Virus Genes 29:3, 303–309, 2004

Molecular Characterization of Two Distinct Begomoviruses from Papaya in China*

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Received April 25, 2004; Accepted May 13, 2004

Abstract. Six papaya samples showing downward leaf curling were collected in Guangdong and Guangxi provinces, China. The result of TAS-ELISA showed they were all infected by geminiviruses. Comparison of partial DNA-A sequences reveals that these virus isolates can be classified into two groups. Group I includes isolates G2, G4, G5, G28 and G29 from Guangxi province, while isolate GD2 from Guangdong province belongs to Group II. The complete DNA-A sequence of G2 and GD2 were characterized. Sequence comparisons showed that the DNA-A of G2 and GD2 were most closely related to that of *Ageratum yellow vein China virus*-[Hn2] and *Ageratum yellow vein virus,* respectively, with 83.4 and 75.2% nucleotide sequence identity, while DNA-A sequence between G2 and GD2 had only 73.4% sequence identity. The molecular data suggests that G2 and GD2 are two distinct begomoviruses, for which the name *Papaya leaf curl China virus* (PaLCuCNV) for G2 and *Papaya leaf curl Guangdong virus* (PaLCuGDV) for GD2 are proposed. Comparison of individual encoded proteins showed the coat protein of G2 and GD2 shared highest amino acid sequence identity (97.7 and 94.2%, respectively) with that of *Pepper leaf curl virus*-[Malaysia] (PepLCV-[MY]), suggesting the CP of these viruses may have identical ancestor.

Key words: begomovirus, DNA-A, nucleotide sequence, *Papaya leaf curl virus*

Introduction

Geminiviruses are a family of plant viruses characterized by having circular single-stranded DNA genomes packaged within twinned icosahedral particles. Based on the genome organization and biological properties, geminiviruses are currently divided into four genera: *Mastrevirus, Curtovirus, Topocuvirus,* and *Begomovirus* [1]. Begomoviruses are transmitted by the whitefly *Bemisia tabaci* to a wide range of dicotyledonous plants. Most begomoviruses have bipartite genomes known as DNA-A and DNA-B components. Some begomoviruses, such as *Ageratum yellow vein virus* (AYVV), *Tomato yellow leaf curl virus* (TYLCV) and *Cotton leaf curl Multan virus* (CLCuMV), have only a single component equivalent to DNA-A of bipartite begomoviruses [2,3], and novel DNA satellite, DNA β, was found to be associated with monopartite begomoviruses such as AYVV, *Bhendi yellow vein mosaic virus* (BYVMV), CLCuMV and *Tomato yellow leaf curl China virus* (TYLCCNV) [3–5].

Geminiviruses cause significant yield losses to many crop plants throughout the world [6]. In China, many begomoviruses were isolated from different hosts such as tobacco, squash, tomato and *Malvastrum coromandelianum* [5,7]. Papaya leaf curl disease has been reported in recent years in south of China, and the incidence and severity of the disease has increased rapidly in Guangxi and Guangdong provinces. In this paper we report that two distinct begomoviruses are associated with papaya leaf curl disease in China.

*The GenBank accession numbers of the sequences reported in this paper are AJ558122 and AJ558123.

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Materials and Methods

Source of Virus

Papaya samples showing leaf curling, vein thickening and leaf chlorosis symptoms were collected from papaya (*Carica papaya* L.) in Guangxi and Guangdong provinces of China in November 2002 (Table 1).

TAS-ELISA

Triple antibody sandwich ELISA (TAS-ELISA) was done as described [8]. The monoclonal antibodies (MAbs) of *African cassava mosaic virus* (ACMV), *Indian cassava mosaic virus* (ICMV) and *Okra leaf curl virus* (OLCV) were kindly provided by B. D. Harrison, Scottish Crop Research Institute, UK.

Total DNA Extraction, PCR and Cloning

Nucleic acids were extracted from symptomatic papaya leaves following the method of Xie et al. [9]. Partial products about 500 bp covering part of the intergenic region and AV2 gene of DNA-A were amplified with degenerate primers PA and PB [9]. On the basis of the determined sequences of 500 bp fragments, primer pairs Y6F1/TLCV/R and GD2F/GD2R were designed for amplification the whole DNA-A of isolates G2 and GD2, respectively. PCR was done as described [8]. PCR products with the expected size were purified and cloned into pGEM-T Easy Vector (Promega), and sequenced using the automated model 377 DNA sequencing system (Perkin-Elmer). Primers used for PCR and sequencing are shown in Table 2.

Table 1. The location of papaya samples

Sample	Region	Province	Accession numbers in GenBank	
			Partial DNA-A	DNA-A
G2	Nanning	Guangxi		AJ558122
G4	Nanning	Guangxi	AJ632298	
G5	Nanning	Guangxi	AJ632299	
G28	Tiandong	Guangxi	AJ632300	
G29	Tiandong	Guangxi	AJ632301	
GD2	Guangzhou	Guangdong		AJ558123

Table 2. Primers used for PCR and sequencing

Primers	Sequence (5′ → 3′)[a]	Position in G2 or GD2 DNA-A
Primers used for DNA-A cloning		
PA	taatattacckgwkgvccsc	2734–13 nts in G2
PB	tggacyttrcawggbccttcaca	515–493 nts in G2
Y6F1	accggatgtacagaagccctga	458–479 nts in G2
TLCV/R	atctgctggtcgcttcgacat	309–289 nts in G2
GD2F	gtgggatccacttctgaacgag	117–138 nts in GD2
GD2R	agtggatcccacatttttgaag	128–107 nts in GD2
Primers used for DNA-A sequencing		
H2F	gaattctgcattctttaatgcc	160 1–1622 nts in G2
Y68R2	aataagctttgaggagcg	79–62 nts in G2
Y6R2	ggaagccagttcaaattaaagg	1723–1702 nts in GD2
Y6F2	cctttaatttgaactggcttcc	1702–1723 nts inGD2
TLCV/F1	gtgactggtggaacaatatgc	833–852 nts in GD2
TLCV/F3	atcttgaatagaggggatt	1358–1377 nts in GD2

[a]B = C, T or G, K = G or T, R = A or G, S = C or G, V = A, C or G, W = A or T, Y = C or T.

DNA Sequence Analysis

The sequences were assembled and analyzed with the aid of DNAStar software (Madison. Wis., USA). Phylogenetic trees were constructed using the full optimal alignment and neighbour-joining method options with 1000 bootstrap replications available in DNAMAN version 5.0 (Lynnon Biosoft, Quebec, Canada). The database accession numbers of the begomovirus DNA-A sequences used for comparison or dendrogram construction were: *African cassava mosaic virus*-Uganda severe (ACMV-UGSVr, AF126802), *Ageratum yellow vein virus* (AYVV, X74516), AYVV-[Tomato] (AYVV-[To], AB100305), *Ageratum yellow vein China virus*-[Hn2] (AYVCNV-[Hn2], AJ495813), *Bean golden yellow mosaic virus*-[Puerto Rico] (BGYMV-[PR], M10070), *Beet curly top virus* (BCTV, X04144), *Cotton leaf curl Kokhran virus*-[72b] (CLCuKV-[72b], AJ002448), *Cotton leaf curl Multan virus*-[Okra] (CLCuMV-[OK], AJ002459), *Euphorbia leaf curl virus* (EuLCV. AJ558121), *Indian cassava mosaic virus* (ICMV, Z24758), *Malvastrum yellow vein virus*-[Y47] (MYVV-[Y47], AJ457824), *Bhendi yellow vein mosaic virus*-[301] (BYVMV-[301], AJ002453), *Okra yellow vein mosaic virus*-[201] (OYVMV-[201], AJ002451), *Papaya leaf curl virus* (PaLCuV, Y15934), *Pepper leaf curl virus* (PepLCV, AF134484), PepLCV-[Malaysia] (PepLCV-[MY], AF414287), *Squash*

leaf curl China virus (SLCCNV, AB027465), *Squash leaf curl Yunnan virus* (SLCYV, AJ420319), *Tobacco curly shoot virus*-[Y1] (TbCSV-[YI], AF240615), *Tobacco leaf curl Yunnan virus* – [Y3] (TbLCYNV-[Y3], AF240674), *Tomato golden mosaic virus*-Yellow vein (TGMV-YV, K02029), *Tomato leaf curl China virus*-[G18] (ToLCCNV-[G18], AJ558119), *Tomato leaf curl Karnataka virus* (ToLCKV, U38239), *Tomato leaf curl Taiwan virus* (ToLCTWV, U88692), *Tomato leaf curl Vietnam virus* (ToLCVV, AF264063), *Tomato yellow leaf curl china virus* (TYLCCNV, AF311734) and *Tomato yellow leaf curl Thailand virus*-[1] (TYLCTHV-[1], X63015).

Results

Symptoms

Papaya leaf curl disease was easily found in papaya gardens in Guangxi and Guangdong provinces. 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 fields (Fig. 1). Diseased papaya plants produced small and distorted fruits. In some papaya gardens, almost all plants were destroyed by leaf curl disease, so farmers had to abandon the gardens.

Fig. 1. Papaya showing downward leaf curling and stunt symptoms.

Epitope Profiles

Fresh extracts of papaya leaves were tested by TAS-ELISA with MAbs raised against particles of ACMV, ICMV and OLCV. The results obtained with MAbs showed that all the six papaya samples were infected with begomoviruses and their epitope profiles can divide into two types (Table 3). Type 1 includes three isolates (G2, G4, G5) originated from Nanning, which reacted strongly with MAbs SCR 20 and 23 of ACMV, SCR 66 of ICMV, SCR 106 of OLCV, weakly or moderately with SCR 17 of ACMV, SCR 54, 55, 56 and 58 of ICMV. Isolates G28, G29 collected from Tiandong can fall into type 2. This group reacted strongly with SCR 18, 20 and 23 of ACMV, SCR 54 and 56 of ICMV, weekly with SCR 17 of ACMV, SCR 55 and 66 of ICMV, SCR 106 of OLCV. GD2 collected from Guangzhou strongly reacted with SCR 18 of ACMV, reaction with other Mabs was not tested because of limited leaf material.

Genomic Organization of DNA-A

The fragment about 500 bp was amplified with the degenerate primers PA and PB in six papaya samples. Comparisons of these sequences showed that the nucleotide sequence identity ranged from 66.6 to 97.9% among these six isolates. The six isolates could fall into two groups. Group I includes 5 isolates (G2, G4, G5, G28, G29), the sequences within the group are closely related and share 89–98% nucleotide sequence identity. GD2 shares lower sequence identities (67–71%) with

Table 3. Reactions of geminivirus isolates from papaya in China in TAS-ELISA with monoclonal antibodies

	17	18	20	23	54	55	56	58	60	62	66	68	106
G2	2	0	3	3	3	1	2	2	0	0	3	0	3
G4	1	0	3	3	2	2	2	0	0	0	3	0	3
G5	0	0	3	3	2	1	0	0	0	0	3	0	3
G28	1	3	3	3	3	1	3	0	0	0	2	0	1
G29	1	3	3	3	3	1	3	0	0	0	2	0	1
GD2	–	3	–	–	–	–	–	–	–	–	–	–	–

MAbs SCR 17–23 were raised against ACMV, SCR 53–66 against ICMV, SCR102–106 against OLCV. Scores represent A_{405} ranges as follows: 0 (< 0.3), 1 (0.3–0.6), 2 (0.61–1.20), 3 (1.21–1.80), 4 (> 1.8). –: not tested.

isolates in Group I, it could be classified as Group II.

Based on the comparison of the determined sequences, representative isolates G2 and GD2 from Groups I and II were chosen to be sequenced completely. The complete DNA-A sequence of G2 and GD2 were determined to be 2740 and 2742 nucleotides (nts), respectively. Both of G2 and GD2 DNA-A have a genomic organization of typical begomovirus originating from old world, with two ORFs [AV1(CP) and AV2] in virion-sense 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 at 2661–2664 nts in G2 and at 2655–2658 nts in GD2; and repeated iteron sequence TTGGT in GD2 (nucleotides 2591–2595, repeated at nucleotides 2645–2649) and GGGTC in G2 (nucleotides 2615–2619, repeated at nucleotides 2650–2654) upstream to the 5′ side of the TATA motif. The DNA-A and IR sequence identities between G2 and GD2 were only 73.4 and 41.3%, respectively (Table 4), suggesting that each represents a different virus species. G2 and GD2 had the highest amino acid sequence identity for CP (95.3%), while relatively lower sequence identities were found for AV2 and AC1–AC4 (43.3–79.6%).

Affinities to other Begomoviruses

Sequence similarity searches were performed using the BLAST program (http://www.nchi.nlm.nih.gov/). The results for relatively closely related begomoviruses were listed in Table 4. The complete nucleotide sequence of G2 shares the highest identity (83.4%) with AYVCNV-[Hn2], whereas less than 80.6% identities were found with other begomoviruses. The IR is the most great variation region of DNA-A. G2 has an IR of 269 nts, and shares 26.0–71.4% sequence identities with that of other begomoviruses. When individual encoded proteins were compared, G2 had the highest amino acid sequence identity with PepLCV-[MY] for CP (97.7%) and AV2 (84.5%), AYVCNV-[Hn2] for AC1 (92.8%), AC2 (95.6%) and AC3 (94.0%), and TbCSV-[Y1] for AC4 (94.8%). Different parts of the genome of G2 are related to different virus, indicating that G2 may originate from different viruses.

The percentages of identity of nucleotide sequence or amino acid sequences of encoded proteins between GD2 and other begomoviruses

Table 4. Pairwise comparisons of the nucleotide sequence identities of DNA-A and amino acid sequence identities of encoded proteins between G2 and other begomoviruses

Virus	DNA-A[a]	IR[a]	AV1(CP)[b]	AV2[b]	AC1(Rep)[b]	AC2[b]	AC3[b]	AC4[b]
GD2	73.4	41.3	95.3	76.1	79.6	71.3	68.9	43.3
AYVV	80.6	64.7	89.9	75.0	85.0	89.6	89.6	40.8
AYVCNV-[Hn2]	83.4	71.4	89.1	57.3	92.8	95.6	94.0	88.7
EuLCV	68.5	37.5	82.1	62.4	78.6	58.5	66.7	46.4
MYVV-[Y47]	71.5	66.9	81.7	62.9	83.2	56.6	61.5	84.7
PaLCuV	66.8	40.9	82.4	71.8	70.4	59.7	65.7	36.5
PepLCV-[MY]	78.2	59.9	97.7	84.5	82.0	64.9	72.4	72.2
SLCCNV	62.8	26.0	81.6	58.1	67.9	47.0	60.7	11.2
SLCYV	65.4	49.1	79.3	66.1	68.7	67.9	58.5	35.1
TbCSV-[Yl]	77.1	69.5	83.2	71.8	82.5	58.2	64.9	94.8
TbLCYNV-[Y3]	76.1	48.0	78.8	71.8	79.6	91.9	91.0	46.9
ToLCCNV-[G18]	72.6	63.0	75.9	67.2	83.7	60.7	65.7	82.5
ToLCV-IN2	79.6	63.7	90.7	75.0	83.7	88.9	91.8	43.8
ToLCTWV	79.5	57.2	94.6	82.3	84.8	72.6	69.4	82.5
TYLCCNV	76.1	58.7	82.8	78.3	84.7	70.9	71.6	80.4
TYLCTHV-[1]	74.8	66.2	79.7	69.6	84.7	71.6	71.6	87.6

[a]Nucleotide sequence identity.
[b]Amino acid sequence identity.

Table 5. Pairwise comparisons of the nucleotide sequences identities of DNA-A and amino acid sequence identities of encoded proteins between GD2 and other begomoviruses

Virus	DNA-A[a]	IR[a]	AV1(CP)[b]	AV2[b]	AC1(Rep)[b]	AC2[b]	AC3[b]	AC4[b]
AYVV	75.2	45.2	90.3	78.4	85.6	66.7	68.7	75.3
AYVCNV-[Hn2]	72.0	46.1	89.9	63.2	82.6	73.3	70.1	45.4
EuLCV	71.9	44.6	80.9	65.0	85.6	63.0	65.9	85.6
MYVV-[Y47]	67.8	46.0	79.8	64.7	77.7	59.6	62.2	42.3
PaLCuV	67.8	49.1	81.2	75.2	69.8	66.4	63.4	45.9
PePLCV-[MY]	73.2	45.8	94.2	75.9	78.9	66.4	76.1	46.4
SLCCNV	64.9	26.6	79.7	65.0	70.0	54.5	60.0	17.5
SLCYV	65.0	45.8	78.1	70.5	70.1	66.4	58.5	43.6
TbCSV-[Y1]	70.1	50.6	82.0	76.1	74.5	64.9	61.2	45.4
TbLCYNV-[Y3]	71.0	45.0	77.6	76.1	82.0	70.4	68.7	79.2
ToLCVV	74.4	54.2	93.4	78.4	80.4	65.2	60.4	44.3
ToLCCNC-[G18]	68.6	44.7	75.1	73.3	80.9	65.9	62.7	48.5
ToLCTWV	73.2	53.0	91.8	72.6	80.4	69.6	67.9	47.4
TYLCCNV	70.2	47.2	81.6	72.2	80.8	71.6	70.9	46.4
TYLCTHV-[1]	67.7	47.6	78.9	73.2	78.3	70.1	70.9	44.3

[a]Nucleotide sequence identity.
[b]Amino acid sequence identity.

are listed in Table 5. GD2 is most closely related to AYVV, with 75.2% nucleotide sequence identities for DNA-A and 85.6%, 78.4% amino acid sequence identities for AC1 and AV2, respectively. GD2 has the highest amino acid sequence identities with PepLCV-[MY] for CP (94.2%) and AC3 (76.1%), AYVCNV-[Hn2] for AC2 (73.3%) and EuLCV for AC4 (85.6%).

Phylogenetic analysis was performed based on a multiple alignment of DNA-A sequences of G2, GD2 and 25 other related geminiviruses (Fig. 2). G2 and GD2 cluster together with begomoviruses infecting ageratum, tomato, pepper, squash and tobacco in Asia.

Discussion

Mosaic disease caused by *Papaya ringspot virus* had been reported in papaya in China for many years [10], while other viruses were not reported any more. In recent year, leaf curling disease in papaya is very common in Guangxi and Guangdong provinces, the disease has become a major constraint for papaya production in some parts of Guangxi and Guangdong provinces. Based on TAS-ELISA and PCR detection, we demonstrate that six papaya leaf curl samples were infected by begomoviruses. The full-length DNA-A of isolates G2 and GD2 shares the highest nucleotide sequence identities with AYVCNV and AYVV (83.4 and 75.2%, respectively). Begomoviruses are classified based on genome sequence, especially DNA-A sequence [11]. In general, begomoviruses sharing a overall DNA-A nucleotide sequence identities less than 89% are considered to be distinct begomoviruses [1]. Therefore, isolates G2 and GD2 should be considered as two distinct, begomoviruses. The symptoms induced by G2 and GD2 in papaya were all downward leaf curling and vein thickening, so we propose that G2 be named as *Papaya leaf curl China virus* (PaLCuCNV) and GD2 as *Papaya leaf curl Guangdong virus* (PaLCuGDV).

Although isolates G2 and GD2 were collected from the adjacent regions, Guangxi and Guangdong province, and both had papaya leaf curl symptom, the DNA-A of G2 and GD2 only share 73.4% nucleotide sequence identity, indicating a genetic diversity among begomoviruses infecting papaya, so it is impossible to characterize virus only by the biological symptoms. To detect a DNA-B or DNAβ component, the primer pairs PCRc1/PBL1v2040 and beta01/beta02 specific for DNA-B and DNAβ [12,13], respectively, were used for PCR reaction, and no amplified products were found in all the six isolates. Construction of infective clones of G2 and GD2 DNA-A is

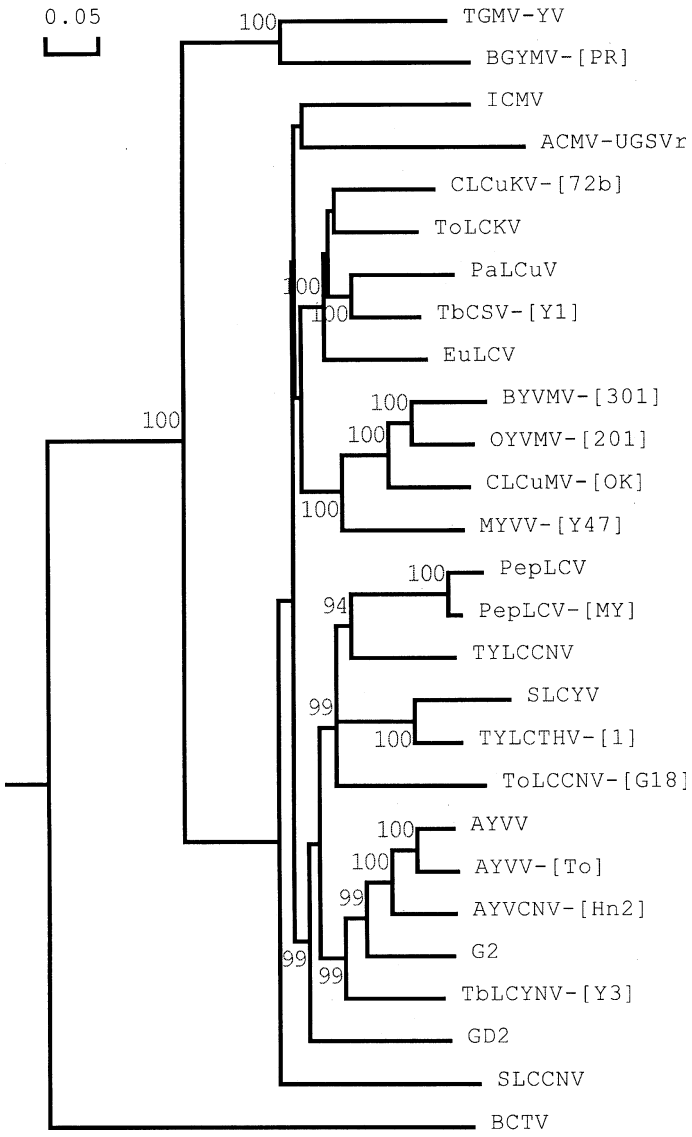

Fig. 2. Dendrograms showing the relationships among tomato-infecting begomoviruses and other representative geminiviruses based on multiple alignments of nucleotide sequences of DNA-A. The dendrograms were constructed by the neighbour-joining method of DNAMAN and bootstrapped 1000 times. Bootstrap scores exceeding 50% are placed at major nodes, nodes lacking a score are considered dubious. Horsizontal distances are proportional to sequence distances; vertical distances are arbitrary. The trees were rooted on the sequence of BCTV DNA-A.

necessary to know the monopartite or bipartite nature for these two begomovirus species.

Geminiviruses have caused significant yield losses to many crops in the world in the recent decade. In China, we have characterized many distinct begomoviruses, the outbreaks of B biotypes of whitefly together with the existence of many begomovirus species may indicate that begomoviruses will emerge as a threat in China in the near future.

Acknowledgments

This work was supported by National Outstanding Youth Foundation (Grant No. 30125032) and

Natural Science Foundation of China (Grant No. 30370927).

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