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Identification of a New Potyvirus Associated with Chlorotic Vein Banding Disease of *Spathiphyllum* spp., in Andhra Pradesh, India

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The genome of a potyvirus isolate associated with chlorotic spots and vein banding symptoms on *Spathiphyllum* spp., in Andhra Pradesh state, India was amplified by RT-PCR using degenerate potyvirus primers, amplicons cloned, and sequence (1.6 kb) analyzed. This virus isolate shared maximum identity of 74.8% and 80.2% at coat protein (CP) gene nucleotide (906 nucleotides) and amino acid (302 amino acids) levels, respectively with *Dasheen mosaic virus* (DsMV)-M13 isolate reported from China. But its 3'-UTR (258 nucleotides) had maximum identity of 62.5% with DsMV-Vietnam isolate. The deduced molecular weight of CP is 33.57 kDa and it contained DAG triplet in its N-terminal region. In CP amino acid based phylogenetic analysis, this virus isolate represented a separate branch but closer to DsMV isolates cluster. Based on the molecular criteria set for the discrimination of species and genus in the *Potyviridae* family, the present virus isolate was identified as a distinct virus species in the genus *Potyvirus* and proposed the name Spathiphyllum chlorotic vein banding virus (SCVbV).

Keywords **:** genome sequence analysis, potyvirus, *Spathiphyllum* spp.

Aroids found naturally in different habitats in tropical and subtropical regions of the world. They are cultivated through vegetative propagules (corms, cormels, tubers, stem pieces) and or by micropropagation as food crops, foliage or flowering ornamentals, aquarium, and medicinal plants. *Spathiphyllum* spp. (Peace lily) are commonly cultivated as foliage ornamentals.

Eight viruses (*Bean yellow mosaic virus*, BYMV; *Calla lily latent virus*, CLLV; *Dasheen mosaic virus*, DsMV; *Konjac mosaic virus*, KoMV; Peace lily mosaic virus, PeLMV; *Soybean mosaic virus*, SMV; *Turnip mosaic virus*, TuMV; and *Zantedeschia mild mosaic virus*, ZaMMV) that belong to the genus *Potyvirus,* family *Potyviridae* have been reported to naturally infect different aroids world wide (Chen et al., 2003; Chen et al., 2006; Huang and Chang, 2005; Kwon et al., 2002; Nishiguchi, 2006; Pham et al., 2002; Shi et al., 2005; Zettler et al., 1978; GenBank A/C DQ851494). Among these potyviruses, DsMV is world wide in distribution and naturally infects several aroid and orchid plant species (Farrcyrol et al., 2006; Zettler et al., 1978). Many of the above potyviruses have been reported to induce chlorotic spotting, chlorotic ring spotting, chlorotic vein banding, chlorotic feathery vein mottling, mosaic, leaf distortion/malformation, and reduction in the vigor of growth of aroid plants. Further, they decreased the rooting of virus contaminated aroid propagules.

In India, the virus infecting aroid plants, presumed to be DsMV based on external symptoms for a long period, was later confirmed (Ahlawat et al., 2003; Pandit et al., 2001; Ram et al., 2003; GenBank accession number FJ160764). In addition to DsMV, a carla-like virus has been suspected to infect *Amorphophallus paenifolia* (Elephant foot yam) in Assam, India (Ahlawat et al., 2003). Very recently, the virus isolates associated with natural infections of *Colocasia esculenta*, *Caladium*, and *Dieffenbachia* spp., in Andhra Pradesh state, India were found to be KoMV (Padmavathi et al., 2010). Aroid foliage ornamentals are common in indoor and outdoor gardens and nurseries in Andhra Pradesh state. Symptoms characteristic of virus infections have been noticed on *Spathipyllum* spp., plants. The initial studies have revealed the association of a potyvirus isolate with such infections (data not shown). To confirm this, the total RNA isolated from *Spathiphyllum* spp., showing chlorotic spotting and vein banding was amplified by RT-PCR using degenerate potyvirus primers (Gibbs and McKenzie, 1997), amplicons were cloned, and sequence analyzed to determine the correct identity of the virus.

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Materials and Methods

Virus sources and maintenance. The ornamental plant nurseries around Rajahmundry, (East Godavari district) and in and around Tirupati, Chittoor district, Andhra Pradesh state were surveyed to record the occurrence of virus infections on aroid ornamentals. The leaves and vegetative propagules of six representative symptomatic (Fig. 1A) and asymptomatic *Spathiphyllum* spp., samples were collected for screening by direct antigen coating - enzyme linked immunosorbent assay (DAC-ELISA) (Clark and Bar-Joseph, 1984) using Peanut green mosaic potyvirus antiserum (Dr. P. Sreenivasulu, corresponding author). The ELISA-positive (A_{405} values ranged from 0.19 to0.45) and negative samples were propagated through vegetative propagules in the wire mesh house of the Department of Virology for further analysis.

Isolation of total RNA, RT-PCR, and cloning. The total RNA was extracted from ELISA-positive *Spathiphyllum* spp. as well as from ELISA-negative samples using Trizol reagent (Gibco BRL, UK) according to the manufacturer's protocol and the isolated RNA was resuspended in 20 µl of RNase-free water. 5 µl of RNA was initially denatured at 55-60°C for 10 min and set for first strand cDNA synthesis involving oligo $d(T)_{17}$ primer (Promega, Madison, Wisconsin, USA) and M-MuLV-RT (Fermentas, Burlington, Ontario, Canada) according to the manufacturer's protocol. The second strand synthesis was performed by taking 1.0 µl of cDNA in a 25 µl reaction mixture involving 2.5 µl of 10 X PCR buffer, 25 mM $MgCl_2$, 0.5 µl of 10 mM dNTP mix, 1U of Taq DNA polymerase (Fermentas), and 20 pmol of the degenerate potyvirus primers, i.e., potyvirid primer 1: 5'-CACGGATCCCGGG $(T)_{17}$ (AGC)-3' and potyvirid primer2: 5'-ACCACAGGATCCGG(TCG)AA(CT)AA(CT) AGCGG(GTA)CA(AG)CC-3' as reported by Gibbs and McKenzie (1997). The above PCR reaction was subjected to initial denaturation of 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min and final extension of 72°C for 10 min.

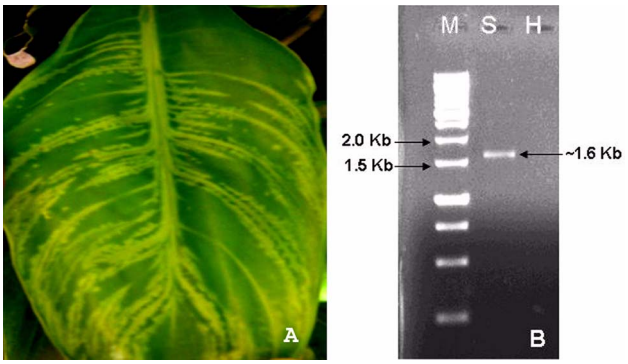

Fig. 1. (A) Chlorotic vein banding symptoms on *Spathiphyllum* spp., leaf. (B) RT-PCR amplicons resolved by 1% agarose gel electrophoresis. M: 1 Kb DNA marker, S: Infected *Spathiphyllum* spp., H: Healthy *Spathiphyllum* spp.

The amplified products were electrophoresed along with 1 Kb DNA ladder (Fermentas) on 1% agarose gel and the amplified products were gel extracted using the Qiaquick gel extraction kit (Qiagen, Hilden, Germany). The gel extracted PCR products were cloned into pGEMT-Easy vector (Promega, Madison, Wisconsin, USA) and transformed into *Escherichia coli* $DH_5\alpha$ cells. The recombinant clones were initially selected by blue-white colony screening and later confirmed by restriction enzyme digestion analysis using *Eco*R1 and *Bam*H1. (Sambrook and Russell, 2001).

Genome sequence analysis. The confirmed recombinant clone was sequenced by availing a commercial sequencing facility (MWG Biotech, Bangalore, India). The partial genome sequence of the virus isolate obtained from *Spathiphyllum* spp., was initially BLAST [United States National Library of Medicine (NLM), Bethesda, MD, UDA] analysed using the NCBI public data base. Further sequence analysis was performed using the Molecular Evolutionary Genetics Analysis (MEGA) version 4.0 (Tamura et al., 2007). The sequence identity of the virus isolate studied herein was calculated with other aroid infecting potyviruses and a few other representative viruses of the genus *Potyvirus* at the coat protein (CP) gene nucleotide, CP amino acid, and 3'-UTR levels.

The protease cleavage site at the NIb/CP of the present virus isolate was determined by comparing the already established NIb/CP cleavage sites of other reported potyviruses (Adams et al., 2005a).

Results and Discussion

The total RNA isolated from *Spathiphyllum* spp., when subjected to RT-PCR using degenerate potyvirus primers yielded amplicons of ~1.6 Kbp (Fig. 1B). The amplicons corresponded to 3'-UTR, complete CP gene, and part of NIb gene. When the recombinant pGEMT-Easy vector having the insert was digested with *Eco*R1 and *Bam*H1, ~1.6 kbp insert released.

The generated sequence of the positive clone, when initially BLAST analyzed using NCBI public data base, revealed maximum identity with DsMV-M13 isolate reported from China. It showed maximum identity of 74.8% and 80.2% at CP gene nucleotide and amino acid levels, respectively with DsMV-M13 isolate. Where as at 3'-UTR (258 nt) nucleotide level, it had maximum identity of 62.5% with DsMV isolate from Vietnam (Table 1). *Spathiphyllum* spp., were found to be susceptible to DsMV

Table 1. Per cent identity of CP gene and 3'-UTR of a potyvirus isolate associated with chlorotic vein banding symptoms on *Spathiphyllum* spp., in Andhra Pradesh, India with other potyviruses*

Virus	GenBank accession numbers	% CP gene nucleotide identity	% CP amino acid identity	% 3'-UTR identity
Spathiphyllum chlorotic vein banding virus	GQ421462	100	100	100
DsMV-Kerala	FJ160764	71.9	78.5	59.6
DsMV-Florida	(U00122)	70.1	74.1	61.0
DsMV-Florida	U08124	72.7	78.5	60.6
DsMV-M13	NC003537	74.8	79.9	60.2
DsMV-Vietnam	DQ925466	68.3	74.6	62.5
DsMV-Vn/CE2	DQ925465	73.4	79.2	61.3
DsMV-Vn/CE1	DQ925464	71.4	71.4	61.0
DsMV-TW	AJ298036	74.3	80.1	60.0
BCMV-NL	EU713858	58.1	65.4	32.0
SMV	S42280	57.9	64.2	39.0
WMV-2	D13913	57.8	63.3	40.0
ZaMMV	AY626825	54.8	54.9	37.5
PeLMV-Haiphong, (Vietnam)	DQ851494	53.6	53.0	30.3
KoMV-Cal-India	EU924640	48.8	48.6	17.3
KoMV-Col-India	EU979524	48.6	48.6	15.1
KoMV-Dief-India	EU924639	48.8	48.6	17.3
KoMV-S.Korea	AB081519	48.4	48.6	15.1

*BCMV: *Bean common mosaic virus*, DsMV: *Dasheen mosaic virus*, KoMV: *Konjac mosaic virus*, PeLMV: Peace lily mosaic virus, SMV: *Soybean mosaic virus*, WMV-2: *Watermelon mosaic virus*-2, ZaMMV: *Zantedeschia mild mosaic virus*.

and PeLMV infections (Zettler et al., 1978 and GenBank A/C DQ851494). In India, DsMV was reported to infect four aroid ornamental (*Aglaonema* spp., *Philodendron* spp., *Colocasia esculenta* and *Zantedeschia* spp.) plants (Ram et al., 2003). The present virus isolate shared identity of 71.9% (CP gene nucleotide), 78.2% (CP amino acid), and 59.6% (3'-UTR) with recently reported DsMV-elephant foot yam isolate from Kerala state, India (Table 1). The deduced size of CP gene of this isolate is 906 nucleotides in length encoding 302 amino acids with a molecular weight of 33.57 kDa. The CP amino acid based phylogenetic analysis revealed that the virus isolate from *Spathiphyllum* spp., clustered along with DsMV isolates but as a distinct branch (Fig. 2). Like several other aphid transmitted potyviruses, the CP of this virus also contained DAG triplet in its amino terminus. The NIb/CP cleavage site identified is Q/A.

According to the molecular criteria suggested by Adams et al. (2005b), different potyvirus species within the genus have CP gene nucleotide percent identity between 35.6 to

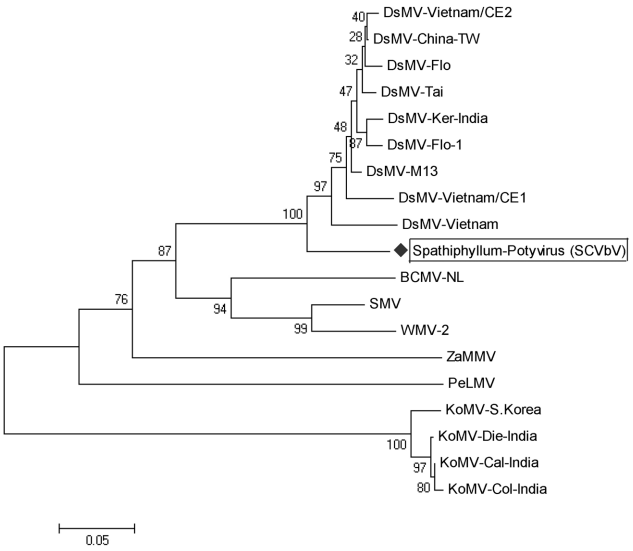

Fig. 2. Spathiphyllum chlorotic vein banding virus isolate (SCVbV) CP amino acid based phylogenetic tree showing the relationships with other potyviruses of the genus *Potyvirus*. The virus acronyms and GenBank A/C numbers are given in Table 1. The phylogenetic tree was constructed using MEGA 4.0. The values at the forks indicate the number of trees that this grouping occurred after bootstrapping the data. The scale bar shows the number of substitutions per base.

81.1, CP amino acid percent identity between 13.2 to 88.6, and 3'-UTR nucleotide percent identity between 30.9 to 84. Based on these criteria, the potyvirus isolate associated with prominent chlorotic vein banding symptoms on *Spathiphyllum* spp., in Andhra Pradesh, India was identified as a distinct virus species in the genus *Potyvirus*, and authors proposed the name Spathiphyllum chlorotic vein banding virus (SCVbV). Further work on biological properties of the virus and its full length genome sequence analysis may substantiate its exact identity within the genus *Potyvirus*.

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