NANOVIRUS-LIKE DNA COMPONENT ASSOCIATED WITH THE MALVASTRUM YELLOW MOSAIC VIRUS

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Received October 9, 2007; accepted January 28, 2008

Summary. – Nanovirus-like DNA components signed DNA1 are single-stranded molecules associated with monopartite begomovirus-satellite complexes. DNA1 molecules ranging in size from 1373–1385 nucleotides were detected in 5 isolates Hn36, Hn38, Hn39, Hn44, and Hn45 of Malvastrum yellow mosaic virus (MalYMV) isolated in Hainan, China. DNA1 molecules of all isolates contained three characteristics: a single ORF, conserved hairpin structure, and A-rich region. Nucleotide sequence comparison showed that DNA1 molecules of 5 examined isolates shared high sequence identity (71.8–98.8%). In comparison with other begomovirus DNA1 and nanovirus-associated DNA, the sequence identities 59.6–76.3% and 27.2–38.4%, respectively, were found.

Key words: Malvastrum yellow mosaic virus; DNA1; geminiviruses; nanoviruses

Introduction

The family *Geminiviridae* contains plant viruses with circular single-stranded DNA genome encapsidated in unique twinned particle. A greater part of geminiviruses belong to the genus *Begomovirus*. The majority of begomoviruses contain two components of DNA referred to as DNA A and DNA B that are essential for virus proliferation (Fauquet *et al*., 2005). Some of the begomoviruses have only a single genomic component, equivalent to the DNA A of their bipartite virus counterparts (Lazarowitz, 1992).

Recently, a novel satellite DNA referred to as DNA ß was found in some monopartite begomoviruses. DNA ß is approximately half the size of the viral genomic DNA A and apart from the nonanucleotide sequence (TAATATTAC), it has no sequence identity to either DNA A or DNA B. DNA ß requires DNA A for the replication, encapsidation, insect transmission, and movement in plants (Xiong *et al*., 2007; Wu and Zhou, 2007). It encodes an ORF (ßC1) on the complementary-sense strand that plays an essential role in the symptom induction and host adaptation (Cui *et al*., 2004; Saeed *et al*., 2005; Saunders *et al*., 2004).

Furthermore, a circular, single-stranded DNA molecule labeled as DNA1 has been identified to associate with geminiviruses. The DNA1 satellite-like molecules are approximately half the size of begomovirus DNA A or DNA B and encode a rolling-circle replication initiator protein with similarity to that of nanoviruses (Briddon *et al*., 2004; Bull *et al*., 2003; Xie *et al*., 2004). DNA1 molecules are capable of self-replication in host plants and are not required for disease development (Wu and Zhou, 2005).

MalYMV is a distinct begomovirus identified in China (Guo *et al*., 2007). MalYMV is most closely related to the Malvastrum yellow vein virus (MYVV) with 81% nucleotide sequence identity (Jiang and Zhou, 2004). In this paper, we report the cloning and sequencing of DNA1 molecules associated with 5 MalYMV isolates and their comparison with DNA1 molecules of other begomoviruses and with nanovirus DNAs.

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Abbreviations: MalYMV = Malvastrum yellow mosaic virus; Rep = replication-associated protein; CLCuMV = Cotton leaf curl Multan virus; TYLCCNV = Tomato yellow leaf curl China virus

Materials and Methods

Virus isolates. Five plants *Malvastrum coromandelianum* exhibiting the yellow mosaic symptoms were collected in fields of Danzhou, Hainan Province. The virus samples were identified as the isolates of MalYMV (Guo *et al*., 2007) and designated as Hn36, Hn38, Hn39, Hn44, and Hn45.

DNA extraction, PCR. Total DNA was extracted from tissues of naturally infected symptomatic plants as described previously (Zhou *et al*., 2001). DNA1 molecules were produced by PCR-mediated amplification with universal primers UN101/UN102 (Bull *et al*., 2003). The PCR products were recovered, purified, cloned into pGEM-T vector (Promega), and sequenced using the automated model 3730 DNA sequencing system (Perkin Elmer).

Sequence determination and sequence analysis. Sequence data for analysis were assembled and analyzed by the DNAStar Software (DNAStar Inc.) and multiple sequence alignments were performed using DNAMAN Version 4.0 for Windows (Lynnon Biosoft). A phylogenetic tree was constructed using the neighbor-joining method provided by DNAMAN with 1000 bootstrap trials. Vertical branches are arbitrary, horizontal branches are proportional to the calculated mutation distances. The numbers at each branch indicate the percentage of 1000 bootstrap replications, which supports the grouping at the node. Bootstrap scores exceeding 90% are placed at the major nodes. All compared sequences were obtained from GenBank (Table 1).

Table 1. Accession numbers of DNA1 molecules of begomoviruses and nanovirus-associated DNAs used for the comparison

Begomoviruses	Abbreviation	Acc. No.
Tobacco curly shoot virus	TbCSV	AJ579346
Tomato yellow leaf curl China virus	TYLCCNV	AJ579354
Ageratum yellow vein Kenya virus	AYVKEV	AJ512963
Cotton leaf curl Multan virus	CLCuMV	AJ512957
Okra leaf curl virus	OLCV	AJ512954
Sida yellow vein Vietnam virus	SiYVVNV	DQ641718
Nanoviruses		
Banana bunchy top virus	BBTV	AF216221
Coconut foliar decay virus	CFDV	M29963
Faba bean necrotic yellow virus	FBNYV	X80879
Milk vetch dwarf virus (component C1)	MVDV-C1	AB000920
Subterranean clover stunt virus (component C6)	SCSV-C6	U16735

Results and Discussion

Genomic organization of DNA1 molecules associated with MalYMV

PCR was carried out in attempts to detect a putative DNA1 molecule in MalYMV samples using abutting primers UN101 (AAGCTTGCGACTATTGTATGAAAGAGG) and UN102 (AAGCTTCGTCTGTCTTACGAGCTCGCTG) designed from highly conserved regions of the replication-associated protein (Rep) encoding genes of DNA1 molecules. All collected MalYMV isolates Hn36, Hn38, Hn39, Hn44, and Hn45 contained the DNA1 molecules. The complete nucleotide sequences of DNA1 molecules were 1373–1385 nts in length (Acc. Nos. AM236763–AM236767). The DNA1 molecules are marked according to their isolates as Hn36 DNA1, Hn38 DNA1, etc.

The isolated DNA1 molecules contained conserved nonanucleotide sequence TAGTATTAC, a region high in adenine (A-rich) and a single Rep ORF on the virion-sense DNA strand. The nonanucleotide sequence TAGTATTAC is common to nanoviruses and similar to the sequence TAATATTAC of geminiviruses. For both geminiviruses and nanoviruses, this sequence contains the nick site for initiation of virion-sense DNA replication. The A-rich region of examined five DNA1 molecules is approximately 140–159 nts long with an A content of 49–51% and is located in all the DNA1 components immediately downstream of the Rep gene. This region is the only one that distinguishes the begomovirus associated DNA1 components from Rep-encoding components of nanoviruses. The highly conserved nucleotide sequences encompassed Rep ORF with the capacity to encode a protein of 315 amino acids (approx. 36.6 kDa) that resembled Rep of nanoviruses. A consensus TATA box appeared upstream of the Rep ORF and several consensus transcript polyadenylation signals (AATAAA) occurred downstream of the Rep ORF.

Nucleotide sequence comparisons showed that examined five DNA1 molecules could be divided into two groups. Group 1 contained DNA1 molecules from isolates Hn36, Hn38, and Hn44 sharing 98.0–98.8% sequence identity. Group 2 included DNA1 molecules from Hn39 and Hn45 isolates with 83.1% sequence identity. Only 71.8–77.5% nucleotide sequence identities were found between group 1 and group 2 (Table 2).

Table 2. Sequence identities (%) of complete nucleotide (top right) and Rep amino acid (bottom left) sequences between DNA 1 molecules of MalYMV isolates

Isolate	Hn36	Hn38	Hn44	Hn39	Hn45
Hn36	100.0	98.8	98.0	77.1	71.8
Hn38	99.1	100.0	98.2	77.1	72.0
Hn44	98.7	99.4	100.0	77.5	72.1
Hn39	89.9	90.2	90.5	100.0	83.1
Hn45	90.2	90.5	90.2	97.5	100.0

Sequence analysis showed that the identity of the amino acid sequences of the Reps were higher than the identity of the full-length nucleotide sequence among the five DNA1

components. In the group 1, Rep amino acid sequence identity was 98.7–99.4% and in the group 2 was 97.5%. Amino acid sequence identity found between the Reps of group 1 and group 2 was 89.9–90.5% (Table 2).

Sequence identities of Hn36 DNA1, Hn39 DNA1 with other DNA1 of begomoviruses and with nanovirus-associated DNA

In addition, multiple alignments of the nucleotide sequences and the amino acid sequences of Rep were performed between Hn36 DNA1, Hn39 DNA1, and previously reported begomoviruses and nanoviruses DNA1 molecules (Table 3). Nucleotide sequence comparison showed that Hn36 DNA1 had the highest sequence identity (76.3%) with Cotton leaf curl Multan virus (CLCuMV) DNA1. The sequence identities of 59.9–71.5% were detected with other reported DNA1 molecules. Hn39 DNA1 shared 72.9% identity with DNA1 of Tomato yellow leaf curl China virus (TYLCCNV) and 59.6–71.9% sequence identities with other DNA1 molecules. Only 27.2–38.4% nucleotide sequence identity was found between Hn36 DNA1, Hn39 DNA1, and nanoviruses. Relative higher amino acid sequence identity (80.3–88.6%) were found for the Rep among various reported DNA1 molecules, whereas only 31.0–40.6% in comparison with the nanoviruses.

The phylogenetic trees were constructed according to the alignments of genomic nucleotide sequences and amino acid sequences of Reps of DNA1 associated with begomoviruses and nanoviruses. The relationship dendrogram of the full-length nucleotide and amino acid sequences of their Reps showed that the five examined DNA1 molecules were divided into two small branches representing group 1 and group 2 and clustered together with other geminiviruses DNA1 molecules, whereas DNA1 molecules of nanoviruses formed the separate branches (Fig. 1A,B).

Table 3. Comparison of sequence identities (%) of complete nucleotide and Rep amino acid sequences between Hn36 DNA1, Hn39 DNA1, other begomovirus DNA1 molecules and nanovirus-associated DNAs

Virus		Hn36	Hn36	Hn39	Hn39
		DNA1	Rep	DNA1	Rep
Begomoviruses	TbCSV	69.6	88.6	71.9	88.3
	TYLCCNV	68.1	88.3	72.9	88.6
	AYVKEV	59.9	80.3	59.6	82.9
	CLCuMV	76.3	88.6	69.0	88.6
	OLCV	71.5	85.7	69.8	86.7
	SiYVVNV	66.7	85.7	66.5	86.7
Nanoviruses	SCSV-C6	31.9	36.5	30.3	35.1
	BBTV	31.8	33.3	32.4	33.0
	CFDV	27.2	31.0	29.3	32.1
	FBNYV	34.7	36.3	33.2	36.0
	MVDV-C1	38.4	40.6	33.9	39.9

Description of the virus abbreviations is presented in Table1.

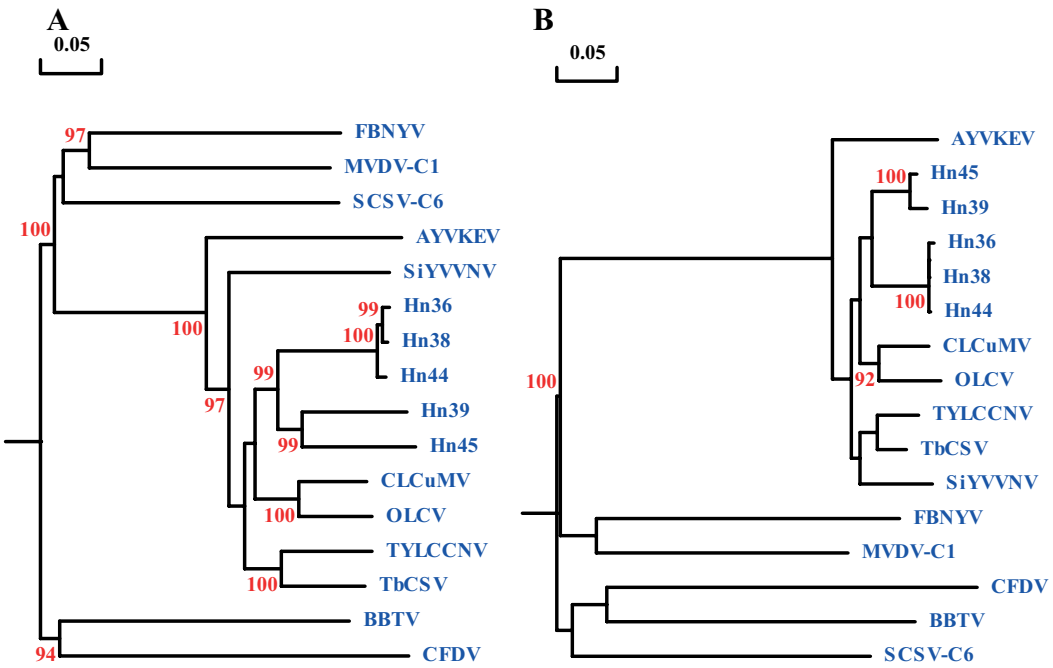

Fig. 1

Neighbor-joining phylogenetic dendrograms based on alignments of the complete nucleotide sequences of DNA1 components of begomoviruses (A) and the predicted amino acid sequences of their Rep genes (B) with nanovirus-associated DNAs

The function of DNA1 is not clear yet, but it is evident that DNA1 functionally interact with geminivirus satellite complexes resulting in symptom alteration and reduction of the level of viral DNA and DNA ß (Mansoor *et al*., 1999; Wu and Zhou, 2005; Saunders and Stanley, 1999). The specific role of DNA1 in evolution of the virus, disease development, and virus life cycle has to be characterized further.

Acknowledgements. This work was supported by the grant No. 30671360 from the National Natural Science Foundation of P.R. China. We thank Prof. Xueping Zhou for his valuable advice and review of the manuscript.

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