Molecular Characterization of Begomoviruses Associated with Papaya Leaf Curl Disease in India

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Abstract

For molecular characterization of papaya leaf curl causing begomoviruses, papaya samples showing symptoms of leaf curl, vein clearing, enations and reduced leaf size were collected from Kodur of Andhra Pradesh, Bangalore of Karnataka, Coimbatore of Tamil Nadu, Lucknow of Uttar Pradesh and Delhi States of India. Using primers specific to begomovirus DNA-A used the total DNA isolated from these samples for begomovirus infection. For molecular differentiation of papaya leaf curl isolates, cloning and sequencing of PCR amplified fragments was done. The complete DNA-A sequence of four papaya leaf curl isolates was characterized. Comparative sequence analysis with 21 other begomoviruses has indicated that the PaLC. Coimbatore isolate has 77.5% nucleotide identity with *Ageratum enation virus* (AEV), PaLCV.Kodur has 90.3% nucleotide identity with *Croton yellow vein mosaic virus* (CrYVMV), PaLC.UP13 isolate has 94.7% nucleotide identity with *Papaya leaf curl virus* (PaLCV) and PaLC.New Delhi isolate has 95.5% nucleotide identity with *Tomato leaf curl New Delhi virus* (ToLCNDV). Phylogenetic analysis and nucleotide comparison has indicated that in India, papaya is infected with more than three distinct begomoviruses. PaLC.Coimbatore isolate has nucleotide identity less than 89% with another known begomoviruses and named as *Papaya leaf curl Coimbatore virus* (PaLCCoV) and considered as a new begomovirus infecting papaya in India.

INTRODUCTION

Geminiviruses are a family of plant-infecting viruses with circular single-stranded DNA genomes encapsidated into small twinned icosahedral virions. The family Geminiviridae is divided into four genera (*Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus*), which are differentiated based on their genome structure; the host plants they infect and the type of insect vector (Fauquet et al., 2008). Members of the genus *Begomovirus* have monopartite (one ~2.8 kb DNA component) or bipartite (two ~2.6 kb DNA components) genomes, infect dicotyledonous plants and are transmitted by whiteflies (*Bemisia tabaci* Genn.) (Pradeep et al., 2005). The genomic component of the bipartite *Begomovirus* (DNA-A), usually the largest of the two components, encodes proteins involved in DNA replication, controls of gene expression and insect transmission. DNA-B encodes products required for inter-cellular and intra-cellular movement of the virus in the tissues of plant hosts (Lazarowitz, 1992; Stanley et al., 2005). Begomoviruses are one of the largest groups of plant viruses and cause economically important diseases of many vegetable and fibre crops (Rojas et al., 2005; Varma and Malathi, 2003).

Papaya (*Carica papaya* L.) is an important fruit crop grown in India, mainly in Andhra Pradesh, Karnataka, Tamil Nadu, West Bengal, Maharashtra, Gujarat, Orissa and Bihar. In the North India papaya production is seriously affected due to *Papaya leaf curl virus*. Papaya leaf curl disease was first reported in India (Thomas and Krishnaswamy, 1939) caused by *Begomovirus*, *Papaya leaf curl virus* (PaLCV) (Saxena et al., 1998). Papaya leaf curl disease which is serious in North India (Nariani, 1956) was recently

noticed in different papaya growing states of Southern India. Begomoviruses have been reported infecting chilli, tomato, cucurbits, legumes, cotton and cassava (Kirthi et al., 2004; Varma and Malathi, 2003; Reddy et al., 2005). Here we describe the molecular characterization of begomoviruses associated with papaya leaf curl disease.

MATERIALS AND METHODS

Virus Isolates

Virus isolates PaLCV Bangalore, PaLCV-Coimbatore, PaLCV-Kodur, PaLCV-New Delhi, and PaLCV-Lucknow were collected from field samples of papaya plants showing leaf curl, crinkling, enation and stunting of plants. The infected papaya samples similar to the leaf curl disease were collected from Kodur in Andhra Pradesh, Coimbatore in Tamil Nadu, Bangalore in Karnataka, New Delhi in Delhi and Lucknow in Uttar Pradesh state, samples were processed for whitefly transmission and DNA isolation.

DNA Extraction, PCR Amplification, Cloning and Sequence Analysis

Nucleic acids were extracted from symptomatic papaya leaves following the method of Xie et al. [9]. Partial products about 700 bp covering part of the intergenic region and AV2 gene of DNA-A were amplified with degenerate primers MKIF1:5'-CCCTGAATGTTYGGATGGAA-3'and MKIR1:5'-CGGGCGTAAAATGACGAT-3'. On the basis of the determined sequences of 700 bp fragments, primer pairs PaLCF; 5'-GTAAGGTGCAGTCTTTTGATGC-3'/PaLCR:5'-CACYTTACATGGGCCTTCACAT C-3' and ToLCNDF:5'-CACAAACATGTGGGATCCATTATTGC-3'/ToLCNDR:5'-GTGGATCCAAACTTGGTGAGCAAGTC-3' were designed for amplification the whole DNA-A of isolates PaLC.Coimbatore, PaLC.Kodur, PaLC.UP13 and PaLC.New Delhi, respectively. PCR was done as described (Ma et al., 2005). PCR products with the expected size were purified and cloned into the PCR amplified product was purified from the gel piece using Qiaquick kit (Qiagen, USA) and then ligated to the vector pTZ57R (MBI Fermentas Inc., Germany). The *E. coli* strain DH5α was transformed with the ligated mix and the resulting colonies carrying recombinant vector was selected. Clones carrying the 2.7 kb insert were confirmed for the presence of insert by restriction digestion of the plasmid and PCR amplification. The clone carrying the 2.7 kb insert was sequenced using the automated model 377 DNA sequencing system (Perkin-Elmer).

DNA Sequence Analysis

The sequences were assembled and analyzed with the aid of DNAStar software (Madison. Wis., USA). The percent identity between aligned sequences was calculated using Clustal X (Thompson et al., 1994) and identity matrix modules of the Bioedit software (Hall, 1999). Multiple sequence alignments were made using Clustal X and the phylogenetic tree constructed by the neighbour joining method (Saitou and Nei, 1987). The data set was subjected to 1000 bootstrap replicates and the tree was drawn using TREEVIEW (Page, 1996). The database accession numbers of the begomovirus DNA-A sequences used for comparison or dendrogram construction were: Ageratum enation virus.Lucknow (AEV.Lucknow, EU867513), Chilli leaf curl virus.Papaya.AD (ChLCV.Papaya.AD, DQ989326), Chilli leaf curl virus.Multan (ChLCV.Multan, AF336806), Croton yellow vein mosaic virus.Bangalore (CrYVMV.Ban, AJ507777), Cotton leaf curl Rajasthan virus-[Sirsa] (CLCuRV.[Sirsa], AY765254), Euphorbia leaf curl virus.Pusa (EuLCV.Pusa, EU194914), Bhendi yellow vein mosaic virus-[Madurai] (BYVMV-[Madurai], AF241479), Papaya leaf curl China virus.[G10] (PaLCCV.[G10], AJ558125), Papaya leaf curl China virus.ZM1 (PaLCCV.ZM1, EU874386), Papaya leaf curl Guangdong virus.[GD2] (PaLCGuV.[GD2], AJ558122). Papaya leaf curl virus.Lucknow (PaLCuV.Lucknow, Y15934), Papaya leaf curl virus.Pakistan (PaLCV.Pk, AJ436992), Tomato leaf curl Bangalore virus.Ban5 (ToLCBV.Ban5, AF295401), Tomato leaf curl Iran virus.Iran (ToLCIV.Iran, AY297924), Tomato leaf curl Gujarath virus.Vadodara (ToLCGV.Vadodara, AF413671), Tomato leaf curl Joydevapur

virus.Chilli (ToLCJV.Chilli, EF194765), Tomato leaf curl Karnataka virus.Karnataka (ToLCKV.Karnataka, U38239), Tomato leaf curl New Delhi virus.Chilli pepper (ToLCNDV.Chillipepper, DQ116880), Tomato leaf curl New Delhi virus.Potato (ToLCNDV.Potato, AY286316), Tomato leaf curl New Delhi virus.Papaya.PD (ToLCNDV.Papaya.PD, DQ989325) and Maize streak virus.A.South Africa (MSV.A.South Africa, Y0085).

RESULTS AND DISCUSSION

Symptoms

Papaya leaf curl disease was easily found in papaya gardens in major papaya growing states of India especially in Andhra Pradesh, Karnataka, Tamil Nadu, Delhi and Uttar Pradesh states. The infected papaya plants showed downward curling of leaves accompanied by vein thickening. The more seriously infected plants tend to develop twisted petioles and stunting in field. Diseased papaya plants either no fruits or produced small and distorted fruits.

Genomic Organization of DNA-A

The fragment about 700 bp was amplified with the degenerate primers PA and PB in eight papaya samples. Comparisons of these sequences showed that the nucleotide sequence identity ranged from 65.6 to 96.9% among these six isolates. The six isolates could fall into four groups. Group I includes 2 isolates (UP8 and UP13), the sequences within the group are closely related and share 93–98% nucleotide sequence identity. Group II includes 2 isolates (PaLC.Ban and PaLC.Kodur), the sequences within the group are closely related and share 93–97% nucleotide sequence identity. The other isolates PaLC.New Delhi and PaLC.Coimbatore, each one a different group having less than 72.3% identity with other groups. Based on the comparison of the determined sequences, representative isolates PaLC.Bangalore, PaLC.Coimbatore, PaLC.Lucknow and PaLC.New Delhi were chosen to be sequenced completely using overlapping primers. The complete DNA-A sequence of PaLC.Coimbatore, PaLC.Kodur, PaLC.Lucknow and PaLC.New Delhi were determined to be 2753, 272758, 2455 and 2740 nucleotides (nts), respectively. All the isolates DNA-A have a genomic organization of typical begomovirus originating from old world, with two ORFs [AV1 (CP) and AV2] in virionsense DNA and four ORFs [AC1 (Rep), AC2, AC3 and AC4] in complementary-sense DNA, separated by an intergenic region (IR). The IR contains various features characteristic of begomoviruses: a putative stem-loop structure with the conserved nonanucleotide sequence TAATATTAC in the loop; a TATA motif in all the isolates and repeated iteron sequence TTGGT in PaLC.Coimbatore, PaLC.Kodur and PaLC.New Delhi and GGGTC in PaLC.UP13. Similar iteron sequences were found in PaLCCV and PaLCGuV (Wang et al., 2004).

Relationship with Other Begomoviruses

Sequence similarity comparisons with other previously reported begomoviruses show that PaLC.Coimbatore isolate has highest similarity of 77.5% with Ageratum enation virus.Lucknow (EU867513) at DNA.A and with other begomoviruses it is less than 75%. When individual encoded proteins were compared PaLC.Coimbatore had the highest amino acid sequence identity with CrYVMV.Ban for CP (92.1%) and for AC3 (79.8%), CLCuRV.Sirsa for AV2 (73.5%) and AC4 (63.5%), EuLCV.Pusa for AC1 (86.7%), and ToLCIV.Iran for AC2 (82.8%) (Table 1). Different parts of the genome of PaLC.Coimbatore are related to different viruses, suggesting that different parts of the genome have different ancestors as observed for *Euphorbia leaf curl virus* (ELCV) (Ma et al., 2004).

The complete nucleotide sequence of PaLC.Kodur shares the highest identity (90.3%) with CrYVMV-[Ban], whereas less than 88.4% identities were found with other begomoviruses. The IR is the most great variation region of DNA-A. PaLC.Kodur has an

IR of 269 nts, and shares 88.9% with CrYVMV-[Ban] and 44.0-82.7% sequence identities with that of other begomoviruses. When individual encoded proteins were compared, PaLC.Kodur had the highest amino acid sequence identity 90.3% with CrYVMV-[Ban] for CP (98.8%), AV2 (94.0%), AC2 (93.2%) and AC3 (98.5%) and ToLCGV.Vadodara for AC1 (92.7%), and AC4 (88.6%) (Table 2).

The complete nucleotide sequence of PaLC.UP13 shares the highest identity (94.7%) with PaLCV-Lucknow, whereas less than 85.1% identities were found with other begomoviruses. The IR is the most great variation region of DNA-A. PaLC.UP13 has an IR of 269 nts, and shares 88.8% with PaLCV-Lucknow and 43.6-79.2% sequence identities with that of other begomoviruses. When individual encoded proteins were compared, PaLC.UP13 had the amino acid sequence identity 90.6% for CP, AV2 (96.0%), AC1 (98.6%), AC2 (98.5%), AC3 (94.0%) and AC4 (95.2%) with PaLCV-Lucknow (Table 3).

The complete nucleotide sequence of PaLC.New Delhi shares the highest identity (95.5%) with ToLCNDV-Chilli, whereas less than 72.8% identities were found with other begomoviruses. The IR is the most great variation region of DNA-A. PaLC.New Delhi has an IR of 269 nts, and shares 96.8% with ToLCNDV-Papaya and 46.3-57.2% sequence identities with that of other begomoviruses. When individual encoded proteins were compared, PaLC.New Delhi had the amino acid sequence identity 98.4% for CP, AV2 (95.5%), AC1 (96.1%), AC2 (92.8%), AC3 (92.6%) and AC4 (86.2%) with ToLCNDV-Chilli (Table 4).

Phylogenetic Analysis

Phylogenetic analysis was performed based on a multiple alignment of DNA-A sequences of PaLC.Coimbatore, PaLC.Kodur, PaLC.UP13 and PaLC.New Delhi and 21 other related geminiviruses (Fig. 1). PaLC.New Delhi cluster together with *Tomato leaf curl New Delhi virus* infecting chilli, cucurbits, potato, and tomato and has more than 90% similarity with these isolates indicating this isolate is *Tomato leaf curl New Delhi virus*. PaLC.Coimbatore clustered as independent group together with *Bendhi yellow vein mosaic virus* and *Cotton leaf curl Rajasthan virus*, which are having less than 80% nucleotide identity. Therefore this isolate is a new begomovirus infecting papaya. PaLC.Kodur clustered together with *Croton yellow vein mosaic virus*, which has 90.3% similarity indicating papaya as new host for this virus. PaLC.UP13, which was collected from Uttar Pradesh cluster together with Papaya leaf curl virus-infecting papaya and has more than 94.7% similarity with this isolate.

Begomoviruses are classified based on genome sequence, especially DNA-A sequence (Stanley et al., 2005). In general, begomoviruses sharing an overall DNA-A nucleotide sequence identities less than 89% are considered to be distinct begomoviruses (Fauquet et al., 2008). Therefore, PaLC.Coimbatore should be considered as distinct begomovirus. The symptoms induced by this isolate in papaya was similar to other isolates and we propose that PaLC.Coimbatore be named as *Papaya leaf curl Coimbatore virus* (PaLCCoV). The isolates PaLC.Kodur, PaLC.UP13 and PaLC.New Delhi have more than 90% sequence identity and were considered as Croton yellow vein mosaic virus-papaya, Papaya leaf curl virus-UP13 and Tomato leaf curl New Delhi virus-ND. The DNA-A of the four viruses shares less than 85% homology, indicating a genetic diversity among begomoviruses infecting papaya, so it is impossible to characterize virus only by the biological symptoms. Further all these isolates have sequence homology less than 76.2% with *Papaya leaf curl virus* (PaLCV.TW) from Taiwan (Chang et al., 2003), *Papaya leaf curl China virus* (PaLCCV) and as *Papaya leaf curl Guangdong virus* (PaLCGuV) from China (Cai et al., 2005; Wang et al., 2004) which all are other begomoviruses infecting papaya, indicating Indian isolates were different begomoviruses.

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Tables

Table 1. Pairwise comparisons of the nucleotide sequence identities of DNA-A and amino acid sequence identities of encoded proteins between PaLC.Coimbatore and other begomoviruses.

Virus	DNAA	IR	AV2	AV1	AC1	AC2	AC3	AC4
AF241479|BYVMV-Madurai	75.9	68.9	72.7	76.9	82.8	55.2	70.8	70.0
AY765254|CLCuRV.Sirsa	76.7	**77.3**	**73.5**	77.3	81.4	56.6	70.8	**63.5**
AJ507777|CrYVMV.Ban	76.5	45.7	65.2	**92.1**	75.9	78.3	**79.8**	37.5
AY297924|ToLCIV.Iran	78.0	54.0	71.3	91.4	81.1	**82.8**	78.3	46.3
AF413671|ToLCGV.Vadodara	77.3	51.2	69.5	91.7	83.6	82.0	76.8	45.3
U38239|ToLCKV.Karnataka	76.4	55.2	69.4	77.3	83.9	81.3	79.1	49.4
Y15934|PaLCV.Lucknow	73.1	48.6	66.9	76.9	74.2	78.3	74.6	35.4
AJ436992|PaLCV.Pk.cotton	73.3	45.6	66.1	77.3	75.3	80.5	79.1	38.5
EU867513|AEV.Lucknow	**77.5**	53.5	66.6	773	84.4	79.8	75.3	6.35
EU194914|EuLCV.Pusa.Bihar	76.7	50.3	60.0	77.7	**86.7**	79.1	76.8	65.0
DQ989326|ChLCV.Papaya.AD	76.2	53.0	66.9	76.9	83.1	81.3	76.8	45.3
EF194765|ToLCJoV.Chilli	75.0	50.1	64.4	76.5	81.7	82.0	75.3	45.3
AF295401|ToLCBV.Ban5	74.9	51.4	65.2	72.6	83.6	78.3	74.6	43.2
EU874386|PaLCCV.ZM1	72.4	53.0	68.1	82.4	77.0	63.7	6.79	41.2
AJ558125|PaLCCV.[G10]	71.9	49.4	65.5	82.1	76.7	61.4	70.1	44.3
AJ558122|PaLCGuV.[GD2]	72.9	53.6	69.8	80.9	80.1	68.1	67.1	61.4
DQ116880|ToLCNDV.Chillipepper	70.4	46.8	63.4	77.3	77.5	52.5	60.2	37.5
DQ989325|ToLCNDV.Papaya.PD	70.6	47.2	61.7	76.9	70.2	65.6	71.3	37.5

Table 2. Pairwise comparisons of the nucleotide sequence identities of DNA-A and amino acid sequence identities of encoded proteins between PaLC.Kodur and other begomoviruses.

Virus	DNAA	IR	AV2	AV1	AC1	AC2	AC3	AC4
AF241479|BYVMV-Madurai	71.9	44.0	63.6	79.2	78.7	60.8	73.8	48.0
AY765254|CLCuRV.Sirsa	73.7	44.2	65.2	79.6	80.9	62.6	73.1	48.9
AJ507777|CrYVMV.Ban	**90.3**	**88.9**	**94.0**	**98.8**	80.8	**93.2**	**98.5**	35.4
AY297924|ToLCIV.Iran	88.4	75.0	88.9	96.8	87.8	89.5	89.5	87.6
AF413671|ToLCGV.Vadodara	86.1	77.0	847	94.9	**92.7**	88.0	79.8	**88.6**
U38239|ToLCKV.Karnataka	84.2	72.0	88.1	81.6	91.6	86.5	88.8	82.4
Y15934|PaLCV.Lucknow	81.3	82.7	84.7	81.2	78.6	86.5	87.3	34.3
AJ436992|PaLCV.Pk.cotton	81.5	78.5	86.4	82.0	80.8	91.7	89.5	35.4
EU867513|AEV.Lucknow	81.3	66.4	88.9	81.6	82.8	85.8	87.3	48.9
EU194914|EuLCV.Pusa.Bihar	81.7	71.0	80.5	82.4	86.1	87.3	89.5	51.0
DQ989326|ChLCV.Papaya.AD	79.8	65.5	81.3	79.2	86.1	84.3	75.3	73.1
EF194765|ToLCJoV.chilli	80.3	63.5	85.9	80.8	86.1	85.8	78.3	72.1
AF295401|ToLCBV.Ban5	78.1	53.8	66.9	75.3	85.8	81.3	82.0	73.1
EU874386|PaLCCV.ZM1	76.0	64.3	73.1	81.7	82.3	65.1	67.1	79.3
AJ558125|PaLCCV.[G10]	75.8	60.8	71.4	82.4	82.5	62.2	67.1	77.3
AJ558122|PaLCGuV.[GD2]	72.6	53.5	78.9	80.1	80.1	68.8	63.4	46.8
DQ116880|ToLCNDV.Chillipepper	71.0	51.4	65.2	80.4	77.8	56.8	60.2	37.5
DQ989325|ToLCNDV.Papaya.PD	72.0	52.2	64.4	79.2	69.4	72.3	80.1	38.5

Table 3. Pairwise comparisons of the nucleotide sequence identities of DNA-A and amino acid sequence identities of encoded proteins between PaLC.UP13 and other begomoviruses.

Virus	DNAA	IR	AV2	AV1	AC1	AC2	AC3	AC4
AF241479|BYVMV-Madurai	70.8	43.6	69.4	92.9	70.5	60.8	68.6	38.0
AY765254|CLCuRV.Sirsa	72.3	46.2	71.0	93.3	71.0	62.0	70.1	54.1
AJ507777|CrYVMV.Ban	84.9	79.2	89.8	83.5	88.3	83.5	87.3	87.0
AY297924|ToLCIV.Iran	78.7	69.0	88.9	82.0	71.7	86.5	85.8	29.8
AF413671|ToLCGV.Vadodara	77.7	68.7	83.8	81.2	75.6	82.8	78.3	30.9
U38239|ToLCKV.Karnataka	81.5	68.7	**92.3**	97.2	74.5	83.5	85.0	32.9
Y15934|PaLCV.Lucknow	**94.7**	**88.8**	90.6	96.0	**98.6**	**98.5**	**940**	**95.2**
AJ436992|PaLCV.Pk.cotton	89.2	79.4	**923**	96.4	91.1	88.0	84.3	89.4
EU867513|AEV.Lucknow	83.0	62.7	91.5	**97.6**	77.5	88.0	85.0	54.1
EU194914|EuLCV.Pusa.Bihar	85.1	67.3	80.5	96.4	77.8	97.7	**94.7**	41.0
DQ989326|ChLCV.Papaya.AD	80.8	71.8	88.9	94.9	76.4	.83.5	74.6	36.0
EF194765|ToLCJoV.chili	80.7	64.7	89.2	96.0	75.0	828	805	28.8
AF295401|ToLCBV.Ban5	76.7	55.8	69.4	88.2	75.0	79.8	82.0	31.9
EU874386|PaLCCV.ZM1	73.3	64.6	77.3	84.4	70.7	63.7	64.9	30.9
AJ558125|PaLCCV.[G10]	73	61.4	73.9	84.8	70.7	60.7	64.9	27.8
AJ558122|PaLCGuV.[GD2]	72.4	54.8	79.8	83.6	70.4	69.6	62.6	40.6
DQ116880|ToLCNDV.Chillipepper	71.0	52.7	68.6	93.7	67.3	56.1	59.5	24.7
DQ989325|ToLCNDV.Papaya.PD	72.5	52.5	67.7	93.3	59.2	70.1	77.2	28.2

Table 4. Pairwise comparisons of the nucleotide sequence identities of DNA-A and amino acid sequence identities of encoded proteins between PaLC.New Delhi and other begomoviruses.

Virus	DNAA	IR	AV2	AV1	AC1	AC2	AC3	AC4
AF241479|BYVMV-Madurai	69.4	46.3	57.0	90.6	74.9	52.4	61.0	36.0
AY765254|CLCuRV.Sirsa	70.1	47.4	57.0	90.6	76..0	52.0	61.7	43.5
AJ507777|CrYVMV.Ban	68.8	51.4	61.8	8.04	68.1	60.4	64.7	28.2
AY297924|ToLCIV.Iran	70.4	50.1	66.0	79.6	75.0	55.3	62.5	38.1
AF413671|ToLCGV.Vadodara	70.8	51.1	669	777	781	56.1	59.5	36.0
U38239|ToLCKV.Karnataka	72.8	52.0	65.2	93.7	77.5	532	63.9	37.1
Y15934|PaLCV.Lucknow	69.9	50.3	65.2	91.0	65.6	56.8	.63.9	25.8
AJ436992|PaLCV.Pk.cotton	69.8	48.0	65.2	91.7	673	58.2	65.4	25.8
EU867513|AEV.Lucknow	72.7	54.8	65.8	92.1	75.0.	55.3	63.2	43.5
EU194914|EuLCV.Pusa.Bihar	72.3	55.8	61.6	91.7	75.9	57.5	66.1	36.0
DQ989326|ChLCV.Papaya.AD	72.2	50.4	63.5	91.4	76.7	53.9	65.4	43.2
EF194765|ToLCJoV.Chilli	71.8	52.0	63.6	92.5	75.6	54.6	64.7	37.1
AF295401|ToLCBV.Ban5	71.1	51.6	56.5	87.5	76.7	55.3	63.2	39.1
EU874386|PaLCCV.ZM1	69.4	57.2	56.0	82.1	76.2	50.7	62.5	37.1
AJ558125|PaLCCV.[G10]	70.3	55.8	54.3	81.7	76.7	51.4	63.9	38.1
AJ558122|PaLCGuV.[GD2]	70.8	53.5	65.5	80.1	77.6	55.7	60.2	36.4
DQ116880|ToLCNDV.Chillipepper	**95.5**	90.2	**95.5**	**98.4**	**96.1**	**92.8**	**92.6**	**86.2**
DQ989325|ToLCNDV.Papaya.PD	90.8	**96.3**	93.7	97.6	86.7	74.8	71.3	81.0

Figures

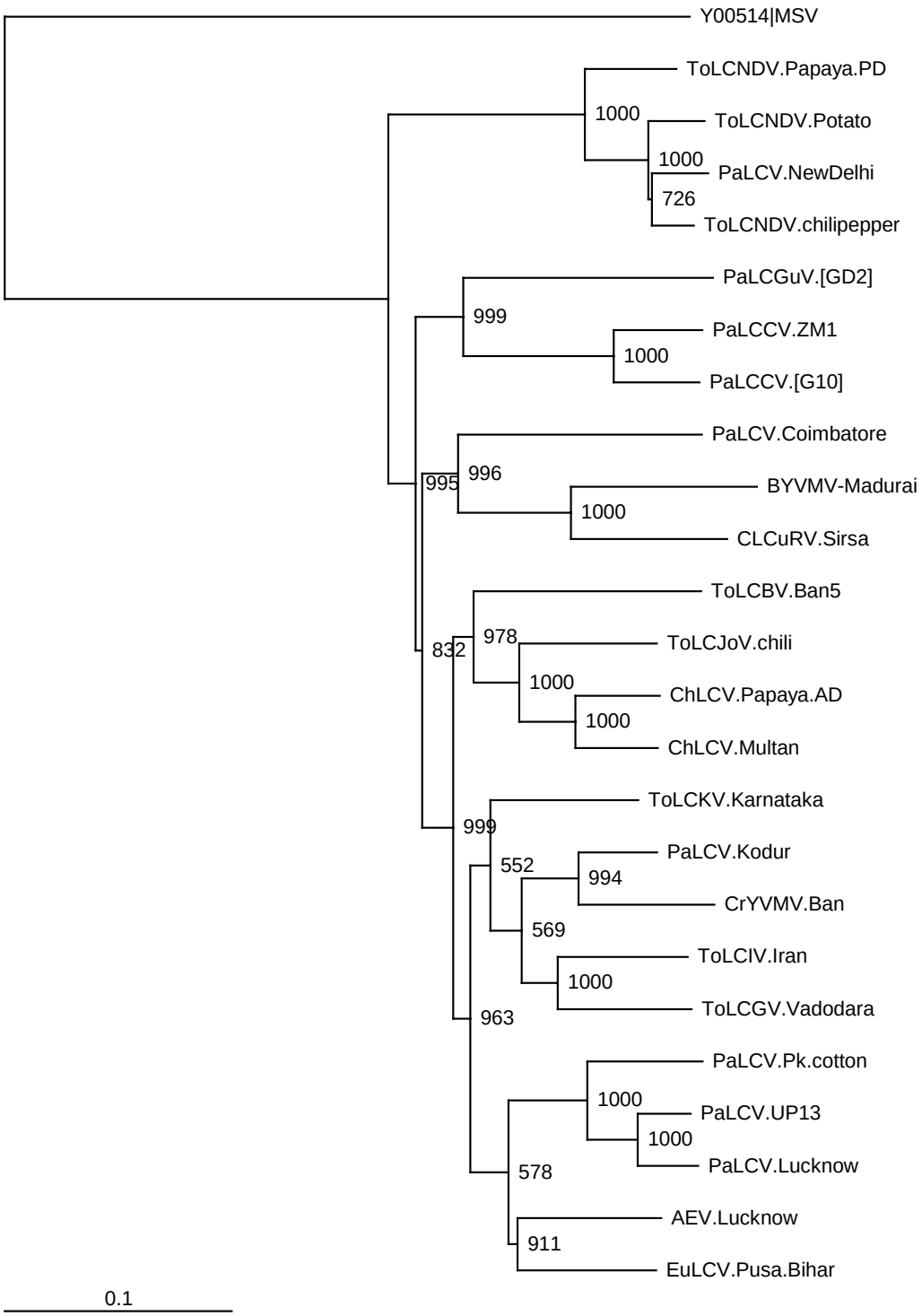

Fig. 1. Dendrograms showing the relationships among papaya-infecting begomoviruses and other representative geminiviruses based on multiple alignments of nucleotide sequences of DNA-A. The trees were rooted on the sequence of MSV.A.