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MOLECULAR CHARACTERIZATION OF TOMATO LEAF CURL HAINAN VIRUS AND TOMATO LEAF CURL HANOI VIRUS IN VIETNAM

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ABSTRACT

Three begomovirus isolates were detected from tomato and papaya plants showing leaf curling symptoms in the Red River Delta of northern Vietnam. The complete genomes of three isolates, which were assigned as tomato-89, tomato-100 and papaya-31, were amplified, cloned, sequenced and deposited in the GenBank. Molecular characterization of the tomato-89 and papaya-31 isolates evidenced that they were Tomato leaf curl Hainan virus (ToLCHnV), a recently identified virus from southern China, and as such this virus was detected in Vietnam for the first time. More importantly, papaya, a novel natural host of this virus, was also identified. Analyses also indicated ToLCHnV has evolved from Vietnam. Molecular characterization of the tomato-100 isolate justified it as a putatively novel virus. The virus was named Tomato leaf curl Hanoi virus (ToLCHanV), which has apparently emerged through recombination with Papaya leaf curl China virus (PaLCuCNV) and Ageratum leaf curl virus (ALCV).

Key words: papaya, begomovirus, recombination

INTRODUCTION

The *Begomovirus* genus is the largest of the family *Geminiviridae*, containing 196 species recognised officially by International Committee on Taxonomy of Viruses (ICTV) in 2009 (http://ictvonline.org/). All begomoviruses are naturally transmitted through whitefly (*Bemisia tabaci*) and infect a wide range of dicotyledon plants (Seal et al., 2006). Some of the most important begomoviruses are those that infect cassava (Legg and Fauquet, 2004), cotton (Briddon, 2003; Briddon and Markham, 2000) and tomato (Moriones and Navas-Castillo, 2000).

Begomovirus genomes are circular single-stranded (ss) DNA molecules of approximately 2.7 kb, encapsidated within twinned (geminate) icosahedral virions. Begomoviruses have either bipartite genomes (DNA-A and DNA-B), or monopartite genomes equivalent to DNA-A. Genome of monopartite viruses and DNA-A of bipartite viruses typically has six open reading frames (ORF). DNA-B of bipartite begomoviruses has two ORFs (Stanley et al., 2005).

Based on phylogenetic relatedness and genomic features, begomoviruses can be divided into two groups, the Old World (Europe, Africa, Asia) and the New World (The Americas) viruses. All New World (NW) begomoviruses are bipartite, whereas both bipartite and monopartite begomoviruses are present in the Old World (OW). It was thought that NW begomoviruses arose more recently than OW viruses, evolving after continental separation of the Americas from Gondwana (Rybicki, 1994). However, the finding of two bipartite begomoviruses infecting jute (*Corchorus capsularis*) from Vietnam, Corchorus yellow vein virus (CoYVV) and Corchorus golden mosaic virus (CoGMV), that share several features with other NW bipartite begomoviruses (Ha et al.,

2006; Ha et al., 2008), supporting the hypothesis that NW-like viruses were present in the OW prior to continental separation and that South China including the South-East Asia, and Vietnam in particular, is major centre of origin and diversity of begomoviruses (Briddon et al., 2010; Ha et al., 2006; Ha et al., 2008; Nawaz-ul-Rehman and Fauquet, 2009).

Recently, a large number of begomoviruses have been detected and characterised from a wide range of crops and weeds in Vietnam (Blawid et al., 2008; Green et al., 2001; Ha et al., 2006; Ha et al., 2008; Revill et al., 2003). In this paper, we report the identification and molecular characterization of two other begomoviruses infecting tomato and papaya in Vietnam.

MATERIALS AND METHODS

Plant samples and DNA extraction

Tomato and papaya samples exhibiting the symptoms typical for begomovirus infection such as leaf curling were collected in Hanoi and Hungyen provinces, respectively, of northern Vietnam. The leaf samples were dried using self-indicating silica gel and stored at room temperature until use. Total DNA was extracted from the dried leaf samples using a CTAB method (Doyle and Doyle, 1987). Briefly, approximately 20 mg of dried tissues were transferred into a 1.5 mL eppendorf tube and homogenized with 500 µL extraction buffer containing 2 M NaCl, 25 mM EDTA, 100 mM Tris-HCl, 2 % PVP and 2 % cetyl trimethyl ammonium bromide (CTAB) using a plastic pestle. The total DNAs were extracted twice with chloroform:isoamyl alcohol (24:1) and pelleted with cold propanol. DNA pellets were washed twice with 70 % ethanol, air dried for 30 mins and dissolved in 50 µL TE buffer and stored at -20 ^{O}C.

Polymerase chain reaction (PCR)

Begomoviruses were detected from diseased plants by PCR using degenerate and specific primers (Table 1). PCRs were performed using a DreamTaq polymerase (Fermentas). From the sequences amplified by degenerate primers, two back-to-back primers, Hai50F and Hai50R (Table 1), were designed to amplify the complete genomes of three virus isolates in this study using an Expand Long Template PCR System (Roche) with buffer No.3 following producer's recommendation.

Table 1. Primers used in this study

Primers	Sequence (5' – 3')*	Use
TYLCVNV-Sp-F1	TGTGTTACATATTCTGTGTTTTCC	To detect TYLCVNV, 1386 bp
TYLCVNV-Sp-R1	AAATACATCAAAATCTGCAGAGAGC	
ToLCVV-Sp-F2	GACCAGTCTGAAGGTGTGAGTTC	To detect ToLCVV, 454 bp
ToLCVV-Sp-R2	ACTCAAGCTATAAAGAATACCTAGAC	
BegoAFor1	TGYGARGGiCCiTGYAARGTYCARTC	To detect begomoviruses†, ~ 1.2 kb
BegoARev1	ATHCCMDCHATCKTBCTiTGCAATCC	
Hai50F	GGACTTGTATTGTGATGATGTCG	To amplify the complete genome of three virus isolates in this study
Hai50R	CCAATTCAATTACAACCTGAGG	
HaiSeqF	TTGATTGCCTCGGCATATGC	To sequence the complete genome of three virus isolates in this study
HaiSqR	GACCTCCTTTTGTTTGTGAC	

* In the primer sequences, I = Inosine, Y = C/T, R = G/A, W = A/T, V = A/C/G, S = C/G and D =A/G/T.
† From Ha *et al.,* (2006).

Cloning and sequencing

Amplicons were purified from agarose gels using PureLink™ Quick Gel Extraction Kit (Invitrogen) and cloned using InsTAclone™ PCR Cloning Kit (Fermentas) and *E. coli* XL1-Blue competent cells (Stratagene) following producer's recommendation. Cloned plasmids were purified using an AccuPrep Plasmid Mini Extraction Kit (Bioneer) and inserts were verified by restriction digestion. One or two clones for each sample were sequenced in both orientations using the ABI Prism®Bigdye™ Terminator Kit (PE Applied Biosystem), air dried and sent to the Institute of Biotechnology at Hanoi for reading.

Sequence analysis

The genomic sequences were assembled from contiguous sequences using the Seqman program (DNASTAR). ORFs were identified using the Vector NTI Suite7 program. Sequences were aligned with the ClustalX program (Thompson et al., 1997). Sequence identities were calculated using the ''Sequence Identity Matrix'' option in BioEdit program version 7.05 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The phylogenetic trees were constructed from the ClustalX-aligned sequences using a MEGA version 4 (Tamura et al., 2007) using a neighbour-joining (NJ) method with the Kimura 2-Parameter model for estimating the distances. All phylogenetic analyses were bootstrapped with 1000 replicates. Recombination was analysed using the Recombination Detection Program version 2.0 (RDP2) (Martin et al., 2005), also available online (http://darwin.uvigo.es/rdp/rdp.html).

Virus taxonomy

Novel virus species names were assigned by using the rules proposed by the ICTV Geminiviridae Study Group (Fauquet et al., 2008). Demarcation of viral species was based on overall sequence comparisons, using a threshold of 89 % nucleotide sequence identity (Fauquet et al., 2008).

RESULTS

Identification of begomoviruses by PCR

Prior to implementation of this work, only Tomato yellow leaf curl Vietnam virus (TYLCVNV) and Tomato leaf curl Vietnam virus (ToLCVV) were identified to infect tomato in northern Vietnam (Green et al., 2001; Ha et al., 2008). To see if other tomato begomoviruses are present in northern Vietnam, tomato plants showing symptoms typical for the yellow leaf curl disease were collected. In a PCR tests, two tomato samples, assigned as tomato-89 and tomato-100, collected in Gialam and Thanhtri districts of Hanoi province, respectively, were positive with degenerate primers (BegoAFor1/BegoARev1) but negative with specific primers (TYLCVNV-Sp-F1/TYLCVNV-Sp-R1 and ToLCVV-Sp-F2/ToLCVV-Sp-R2), suggesting they might be unidentified begomovirus(es) of tomato in northern Vietnam. The BegoAFor1/BegoARev1 PCR products (~ 1.2 kb) of the two isolates were then directly sequenced using PCR primers. Blast search showed tomato-89 and tomato-100 shared 99 and 97 % nucleotide sequence identities with Tomato leaf curl Hainan virus (ToLCHnV) and Ageratum leaf curl virus (ALCV), respectively.

As Papaya leaf curl China virus (PaLCuCNV) was detected from tobacco in Vietnam (Ha et al., 2008) so field surveys were performed throughout northern Vietnam to find if this virus infects papaya. However, we observed only one papaya plant in the Yenmy district of Hungyen province, assigned as papaya-31, showing leaf curling and stunting symptoms similar to those caused by PaLCuCNV or Papaya leaf curl Guangdong (PaLCuGDV) on papaya in China (Wang et al., 2004)

(Fig. 1). PCR test using degenerate primers on this sample was positive. Interestingly, the nucleotide sequence of the amplified fragment was 97 % identical with the genome of ToLCHnV.

Fig. 1. Leaf curling symptom of papaya from Vietnam (a and b, sample papaya-31) and from China (c, Wang et al., 2004)

Cloning and sequencing complete genomes

As the identity of a begomovirus is precisely identified only on the basis of complete genome, so tomato-89, tomato-100 and papaya-31 isolates were further analysed by cloning the complete genomes. Two adjacent out-warding primers, Hai50F and Hai50R, were designed based on the conserved sequence of the fragments amplified with degenerate primers. Using these back-to-back primers, the complete genomes of the three isolates were obtained. The PCR bands were then gel purified, cloned and sequenced. Finally, the complete sequences of the three isolates were obtained and deposited in the GenBank with the accession numbers HQ162268 (papaya-31), HQ162269 (tomato-89) and HQ162270 (tomato-100).

Genomic characterization

The complete genomes of tomato-89, tomato-100 and papaya-31 were 2743, 2740 and 2748 nucleotides (nts), respectively. The three genomes had organization typical for that of OW monopartite begomoviruses (Fig. 2). On the complementary-sense strand, each genome had four ORFs, which are C1 encoding the replication initiation protein (Rep), C2 encoding the transcriptional activator (TrAP), C3 encoding the replication enhancer (REn) and C4 encoding the C4 protein. As a general rule among begomoviruses, the C2 and C4 ORFs of each isolate were located on the same reading frame. On the virion-sense strand, each genome had two ORFs, which are V1 encoding the coat protein (CP) and V2 encoding the V2 protein.

Analysis of the intergenic region (IR) locating between the 5' terminuses of the V2 and C1 ORFs revealed structures and sequences characteristic to those of begomoviruses. The IRs were 273-280 nts in length, each contained an origin of replication (*ori*) region. The *ori* region of each isolate encompass a 33 nts identical stem-loop structure, GCGGCCATCCGTATAATATTACCGGATGGCCGC, whose the sequence TAATATTAC within the loop was identical among all reported begomoviruses.

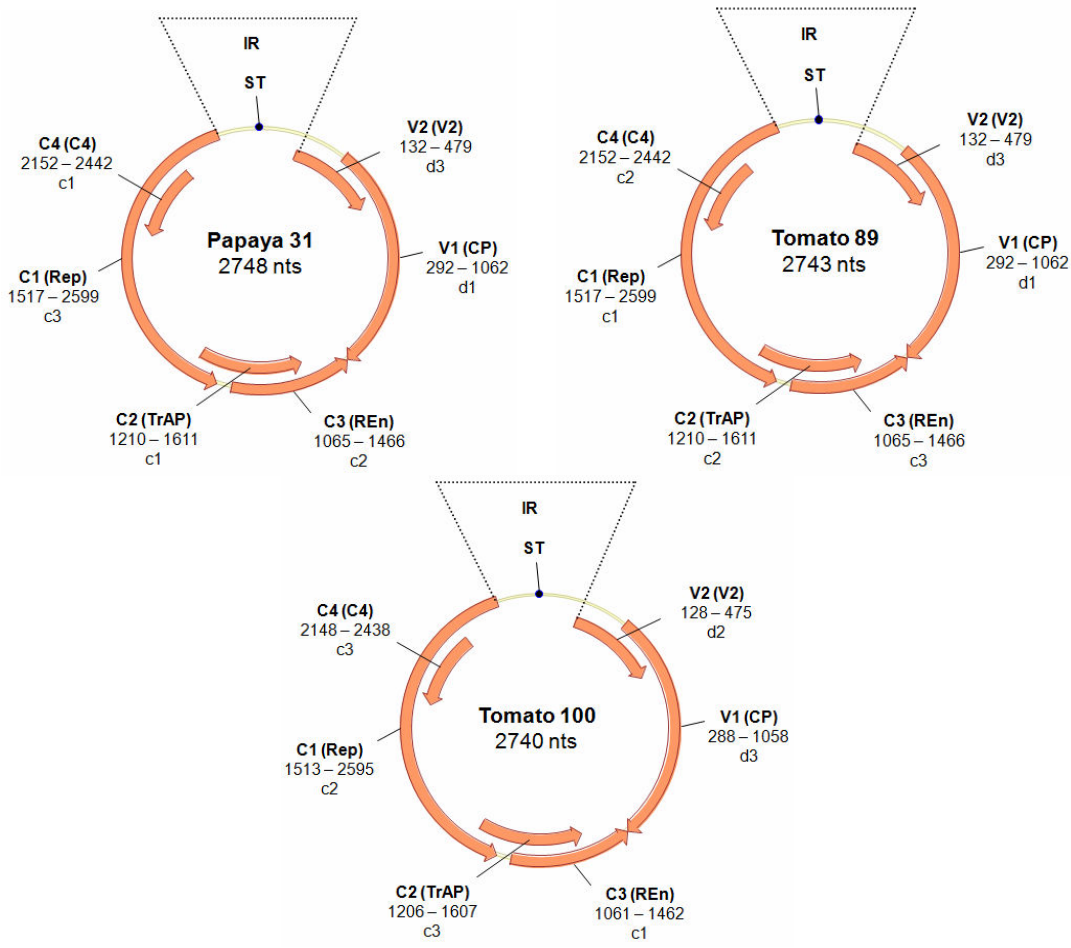

Fig. 2. Genome organization of three virus isolates. IR, intergenic region; ST, stem-loop structure; nts, nucleotides. The ORFs (with corresponding proteins in parentheses), their position on genomes and reading frames with c and d indicating complementary-sense and virus-sense orientation, respectively, are shown.

The *ori* region of begomoviruses contains iterated DNA sequences (iterons), whose sequence, number, orientation and spacing are species-specific. Furthermore, the iteron sequence correlates with a Rep N-terminal region, namely iteron-related domain (IRD) (Arguello-Astorga et al., 1994; Arguello-Astorga and Ruiz-Medrano, 2001). Scanning the *ori* and Rep N-terminal regions showed papaya-31 and tomato-89, considered to be isolates of ToLCHnV based on overall nucleotide identity as mentioned later, differed in the iteron and IRD characteristics. Papaya-31 had the iteron/IRD features identical with those of ToLCHnV with two iterons (GGTGT) upstream of the TATA box and the IRD sequence being MPPPKKFLIN. In contrast, tomato-89 had three iterons (GGTGT) upstream of the TATA box and one complementary iteron (ACACC) downstream of the TATA box. The IRD sequence of tomato-89 was also different by 3 amino acids (MAPPNKFRIN) compared with that of papaya-31. Compared with known begomoviruses, the iteron/IRD features of tomato-89 were similar with those of ToLCVV and Tomato yellow leaf curl Thailand virus (TYLCTHV). The iteron/IRD features of tomato-100 were identical with those of PaLCuCNV and similar with those of tomato-89, ToLCVV and TYLCTHV, except that tomato-100 had only two iterons.

Analysis of the putative amino acid (aa) sequences of two largest proteins, Rep and CP, of the three isolates also revealed many well characterized functional motifs or residues such as motifs I, II, III, P-loop and α helix 4 on the Rep protein and the nuclear localizing signals (positively charged aa residues) on the CP protein. These all motifs are required for replication of begomiviruses (Kunik et al., 1998; Laufs et al., 1995; Orozco et al., 1997; Unseld et al., 2001).

Sequence comparison

Sequence comparison over complete genomes showed payaya-31 and tomato-89 shared 93.7 % nt sequence identity with each other, indicating they are member of same species. Compared with six most closely related GenBank viruses, ToLCVV, PaLCuCNV, ALCV, TYLCVNV and TYLCTHV, the two isolates shared highest sequence identities (95.9 and 93 %, respectively) with ToLCHnV but less than 87 % with the five remaining GenBank viruses (Table 2).

The complete genome of tomato-100 were 87.7, 87.6 and 87 % nt identical with that of tomato-89, ToLCHnV and PaLCuCNV, respectively, and less than 87 % with that of other viruses, suggesting that tomato-100 belongs to a distinct species.

Interestingly, tomato-100 had very high CP sequence identity (97.4 and 99.2 % at nt and aa levels, respectively) but only 84.9 % overall identity with ALCV. Similarly, tomato-100 shared very high IR sequence identity (92 %) but only 87 % overall identity with PaLCuCNV. These differences suggest tomato-100 may be a recombinant virus.

Phylogenetic analysis

A phylogenetic tree was constructed based on the complete genomes of monopartite viruses or DNA-A of bipartite viruses (Fig. 3). The analysed sequences were either present in Vietnam or most closely related in Blast searches or geologically representative. In total, 40 GenBank sequences representative for 32 species were used in the analysis.

As shown in the tree, tomato-89, papaya-31 and two isolates of ToLCHnV from China, HaNHK7 and HaNHK8, formed a distinct species cluster that was well supported by bootstrap analysis (100 %). Interestingly, in this cluster, the two Vietnam isolates were distal and basal to the two China isolates, suggesting ToLCHnV has been present in Vietnam for a considerable period. Tomato-100 formed a distinct branch independent with other begomoviruses but more close to the ToLCHnV cluster.

Table 2. Sequence comparison of the complete genome, IR and selected ORFs of the three virus isolates identified in this study and the most closely related GenBank viruses showing nt, nucleotide level and aa, amino acid level.

GenBank virus	Sequence identity (%)					
	Genome (nt)*	IR (nt)	CP (nt)	CP (aa)†	Rep (nt)	Rep (aa)
			Papaya-31			
ToLCHnV-FN434083	95.9	91.8	95.3	96.5	96.6	97.8
Tomato-89	93.7	83.3	98.4	97.3	90.8	92
Tomato-100	86.3	64.3	91.3	96.5	88.6	91.7
ToLCVV-AF264063	83.1	68.6	92.1	95.3	86.2	87
ALCV-AJ851005	82.9	77.2	90.9	96.5	77.2	83.1
TYLCVNV-DQ641697,	82.4	69.7	92.7	95.3	76.6	82.8
PaLCuCNV-AJ876548	82.3	61.7	92	96.9	80.7	84.5
TYLCTHV-AY514630‡	77.4	67.5	72.5	78.1	85.3	87.5
			Tomato-89			
Papaya-31	93.7	83.3	98.4	97.3	90.8	92
ToLCHnV-FN434083	93	82.2	96.5	99.2	91.2	92.2
Tomato-100	87.7	71	92	99.2	89.6	92
ToLCVV-AF264063	86.3	83.2	93.1	98.1	89.4	90.6
PaLCuCNV-J876548	84.6	72	92.7	99.6	81.9	85
ALCV-AJ851005	83.4	79	91.7	99.2	78.9	83.4
TYLCVNV-DQ641697	83.3	71.9	93.6	98.1	77.6	81.7
TYLCTHV-AY514630	78.9	73.1	73.2	80.5	87.9	90.3
			Tomato-100			
Tomato-89	87.7	71	92	99.2	89.6	92
ToLCHnV-FN434083	87.6	65.1	91.3	98.4	90.4	92.2
PaLCuCNV-AJ876548	87	92	95.7	99.6	84.5	86.7
Papaya-31	86.3	64.3	91.3	96.5	88.6	91.7
ALCV-AJ851005	84.9	60.3	97.4	99.2	77.3	82.8
ToLCVV-AF264063	82.6	73.5	90.5	97.7	86.1	87.5
TYLCTHV-AY514630	80	76.5	71.2	80.1	89.4	92.5
TYLCVNV-DQ641697	78.9	64	90.1	98.1	74.5	80.6

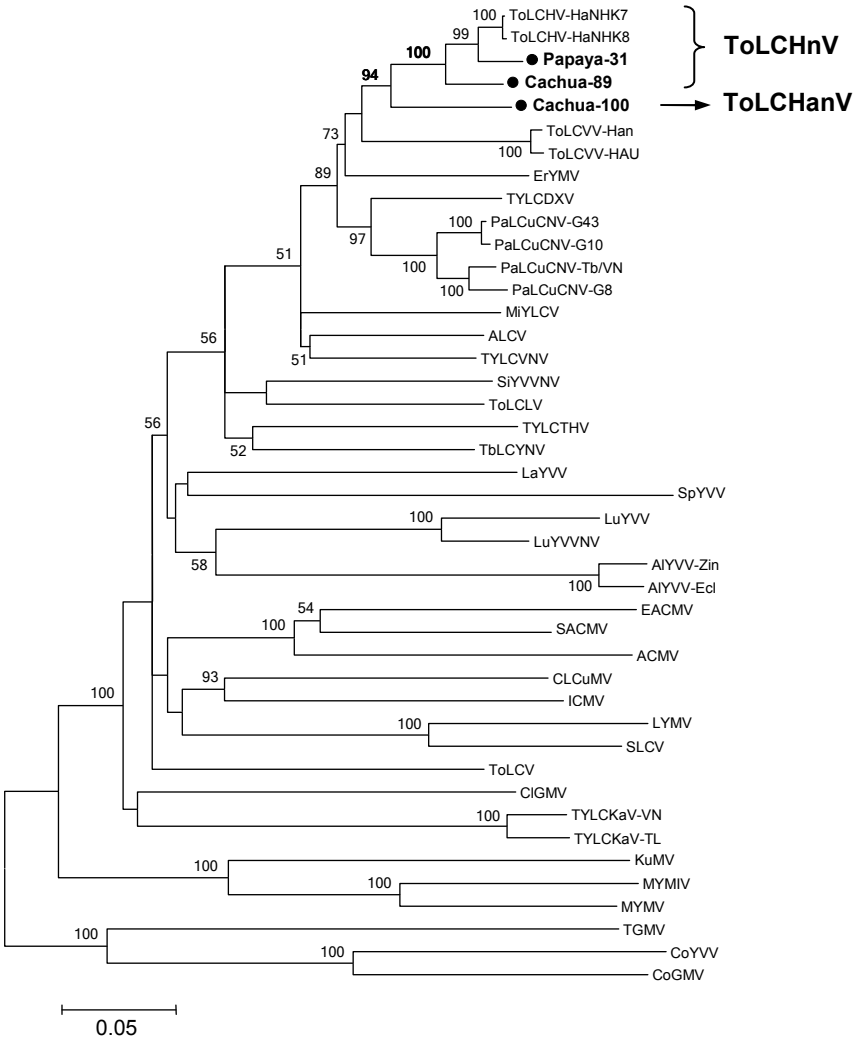

Fig. 3. The Neighbor-Joining bootstrap consensus tree based on the complete genomes. Only bootstrap percentages higher than 50% (1000 replicates) are shown. The genetic distances were computed using the Kimura 2-parameter method. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The names and accession numbers of all GenBank sequences are: African cassava mosaic virus (ACMV: AF126802), Ageratum leaf curl virus (ALCV: AJ851005), Alternanthera yellow vein virus (AlYVV-Ecl: DQ641704, -Zin: DQ641703), Clerodendrum golden mosaic virus (ClGMV: DQ641692), Corchorus golden mosaic virus (CoGMV: DQ641688), Corchorus yellow vein virus (CoYVV: AY727903), Cotton leaf curl Multan virus (CLCuMV: AJ002459), East African cassava mosaic virus (EACMV: AF126806), Erectites yellow mosaic virus (ErYMV: DQ641698), Indian cassava mosaic virus (ICMV: AJ314739), Kudzu mosaic virus (KuMV: DQ641690), Lindernia anagallis yellow vein virus (LaYVV: DQ641701), Loofa yellow mosaic virus (LYMV: AF509739), Ludwigia yellow vein Vietnam virus (LuYVVNV: DQ641699), Ludwigia yellow vein virus (LuYVV: DQ641708), Mimosa yellow leaf curl virus (MiYLCV: DQ641695), Mungbean yellow mosaic India virus (MYMIV: AY271893), Mungbean yellow mosaic virus (MYMV: AJ132575), Papaya leaf curl China virus (PaLCuCNV-G10: AJ558125, -G43: AJ876548, -G8: AJ558124, -Tob/VN: DQ641700), Sida yellow vein Vietnam virus

(SiYVVNV: DQ641696), South African cassava mosaic virus (SACMV: AF155806), Spilanthes yellow vein virus (SpYVV: DQ641694), Squash leaf curl China virus (SLCCNV: AF509743), Tobacco leaf curl Yunnan virus (TbLCYNV: AJ566744), Tomato golden mosaic virus (TGMV: K02029), Tomato leaf curl Hainan virus (ToLCHnV-HaNHK7: FN256261, -HaNHK8: FN434083), Tomato leaf curl Laos virus (ToLCLV: AF195782), Tomato leaf curl Vietnam virus (ToLCVV-Han: AF264063, -HUA: DQ641705), Tomato leaf curl virus (ToLCV: S53251), Tomato yellow leaf curl Dangxa virus (TYLCDXV: EU189150), Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV-TL: AF511529, -VN: DQ169054), Tomato yellow leaf curl Thailand virus (TYLCTHV: AY514630), Tomato yellow leaf curl Vietnam virus (TYLCVNV: DQ641697).

Recombination analysis

Recombination analysis could not find any statistically significant recombinant fragments in association with tomato89 and papaya-31. However, two putative recombinant fragments were identified in association with tomato-100 (Table 3, Fig. 4).

The first fragment of tomato-100 was 1157 nt in length and encompassed the entire V2 and V1 ORFs and one third of the C3 ORF. This fragment shared 97.5 % sequence identity with the cognate region of ALCV (1157 nt in length), while the two viruses were only 84.9 % overall identical.

The second fragment of tomato-100 was 1537 nt in length and encompassed half of the C1 ORF, the entire IR and most of the V1 ORF. This fragment shared 96.9 % sequence identity with the cognate region of PaLCuCNV (1574 nt in length), while the two viruses were only 87 % overall identical.

Table 3. Sequence identities (%) of the recombinant fragments of Tomato-100 and cognate partners

Partner	First fragment (111-1267)*	Second fragment (2097 – 893)*	Complete genome
PaLCuCNV-AJ876548	-	96.9	87.0
ALCV-AJ851005	97.5	-	84.9

* The numbers in parenthesis are nucleotide positions in the tomato-100 genome

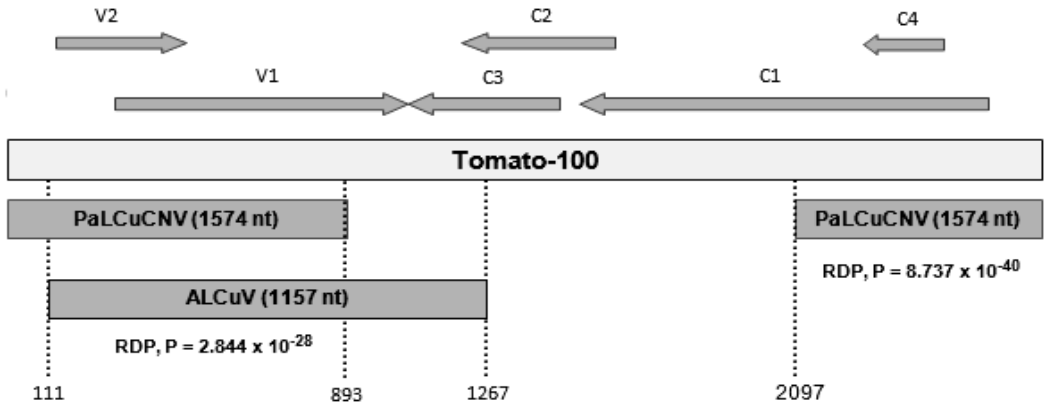

Fig. 4. Schematic representation of the recombinant regions relating to tomato-100. Break points, sizes of the recombinant fragments (derived from the tomato-100 genome) are indicated. Original detection method and multiple comparison-corrected P values (Martin et al., 2005) of the recombinant fragments are shown. Data set encompasses nine ClustalX-aligned sequences: Papaya-31, tomato-89,

tomato-100, ToLCHnV-FN434083, ToLCVV-AF264063, ALCV-AJ851005, TYLCVNV-DQ641697, PaLCuCNV-AJ876548 and TYLCTHV-AY514630.

DISCUSSION

Justification of taxonomic position of papaya-31 and tomato89 viruses was straightforward as they shared overall sequence identities of 95.9 and 93.7 %, respectively, with ToLCHnV, a tomato monopartite begomovirus that has recently been characterised from Hainan island of southern China (Zhang et al., 2010). These identities are much higher than the 89 % threshold for begomovirus classification. As such, it is for the first time ToLCHnV was identified and characterised in Vietnam. More importantly, papaya was also identified as another natural host of this virus.

Similarly to the previous observation (Zhang et al., 2010), the alfasatellite and betasatellite components were not detected from tomato-89 and payaya-100 samples in PCR tests using degenerate primers designed by Ha *et al*. (2008). Alfasatellites and betasatellites are circular ssDNA molecules, approximately 1.35 kb in length and often found in association with many OW monopartite begomoviruses (Briddon and Stanley, 2006; Nawaz-ul-Rehman and Fauquet, 2009). Without an involved betasatellite, it is difficult to explain why this virus infect papaya as betasatellites have been shown to suppress the host defence (Briddon and Stanley, 2006; Cui et al., 2005), which may help virus to adapt a new host. It is more likely that ability of ToLCHnV to infect papaya is involved in the whitefly vector. As papaya is not preferred host of this vector, the infection of the papaya-31 plant might result from a rare event, in which viruliferous whiteflies fed on the plant in a "no choice" condition and transmitted virus. This may explain why no more diseased papaya plants have been observed, although another papaya begomovirus, PaLCuCNV, was detected from tobacco in 2005 (Ha *et al.*, 2008) and recently from ageratum (data not shown) in northern Vietnam.

Evidences from this study such as (i) the low overall sequence identifies of the two Vietnam isolates, papaya-31 and tomato-89, compared with each other (less than 94 %) and with the China isolates (less than 96 %), (ii) the finding of the two different hosts (tomato and papaya), (iii) the difference in the iteron and IRD features between the two Vietnam isolates, and (iv) the distal positions of the Vietnam isolates in the phylogenetic tree compared with each other and with the China isolates, indicate that ToLCHnV is more diverse in Vietnam than in China and that the virus has evolved from Vietnam rather than from China. It would be worthwhile to note that geological proximity between Hainan island of China and the Red River Delta of Vietnam, where isolates of ToLCHnV were identified, may facilitate migration of the whitefly vectors between the two regions.

We had difficulty in determination of the taxonomic status of the tomato-100 isolate. This isolate had highest overall nt identity (87.7 %) with ToLCHnV. Fauquet et al. (2008) reported that most viruses with approximately 87 % identity may be recombinants. Indeed, computing analysis detected two putative recombinant fragments in association with tomato-100. The biggest recombinant fragments covered over half of the genome including the *ori* and Rep N terminal regions, which contains species-specific factors essential for replication. Sequence analyses of the recombinant fragment suggest that tomato-100 would have higher affinity, in terms of *trans*-replication, with PaLCuCNV (Arguello-Astorga et al., 1994; Arguello-Astorga and Ruiz-Medrano, 2001; Fontes et al., 1994a; Fontes et al., 1994b; Hanley-Bowdoin et al., 2000; Orozco et al*.,* 1997).

Strict application of the 89% taxonomy rule, phylogenetic analysis, sequence comparison and analysis of species-specific factors relating to replication, supported the classification of tomato-100 as a distinct virus. The virus was, therefore, tentatively named Tomato leaf curl Hanoi virus and abbreviated as ToLCHanV. Interestingly, a betasatellite molecule was found in association with this virus from tomato-100 plant (data not shown). More work needs to be done to characterise the biological features of ToLCHanV and elucidate the role of the related betasatellite molecule.

CONCLUSION

In this study, three begomovirus isolates, tomato-89, tomato-100 and papaya-31, were detected from tomato and papaya as shown by the isolate names. The complete genomes of the three isolates were amplified, cloned and sequenced. Molecular characterization of the tomato-89 and papay-31 isolates identified they are ToLCHnV, a recently identified virus from southern China. It is the first time ToLCHnV has been detected in Vietnam; and papaya is the recovered second host.

Molecular characterization of the tomato-100 isolate justified it as a putatively novel virus. The virus was named Tomato leaf curl Hanoi virus (ToLCHanV), which has apparently emerged through recombination.

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