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Molecular characterization of Ramie mosaic virus isolates detected in Jiangsu and Zhejiang provinces, China

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Summary. – Virus isolates were obtained from three ramie samples (*Boehmeria nivea* L.) showing yellow mosaic symptoms collected in Jiangsu and Zhejiang provinces, China. Comparison of partial DNA-A fragments amplified with begomovirus universal primers PA/PB revealed that these viral isolates shared a high sequence identity. The complete DNA-A sequences of two isolates J4 and Z1 were determined to be 2736 and 2737 nts, respectively, sharing 94.7% nucleotide sequence identity with each other. Also, the DNA-B components were identified for J4 and Z1 isolates and comprised 2717 and 2719 nts, respectively, sharing 88.6% nucleotide sequence identity with each other. Furthermore, sequence alignment and phylogenetic analysis showed that J4 and Z1 isolates had the highest sequence identities (93.6–94.7%) with isolates of Ramie mosaic virus (RamMV) for DNA-A. These molecular data suggested that J4 and Z1 may be two different isolates of RamMV. This is the first report about the occurrence of a bipartite begomovirus in these regions of China.

Keywords: begomovirus; ramie; bipartite; DNA-A; DNA-B

Introduction

Geminiviruses are plant viruses that are characterized by the small circular single-stranded DNA (ssDNA) genomes, encapsidated in twinned icosahedral particles (Lazarowitz, 1992). Based on the insect vector, genome organization, and host range, the family *Geminiviridae* is divided into four genera *Begomovirus*, *Mastrevirus*, *Curtovirus*, and *Topocuvirus* (Fauquet *et al*., 2003). Most geminiviruses belong to the genus *Begomovirus*. For the most part, they are emerging viral pathogens that infect dicotyledonous plants and are transmitted by the whitefly *Bemisia tabaci*. Many begomoviruses have a bipartite genome consisting of DNA-A and DNA-B component. Both components of the genome have a noncoding common region (CR) sequence of approximately 200 nts in length that is highly conserved among species (Hanley-Bowdoin *et al*., 1999). However, a number of begomoviruses found in the Old World have only a single genomic DNA component equivalent to the DNA-A of the bipartite genome (Saunders *et al*., 2000). Many monopartite begomoviruses are associated with a satellite DNA termed DNA β (Jose and Usha, 2003; Guo *et al*., 2008).

In China, begomoviruses have been reported to infect a wide variety of crops, weeds, and ornamental plants (Xie and Zhou, 2003; Ma *et al*., 2004; Xiong *et al*., 2007). In order to provide a better understanding of the genomic variation and evolution of begomoviruses in China, different plant species collected from the different parts of China were

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Abbreviations: AYVCNV = Ageratum yellow vein China virus; ClGMCNV = Clerodendrum golden mosaic China virus; ClGMV = Clerodendrum golden mosaic virus; CR = common region; ICMV = Indian cassava mosaic virus; IR = intergenic region; LYMV = Loofa yellow mosaic virus; MiYLCV = Mimosa yellow leaf curl virus; MYMV = Mungbean yellow mosaic virus; PaLCuCNV = Papaya leaf curl China virus; RamMV = Ramie mosaic virus; SiYMV = Sida yellow mosaic virus; SLCCNV = Squash leaf curl China virus; SLCPHV = Squash leaf curl Philippines virus; StaLCV = Stachytarpheta leaf curl virus; ToLCGV = Tomato leaf curl Gujarat virus; ToLCHV = Tomato leaf curl Hsinchu virus; ToLCNDV = Tomato leaf curl New Delhi virus; TYLCGDV = Tomato yellow leaf curl Guangdong virus; TYLCTHV = Tomato yellow leaf curl Thailand virus

screened for viral infection. Here, we report the molecular characterization and identification of two RamMV isolates J4 and Z1 infecting ramie plants in Jiangsu and Zhejiang provinces, China.

Materials and Methods

Virus isolates. In 2008, virus isolates J4, J5, and Z1 were collected in Yixing, Jiangsu province and Yuhang, Zhejiang province of China, respectively, from the field ramie (*Boehmeria nivea* L.) samples exhibiting yellow mosaic symptoms.

Total DNA extraction, amplification, and cloning. Total DNA was extracted according to Xie *et al*. (2002). PCR was performed from the total DNA using the degenerate primer pair PA and PB, which were designed to amplify part of IR, AV2 and AV1 genes of DNA-A (Zhou *et al*., 2003). Based on the determined sequences, primer pairs J4F (5'-CATGTATCGGAAGCCCAAGATG-3') and J4R (5'-ATGGGCCTGTACGTCCAAGC-3') were designed to obtain the full-length DNA-A of J4 and Z1 isolates. To amplify the viral DNA-B components, the primers J4BF1 (5'-AGATTCACCGGTG GAGCTGT-3') and J4BR1 (5'-TCAGGGCATTCTTGGACACG-3') were designed to amplify a 706 bp fragment comprising the BV1 sequence. PCR products of the expected size were cloned and sequenced. Based on the partial DNA-B sequence obtained, the overlapping primers J4BF2 (5'-TTCCACTTTGCGCGTGCGTG-3') and J4BR2 (5'-AACACGCGCCCTTCTCCAGT-3') were designed for amplification of the entire DNA-B molecules of J4 and Z1 isolates. PCR products were purified and cloned into pGEM-T Easy Vector (Promega) and then sequenced using the automated model 377 DNA sequencing system (Perkin-Elmer).

Sequence analysis. Sequence data were assembled and analyzed with the aid of the DNAStar software version 6.0 and DNAMAN version 5.2.2 (Lynnon Biosoft). Similarity searches with begomovirus sequences were performed using BLAST (http://www.ncbi.nlm.nih.gov/). Multiple sequence alignments were performed by the CLUSTAL V method of MegAlign in DNAStar. The phylogenetic trees were obtained using the neighbor-joining method with 1000 bootstrap replications available in DNAMAN. Vertical branches are arbitrary, horizontal branches are proportional to calculated mutation distances. Bootstrap scores exceeding 50% are placed at major nodes; nodes lacking a score are considered dubious. The database Acc. Nos. of begomovirus DNA-A sequences used for the comparison and phylogenetic analysis were: AYVCNV (AJ564744), ClGMCNV-[Fz7] (FJ011668), ClGMV (DQ641692), ICMV-[Mah] (AJ314739), LYMV (AF509739), MiYLCV (DQ641695), MYMV (DQ400848), PaLCuCNV-[G2] (AJ558123), RamMV-[F3] (EF125190), RamMV-[Hn] (NC_010791), SiYMV-[CN] (AM048837), SLCCNV-[B] (AF509743), SLCCNV-[G25] (AM260206), SLCPHV (AB085793), StaLCV-[Hn6.1] (AJ564742), ToLCGV-[Var] (AY190290), ToLCHV (DQ866131), ToLCNDV-[PK] (AJ620187), TYLCGDV-[G32] (GQ169042) and TYLCTHV-[2] (AF141922). For the phylogenetic analysis and sequence comparisons of DNA-B, database Acc. Nos. of the sequences were: ClGMCNV-[Fz7] (FJ011669), ClGMV (DQ641693), ICMV-[Mah] (AJ314740), LYMV (AF509740), MYMV (DQ400849), RamMV-[F3] (FJ874926), RamMV-[Hn] (NC_010792), SLCCNV-[B] (AF509742), SLCCNV-[G25] (AM260208), SLCPHV (AB085794), ToLCGV-[Var] (AY190291), ToLCNDV-[PK] (AJ620188), and TYLCTHV-[2] (AF141897). For abbreviations see the corresponding section on the front page.

Results

Molecular characterization of DNA-A

The 500 bp partial DNA-A fragments of isolates J4, J5, and Z1 amplified by the degenerate primers were sequenced. Alignment of the resulting sequences (GenBank Acc. No. FN396969, FN396971, and FN396973, respectively) showed that nucleotide sequence identities ranged from 91.6 to 99.2% among these isolates. Then, the isolates J4 and Z1 were chosen at random for a full-length DNA-A sequencing. The complete DNA-A sequences of J4 and Z1 isolates were 2736 and 2737 nts in length, respectively. Both DNA-A of J4 and Z1 had a genomic organization of a typical begomovirus encoding two ORFs (AV1 and AV2) in the virion-sense DNA and four ORFs (AC1, AC2, AC3, and AC4) in the complementary-sense DNA separated by IR. The IR had a putative stem-loop structure with the conserved nonanucleotide sequence TAATATTAC in the loop. The complete DNA-A sequence identity between J4 and Z1 was 94.7% (Table 1).

Sequence similarity search showed that J4 and Z1 DNA-As had the highest sequence identities (93.6–94.7%) with the isolates of Ramie mosaic virus (RamMV-[F3], Acc.

Table 1. Nucleotide sequence identities of DNA-A and DNA-B components and amino acid sequence identities of predicted proteins between the isolate J4 and closely related begomoviruses

Begomovirus	DNA-A[a]	DNA-B[a]	AV1[b]	AV2[b]	AC1[b]	AC2[b]	AC3[b]	AC4[b]	BV1[b]	BC1[b]
RamMV-[Z1]	94.7	88.6	99.6	95.9	96.1	97.0	91.1	81.8	97.5	97.1
RamMV-[F3]	94.4	90.6	96.5	92.6	97.0	95.1	94.8	81.5	98.6	98.6
RamMV-[Hn]	93.6	91.2	96.5	93.4	96.4	96.5	93.3	74.2	95.7	98.9
ToLCHV	94.7	NA	97.3	95.9	95.3	97.8	94.0	83.1	NA	NA

[a]Nucleotide sequence identity. [b]Amino acid sequence identity. NA = not available at the GenBank. For abbreviations see the corresponding section on the front page.

No. EF125190 and RamMV-[Hn], Acc. No. NC_010792). When individual proteins were compared among the different RamMV isolates, high amino acid sequence identities were found for AV1, AV2, AC1, AC2, and AC3, while relatively lower sequence identity was found for AC4 (Table 1).

Molecular characterization of DNA-B

The isolates RamMV-[F3] and RamMV-[Hn] were reported to be bipartite begomoviruses, so primer pairs J4BF2/J4BR2 were designed based on the two DNA-B sequences. The DNA-B components were obtained from the ramie samples. The complete DNA-B sequences of J4 (Acc. No. FN396970) and Z1 (Acc. No. FN396972) were determined to be 2717 and 2719 nts, respectively. Each sequence possessed two ORFs, one (BV1) in the virion-sense DNA and the other (BC1) in the complementary-sense DNA, separated by an IR containing a CR region shared with DNA-A. The CR had a putative stem-loop structure with the conserved nonanucleotide sequence TAATATTAC in the loop. The nucleotide sequence identities between the CRs of DNA-A and DNA-B of J4 and Z1 isolates were 99% and 96%, respectively. The DNA-B sequence identity between J4 and Z1 isolates was 88.6% (Table 1). Furthermore, J4 and Z1 isolates shared high amino acid sequence identities for BV1 (97.5%) and BC1 (97.1%).

BLAST search in the GenBank database showed that J4 and Z1 DNA-Bs were most closely related to those of RamMV-[Hn] and RamMV-[F3], with 88.0-91.2% nucleotide sequence identities, respectively. When individual encoded proteins were compared among the different RamMV isolates, J4 had the highest amino acid sequence identities with RamMV-[F3] for BV1 (98.6%), RamMV-[Hn] for BC1 (98.9%) (Table 1).

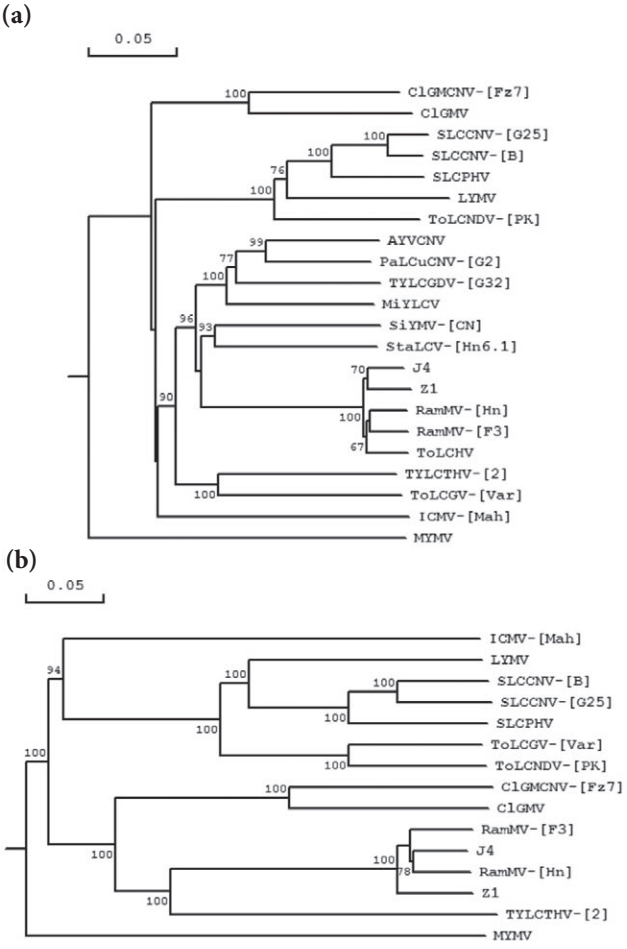

Fig. 1

Dendrograms based on the alignments of DNA-A (a) and DNA-B (b) sequences of J4 and Z1 isolates with other begomoviruses

Affinities to other begomoviruses

The phylogenetic analysis based on the DNA-A and DNA-B nucleotide sequences of J4, Z1, and other previously reported begomoviruses was shown in Fig. 1. In accordance with the relationships predicted by DNA-A sequence comparisons (Table 1), DNA-A of J4 and Z1 isolates clustered together with those of RamMV-[F3], RamMV-[Hn], and ToLCHV, and were distantly related to other begomoviruses (Fig. 1). A similar relationship appeared in the phylogenetic tree based on the DNA-B nucleotide sequences.

Discussion

Since 2006, monopartite begomoviruses were detected in field-grown tomato showing yellow leaf curl disease in several regions of Zhejiang province, China (Mugiira *et al*., 2008). However, to date, no bipartite begomoviruses were reported in the same regions. Based on PCR detection we demonstrated that three virus samples collected in Jiangsu and Zhejiang provinces from the diseased ramie plants contained a bipartite begomovirus. A search in the GenBank database showed that the full-length DNA-A components of isolates J4 and Z1 shared the highest nucleotide sequence identity (about 94%) with two isolates of an unassigned begomovirus (RamMV-[F3] and RamMV-[Hn]). Generally, DNA-A component of begomovirus isolate showing sequence identity below the threshold value of 89% demarcates a distinct begomovirus species, while isolate showing more than 89% sequence identity is considered to be isolate of the same species (Fauquet *et al*., 2003). According to the above criteria, the isolates J4 and Z1 were considered as two different isolates of RamMV. For the first time our results showed the presence of bipartite begomoviruses in these regions of China.

A search in the GenBank database showed that the bipartite RamMV was firstly detected in ramie plants in Hunan, China. The tobacco-infecting strain of RamMV was further identified in Fujiang, China. The sampling places for RamMV in Zhejiang and Jiangsu provinces were quite isolated without crops within 100 meters and no begomovirus-like symptomatic plants were observed nearby. Hence, we think that RamMV is indigenous in these regions of China.

Similarity search in the GenBank database revealed that ToLCHV DNA-A segment (DQ866131) obtained from diseased tomato of Taiwan, shared approximately 94% sequence identity with DNA-A components of RamMV, suggesting that ToLCHV and RamMV belong to the same species. As RamMV was found to infect ramie in many provinces of China, we supposed that it was very likely that ramie might serve as an original host of ToLCHV. Therefore, we suggest to change the term Tomato leaf curl Hsinchu virus to the term Ramie mosaic virus.

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References

Fauquet CM, Bisaro DM, Briddon RW, Brown JK, Harrison BD, Rybicki EP, Stenger DC, Stanley J (2003): Revision of taxonomic criteria for species demarcation in the Geminiviridae family, and an updated list of begomovirus species. Arch. Virol. 148, 405–421. doi:10.1007/s00705-002-0957-5

Guo W, Jiang T, Zhang X, Li GX, Zhou XP (2008): Molecular variation of satellite DNAβ molecules associated with Malvastrum yellow vein virus and their role in pathogenicity. Appl. Envirol. Microbiol. 74, 1909–1913. doi:10.1128/AEM.02461-07

Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, Robertson D (1999): Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. Crit. Rev. Plant Sci. 18, 71–106. doi:10.1016/S0735-2689(99)00383-4

Jose J, Usha R (2003): Bhendi yellow vein mosaic disease in India is caused by association of a DNAβ satellite with a begomovirus. Virology 305, 310–317. doi:10.1006/viro.2002.1768

Lazarowitz SG (1992): Geminiviruses – genome structure and gene function. Crit. Rev. Plant Sci. 11, 327–349.

Ma XY, Cai JH, Li GX, Qin BX, Zhou XP (2004): Molecular characterization of a distinct begomovirus infecting Euphorbia pulcherrima in China. J. Phytopathol. 152, 215–218. doi:10.1111/j.1439-0434.2004.00832.x

Mugiira RB, Liu SS, Zhou XP (2008): Tomato yellow leaf curl virus and Tomato leaf curl Taiwan virus Invade South-east Coast of China. J. Phytopathol. 156, 217–221. doi:10.1111/j.1439-0434.2007.01345.x

Saunders K, Bedford ID, Briddon RW, Markham PG, Wong SM, Stanley J (2000): A unique virus complex causes ageratum yellow vein disease. Proc. Natl. Acad. Sci. USA 97, 6890–6895. doi:10.1073/pnas.97.12.6890

Xie Y, Zhou XP (2003): Molecular characterization of Squash leaf curl Yunnan virus, a new begomovirus and evidence for recombination. Arch. Virol. 148, 2047–2054. doi:10.1007/s00705-003-0153-2

Xie Y, Zhou XP, Zhang ZK, Qi YJ (2002): Tobacco curly shoot virus isolated inYunnan is a distinct species of begomovirus. Chin. Sci. Bull. 47, 197–200. doi:10.1360/02tb9047

Xiong Q, Fan SW, Wu JX, Zhou XP (2007): Ageratum yellow vein China virus is a distinct begomovirus species associated with a DNAβ molecule. Phytopathology 97, 405–411. doi:10.1094/PHYTO-97-4-0405

Zhou XP, Xie Y, Peng Y, Zhang ZK (2003): Malvastrum yellow vein virus, a new begomovirus species associated with satellite DNA molecule. Chin. Sci. Bull. 48, 2205–2209. doi:10.1360/03wc0272