

First report of two distinct badnaviruses associated with *Bougainvillea spectabilis* in India

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Abstract Two distinct types of symptoms, severe yellow mosaic and chlorotic vein-banding, were observed on *Bougainvillea spectabilis* at two different locations in India. Electron microscopy revealed bacilliform virus particles in leaf samples from both places. PCR amplification, cloning, sequencing and sequence analysis of reverse transcriptase and ribonuclease H coding region in open reading frame 3 indicated that two distinct badnaviruses are associated with *B. spectabilis* in India.

Keywords *Badnavirus* · *Bougainvillea spectabilis* · Delhi · Tirupati · PCR

Bougainvillea spectabilis Willd (flower paper) is a widely grown ornamental plant in India. During 2008, *B. spectabilis* plants developed distinct severe yellow mosaic (SYM) in parts of Tirupati (Fig. 1a). Subsequently in 2009, a chlorotic vein-banding (CVB) was noticed on leaves of *B. spectabilis* on the campus of the Indian Agricultural Research Institute, New Delhi (Fig. 1b). Leaf dip electron microscopy of symptomatic bougainvillea leaves revealed the association of bacilliform virus particles measuring 120–150 nm × 20 nm (Fig. 2). Previously, bacilliform virus particles have been shown to be associated with bougainvillea plants with CVB in Brazil (Rivas et al. 2005) and mottling, chlorosis, vein-banding and stunting in Taiwan (Tsai et al. 2005).

To confirm the virus identity, symptomatic leaves of bougainvillea collected from Tirupati and Delhi were

subjected to polymerase chain reaction (PCR) amplification using two degenerate *Badnavirus* primers viz. BadnaFP (5'ATGCCITTYGGIAARAAYGCICC3') and BadnaRP (5'CCAYTTTRCAIACISCICCCCAICC3') corresponding to putative ribonuclease H (RNase H) and reverse transcriptase (RT) coding regions (Yang et al. 2003). Both samples yielded an amplicon of approximately 600 bp, and no amplification was observed in healthy, symptomless bougainvillea (Fig. 3). The amplicons were cloned in pGEM-T easy vector (Promega, Madison, WI, USA) and sequenced. BLAST analysis of the 577 bp of RNase H and RT nucleotide sequences of viral DNA from bougainvillea with SYM symptoms from Tirupati (accession GQ254410) had maximum sequence identity of 73% in open reading frame 3 (ORF 3) of *Bougainvillea spectabilis* chlorotic vein-banding virus (BsCVBV) (accessions EU034539, DQ347841, and DQ103759). An identical sequence size of amplified viral DNA isolated from bougainvillea with CVB symptoms from Delhi (accession GQ254411) had 72% sequence identity with the sequences of same three BsCVBV isolates. Nucleotide and amino acid sequences of putative RT and RNase H region of ORF 3 of these two badnaviruses were analyzed using Bio Edit version 7.0.4 for sequence identities with other badnaviruses. The two badnaviruses infecting bougainvillea in India shared an identity of 76.2% in nucleotide and 88.6% in amino acid sequences with each other. Comparison of nucleotide and amino acid sequences of badnaviruses infecting bougainvillea from India and Taiwan showed identities in the range of 72.3–73.5% for nucleotides and 83.4–84.9% in amino acids of RT and RNase H. Because only 465 bp in the RT and RNase H regions was available for the nucleotide sequence of BsCVBV from Brazil, we compared it separately with the Indian and Taiwan isolates of BsCVBV; the Indian isolates were found to have nucleotide identities of

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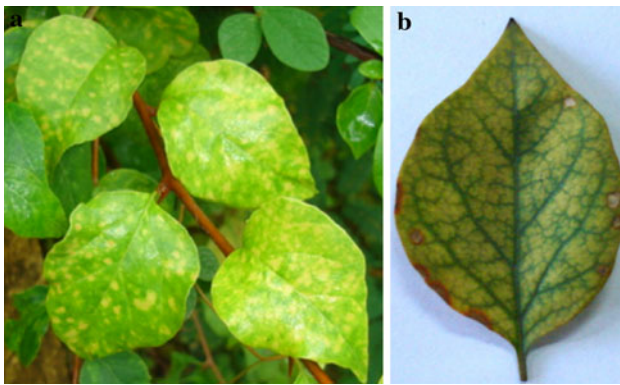


Fig. 1 a Severe yellow mosaic on leaf of *Bougainvillea spectabilis* (Tirupati). b Chlorotic vein-banding on leaf of *Bougainvillea spectabilis* (Delhi)

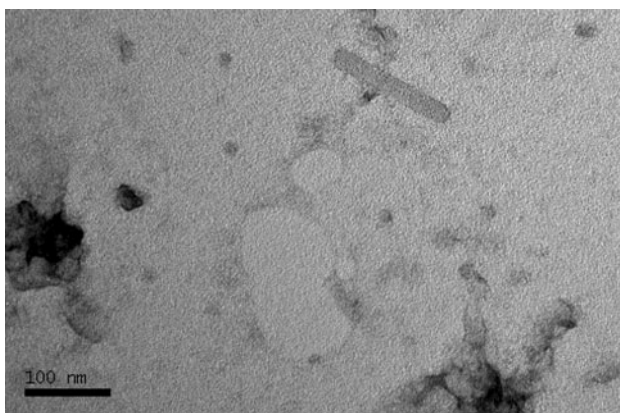


Fig. 2 Electron micrograph of bacilliform virus particle in negatively stained preparation

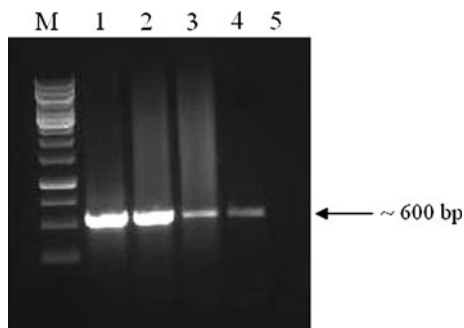


Fig. 3 Gel electrophoresis of PCR amplification of RT and RNase H region of *Badnavirus* infecting *Bougainvillea spectabilis*. Lane M 100-bp DNA ladder, lanes 1 and 2 Tirupati isolate, lanes 3 and 4 Delhi isolate, and lane 5 asymptomatic healthy control

71.4–71.6% with the Brazil isolate and 71.6–73.3% with Taiwan isolates. The amino acid sequence identity was in the range of 77.7–78.3% with Brazil isolate and 82.7–84.5% with Taiwan isolates (Table 1). Further

Table 1 Pairwise comparison (%) of nucleotide (465 bp) and amino acid sequences of putative RT and RNase H region among *Bougainvillea spectabilis* chlorotic vein-banding virus (BsCVBV) isolates of Taiwan, Brazil and India

AA (%)	NT (%)				
	BsCVBVDelhi	BsCVBVTirupati	BsCVBV1	BsCVBV2	BsCVBV3
BsCVBVDelhi		75.2	71.6	71.8	71.4
BsCVBVTirupati	88.8		73.1	73.3	71.6
BsCVBV1	82.7	83.3		97.2	80.6
BsCVBV2	83.9	84.5	98.7		81.7
BsCVBV3	78.3	77.7	85.1	85.8	

NT nucleotide, AA amino acid, *BsCVBVDelhi* tentative name for Delhi isolate (accession GQ254411), *BsCVBVTirupati* tentative name for Tirupati isolate (accession GQ254410), *BsCVBV1* *Bougainvillea spectabilis* chlorotic vein-banding virus from Taiwan (accession EU034539), *BsCVBV2* *Bougainvillea spectabilis* chlorotic vein-banding virus from Taiwan (accession DQ347841), *BsCVBV3* *Bougainvillea spectabilis* chlorotic vein-banding virus from Brazil (accession AY532653)

comparison of two badnaviruses infecting bougainvillea from India with badnaviruses infecting other crops showed identities in the range of 59.2–64.6% in nucleotide and 58–64.7% in amino acid sequences of RT and RNase H (Table 2).

The nucleotide and amino acid sequence of badnaviruses infecting bougainvillea and other crops were aligned using the programmes Bio Edit version 7.0.4 and Clustal_W (Hall 1999). Phylogenetic analyses were performed with the programme MEGA tool version 4 (Tamura et al. 2007). Phylogenetic trees were generated with neighbour-joining (NJ) method and viewed with the NJ plot program (Tamura et al. 2007). Phylogenetic tree of nucleotides and amino acid indicated that both the Delhi and Tirupati isolates clustered with Taiwan isolates of BsCVBV and badnaviruses infecting citrus (*Citrus mosaic virus*; CMBV), dracaena (*Dracaena mottle virus*; DrMV), cacao [*Cacao swollen shoot virus*; CSSV], dioscorea [*Dioscorea bacilliform virus*; DBV], piper (*Piper yellow mottle virus*; PYMoV), kalanchoe (*Kalanchoe top-spotting virus*; KTSV) and banana [*Banana streak Mysore virus*; BSMYV] (Fig. 4a, b). Badnaviruses infecting taro (*Taro bacilliform virus*; TaBV) and sugarcane (*Sugarcane bacilliform IM virus*; SCBIMV) grouped separately.

The pairwise comparison and phylogenetic analysis indicated that bacilliform virus particles associated with *Bougainvillea spectabilis* from two different geographical locations in India may be two distinct badnaviruses because they shared nucleotide identity of less than 80% with each other within the RT and RNase H region. They also had less than 80% identity with other badnaviruses infecting bougainvillea reported previously from Brazil and Taiwan, as per criteria used for demarcating species in genus *Badnavirus* (Hall et al. 2005). Based on this, the two badnaviruses infecting *B. spectabilis* from Tirupati and Delhi may be two new tentative species of genus

Table 2 Pairwise comparison (%) of nucleotide and amino acid sequences of putative RT and RNase H region among definitive and tentative *Badnavirus* species

AA (%)	NT (%)													
	BsCVBV Delhi	BsCVBV Tirupati	BsCVBV1	BsCVBV2	BSMyV	CSSV	KTSV	PYMV	SCIMBV	CMBV	DBV	TaBV	DrMV	ComYMV
BsCVBVDelhi		76.2	72.3	72.5	64.0	61.1	61.7	59.2	60.5	62.1	61.6	62.9	64.6	62.0
BsCVBVTirupati	88.6		73.5	73.5	64.3	60.2	61.2	60.8	60.7	62.1	62.8	62.1	64.6	60.6
BsCVBV1	83.9	83.4		96.5	66.0	59.3	61.6	60.1	60.5	62.3	65.9	63.1	63.4	58.9
BsCVBV 2	84.9	84.4	98.9		65.4	59.5	60.7	59.2	60.0	61.4	64.8	62.8	64.0	58.2
BSMyV	62.6	63.2	63.2	63.2		61.8	67.7	65.5	60.9	63.6	68.2	60.7	63.2	60.6
CSSV	59.0	59.5	59.5	60.1	61.1		59.4	64.0	57.3	64.8	63.2	63.0	60.8	59.3
KTSV	58.0	59.0	60.6	60.6	73.5	56.9		64.3	58.5	64.1	64.5	59.2	60.6	62.0
PYMV	61.6	64.2	61.1	61.1	72.5	63.2	65.8		61.7	64.0	66.4	62.8	61.1	63.7
SCIMBV	58.5	59.0	58.5	59.0	62.6	57.5	54.9	60.1		59.3	62.3	62.3	58.2	61.5
CMBV	62.1	61.6	63.2	63.2	69.4	68.3	64.2	65.8	58.5		66.2	58.4	60.0	60.0
DBV	61.6	62.1	63.2	63.2	73.0	65.2	65.8	67.8	62.6	67.3		61.6	62.9	61.5
TaBV	61.6	63.7	62.6	62.6	62.1	63.7	59.5	64.7	62.1	59.0	64.7		60.0	59.3
DrMV	63.2	64.2	63.2	63.2	66.3	59.5	65.2	64.2	58.0	62.6	64.7	60.6		57.7
ComYMV	64.7	63.7	62.1	63.2	66.3	55.9	59.0	63.7	62.1	62.6	65.2	60.6	63.2	

NT, nucleotide; AA, amino acid; BsCVBVDelhi, tentative name for Delhi isolate (accession GQ254411); BsCVBVTirupati, tentative name for Tirupati isolate (accession GQ254410); BsCVBV1, Bougainvillea spectabilis chlorotic vein-banding virus from Taiwan (accession EU034539); BsCVBV2, Bougainvillea spectabilis chlorotic vein-banding virus from Taiwan (accession DQ347841); BSMyV, *Banana streak Mysore virus* (accession NC_006955); CSSV, *Cacato swollen shoot virus* (accession NC_001574); KTSV, *Kalanchoe top-spotting virus* (accession NC_004540); PYMV, *Piper yellow mottle virus* (accession DQ836237); SCIMBV, *Sugarcane bacilliform IM virus* (accession NC_003031); CMBV, *Citrus mosaic virus* (accession NC_003382); DBV, *Dioscorea bacilliform virus* (accession DQ822073); TaBV, *Taro bacilliform virus* (accession NC_004450); DrMV, *Dracaena mottle virus* (accession NC_008034); ComYMV, *Commelina yellow mottle virus* (accession NC_001343)

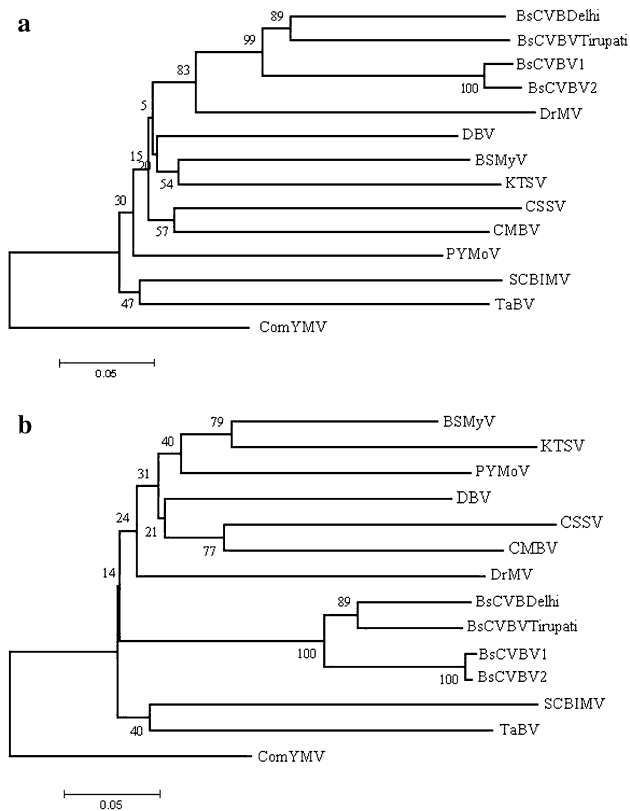


Fig. 4 **a** Phylogenetic neighbour-joining tree of nucleotide sequences of putative RT and RNase H region of 14 definitive and tentative badnaviruses using MEGA programme version 4. **b** Phylogenetic neighbour-joining tree of amino acid sequences of putative RT and RNase H region of 14 definitive and tentative badnaviruses using MEGA programme version 4

Badnavirus in India. The study has implication in determining diversity in badnaviruses infecting bougainvillea, their diagnostics and certification of plant material in exchange programmes.

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Appendix

BsCVBDelhi, tentative name for Delhi isolate (accession GQ254411); BsCVBVTirupati, Tentative name for Tirupati isolate (accession GQ254410); BsCVBV1, Bougainvillea spectabilis chlorotic vein-banding virus from Taiwan (accession EU034539); BsCVBV2, Bougainvillea spectabilis chlorotic vein-banding virus from Taiwan (accession DQ347841); BSMyV, *Banana streak Mysore virus* (accession NC_006955); CSSV, *Cacao swollen shoot virus* (accession NC_001574); KTSV, *Kalanchoe top-spotting virus* (accession NC_004540); PYMoV, *Piper yellow mottle virus* (accession DQ836237); SCBIMV, *Sugarcane bacilliform IM virus* (accession NC_003031); CMBV, *Citrus mosaic virus* (accession NC_003382); DBV, *Dioscorea bacilliform virus* (accession DQ822073); TaBV, *Taro bacilliform virus* (accession NC_004450); DrMV, *Dracaena mottle virus* (accession NC_008034); ComYMV: *Commelina yellow mottle virus* (accession NC_001343).

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