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SHORT COMMUNICATION

Molecular evidence for the occurrence of tomato leaf curl New Delhi virus on chayote (*Sechium edule*) in southern India

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Abstract During 2012–2014, mosaic disease on chayote in the farmers field of Kodaikanal region (high altitude zone) of Tamil Nadu was observed. The disease was characterized with severe mosaic, cupping and enation on leaves with reduced fruit size. Disease was found to causes an yield loss of more than 60% with the maximum disease incidence of 100% for the past 5 years consecutively. Preliminary serological and molecular screening indicated the association of begomovirus with the disease. Complete nucleotide sequence and phylogenetic analysis of DNA A revealed the identity of the virus as tomato leaf curl New Delhi virus (ToLCNDV). In recombination analysis study, the major parent was identified as ToLCNDV from Pakistan infecting tomato. Thus the present finding confirms expansion of new geographical region and host for ToLCNDV causing mosaic disease on chayote from Tamil Nadu. To our knowledge this is the first confirmed report for the occurrence of ToLCNDV on chayote in southern India.

Keywords Begomovirus · Tomato leaf curl New Delhi virus · Mosaic · Rolling circle amplification · Cucurbits · Cucurbits virus

Tomato leaf curl New Delhi virus (ToLCNDV) is a member of the family *Geminiviridae* belongs to the Genus *Begomovirus*. ToLCNDV is a bipartite begomovirus constituting DNA A and DNA B as their genomic components and are efficiently transmitted by whitefly, *Bemisia tabaci* to a wide range of host plants. ToLCNDV is causing serious threats to various horticultural crops in the countries of the Asian continent viz., India, Pakistan, Bangladesh, Philippines and Thailand [5, 6, 17]. ToLCNDV was originally reported to infect solanaceous vegetables since 1980s, however in recent days it has extended its host range towards the cucurbitaceous vegetables viz., pumpkin, bottle gourd, bitter gourd, ash gourd, cucumber, ridge gourd, watermelon, long melon and ivy gourd throughout the globe [4, 9, 12, 13, 15]. Predominantly, squash leaf curl China virus (SLCCNV) and ToLCNDV are the two major begomoviruses reported on the cucurbits from India.

Chayote (*Sechium edule*) is an cucurbitaceous vegetable cultivated on a large scale in the high altitude zone (Kodaikanal region) of Tamil Nadu. During 2012–2014, a survey was conducted on mosaic diseases of cucurbits cultivated in Tamil Nadu, severe mosaic disease associated with the chayote crop, which is imposing serious threat to the crop cultivation was observed. Despite being a perennial crop, farmers are cultivating chayote as annual crop in this region because of the occurrence of this disease for the

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past 5 years consecutively. During a survey in and around the Kodaikanal region which include Thanndikudi, Thandiankudisai, Pannaikadu and Pallangi, a severe mosaic disease was recorded with a maximum disease incidence of 100% under natural field conditions. The disease was characterized by severe mosaic, cupping and enation on the leaves with reduced fruit size (Fig. 1). Farmers are facing yield loss of more than 90% because of this mosaic disease [14]. Also diseased fields were observed with enormous whitefly population. Earlier Mandal et al. [7] had recorded the associated of ToLCNDV with chayote yellow mosaic disease from Northern India. The objective of this study was to study the causal virus associated with the mosaic diseases of the chayote crop and to complete the characterization of the virus in Southern India.

Nearly thirty leaf samples showing severe mosaic symptoms were collected from the Thadiankudisai—TN TDK CHOU1 (Kodaikanal region) were subjected to the DAS-ELISA using the squash leaf curl virus (SLCV) antiserum obtained from DSMZ, Germany along with apparently healthy leaf for preliminary screening to test the association of begomoviruses. Infected leaf samples alone showed positive reaction with mean absorbance range between 0.352 and 0.486 whereas healthy samples recorded 0.103. Based on symptoms, presence of whiteflies in infected field and positive result in ELISA, association of begomovirus was suspected with the disease. Hence total DNA was extracted using CTAB method and PCR amplification has been carried out with the specific primer pair (GK ToLCV F/R) to amplify the complete coat protein of associated begomovirus [10]. The PCR resulted in the successful amplification of ~ 950 bp in the symptomatic leaves of chayote plants only but not in the non-symptomatic samples (Fig. 2). The ~ 950 bp fragment was cloned using TOPO T/A cloning kit (Invitrogen Inc., USA) and sequenced. Sequence results of 950 bp spanning complete coat protein showed that 93% identity towards the ToLCNDV (KF551576).

Rolling circle amplification (RCA) was performed to amplify the complete genome of DNA A using Ø29 DNA polymerase (Thermo Scientific, USA) following the standard protocol [16]. RCA product was digested with different restriction endonucleases (*Bam*HI, *Eco*RI, *Hind*III, and *Xba*I). The ~ 2.7 kb fragment was produced by the *Xba*I corresponding to the genome size of the begomovirus and no other enzymes (*Hind*III, *Bam*HI and *Eco*RI) produced. The *Xba*I digested linearized 2.7 kb fragment separated on agarose gel (1%) and were purified using Quick

Fig. 1 Symptoms of severe mosaic on chayote (**i** mosaic, **ii** whiteflies on leaves, **iii** enation and **iv** reduction of fruit size)

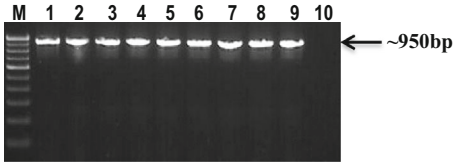

Fig. 2 PCR amplification of yellow mosaic diseased samples of chayote using specific primer pair (GK ToLCV F/R) from different locations of Kodaikanal region. M: 100 bp ladder marker, lanes 1-4: Thanndikudi, lanes 5-6: Thandiankudisai, lanes 7-8: Pannaikadu, lane 9: Pallangi, lane 10: Healthy control

gel extraction kit (Qiagen, Germany) and ligated to *Xba*I linearized pUC19 vector. The ligated product was transformed in *Escherichia coli* strain DH5α, and total of 26 colonies were obtained and all the colonies were subjected for plasmid miniprep extraction. Plasmid DNA was restricted with cloning site enzyme and *Bgl*I with an aim to release the 2.7 kb insert. From the double digestion of recombinant plasmid DNA with *Bgl*I and *Xba*I, two positive clones were obtained; both the clones were sequenced by primer walking.

The sequence of 2739 nucleotides was obtained from both the clones sharing 99.5% identity among themselves. The nucleotide sequence was initially subjected to BLAST and the sequence shared maximum identity of 91% towards ToLCNDV reported from India, Pakistan and Spain. The genome structure was determined using Vector NTI (Invitrogen Inc., USA) and the sequence was submitted in the in the NCBI database (KP191047). The analysis of the sequences shows the genome organization of the present begomovirus isolate resembling old world begomoviruses with two open reading frames (ORFs) on viral sense strand and five ORFs on the complementary sense strand.

Phylogenetic analysis of the complete DNA A nucleotide sequence of ToLCNDV isolate infecting chayote with the other ToLCNDV isolates reported from different parts of the world available in the database was determined using MEGA6.0 software (Fig. 3). Striking feature of the dendrogram is the formation of separate cluster by our isolate (TN TDK CHOU1) with the other isolates reported from Southern India and Spain infecting cucurbits and tomato. Also phylogeograph shows chayote isolate as distinct from other ToLCNDV strains. Based on sequence analysis and considering the ICTV demarcation criteria for begomovirus [3], it is evident that the present isolate of begomovirus, associated with severe mosaic disease of chayote, is an isolate of ToLCNDV.

Sequence identity matrix was generated using Bioedit Sequence Alignment Editor (version 5.0.9). Upon comparison of complete DNA A sequence with other ToLCNDV isolates, it was found to exhibit more than 92% identity with ToLCNDV (KT426903 and KT426906) reported from Karnataka (Supplementary Table 1). Similarly, different ORFs on DNA A such as AV1, AV2, AC2, AC3 and AC5 were showing maximum identity with ToLCNDV infecting ridge gourd from Karnataka. Whereas ORF AC1 was having maximum identity (92.5%) with ToLCNDV (KP235541) from Tamil Nadu infecting chilli and ORF AC4 had 96% identity with other Indian isolates reported on poppy, cucumber, chilli and tomato (KC513822, KC545812, HM007113 and U15016, respectively). The inter-generic region between ORF AV1 and AC1 showed a maximum identity of 89.8% with ToLCNDV (KT426906) (Supplementary Table 1).

The recombination detection programme (RDP4) tool was for detection of probable recombination events, identification of likely parents and localization of possible recombination breakpoints. The analysis was performed with default settings using a 0.05 *P* value cutoff [8]. Two algorithms, GENCONV (P 1.243×10^{-01}) and SiScan (P 7.510×10^{-37}) showed the intraspecific recombination and identified as a ToLCNDV variant. One recombinant fragment (coordinates 40–72 in the IR region) was detected for ToLCNDV-infecting chayote in Tamil Nadu (TN TDK CHOU1). ToLCNDV-PkT5/6 (AF448058) from Pakistan infecting Tomato was indicated as the major parent, and ToLCNDV-RG5 (KT426907) infecting ridge gourd in Karnataka was minor parent. The recombination analysis results suggest that the ToLCNDV (TN TDK CHOU1) isolate evolved from the Pakistan strain and Indian strain by recombination.

In the present study, we report the association of ToLCNDV on the natural occurrence of severe mosaic disease on chayote based on the complete DNA-A sequence for the first time from Southern India. There are reports that showed ToLCNDV (DNA A and B) cause disease in a severe form upon association with DNA β molecule [18]. Since *Xba*I alone produced ~ 2.7 kb fragment in the restriction the digestion, attempt was made for the detection of DNA B and satellites (DNA α and DNA β) through PCR analysis. DNA B was detected in the samples with the two primer pairs (ToLCBD971F 5′-GTGGCAGAACGCCACCATGAACG-3′; ToLCBD2142R 5′-GCTGCGCGGCCAATATGTCAA TAG-3′ and ToLCBD2081F 5′-GCGTACTCWACGCG CTCAGATTG-3′/ToLCBD656R 5′-GTGTTTCACAGA TTTCCTTACGCG-3′) as described by Venkataravanappa et al. [19]. All the tested samples were found to be positive for the presence of DNA B by producing amplification of ~ 1.2 kb with both the primer pairs. Similarly, samples tested were negative in the PCR analysis for the association of α-satellite and β-satellite with the universal primer pairs UN101/UN102 and β01/β02, respectively [1, 2]. The natural occurrence of mosaic disease on chayote in severe form is observed in the successive years under the farmer's field at lower Palani Hills region (Kodaikanal) of Tamil Nadu. Distribution of ToLCNDV on tomato was mainly observed

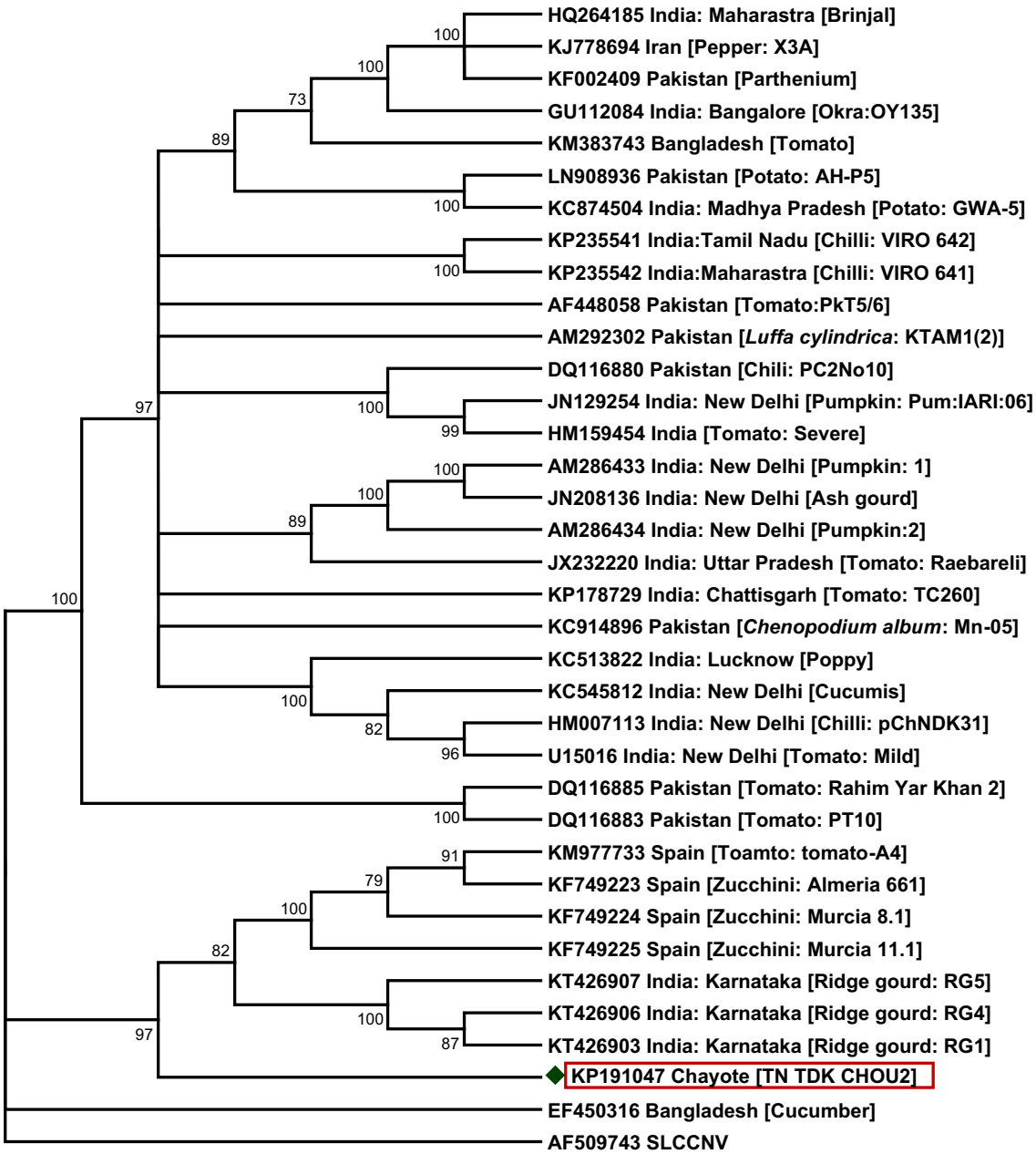

Fig. 3 Phylogenetic analysis of ToLCNDV (TN TDK CHOU1) isolate infecting chayote in Tamil Nadu based on complete DNA A nucleotide sequences with other ToLCNDV isolates. The trees were constructed using the NJ algorithm implemented by MEGA 6.0 with SLCCNV as an outgroup. The bootstrap consensus tree values from 1000 replicates are given at the branch nodes. Branches corresponding to partitions reproduced in less than 70% of bootstrap replicates are collapsed

in North India while in southern part it is present on cucurbits. Patil et al. [11] reported occurrence of ToLCNDV on ridge gourd from South India causing ridge gourd yellow mosaic disease. Similarly [9, 10] had also documented ToLCNDV on bitter gourd and pumpkin. Previously [7] reported the occurrence of ToLCNDV on chayote from North Western India only based on the partial coat protein gene and the present study further confirms the occurrence of ToLCNDV on chayote leading to major threat for the cultivation of chayote in Southern India.

Roy et al. [13] reported that the natural occurrence of ToLCNDV on ash gourd in North India. It found to have its perpetuation even in the absence of main host and its subsequent spread to tomato. In North India, cucurbits are grown mainly during the *Kharif* season as sowing time of tomato coinciding the harvesting of cucurbits. But in Tamil Nadu, cucurbits are cultivated throughout the year concurrently with the tomato. In the epidemiological point of view, the occurrence of ToLCNDV on cucurbits is easily

transmitted by the whitefly from the tomato crops grown near to the cucurbits field. Hence ToLCNDV in Tamil Nadu might be simultaneously perpetuating on both the tomato and cucurbits crops without any interruption. This finding will be helpful in deriving the management strategy for the begomovirus infection on cucurbits.

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References

1. Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID, Dhawan P, Rishi N, Siwatch SS, Abdel-Salam AM, Brown JK. Diversity of DNA β, a satellite molecule associated with some monopartite begomoviruses. Virology. 2003;312:106–21.
2. Bull SE, Briddon RW, Markham PG. Universal primers for the PCR-mediated amplification of DNA 1: a satellite-like molecule associated with begomovirus-DNA β complexes. Mol Biotechnol. 2003;23:83–6.
3. Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X. Geminivirus strain demarcation and nomenclature. Adv Virol. 2008;153:783–821.
4. Ito T, Sharma P, Kittipakorn K, Ikegami M. Complete nucleotide sequence of a new isolate of *Tomato leaf curl New Delhi virus* infecting cucumber, bottle gourd and muskmelon in Thailand. Adv Virol. 2008;153:611–3.
5. Kon T, Dolores M, Bajet NB, Hase S, Takahashi H, Ikegami M. Molecular characterization of a strain of *Squash leaf curl China virus* from the Philippines. J Plant Pathol. 2003;15:535–9.
6. Mandal B. Emerging geminiviral diseases and their management. In: Sharma P, Rajarshi KG, Ikegami M, editors. Emergence of begomovirus diseases in cucurbits in India. New York: Nova Science Publishers Inc.; 2010. p. 167–81.
7. Mandal B, Mandal S, Sohrab SS, Pun KB, Varma A. A new yellow mosaic disease of chayote in India. Plant Pathol. 2004;53:797.
8. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol. 2015;1:vev003. doi:10.1093/ve/vev003.
9. Nagendran K, Mohankumar S, Manoranjitham SK, Karthikeyan G. Molecular detection and characterization of *Tomato leaf curl New Delhi virus* causing mosaic disease on bitter gourd in Tamil Nadu, India. Trends Biosci. 2014;7(23):3925–31.
10. Nagendran K, Kumar SM, Priyanka R, Karthikeyan G. Molecular evidence for the occurrence of tomato leaf curl New Delhi virus and squash leaf curl China virus on volunteer pumpkin plants in Tamil Nadu. Trends Biosci. 2014;7(24):4308–11.
11. Patil CV, Ramdas SV, Premchand U, Shankarappa KS. Survey, symptomatology, transmission, host range and characterization of begomovirus associated with yellow mosaic disease of ridge gourd in southern India. Virus Dis. 2017;28(2):146–55.
12. Phaneendra C, Rao KRSS, Jain RK, Mandal B. *Tomato leaf curl New Delhi virus* is associated with pumpkin leaf curl: a new disease in northern India. Indian J Virol. 2012;23(1):42–5.
13. Roy A, Spoorthi P, Panwar G, Bag MK, Prasad TV, Kumar G, Gangopadhyay KK, Dutta M. Molecular evidence for occurrence of *Tomato Leaf Curl New Delhi Virus* in ash gourd (*Benincasa hispida*) germplasm showing a severe yellow stunt disease in India. Indian J Virol. 2012; doi:10.1007/s13337-012-0115-y.
14. Sangeetha B, Malathi VG, Alice D, Renukadevi P, Suganthy M. Suppression of severe mosaic disease in Chayote (Chow-chow) *Sechium edule*. Sacq. Sw. J Antivirus Antiretrovirus. 2016;8(5):92.
15. Shorab SS, Mandal B, Ali A, Varma A. Molecular diagnosis of emerging begomovirus diseases in cucurbits occurring in northern India. Indian J Virol. 2006;17:88–95.
16. Singh MK, Haq QMR, Singh K, Mandal B, Varma A. Molecular characterization of *Tobacco leaf curl Pusa virus*, a new monopartite begomovirus associated with Tobacco leaf curl disease in India. Virus Genes. 2011;43:296–306.
17. Tahir M, Haider MS. First report of *Tomato leaf curl New Delhi virus* infecting bitter gourd in Pakistan. Plant Pathol. 2007;54:807.
18. Usahrani KS, Srivastava A, Padmalatha KV, Malathi VG. First report of the association of a defective satellite DNA ß molecule with a bipartite genome begomovirus causing potato leaf curl disease in India. J Plant Pathol. 2004;86:177–80.
19. Venkataravanappa V, Reddy CL, Jalali S, Reddy MK. Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. Virus Genes. 2012;44(3):522–35.