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GENETIC DIVERSITY OF TOMATO LEAF CURL VIRUSES (BEGOMOVIRUS COHENI) INFECTING TOMATO

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ABSTRACT

Background: Sequence analyses and phylogenetic comparison indicated that, the five isolates of B. coheni might have evolved by recombination between viruses related to two or more viral ancestors. The isolates were different from north Indian isolates, with variability even within isolates collected from a geographical location, indicating that, there will be continuous variability in geminiviruses.

Objective: To study the genetic diversity of ToLCD belongs to a group of closely related begomoviruses that emerged in the regions of Tamil Nadu, India, and to identify and determine their molecular variability.

Methods: Infected plant samples of ToLCV were collected from S. lycopersicum plants expressing typical symptoms of leaf curling, stunting, distortion, interveinal yellowing and necrosis of older leaves from tomato growing areas of Salem, Erode, Coimbatore, Madurai and Tirunelveli districts of Tamil Nadu. Totally 11 villages were surveyed randomly during 2010-2012 and the plant samples were immediately frozen in liquid nitrogen and stored at -80°C for further studies.

Results: The ToLCD symptoms varied from place to place and were exhibiting mild to severe leaf curling, reduction in leaf size and shortening of internode, upward curling, stunted growth, reduction in leaf size and puckering. The PCR was performed using ToLCV specific primers and fragment length of ~2.4 kb size obtained in 15 of 19 samples tested to confirm the presence of ToLCV. Based on the above work the ToLCV specific primers were designed and analyzed for further study. The complete nucleotide sequences (~2.7 kb) of selected isolates amplified by PCR were found to be monopartite genome and identified as 2 species of Tomato leaf curl viruses. On the basis of nucleotide similarity (89%) the virus from Tomato leaf curl Karnataka virus-CBE 1 and Tomato leaf curl Karnataka virus-SA1 of Tamil Nadu and virus from Tomato leaf curl Gujarat virus- EDE1, Tomato leaf curl Gujarat virus-TRN1 and Tomato leaf curl Gujarat virus-MDU1 have been identified ToLCKV and ToLCGV. The proposed name is ToLCKV and ToLCGV.

Conclusion: This study is a report that reveals the presence of new strains of geminiviruses infecting tomato plants, which have single genomic component and molecular diversity of Tamil Nadu region. Transcription of this single component produces all gene products required to support a complete cycle of infection and transmission of the virus.

KEYWORDS: Disease incidence, genome organization, monopartite genome, phylogenic relationship, Solanum lycopersicum, B. coheni.

INTRODUCTION

Tomato (Solanum lycopersicum L.) is an important vegetable crop widely grown in tropical and sub-tropical regions of the world. In India, documented upto 11% of global tomato production and yielding upto 20.33 million metric tons from 848,710 hectares [Naveen et al., 2025). Several diseases were reported including fungi, bacterial, viral and nematode infections to cause severe yield loss in tomato crop worldwide. Among them Tomato Leaf Curl Disease (ToLCD), caused by Begomovirus coheni, (Tomato leaf curl virus (ToLCV)) (genus Begomovirus, family Geminiviridae, is a major constraint leading to severe yield and quality loss in tomato. The disease is widespread and is a major problem in tomato crop causing yield loss ranging from 27 to 100% depending on the stages of infection (Muniyappa et al., 2000, Prasanna et al., 2015) and are infected with more than 100 monopartite and bipartite begomoviruses in the globe (Souza et al. 2022). However, more than 13 species of begomovirus associated with Tomato Yellow Leaf Curl Disease (TYLCD) is major constraint to tomato production (Yan et al. 2021). Genus Begomovirus, family Geminiviridae, were characterized by their circular and single-stranded DNA (ssDNA). These viruses normally appear as incomplete icosahedral particles and are transmitted through whitefly (Bemisia tabaci Genn.) in a persistent manner (Al-Matroushi et al., 2024).

Geminiviruses are a diverse group of plant viruses with circular single-stranded DNA genome composed of 1 or 2 components of 2700-3000 bp length, DNA-A and DNA-B, which are encapsidated within twinned icosahedral particle of 20×30 nm (Briddon et al., 2003). B. coheni is characterized either as a bipartite genome (DNA-A and DNA-B) or

monopartite genome (DNA-A) (Stanley et al., 2005). The DNA-A and DNA-B are approximately 2.7 kb in size and contain a ~220 bp common region. Based on genome arrangement and biological properties, geminiviruses are classified into 1 of 7 genera; Becurtovirus, Begomovirus, Curtovirus, Eragrovirus, Mastrevirus, Topocuvirus and Turncurtovirus. The bipartite begomoviruses, Tomato yellow leaf curl virus (TYLCV), Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato yellow leaf curl Malaga virus (TYLCMalV) were reported as causal agents of TYLCD (Varma and Malathi, 2003). Four distinct begomoviruses reported to infect tomato crop in India are: Tomato Leaf Curl Karnataka Virus (ToLCKV), Tomato Leaf Curl Bangalore Virus (ToLCBV), Tomato Leaf Curl Gujarat Virus (ToLCGV) and Tomato Leaf Curl New Delhi Virus (ToLCNDV). Also, other tentative begomovirus species seems to be present such as Tomato Leaf Curl India Virus, Tomato leaf curl Rajasthan virus (ToLCRV), Tomato leaf curl Palampur virus (ToLCPaV) and Tomato leaf curl Pune virus (ToLCPuV) (Sahu et al., 2012). TYLCV has three open reading frames (ORFs) in the virion sense, comprising V1 (coat-protein: CP), V2 (pre-coat protein) and V3 (Gong et al. 2021), and six ORFs in the non-virion sense: C1 (replication protein: Rep), C2 (transcriptional activator protein: TrAP), C3 (replication enhancer protein: REn), C4, C5 and C7 (Liu et al. 2023; Zhao et al. 2022). TYLCV proteins, including Rep, TrAP, C4, C5, and C7, play key roles in symptom development, viral movement, pathogenicity and suppression of RNA silencing (Yan et al. 2021). A non-coding intergenic (IR) region is present in the virus genome that assists in the virus replication and transcription. It includes an evolutionarily conserved nonanucleotide ori sequence (TAATATT/AC) located in the stem-loop forming region (Fiallo-Olive and Navas-Castillo 2023).

Monopartite genomes are homologous to the DNA-A of bipartite geminivirus and all viral factors required for viral replication, encapsidation, transmission, and systemic spread are encoded in this genome (Ahmed et al., 1991). Monopartite begomoviruses have been associated with a novel DNA component, the betasatellite, which is approximately half the genome size of the helper virus (Pandey et al., 2010). The first monopartite genomic sequence of Bangalore strain of ToLCBV DNA-A was reported by Hong and Harrison (1995). Similarly, two other sequences have also been reported from Bangalore (Muniyappa et al., 2000). Kirthi et al. (2002) confirmed that ToLCV from south India is only monopartite, and the DNA-A component consists of 6 open reading frames (ORF), 2 virion strands (AV1 and AV2) and 4 complementary strands (AC1, AC2, AC3, AC4) The discovery of ToLCV in the Indian subcontinent, together with its spread, has aggravated the ToLCD situation. A study by Hou and Gilbertson, 1996, towards identifying and understanding the molecular variability and the distribution of TYLCV, a threat to tomato production in Asia and Africa. Generation of genetic diversity in viral populations provides novel opportunities for adaptation to new hosts and changing environmental conditions. Three major forces drive the evolution of viruses viz., mutation, recombination, and re-assortment. Recombination occurs in RNA and DNA genomes and plays a major evolutionary role (Monci et al., 2002).

TYLCV has evolved within populations (intra-or interspecies) due to its rapid mutation rate and the ability of the parental viruses to recombine genetically (Duffy and Holmes 2008; Marchant et al. 2020). These evolutionary factors can lead to the emergence of novel viral genomes or variants, which are subsequently influenced by natural selection, genetic drift and gene flow (García-Arenal and Zerbini 2019; Yan et al. 2021). Based on the information this work was conducted to study the genetic diversity of ToLCD belongs to a group of closely related begomoviruses that emerged in the region of Tamil Nadu, India, to identify and determine their molecular variability.

MATERIALS AND METHODS

Viral sources

Infected plant samples of ToLCV were collected from S. lycopersicum plants expressing typical symptoms of leaf curling, stunting, distortion, interveinal yellowing and necrosis of older leaves from tomato growing areas of Salem, Erode, Coimbatore, Madurai and Tirunelveli districts of Tamil Nadu. Totally 11 villages were surveyed randomly during 2010-2012 (August to December) and the plant samples were immediately frozen in liquid nitrogen and stored at -80°C for further studies.

Total DNA extraction, cloning and sequencing

Nucleic acids were extracted from samples following the GEM-CTAB method (Rouhibakhsh et al., 2008) using 2% β-mercaptoethanol. Viral DNA was initially amplified by PCR using PTV FP: 5’ AGG ACA TGC GAA CCA ATC AT 3’ and PTV RP: 5’CAC TTC CAA AAA ATT TAT TAG AAT TTG3’) and polymerase chain reaction (PCR) was performed for 35 thermocycles with the steps: initial denaturation 94°C for 3 min; denaturation for 30 sec at 94°C; an annealing step for 1 min at 47.3°C; an extension step for 3 min at 72°C, and final extension for 10 min at 72°C. Amplificons of PCR products ~2.4 kb were detected by 1.2% agarose gel electrophoresis. For cloning, the DNA fragments of the PCR were purified with QIAquick Gel Extraction Kit - Qiagen, and cloned into pGEM-T easy vector (Promega; Madison, WI) according to manufacturer instructions. Positive clones were sequenced by primer walking method and analyzed with Blastn program (NCBI, Bethesda, Maryland). Multiple sequence alignment was done with Clustal W (Thompson et al., 1994).

According to the determined sequences, abutting primer pairs FLTV-FP/FLTV-RP were designed and used to amplify the complete viral DNA genome of CBE1, SA1, EDE1, TRN1 and MDU1. The adjoining primers (FLTV: FP 5' GGATCCATTGTTAAACGAGT 3’ and FLTV: RP 5'GGATCCCACATAGTGCGA 3') had a BamHI restriction site at 5’ for excising the full-length genome by restriction digestion. Full length Tomato leaf curl viruses from Salem, Erode, Coimbatore, Madurai and Tirunelveli, strains were detected from genomic DNA amplified by PCR using a high-fidelity DNA polymerase enzyme, Phusion™ from finnzymes. The PCR conditions were initial denaturation 98°C for 2 min; 35 cycles consisting each of a denaturation step for 10 sec at 98°C; an annealing step for 30 sec at 57.1°C; an extension step for 1 min 30 sec at 72°C, and a final extension for 10 min at 72°C.

The PCR products of expected sizes were purified by QIAquick Gel Extraction Kit - Qiagen and cloned into pTZ57R/T using a T/A cloning kit (MBI, Fermentas, Amherst, NY) according to manufacturer instructions. Positive colonies were screened by colony PCR followed by plasmid isolation and restriction digestion. The complete genome sequences of ToLCV isolates (Coimbatore, Erode, Salem, Madurai and Tirunelveli districts of Tamil Nadu, India) were commercially sequenced by primer walking method. All ORFs that could potentially express proteins were used in protein-protein BLAST (BLASTp) (Altschul et al., 1990) searches of complete sequences was carried out using the NCBI database and BLAST program to identify homologous sequences.

Sequence analysis

Sequence data were edited using BioEdit (ver. 7.0) software to derive a consensus sequence for each virus and aligned with a multiple alignment program ClustalX, (ver. 1.81) (Vaucheret, 2006). Conserved domains were identified using the blastp search tool. Phylogenic trees were constructed using the neighbor-joining method using MEGA, ver. 3.1 (Rodriguez-Negretel et al., 2008). Begomovirus sequences were retrieved from the GenBank database (http://www.ncbi.nlm.nih. gov/entrez) for multiple sequence analyses (Table 1).

Table 1. Begomovirus sequences retrieved from the GenBank database for multiple sequence analyses.

Virus isolates	GenBank accession
Tomato leaf curl Gujarat virus (ToLCGV [Nepal])	AY234383
Tomato leaf curl Gujarat virus (ToLCGV [Xant])	FR819708
Tomato leaf curl Gujarat virus (ToLCGV [Dhanbad])	EU573714
Tomato leaf curl Gujarat virus (ToLCGV [Kelloo])	AF449999
Tomato leaf curl Gujarat virus (ToLCGV [Varanasi])	AY190290
Tomato leaf curl Gujarat virus (ToLCGV [Ramgarh])	GQ994098
Tomato leaf curl Karnataka virus (ToLCKV [IKB3])	HM851186
Croton yellow vein mosaic virus (CrYVMV)	EU727086
Papaya leaf curl China virus (PaLCuCNV)	AJ876548
Papaya leaf curl China virus (PaLCuCNV)	DQ641700
Tomato leaf curl Bangalore virus (ToLCBV [Ban5])	AF295401
Chilli leaf curl virus (CLCV)	JN663866
Indian cassava mosaic virus (ICMV [Ker 2])	AJ575819
Sri Lankan cassava mosaic virus (SLCMV [Attur])	KC424490
Mestha yellow vein mosaic virus (MeYVMV [IN:bar 2:06])	EF428256
Bhendi yellow vein mosaic virus (BYVMV)	NC003418
Cotton leaf curl virus (CLCuV)	NC017827
Tomato leaf curl New Delhi virus (ToLCNDV)	FJ468356
Tomato leaf curl New Delhi virus (ToLCNDV [Severe])	U15015
Mungbean yellow mosaic virus (MYMV [Namakkal])	DQ865201
Mungbean yellow mosaic virus (MYMV)	DQ400848
Mungbean yellow mosaic virus (MYMV [Vigna])	AJ132575
Mungbean yellow mosaic virus (MYMV Soybean])	AJ421642
Bean golden yellow mosaic virus (BGYMV)	D00201
Tomato leaf curl Iran virus (ToLCV [Iran])	AY297924
Tomato leaf curl Karnataka virus (ToLCKV [IKH12])	HM803118
Tomato leaf curl virus (ToLCV [Bangalore])	U38239
Tomato leaf curl Karnataka virus (ToLCKV [Bangalore])	FJ514798

RESULTS

Identification, detection and molecular characterization of Tomato leaf curl viruses

The incidence of ToLCV was found to be presented in almost all surveyed regions of Coimbatore, Erode, Madurai, Salem, Dharmapuri and Tirunelveli districts of Tamil Nadu, India. Maximum ToLCD incidence of 95.3% was reported from Dharmapuri district followed by Anthiyur of Erode district (92.6%), Gopinathampatti of Dharmapuri district (91.4%) and a minimum ToLCD incidence of 32.6% was reported from experimental fields at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India (Fig. 1).

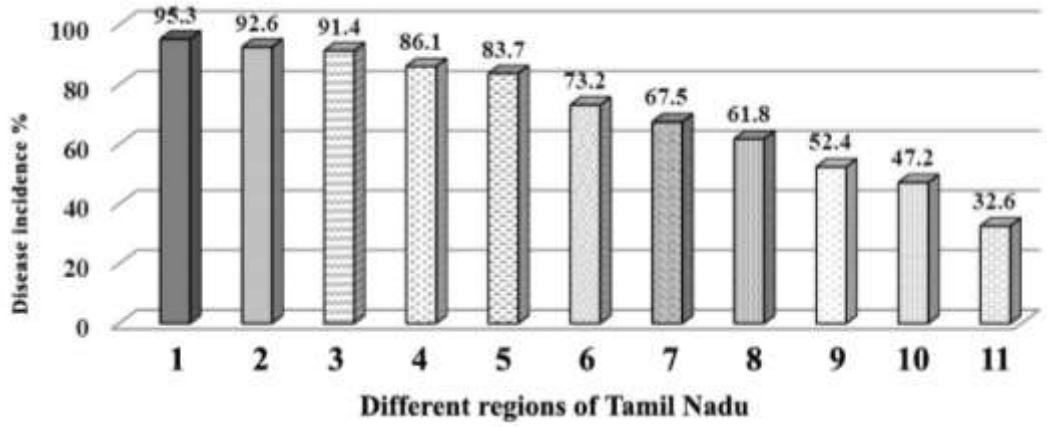


Fig.1. Incidence of Tomato leaf curl disease (ToLCD) in tomato growing areas of Tamil Nadu.

Y-axis indicates the disease incidence from 0-100 in percentage and X-axis physically separated from each other is indicated by a number, i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 representing the locations, i.e., Dharmapuri-1, Erode-2, Dharmapuri-3, Tirunelveli-4, Madurai-5, Salem-6, Coimbatore-6, Tirunelveli-7, Coimbatore-8, Coimbatore-9, Coimbatore-10, Coimbatore-11.

The ToLCD symptoms varied from place to place and were exhibiting mild to severe leaf curling, reduction in leaf size and shortening of internode, upward curling, stunted growth, reduction in leaf size and puckering (Table 2).

Table 2. Symptoms of ToLCD observed during the surveillance in different parts of Tamil Nadu.

Village	District	Cultivars and hybrids	Symptom variation
Marappanaykkanpatti	Dharmapuri	Ruchi gold	Severe leaf curling and yellowing, stunted growth and reduction in leaf size
Anthiyur	Erode	Ruchi gold	Severe leaf curling and yellowing, stunted growth
Gopinathampatti	Dharmapuri	Ruchi gold	Severe leaf curling, stunted growth and shortening of internodes
Valliyur	Tirunelveli	COLCRH 3	Upward curling and yellowing, stunted growth and reduction in leaf size
Usilampatti	Madurai	PKM1	Severe leaf curling and reduction in leaf size
Potaneri	Salem	US618	Upward leaf curl and reduction in leaf size
Madhampatti	Coimbatore	Baghyavan	Leaf curling and puckering symptoms and reduction in leaf size
Puliankudi	Tirunelveli	COLCRH 3	Mild leaf curling, reduction in leaf size, upward curling
Thondamuthur	Coimbatore	DT	Mild leaf curling, upward curling and yellowing,
Narasipuram	Coimbatore	Baghyavan	Leaf curling and stunted growth
TNAU campus	Coimbatore	CO3	Mild leaf curling

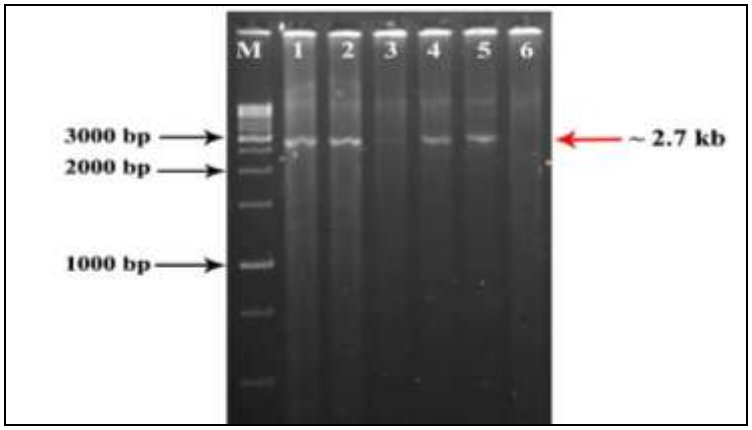
The ToLCV specific primer pair (PTV1FP and PTV1RP) (Table 3) was designed from the conserved region of full-length sequences of ToLCV collected from GenBank database (Accession number: HM851186). The PCR was performed using ToLCV specific primers and fragment length of ~2.4 kb size obtained in 15 of 19 samples tested to confirm the presence of ToLCV. The PCR product from Madhampatti, Coimbatore district, was purified using QIA quick Gel extraction kit (QIAGEN, Valencia, CA), ligated with linearized pGEMT-Easy vector and sequenced. The sequence analysis of Madhampatti isolate indicated 92% identity with ToLCV Iran isolate (AY297924), 91% with ToLCKV (HM803118) and 89% with ToLCNDV (DQ629701).

Table 3. Details of clones and their features.

DNA-A component							
Clone ID	GenBank accession no.	Coding region and their Nucleotide coordinates					
		ORF AV1 (CP)	ORF AV2 (Pre-CP)	ORF AC1 (Rep)	ORF AC2 (TrAP)	ORF AC3 (REn)	ORF AC4 (PTGS)
CBE1	KC713784	304–1074	144–491	2608–1523	1620–1216	1475–1071	2451–2158
EDE1	JX547015	305–1075	145–492	2609–1524	1621–1217	1476–1072	2452–2159
SA1	KF612317	304–1074	144–492	2611–1526	1623–1222	1484–1077	2454–2161
TRN1	KF612318	303–1073	143–490	2610–1525	1622–1215	1477–1070	2453–2160
MDU1	KF612319	303–1073	143–490	2610–1525	1622–1215	1477–1070	2453–2160

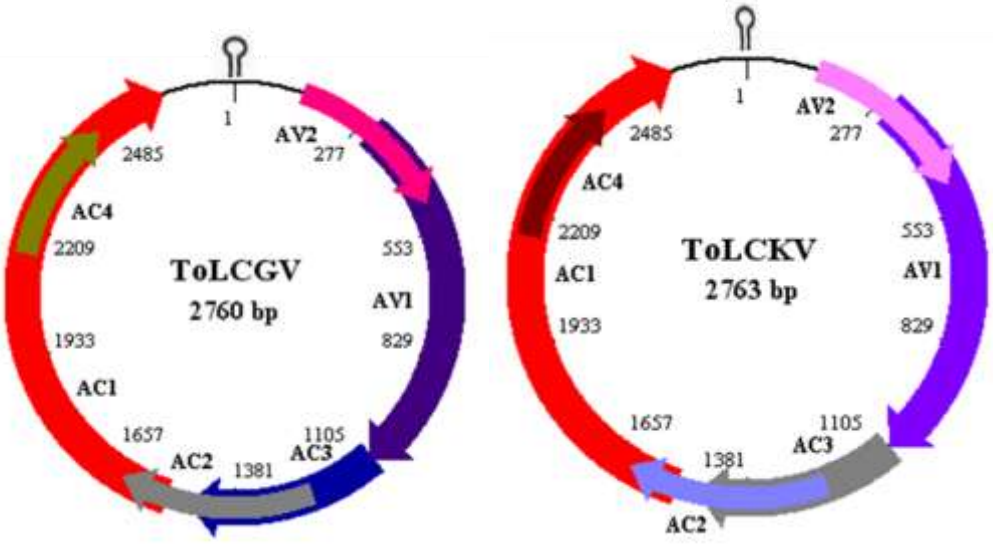
Based on the above work the ToLCV specific primers were designed and analyzed for further study. The complete nucleotide sequences (~2.7 kb) of selected isolates amplified by PCR were found to be monopartite genome and identified as two species of Tomato leaf curl viruses (Fig. 2). The genome length of 2760-2763 nucleotides was referred as DNA-A since they exhibit maximum homology to the DNA-A of formerly characterized begomoviruses. The complete nucleotide sequences (~2.7 kb) from Anthiyur (Erode), Madhampatti (Coimbatore), Potaneri (Salem), Puliankudi (Tirunelveli) and Usilampatti (Madurai), designated as EDE1, CBE1, SA1, TRN1 and MDU1, and sequences of isolates were submitted to NCBI under the accession numbers JX547015, KC713784, KF612317, KF612318 and KF612319. DNA-A sequence of each isolates contained the complementary sense ORFs AC1, AC2, AC3 and AC4 and 2 virion sense ORFs (AV1, AV2) represented as a common genome map of the virus (Fig. 3). Sequence comparisons of genomes and phylogenetic analysis indicated they were more closely related to Old World geminiviruses. Although symptoms were severe, no DNA-B, or betasatellite were found to be associated with the new begomovirus species detected.

Fig. 2. Full length amplification of ToLCV genome in samples collected from tomato growing areas of Tamil Nadu.



Lane M : 1 kb ladder
Lane 1 : ToLCV-EDE1 (Erode)
Lane 2 : ToLCV-CBE1 (Coimbatore)
Lane 3 : ToLCV-SA1 (Salem)
Lane 4 : ToLCV-TRN1 (Tirunelveli)
Lane 5 : ToLCV-MDU1 (Madurai)

Fig. 3. Identified common genome map of the ToLCGVs and ToLCKVs.



Sequence analysis of leaf curl viruses the genomes of Tomato and comparison with other begomoviruses

Sequences EDE1, CBE1, TRN1, SA1 and MDU1 were assembled to identify the DNA-A component and sequences were analyzed individually in BLASTn program. Sequence analysis indicated that clones CBE1 and SA1 had 92% similarity with Tomato leaf curl Karnataka virus (HM851186); EDE1, TRN1, and MDU1 had 95-96% identity with Tomato leaf curl Gujarat virus (AY234383). On the basis of threshold value of 89% set for begomovirus species demarcation by the Geminivirus taxonomy study group (Kings et al., 2011), virus isolates were identified as belonging to the species Tomato

leaf curl Karnataka virus (ToLCKV) and Tomato leaf curl Gujarat virus (ToLCGV) which shared 88% similarity to each other. Clones were designated as ToLCKV-CBE1 (KC713784); ToLCKV-SA1 (KF612317); ToLCGV-EDE1 (JX547015); ToLCGV-TRN1 (KF612318) and ToLCGV–MDU1 (KF612319). On the basis of nucleotide similarity (89%) the virus from Tomato leaf curl Karnataka virus-CBE 1 and Tomato leaf curl Karnataka virus-SA1 of Tamil Nadu and virus from Tomato leaf curl Gujarat virus- EDE1, Tomato leaf curl Gujarat virus-TRN1 and Tomato leaf curl Gujarat virus-MDU1 have been identified as ToLCKV and ToLCGV. The proposed name is ToLCKV and ToLCGV.

The complete nucleotide sequences of DNA-A of Tomato leaf curl isolates had 2760 to 2763 bp and submitted under the GenBank accession numbers and details of coding regions were represented (Table 3). Sequence analysis indicated that, the genome organization was typical of Old World monopartite begomovirus with two ORFs on the viral sense strand; the ORF AV1 (~30 kDa) encodes coat protein (CP) which overlaps with the small ORF AV2 (~13 kDa). The ORFs seen on the complementary sense strand are ORF AC1 (Replication initiator protein, Rep ~40 kDa), ORF AC2 (Transcription activation protein, TrAP ~15 kDa), ORF AC3 (Replication enhancer protein, REn ~15 kDa) and ORF AC4 (PTGS suppressor, ~11 kDa). The intergenic region (IR) separating divergent transcription units on both components harbors the conserved stem-loop structure found in all geminiviruses and the conserved TAATATTAC nonanucleotide sequence that contains the nicking site for initiation of virion-sense DNA replication (Fig. 2) (Stanley, 1995). This region contains a series of cis-acting elements involved in DNA replication and transcription of the AC1 gene (Arguello-Astrorga et al., 1994). All elements are present in the sequences of all isolates of ToLCGVs and ToLCKVs including: (1) an inverted repeat and direct repeats (binding sites for the Rep protein), (2) the TATA box for the AC1 gene, and (3) a conserved stem-loop motif that includes the nonanucleotide sequence nicked by the Rep protein to initiate DNA replication in the origin of replication.

Phylogenetic analysis and sequence comparison with selected begomoviruses

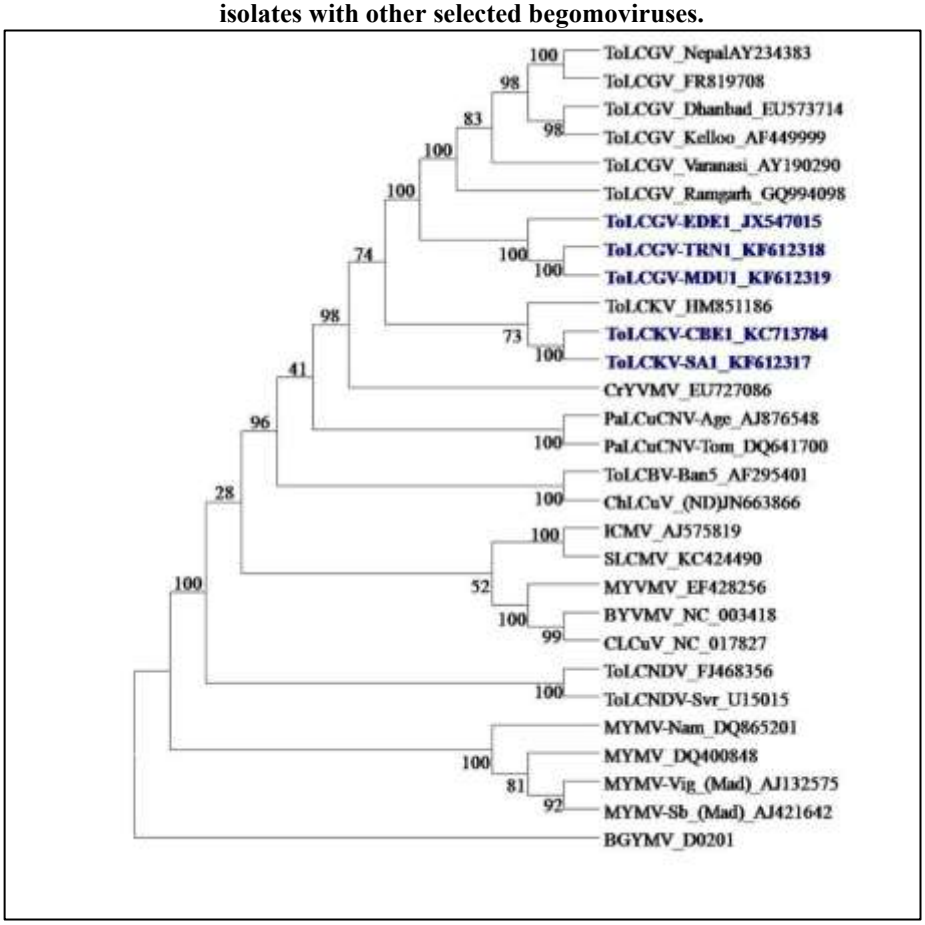
The complete genome sequences of all the five ToLCVs were compared to the sequences from the database (Table 4). Sequences in India were more closely related in Blast searches or geologically represented. All the sequences retrieved from NCBI along with our study isolates subjected to phylogeny analysis produced two different clusters (I and II) (Fig. 4). Two study isolates CBE1 (KC713784) and SA1 (KF612317) were grouped in the larger cluster I and the study isolates EDE1 (JX547015), MDU1 (KF612319) and TRN1 (KF612318) were grouped in cluster II. Cluster I further diverged to form four sub clusters a, b, c, and d. Where sub cluster a diverges into different clades and subclades including the isolates MYMV-Vig (Mad) AJ132575, MYMV-Sb (Mad) AJ421642, MYMV DQ400848, MYMV-Nam DQ865201, BGYMV D0201, ToLCNDV FJ468356, ToLCNDV-Svr U15015, BYVMV NC 003418, ICMV AJ575819, SLCMV KC424490, CLCuV NC 017827, ToLCBV-Ban5 AF295401 ChLCuV (ND)JN663866, PaLCuCNV-Age AJ876548 and PaLCuCNV-Tom DQ641700. Whereas, the isolates CrYVMV EU727086 and ToLCKV HM851186 deviated separately in subcluster b and c respectively. Our study isolates CBE1 (KC713784) and SA1 (KF612317) were grouped in sub cluster d. The isolates CBE1 (KC713784) and SA1 (KF612317) were found to be have more similarity than other study isolates. This may be due to their origination. The cluster II diverged into two subcluster e and f. The sub cluster e deviated into two clades which includes our study isolates MDU1 (KF612319) and TRN1 (KF612318) in clade I and EDE1 (JX547015) in clade 2. While the subcluster f diverted into different clades where all the ToLCGV Ramgarh GQ994098, ToLCGV Nepal AY234383, ToLCGV FR819708, ToLCGV Varanasi AY190290, ToLCGV Dhanbad EU573714 and ToLCGV Kelloo AF449999 isolates are grouped. This phylogeny reveals that, the sample isolated from same host inspite of location or origination are grouped under same sub cluster.

Table 4. Sequence analysis of complete nucleotide sequence of DNA-A component of ToLCV isolates and other selected ToLCV isolates.

GenBank ID/ToLCV isolates	ToLCKV [CBE1]	ToLCGV [EDE1]	ToLCKV [SA1]	ToLCGV [TRN1]	ToLCGV [MDU1]
ToLCKV [CBE1] KC713784	100	88	96	87	88
ToLCGV [EDE1] JX547015)	88	100	88	97	98
ToLCKV [SA1] KF612317	96	88	100	87	87
ToLCGV [TRN1] KF612318	87	97	87	100	99
ToLCGV [MDU1] KF612319	88	98	87	99	100
ToLCGV [Nepal]AY234383	89	95	89	96	96
ToLCGV [Dhanbad] EU573714	89	95	89	96	96
ToLCGV [Kelloo] AF449999	89	95	89	96	96
ToLCGV FR819708	89	94	89	96	96
ToLCGV [Ramgarh] GQ994098	89	94	89	95	96

ToLCGV [Varanasi] AY190290	88	94	88	95	95
ToLCNDV FJ468356	72	71	71	71	71
ToLCNDV-Svr U15015	72	71	71	71	71
ToLCBV-Ban5 AF295401	78	77	78	77	77
ToLCKV HM851186	92	86	92	85	85
BYVMV NC_003418	72	70	72	70	71
ICMV AJ575819	71	70	71	70	70
SLCMV KC424490	71	70	71	70	70
ChLCuV (ND)JN663866	81	80	81	80	80
CLCuV NC_017827	74	72	74	72	72
CrYVMV EU727086	82	78	82	78	78
MYMV DQ400848	63	63	63	62	62
MYMV-Vig (Mad) AJ132575	64	63	64	63	63
MYMV-Sb (Mad) AJ421642	64	63	64	63	63
MYMV-Nam DQ865201	64	64	64	63	63
BGYMV D0201	59	59	59	59	59
PaLCuCNV-Age AJ876548	77	77	77	77	77
PaLCuCNV-Tom DQ641700	77	77	77	76	77

Fig. 4. Phylogenetic dendrogram based on complete nucleotide sequences of DNA-A component of ToLCV isolates with other selected begomoviruses.



DISCUSSION

In the Indian subcontinent, ToLCV is a major problem in tomato cultivation and new strains have been documented which pose a threat to crop productivity (Chakraborty et al., 2003). Tomato leaf curl virus is a whitefly-transmitted geminivirus that causes devastating damage and considered as one of the most important virus in India. ToLCV is widely distributed, so it is important to study the field inspection. The present study revealed that the identification of virus isolate and comparing with the other isolates recorded from different parts in India, in database of GenBank to control the distribution of this virus disease. Early diagnosis of ToLCV was essentially based on distinct geminivirus symptoms observation. Incidence of ToLCV-infection was done on tomato plant cultivated in the fields and glasshouse conditions. This study indicated that tomato plant infected with ToLCV exhibited systemic geminivirus symptoms of severe leaf curling,

reduction in leaf size, shortening of internode, upward curling, stunted growth, reduction in leaf size and puckering depending on cultivar or hybrid as reported by many investigators (Vasudeva and Samraj, 1948; Yassin and Nour, 1965; Sastry and Singh, 1973; Saklani and Mathai, 1977; Saikia and Muniyappa, 1989; Chakraborty et al., 2003). Such results indicate that PCR technique as an effective diagnostic tool and greatly facilitate studies on geminiviruses epidemiology and etiology. The results showed that PCR specific primers for amplification of full length of the DNA components of whitefly transmitted geminiviruses were designed from highly conserved regions of the viral genome identified from nucleotide sequence alignment. The size of the PCR product of full length genome amplified from naturally infected tomato was ~2760-2763 bp. These results were in an agreement with Pandey et al. (2010) who used rolling circle amplification (RCA) to detect full length Tomato leaf curl virus and two monopartite genomes in ToLCV-CTM and ToLCV-K3/K5. The degree of similarity is shared from the full length genome sequences of representatives of other begomoviruses. Similar results were reported by Padidam et al. (1995), where who sequenced genomes of isolates of Tomato leaf curl geminivirus from India (ToLCNDV-mild and ToLCNDV-severe). The variation of upto 46.50% divergence with respect to nucleotide sequence with some north Indian ToLCV, Nasik (AJ 810356); ToLCV, Patna (AJ 810358) isolates, and only with a few south Indian isolates, ToLCV, Maderahalli (AJ810369); ToLCKV, Arsikeri (AY753203) and ToLCV, Aurangabad (AJ 810342) reported by Reddy et al. (2011). On the basis of genome organization and phylogenetic analysis, the virus from Coimbatore and Salem region (ToLCKV-[CBE1(KC713784); SA1(KF612317)]) isolates CBE1 and SA1 were grouped in the Cluster I and sub cluster d. This may be due to their origination. The cluster II diverged into two subcluster e and f. The sub cluster e deviated into two clades which includes our study isolates MDU1 (KF612319) and TRN1 (KF612318) in clade I and EDE1(JX547015) in clade II and is considered as a variant on the basis of DNA-A sequence identity criteria of ICTV (Fauquet et al., 2008) and proposed names are ToLCKV and ToLCGV. Hereafter, the virus isolates are referred to ToLCKV and ToLCGV. Similar investigation made by Shimizu and Ikegami (1999) reported that in total nucleotide sequence comparisons with other geminiviruses, Tomato leaf curl virus was most closely related to ToLCV from Taiwan (TwToLCV) (76% identity), ToLCV from Bangalore (ToLCV-Ban) (74%) and Ageratum yellow vein virus (AYVV) (74%), all possessing a monopartite genome. The ToLCV monopartite virus present only in south India was distinct from the bipartite genome present in north India (Chakraborty et al., 2003). Indian ToLCV isolates are mostly monopartite (DNA-A) in nature with few isolates possessing bipartite (DNA-A and DNA-B) genome organization such as Tomato leaf curl New Delhi virus (ToLCNDV) and Tomato leaf curl Palampur virus (ToLCPalV) (Briddon et al., 2008). The DNA-A components of all viruses in the Asian continent share a common backbone from ToLCV and have integrated other pieces of DNA that have likely originated from other viruses not yet identified (Pandey et al., 2010). Similar study reported the Population distribution of TYLCV was analysed by studying several genetic parameters including genetic diversity, gene flow and genetic differentiation. Broadly, TYLCV-USA populations were grouped into three major categories. Sequences reported very early in 2007–2010 were considered as Population I, Population II was defined by the sequences obtained from 2014 to 2016, and Population III encompassed TYLCV sequences that have emerged in the last few years, specifically from 2019 to 2022.

CONCLUSION

In south India, a rapid displacement of ToLCKV/ToLCGV by CBE1/SA1 and EDE1/TRN1/MDU1-like isolates occurred and is increasing in tomato cultivation. There is wide sequence variability among ToLCV isolates from south India. There is a need for more information in order to develop effective and sustainable approaches to manage diseases caused by these pathogens. This study is a report that reveals the presence of new strains of geminiviruses infecting tomato plants, which have single genomic component and molecular diversity of Tamil Nadu region. Transcription of this single component produces all gene products required to support a complete cycle of infection and transmission of the virus.

Acknowledgement:
Author would like to express thanks to UGC fellowship for completing the task in Ph.D. Project.

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