MOLECULAR CHARACTERIZATION OF A NEW BEGOMOVIRUS INFECTING *SENECIO SCANDENS* IN CHINA

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Summary. – Three begomovirus isolates, G46, G83 and G84 from *Senecio scandens* showing yellow mosaic symptoms were collected from Guangxi Province, P.R. China. The isolates were detected by PCR using universal primers for begomoviruses. Comparison of partial DNA-A sequences (~ 500 bp) of the isolates revealed their 98.7–99.1% identity. The isolate G46, chosen for complete DNA-A sequencing, consisted of 2746 nt and had a typical genomic organization of begomoviruses. The G46 DNA-A had the highest sequence identity (72.4%) with that of Ageratum leaf curl virus among begomoviruses. The molecular data suggest that the isolate G46 is a new begomovirus (species), for which the name Senecio yellow mosaic virus (*Senecio yellow mosaic virus*) is proposed.

Key words: begomovirus; cloning; DNA-A; PCR; phylogenetic analysis; sequencing; Senecio yellow mosaic virus; Senecio scandens

Introduction

Geminiviruses are a large family of plant viruses (*Geminiviridae)* that infect a broad variety of plants and cause significant economic losses worldwide. The most economically important diseases are caused by members of the genus *Begomovirus*, which comprises more than 100 species that infect dicotyledonous plants and are transmitted by the whitefly *Bemisia tabaci* (Brown, 1994; Fauquet *et al.*, 2003). Most of begomoviruses have a bipartite genome. They are characteristic by two types of icosahedral virions containing two types of circular single-stranded DNA genome segments, DNA-A and DNA-B, respectively, each ranging in size from 2.5 kb to 3.0 kb (Lazarowitz, 1992). Both types of virus particles are essential for virus proliferation (Hanley-Bowdoin *et al.*, 1999). A few species of begomoviruses have a monopartite genome, a single DNA molecule resembling DNA-A of bipartite begomoviruses, which encodes for all functions necessary for viral replication and movement in the plant (Navot *et al.*, 1991). Recently, some monopartite begomoviruses, such as Ageratum yellow vein virus (AYVV) and Cotton leaf curl Multan virus (CLCuMV), have been shown to be associated with a satellite-like molecule, referred to as DNAβ (Saunders *et al.*, 2000; Briddon *et al*., 2001). DNAβ depends on a helper virus for replication and encapsidation, and is essential for induction of characteristic symptoms (Cui *et al*., 2004; Saunders *et al*., 2004).

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Abbreviations: ACMV = Africa cassava mosaic virus; AEV = Ageratum enation virus; ALCV = Ageratum leaf curl virus; AYVV = Ageratum yellow vein virus; BCTV = Beet curly top virus; BGYMV = Bean golden yellow mosaic virus; BYVMV = Bhendi yellow vein mosaic virus; ChiLCuV = Chilli leaf curl virus; CLCuKV = Cotton leaf curl Kokhran virus; CLCuMV = Cotton leaf curl Multan virus; CP = coat protein; ELCV = Euphorbia leaf curl virus; IR = intergenic region; MYVV = Malvastrum yellow vein virus; OYVMV = Okra yellow vein mosaic virus; PaLCuCNV = Papaya leaf curl China virus; PepLCBV = Pepper leaf curl Bangladesh virus; SACMV = South African cassava mosaic virus; SbCLV = Soybean crinkle leaf virus; SLCYV = Squash leaf curl Yunnan virus; StaLCV = Stachytarpheta leaf curl virus; TbCSV = Tobacco curly shoot virus; TbLCYNV = Tobacco leaf curl Yunnan virus; TGMV = Tomato golden mosaic virus; ToLCCNV = Tomato leaf curl China virus; ToLCJV = Tomato leaf curl Java virus; TYLCCNV = Tomato yellow leaf curl China virus

In China, many distinct begomoviruses have been characterized from different hosts such as tobacco, squash, tomato, papaya, *Malvastrum coromandelianum* and *Euphorbia pulcherrima* (Zhou *et al*., 2001, 2003; Xie and Zhou, 2003; Li *et al*., 2004; Wang *et al*., 2004; Ma *et al*., 2004). *Senecio scandens* is a Chinese medicinal herb that occurs in southern China and frequently exhibits yellow mosaic symptoms. In this paper, we report on molecular characterization of a new begomovirus infecting *S. scandens* in Guangxi, P.R. China.

Materials and Methods

Virus isolates G46, G83 and G84 were collected from field samples of *S. scandens* plants showing yellow mosaic symptoms in Nanning, Guangxi Province, P.R. China in October 2003.

Total DNA was extracted from leaves of naturally infected symptomatic plants according to Zhou *et al*. (2001).

PCR. Degenerate primers PA and PB were designed to amplify an approximately 500 bp long region covering a part of the intergenic region and AV2 gene of DNA-A (Deng *et al.*, 1994).

Cloning and sequencing. The PCR products were cloned and sequenced. Based on the determined sequence, the overlapping primers G46F (5'-AGAGAACATGGGTGCAACGTC-3', nt 420–440) and G46R (5'-GACATGACATACAGAGCCCAC-3', nt 128–108) were designed to amplify the entire DNA-A of the isolate G46. PCR was done as described by Zhou *et al.* (2001). PCR products of the expected size of 2.4. kb were recovered, cloned into the pGEM-T Easy vector (Promega) and sequenced using an automated ABI sequencer model 3730 (Perkin-Elemer Applied Biosystems).

Sequence analysis was carried out using the DNAStar Software Package (DNAStar). Phylogenetic trees were constructed using full optimal alignment and neighbor-joining method options with 1000 bootstrap replications available in the DNAMAN software, version 5.2.2 (Lynnon Biosoft, Canada). DNA-A sequences of the following geminiviruses (GenBank Acc. Nos) were compared: ACMV (J02057), AEV (AJ437618), ALCV (AJ851005), AYVV (X74516), BGYMV (M10070), BCTV (X04144), BYVMV (AJ002453), ChiLCuV (AF336806), CLCuKV (AJ002449), ELCV (AJ558121), MYVV (AJ457824), OYVMV (AJ002451), PaLCuCNV (AJ558116), PepLCBV (AF314531), SACMV (AJ575560), SbCLV (AB050781), SLCYV (AJ420319), StaLCV (AJ495814), TbCSV (AJ420318), TbLCYNV (AJ512762), TGMV (K02029), ToLCCNV (AJ558118), ToLCJV (AB162141) and TYLCCNV (AJ319675).

Results

Genomic organization of DNA-A

A part of DNA-A of about 500 bp from the three begomovirus isolates (G46, G83 and G84) originating from *S. scandens* was amplified by PCR and sequenced (GenBank Acc. Nos. AJ876550-52, respectively). The three isolates were nearly identical and shared 98.7–99.1% nucleotide sequence identities. The isolate G46 was then chosen for complete DNA-A sequencing. The sequence (Acc. No. AJ876550) consisted of 2746 nt and had an organization typical for begomoviruses originating from the Old World, with two ORFs (AV1 and AV2) in genomic DNA and four ORFs (AC1, AC2, AC3, and AC4) in

Table 1. Nucleotide and amino acid sequence identities of DNA-A and DNA-A encoded proteins between G46 and other begomoviruses

Virus	DNA-A[a]	IR[a]	AV1(CP)[b]	AV2[b]	AC1[b]	AC2[b]	AC3[b]	AC4[b]
AEV	66.4	38.2	78.1	64.3	76.1	49.6	62.7	63.5
ALCV	72.4	47.9	86.4	73.0	81.9	50.4	67.4	71.7
AYVV	71.2	42.6	76.6	67.0	81.9	53.4	65.7	71.4
BYVMV	64.6	29.8	76.6	54.8	78.6	52.6	61.2	64.6
ChiLCuV	65.9	33.5	78.9	61.7	77.7	54.1	64.9	49.5
CLCuKV	66.3	37.1	78.9	64.3	74.2	53.4	61.9	58.0
ELCV	66.6	32.7	77.4	53.0	77.5	53.4	67.4	68.0
MYVV	64.0	41.4	77.7	53.9	74.2	52.6	63.4	41.1
OYVMV	66.1	33.6	77.0	56.5	77.5	51.1	61.9	63.0
PaLCCNV	68.6	40.8	86.0	71.3	78.3	52.6	67.4	37.8
PepLCBV	63.5	34.2	79.3	66.1	67.2	54.9	65.7	43.5
SbCLV	70.3	40.1	82.5	65.2	76.7	54.9	66.4	77.1
SLCYV	61.5	33.1	75.8	64.3	65.3	51.9	54.1	38.3
StaLCV	69.8	42.6	83.7	70.4	78.3	49.6	66.4	74.0
TbCSV	65.7	41.5	78.9	63.5	75.3	51.9	67.2	40.2
TbLCYNV	68.8	41.5	76.6	64.3	78.9	54.1	67.2	72.9
ToLCCNV	66.9	43.1	79.8	69.6	76.9	50.4	59.7	43.3
ToLCJV	70.6	44.5	84.4	63.5	81.7	52.6	63.4	77.1
TYLCCNV	65.4	37.1	79.8	66.1	73.6	51.9	67.4	39.8

[a]Nucleotide sequence identity. [b]Amino acid sequence identity. For the abbreviations see their list on the front page.

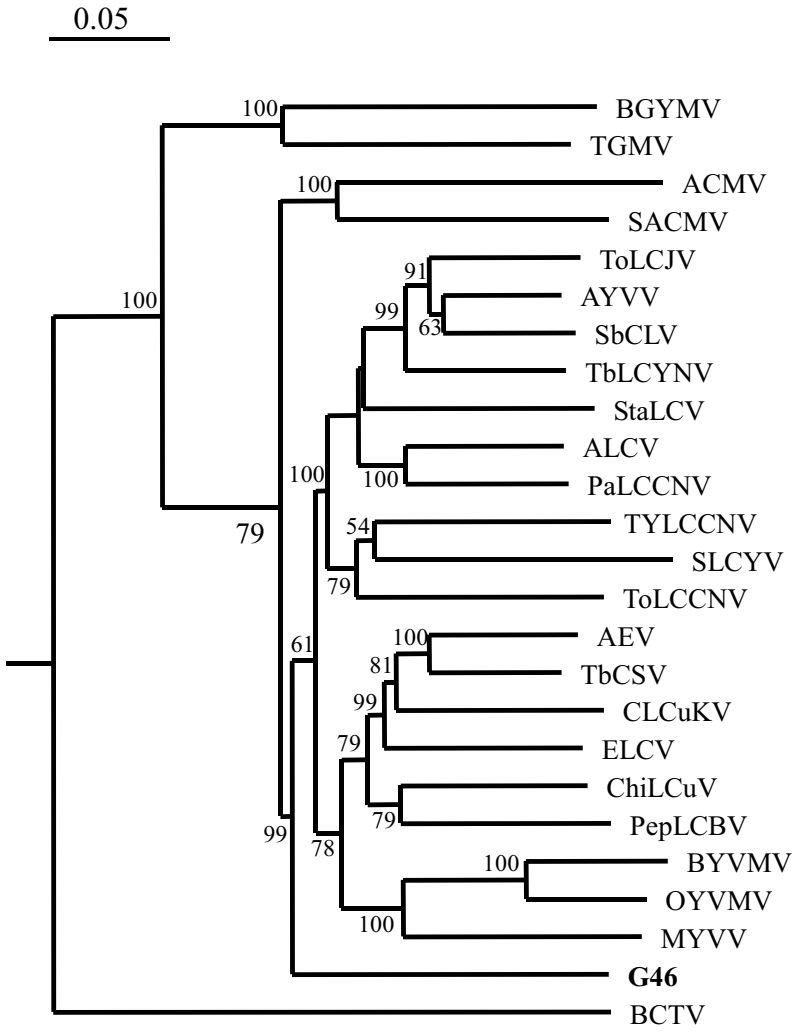

Fig. 1

Phylogenetic tree of begomoviruses infecting *S. scandens* and other begomoviruses based on nucleotide sequences of DNA-A

The bootstrap scores exceeding 50% are placed at major nodes; the nodes lacking the score are considered dubious. Horizontal distances are proportional to sequence distances; vertical distances are arbitrary. The tree was rooted on the respective sequence of BCTV.

complementary DNA, separated by an intergenic region (IR). IR contained 272 nts and exhibited the following features characteristic of begomoviruses: a putative stem-loop structure with the nonanucleotide sequence TAATATTAC in the loop, TATA motif (nt 60–65 of the IR or nt 2657–2662 of the DNA-A sequence), and iteron sequence AGGTCA (nt 2603–2608 and 2648–2653 to the 5'-side of the TATA motif).

Sequence analysis

Similarity of the isolate G46 to other begomoviruses based on complete DNA-A sequences is shown in Table 1. The isolate G46 shared the highest identity (72.4%) with ALCV, while the identity with other begomoviruses was in the range of 61.5–71.2%. In phylogenetic analysis G46 clustered with Asian begomoviruses but formed a unique branch (Fig. 1), suggesting that it is a distinct begomovirus. Furthermore, all begomoviruses from the Old World clustered together, while begomoviruses from the New World formed a separate branch, revealing a geographic basis for phylogenetic relationship.

The IR is a region of DNA-A that shows the greatest sequence variation among geminiviruses (Fontes *et al.,* 1994). The IR of the isolate G46 shares 29.8–47.9% nucleotide sequence identities with those of other begomoviruses. When individually encoded proteins were compared, G46 had the highest amino acid sequence identity with ALCV for coat

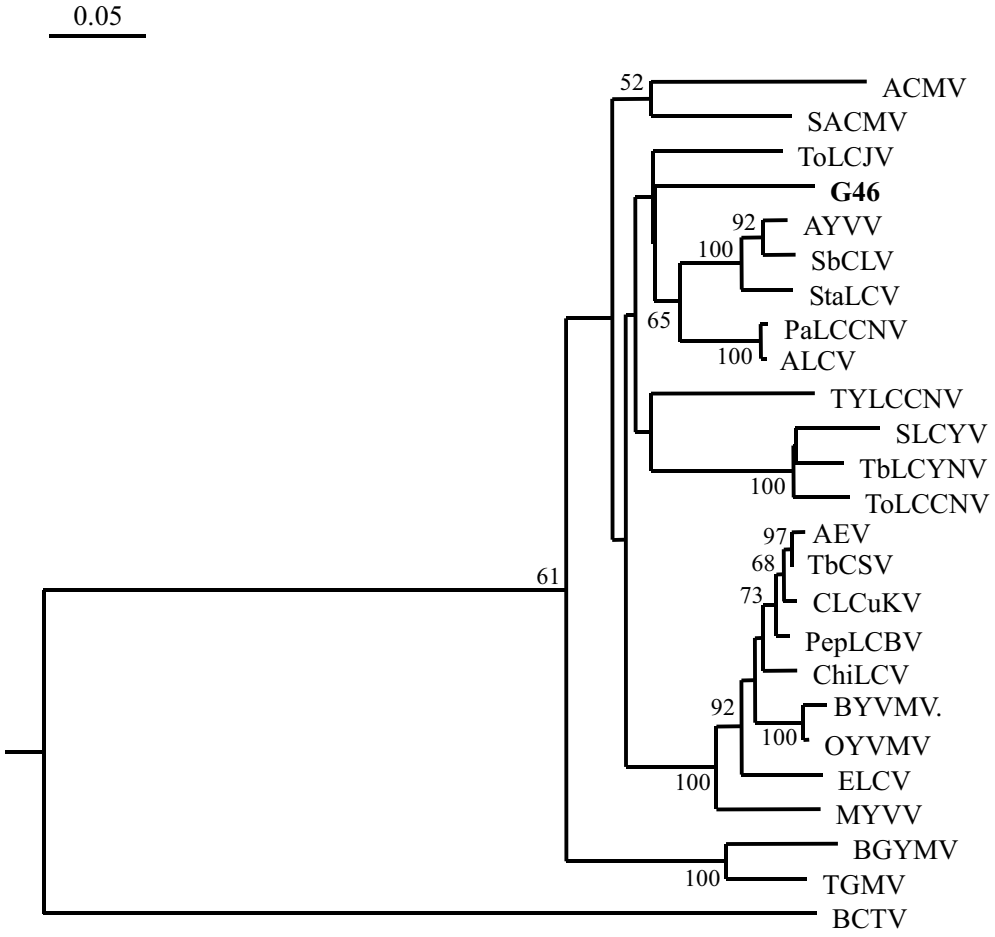

Fig. 2

Phylogenetic tree of begomoviruses infecting *S. scandens* and other begomoviruses based on amino acid sequences of CP

The bootstrap scores exceeding 50% are placed at major nodes; the nodes lacking the score are considered dubious. Horizontal distances are proportional to sequence distances; vertical distances are arbitrary. The tree was rooted on the respective sequence of BCTV.

protein (CP) (86.4%) and AV2 (73%), ALCV and AYVV for AC1 (81.9%), and ToLCJV and SbCLV for AC4 (77.1%). Relatively low amino acid sequence identities were found for AC2 (49.6–54.9%) and AC3 (54.1–67.4%) between G46 and other begomoviruses. In the phylogenetic tree based on CP amino acid sequences G46 clustered together with begomoviruses infecting ageratum, papaya, stachytarpheta and soybean in Asia, suggesting that the CPs of these viruses may have a common ancestor (Fig. 2).

Discussion

The complete DNA-A sequence analysis showed that G46 shared the highest identity (72.4%) with ALCV. It had been accepted that begomoviruses sharing a DNA-A sequence identity less than 89% are considered to be distinct begomovirus species (Fauquet *et al.*, 2003). The significantly lower DNA-A sequence identity of G46 with other begomoviruses suggests that it is a distinct begomovirus species, for which the name *Senecio yellow mosaic virus* (SeYMV) is proposed. The phylogenetic tree based on nucleotide sequences of DNA-A indicates that SeYMV is unique among begomoviruses found in Asia. It implies that SeYMV may differ from other Asian begomoviruses in its evolutionary history.

Our attempts to detect a component equivalent to DNA-B and satellite molecule DNAβ by PCR and Southern blot analysis failed. Preparation of infective clones of G46 DNA-A is necessary for determination of monopartite or bipartite nature of a begomovirus species.

S. scandens is a perennial evergreen shrub occurring in southern China, whose leaf is used for preparation of herbal

medicine. The soup decocted from dry leaf can diminish inflammation and enhance detoxification. Its officinal function can be reduced by infection of the plant with SeYMV resulting in severe yellow mosaic symptom. In China, some begomoviruses have caused recently significant yield losses to crops such as tobacco, papaya and tomato. The presence of diverse begomoviruses together with outbreaks of *B. tabaci* B biotype (Zhang and Luo, 2001) indicates that begomoviruses have already emerged as a serious threat to the future production of crop plants.

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Note of the Editor-in-Chief. The proposed new begomovirus (species), Senecio yellow mosaic virus (*Senecio yellow mosaic virus*) is not listed among viruses (virus species) in the presently valid virus taxonomy (van Regenmortel *et al*., 2000; Fauquet *et al*. 2003). The same pays for the following begomovirus species compareded in phylogenetic analysis: *Ageratum leaf curl virus* (ALCV), *Cotton leaf curl Kokhran virus* (CLCuKV), *Euphorbia leaf curl virus* (ELCV), *Papaya leaf curl China virus* (PaLCuCNV), *Tomato leaf curl China virus* (ToLCCNV), *Tomato leaf curl Java virus* (ToLCJV).

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