.846)

[A]The % identity denotes number of identical sequences (in % of shorter sequence) and value given in brackets is the similarity (relative to maximum similarity).

Abbreviations: ToLCNDV: Tomato Leaf Curl New Delhi Virus; ToLCV: Tomato Leaf Curl Virus, SqLCChV: Squash Leaf Curl China Virus; PLCV: Papaya Leaf Curl Virus; ToLCPaV: Tomato Leaf Curl Palampur Virus; ToLCKaV: Tomato Leaf Curl Karnataka Virus; ToLCRajV: Tomato Leaf Curl Rajasthan Virus; ToLCBaV: Tomato Leaf Curl Bangalore Virus; ChLCV: Chilli Leaf Curl Virus; ToLCGujV: Tomato Leaf Curl Gujarat Virus.

Table 1: Percentage identities in the coat protein gene of the virus isolate from tomato (KU760803) at nucleotide (nt) and amino acid (aa) levels with various Begomovirus isolates based on Genomatix DiAlign programme.

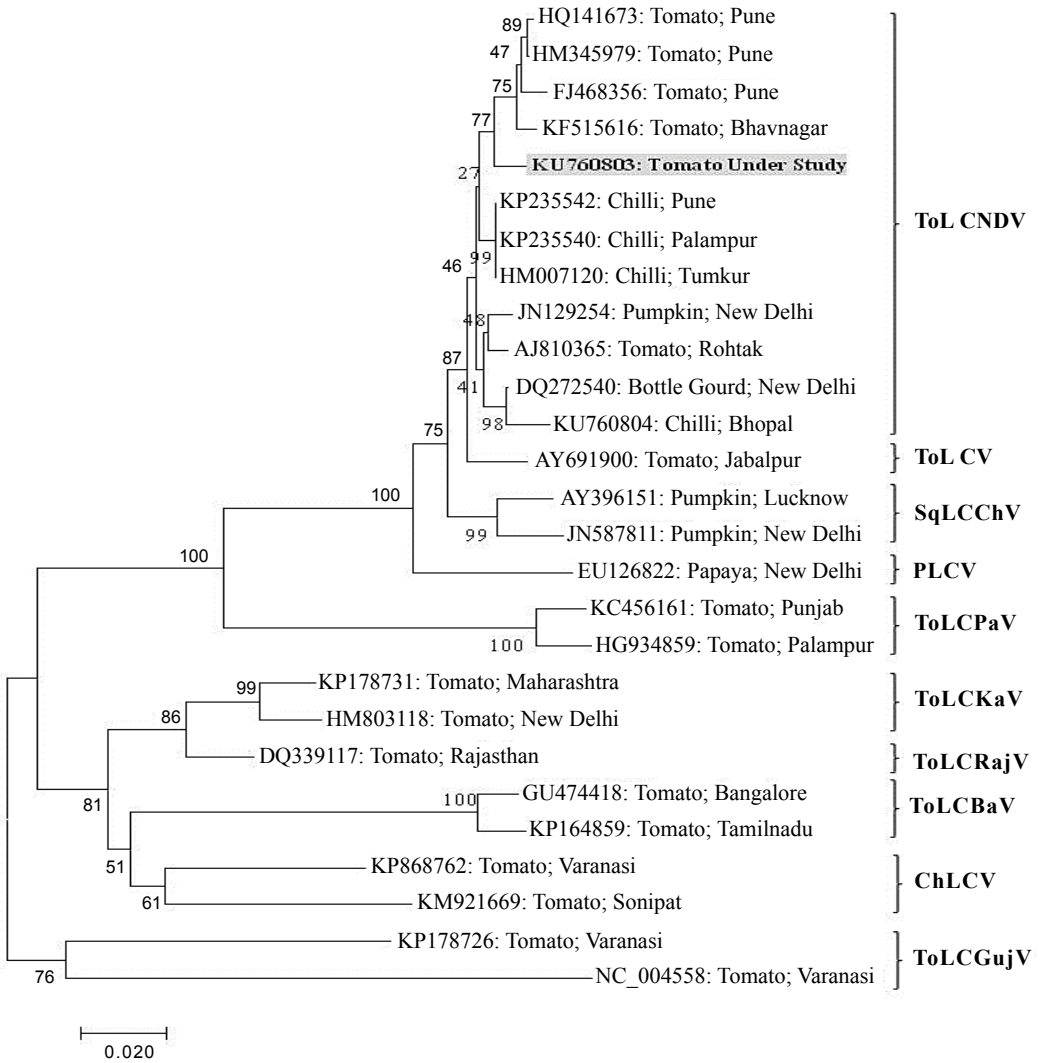

Figure 3: Phylogenetic analysis of complete coat protein (CP) gene of begomovirus isolate under study infecting tomato (KU760803) compared with various begomovirus isolates reported all over the India. The understudy begomoviruses isolate from tomato (KU760803) showed closest relationships with ToLCNDV, close relationships with ToLCVV and distinct relationships other begomovirus isolate (SqLCChV, PLCV, ToLCPaV, ToLCKaV, ToLCRajV, ToLCBaV, ChLCV: ToLCGujV) reported from tomato and other plant species. Phylogenetic analyses tree generated by Molecular Evolutionary Genetics Analysis tool (MEGA v. 7.0) with 1000 replicates bootstrapping, and the tree were generated with the Neighbour joining method and viewed by the NJ plot program.

phylogenetic relationships with ToLCNDV isolates, the Begomovirus understudy associated with leaf curl and blister disease of tomato was identified as isolates of *Tomato leaf curl New Delhi virus*.

According to a review of viruses associated with tomato, several begomovirus species like: *Cucumber mosaic virus* (CMV); *Tomato spotted wilt virus* (TSWV), *Tomato aspermy virus* (TAV); *Tobacco mosaic virus* (TMV); *Tomato bushy stunt virus* (TBSV); *Potato Y virus* (PVY) and *Tomato leaf curl virus* (TLCV) were found to be susceptible and affect the tomato cultivation in worldwide [6-10].

In India ToLCNDV, ToLCV, ToLCBaV, ToLCKaV, ToLCRajV, ToLCGujV have reported on tomato associated with leaf curl disease from North and South India, but there are no report have been found in literature from central region like Madhya Pradesh of India, however, we reported here molecular detection and identification of *Tomato leaf curl New Delhi Virus* (ToLCNDV) based on sequence analysis of its complete coat protein gene associated with leaf curl and blister disease of tomato from central region of Madhya Pradesh at first time of India.

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