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Identification of mixed infection caused by Badnavirus and CMV in Jasmine (*Jasminum multiflorum* Roth)

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The symptoms of chlorotic ring spots and irregular chlorotic patches were recently observed on leaves of landscape-grown jasmine (*Jasminum multiflorum* Roth) in southern parts of India. The causal agent was mechanically transmitted from symptomatic leaves of jasmine to *Nicotiana glutinosa*, *Capsicum annum*, *Solanum lycopersicum*, *Cucurbita pepo* and *Cucumis sativus*. Leaf dip preparations from virus-infected plants in electron microscopy revealed the presence of isometric particles similar to *Cucumber mosaic virus* (CMV) and bacilliform virus particles of badnavirus. The virus reacted specifically with IgG for CMV and Banana streak virus (BSV) in direct antigen coating, enzyme-linked immunosorbent assay. No reaction was observed with ilar-, poty- and tospo-viruses specific IgG. Reverse transcription polymerase chain reaction with total RNA isolated from symptomatic jasmine leaves and infected *N. tabacum* 'Xanthi' using CMV coat protein (CMV CP) specific primers amplified the expected product. The association of badna virus with jasmine was confirmed by PCR amplification, cloning and sequencing of 560 bp amplicon corresponding to the reverse transcriptase and ribonuclease H coding region in open reading frame III. The sequence analysis revealed maximum identity to badna virus group Diascorea bacilliform virus (88.5% at nt level) and CMV group IB (96% at nt level). To our knowledge, this is the first report of CMV and badna mixed infection in jasmine in India.

Keywords: Badnavirus, *Cucumber mosaic virus*, jasmine

Jasmine (*Jasminum* spp.; Family: Oleaceae) is one of the major commercial flower crops of India. Among the different *Jasminum* species, star jasmine (*Jasminum multiflorum* Roth) is grown for their beautiful single-flowered, white blooms, which have no fragrance, as decorative shrubs planted around private gardens and public buildings for their long life, vigor and beauty of the flowers. *Tomato mosaic virus* was the first virus reported infecting *J. multiflorum* species[1]. However, three well-characterized viruses were known to infect other species of Jasmine[2-4]. The objective of the present study was to identify the virus(es) associated with the disease symptoms observed on star jasmine plants.

During the survey conducted in major Jasmine growing areas of South India, the symptoms of chlorotic mottling, ring spots and chlorotic line patterns were noticed from leaf samples of *J. multiflorum* collected from Chintamani, Mandya, Bangalore and Shimoga during April-May 2012 (Fig. 1). The overall incidence ranged less than 1%. *Cucumber mosaic virus* (CMV) was detected in two plants, showing symptoms of irregular chlorotic patches. Badnavirus was detected in one plant showing chlorotic ring spots, while combined infection of both had symptoms of irregular chlorotic patches. The isolate was maintained by vegetative propagation under glasshouse conditions at 25-28°C. As badnavirus is not sap transmissible, only CMV was back transmitted with inoculated plants showing similar symptoms.

Different tests employed to confirm the identity of virus from the source sample include electron microscopy of leaf dip preparation, DAC-ELISA and mechanical inoculation to *Nicotiana glutinosa*, *N. tabacum* Xanthi, *Cucurbita pepo, Cucumis sativa*,

Fig. 1—Infected Jasmine showing symptoms of chlorosis.

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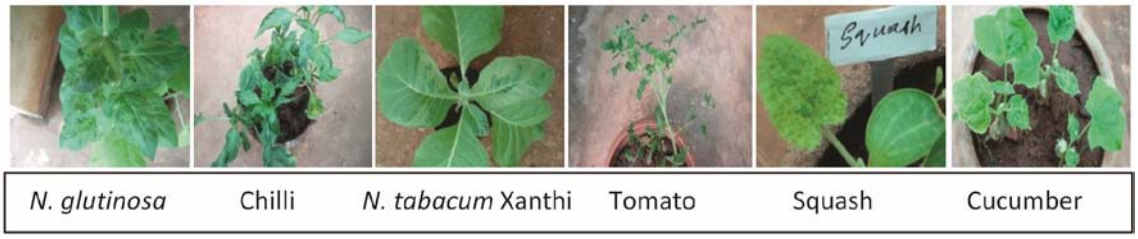

Fig. 2—Mechanical transmission of jasmine virus.

Capsicum annum and *Solanum lycopersicum* to study the host reactions on these species (Fig. 2). Total RNA was extracted from 100 mg symptomatic, fresh jasmine leaf tissue using a Spectrum™ Plant Total RNA Kit (Sigma, Aldrich) and reverse transcribed using MMuLV Reverse Transcriptase (MBI, Fermentas), followed by PCR amplification with CMV CP specific primers (CPF-5´ GCGGATTCATGGACAAATCTGAATCAAC-3´ and CPR-5´ ATGGTACCTCAAACTGGGAGCAGCCCAG -3´) in a Gene Amp PCR system 2400 thermal cycler (Perkin-Elmer, Wallesly, MA, USA). Total genomic DNA was extracted from infected samples using CTAB method and subjected for amplification with degenerate primers designed to amplify the RNAseH region of ORF III, namely Badna F and Badna R (5´-ATGCCITTYGGIITIAARAAYGCICC-3´ & 5´-CCAYTTRCAIACISCICCCCAICC-3´)[5]. The products were cloned and sequenced in duplicate. RT/RNaseH sequences and CMV sequences were aligned using the CLUSTAL W algorithm in MEGA 5.0[6]. Phylogenetic relationships among badnaviruses and cucumber mosaic viruses were estimated using Neighborhood Joining Bootstrap Method (Bootstrap analysis with 1000 replicates) using MEGA 5.0. The BLAST programme was used to identify related sequences from GenBank.

In DAC-ELISA, the sample reacted with CMV and also with the badnavirus antisera (BSV), suggesting the association of a cucumo and badnaviruses in causing infection. However, none of the samples reacted with Groundnut bud necrosis virus (GBNV), Papaya ringspot virus (PRSV) and Tobacco streak virus (TSV) antisera, suggesting the lack of association of a tospo, poty or ilarviruses with the disease. Further, among the antisera to different badnaviruses tested, the sample reacted with Banana streak virus (BSV) antisera, suggesting the association of a virus infecting Jasmine serologically related to badnavirus. These data were also supported by the electron microscopy of leaf dip preparations of diseased leaves, which showed the presence of

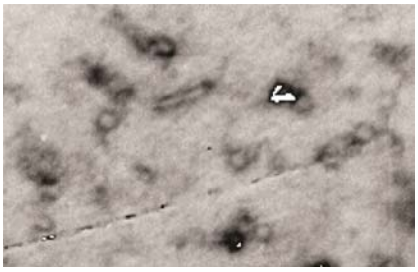

Fig. 3—Electron microscopic observation of CMV and badnavirus in jasmine.

bacilliform-shaped particles measuring about 30 nm×120 nm in size, although particle concentration was very low along with isometric particles (Fig. 3).

Based on the consensus sequences located in the reverse transcriptase and RNaseH of ORF III domains in badna genome and CP region of CMV, PCR as well as RT-PCR was able to amplify PCR product of approx 600 bp and 700 bp corresponding to the BSV and CMV, respectively in Jasmine. The amplicons cloned and then sequenced revealed 523 bp and 657 bp pertaining to badna and CMV specific primers coding for 176 and 218 aa, respectively. Further the sequences were deposited in the GenBank (Acc. no. KF129061 & KF129062). The present report is the first evidence of mixed infection of CMV in association with badnavirus in Jasmine. Similar association was suspected in *Piper betel* and *P. longum*[7] as well as Canna[8]. The sequence analysis with other related sequence of CMV CP region in GenBank revealed close relation with the CMV isolate AN (98% at nt level and 95% at aa level) and that of Badna revealed genetic relatedness to the *Dioscorea bacilliform virus* (DaBV) isolate PG151 Da (93% at nt level & 95% at aa level). The pairwise comparison of badna sequences revealed an identity of 88.3-88.5 % at nt level and 76-77.5% at aa level with DaBV isolates, whereas CMV Jasmine sequences were 94% identical with Group IB CMV, 91% identical with Group IA and 74% identical with

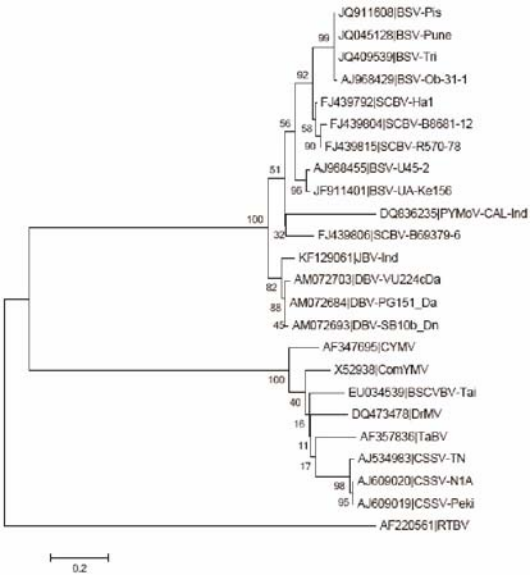

Fig. 4—Phylogenetic tree depicting relationship of badnavirus infecting Jasmine (JBV-Ind) with distinct badnavirus species and their strains based on multiple sequence alignment of a region of ORF III amino acid sequences using Rice tungro bacilliform virus (RTBV) as outgroup. The bootstrap values are shown at the individual node.

Group II sequences. The Phylogenetic analysis using CMV CP sequence data of selected strains of CMV of subgroup IA, IB and II showed the close relationship of Jasmine isolate (CMV-Jasmine) with the strains of CMV of subgroup IB. Similarly phylogeny analysis revealed a close clustering of Jasmine badnavirus (JBV-Ind) with DaBV. The phylogenetic tree constructed favoured the results of sequence similarity showing clustering of JBV-Ind with DaBV isolate PG151_Da and CMV-Jasmine with CMV subgroup IB (Figs 4 & 5). The serological relation of *Dioscorea alata bacilliform virus* (DaBV) with two isolates of banana streak badnavirus (BSV) has earlier been reported[9]. Based on sequence identities and close phylogenetic relationship, the causal viruses of mottle disease in Jasmine were identified as an isolate of CMV belonging to subgroup IB and badnavirus. Thus the etiology of the viral disease in *J. multiflorum* studied might be an association of badnavirus and CMV. Further, the PCR primers used in the present study could serve as efficient, specific early warning diagnostic tool to reduce the risk of spread of the disease to other landscape ornamentals, which may otherwise lead to compromise the aesthetic value of the garden. Since vegetatively propagated plant material can contribute to virus dissemination,

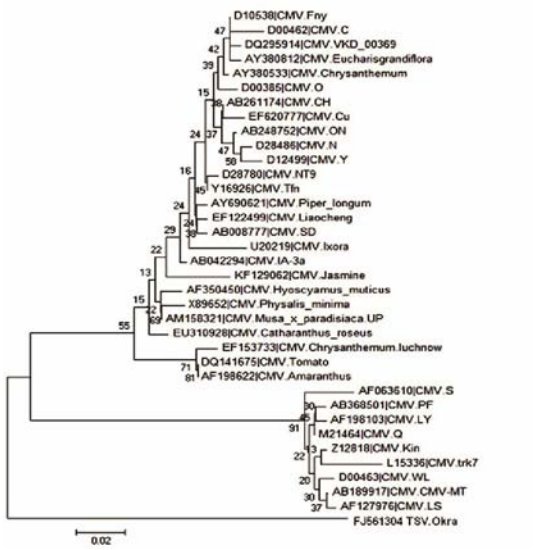

Fig. 5—Phylogenetic tree depicting relationship of CMV CP infecting jasmine (CMV-Jasmine) with CMV and their strains based sequences using Tobacco streak Virus (TSV) as outgroup. The bootstrap values are shown at the individual node.

currently the destruction of infected plants is recommended to prevent the spread of pathogen.

References

1 Kamenova I, Adkins S & Achor D, Identification of *Tomato mosaic virus* infection in jasmine, *Acta Hortic*, 722 (2006) 277-284.

2 Waterworth H E, Purification of arabis mosaic virus isolated from a jasmine plant introduction, *Phytopathology*, 65 (1975) 927-928.

3 Morton C S, Barnett O W & Baxter L W, Damage caused by tobacco ringspot virus to poet's jasmine (*Jasminium officinale*), *Proc. Southern Nurserymen's Assoc Res Congr*, 22 Annu Rep (1976) 111-114.

4 Wilson K I, Chlorotic ringspot of jasmine, *Indian Phytopathol*, 25 (1972) 157-158.

5 Yang I C, Hafner G J, Dale J L & Harding R M, Genomic characterisation of taro bacilliform virus, *Arch Virol*, 148 (2003) 937-949.

6 Tamura K, Peterson D, Peterson N, Stecher G, Nei M *et al*, MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol Biol Evol*, 28 (2011) 2731-2739.

7 Siju S, Bhat A I & Hareesh P S, Identification and characterization of a *Badnavirus* infecting betel vine and Indian long pepper, *J Plant Biochem Biotechnol*, 17 (2008) 73-76.

8 Lockhart B E L, Occurrence of canna yellow mottle virus in North America, *Acta Hortic*, 234 (1988) 69-72.

9 Phillips S, Briddon R W, Brunt A A & Hull R, The partial characterization of a badnavirus infecting the greater Asiatic or water yam (*Dioscorea alata*), *J Phytopathol*, 147 (1999) 265-269.