Characterization of a potyvirus associated with yellow mosaic disease of jasmine (*Jasminum sambac* L.) in Andhra Pradesh, India

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Abstract A virus isolate associated with yellow mosaic disease was purified from commercially cultivated jasmine (*Jasminum sambac*) from Andhra Pradesh, India and it contained flexuous filamentous particles of $\sim 720 \times 13$ nm. The denatured purified virus had single major polypeptide of molecular weight 32 kDa. Complementary DNA representing 1678 nucleotides (nt) of the 3' terminus of viral RNA was cloned and sequenced. Comparisons of complete coat protein (CP) gene nucleotide and amino acid sequences of the present virus isolate with certain reported potyviruses revealed 86.1 and 92.7 % identity, respectively with jasmine potyvirus T (JaVT) reported from Taiwan and less than 70 % with other potyviruses. Based on the phylogenetic analysis of 3' UTR and CP gene, the present virus isolate was identified as an isolate of JaVT that belongs to the genus *Potyvirus* and the name Jasmine yellow mosaic virus-Andhra Pradesh (JaY-MV-AP) is proposed.

Keywords *Jasminum sambac* · Yellow mosaic · Phylogeny · Potyvirus

Jasmine (family *Oleaceae*) is an important commercial floricultural crop in India. Jasmine flowers are very popular in view of its scent and oil. Among the three important species, *Jasminum sambac* is accepted as commercial jasmine and is propagated through stem cuttings [12]. Literature survey indicated that only a few viruses naturally infecting jasmine worldwide are characterized based on limited serological and molecular data [6, 7, 9, 11, 16, 17]. Recently, sequences of *Groundnut bud necrosis virus* (JQ995170) and *Tobacco streak virus* isolates (KC996727, KC996726, KC996725) associated with infections on jasmine in India were deposited in pubmed. Survey of commercial jasmine gardens during 2009 in Chittoor district, Andhra Pradesh, India plants showing yellow mosaic symptoms were found prevalent. Herein, we described the purification, electron microscopy, coat protein analysis and 3' 1678 nt genome sequence that covers 3' untranslated region (UTR), complete coat protein (CP) gene and partial nuclear inclusion protein b (Nlb) gene of the virus associated with yellow mosaic disease of jasmine for correct identification.

Stem cuttings from jasmine plants showing yellow mosaic symptoms (vein clearing followed by yellow mosaic; Fig. 1a, b) were collected from the commercial gardens and established in the green house of Virology Department, Sri Venkateswara University, Tirupati. Virus was purified by following the procedure described by Hema et al. [5]. The final purified virus pellet was resuspended in minimal volume of resuspension buffer (0.02 M HEPES buffer, pH 7.5) for further use. The purified virus preparation was placed on to formvar-coated copper grids, stained with 2 % uranyl acetate (w/v) and observed under JOEL 100 S transmission electron microscope (Japan) at a magnification of 75,000 $\times$ (Indian Institute of Horticultural Research, Bangalore). The virions in purified preparations

The nucleotide sequence reported in this paper has been submitted to GenBank and assigned the accession number FJ543110.

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Fig. 1 Symptoms of yellow mosaic disease on jasmine leaves. **a** Vein clearing and **b** severe mosaic. **c** Electron micrograph of purified flexuous filamentous virus particles associated with yellow mosaic disease of jasmine (magnification $\times 75,000$, Scale bar = 0.4 microns) **d** SDS-PAGE analysis of purified virus preparation associated with yellow mosaic disease of jasmine. *Lane 1* molecular weight markers (Bangalore Genei) and *lane 2* Purified virus preparation

were denatured and the proteins were separated by 12 % SDS-PAGE [8].

RNA was isolated from purified virus preparations using Trizol as per the manufacturer's instructions (Invitrogen, USA). Reverse transcription (RT) was carried out with the Revertaid MMuLV RT (MBI Fermentas, USA) using a degenerate oligo dT reverse primer (oligo dT poty R: 5' CCCAGTCACGACTTTTTTTTTTTTTT 3'). PCR was carried out using 1 μ L of the RT reaction in a reaction mix containing 2.5 units of *Taq* polymerase (MBI Fermentas) with the conditions i.e. 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 45 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The oligonucleotides (M. Krishna Reddy, unpublished data) used for PCR were an oligo dT poty R and a degenerate forward primer based on conserved region of the potyvirus CP gene (potyCP:

5' TGGTGYATHGANAAAYGGNACNTC 3'). A 700 bp fragment was amplified and cloned into the pGEM-T easy vector (Promega, Madison, USA) and authenticity of the recombinant plasmids was confirmed by PCR using M13 forward and reverse primers and sequenced in both directions using an ABI PRISM 3770 DNA sequencer (NCBS DNA sequencing facility, Bangalore). To extend the sequence analysis to regions of the genome upstream of the potyvirus CP region, a second round RT-PCR was carried out using virus gene specific reverse primer (JV RP 5' GCTTTTGGATATTCCACTT 3') and a degenerate forward primer based on the conserved region within the potyvirus NIb protein (potyNIb F: 5' GGICARCCITCIACIGTIGT 3'; M. Krishna Reddy, unpublished data) using reaction conditions as described above but with an annealing temperature of 50 °C. The resulting ~1 kb PCR product was cloned into

Fig. 2 **a** Phylogenetic analysis of the aligned amino acid sequences of the complete coat protein and **b** 3' UTR nucleotide sequences of potyvirus associated with yellow mosaic disease of jasmine (named as JaYMV-AP). The bootstrapped unrooted neighbor-joining trees were constructed using MEGA 4.0.2 package. The values at the forks indicate the percentage of trees in which this grouping occurred after bootstrapping (10,000 replicates). The scale bar shows the number of substitutions per base. Virus acronyms: AWMV (Algerian watermelon mosaic virus), BYMV (Bean yellow mosaic virus), ChiVMV

(Chilly vein mosaic virus), JaVT (*Jasmine virus Taiwan isolate*), JYMV (*Japanese yam mosaic virus*), NYSV (*Narcissus yellow stripe virus*), PPV (*Plum pox virus*), PRSV (*Papaya ring spot virus*), PLDMV (*Papaya leaf distortion mosaic virus*), PTMV (*Peru tomato mosaic virus*), PVY (*Potato virus Y*), ScaMV (*Scallion mosaic virus*), TFW (Thunberg fritillary virus), TuMV (*Turnip mosaic virus*), TVBMV (*Tobacco vein banding mosaic virus*), VVY (*Verbena virus Y*), WTMV (*Wild tomato mosaic virus*), JaYMV-AP (*Jasmine yellow mosaic virus-Andhra Pradesh*)

CloneJet vector (MBI Fermentas) and authenticity of the plasmids was confirmed by PCR and sequencing. Overlapping sequences were assembled and the amino acid sequence was deduced using ExPASy Translate tool [4]. All the procedures for gel extraction and plasmid isolation followed as per Sambrook and Russell [13]. A database search for closely related viral species was carried out using BLAST [3] and generated sequence was compared with 18 reported potyvirus sequences. Values were obtained by analysis with EMBOSS needle program (Needleman-Wunsch algorithm) (European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK), using a gap open cost of 10.0 and gap extension cost of 0.5, for nucleotide sequences, and BLOSUM 62 scoring matrix with gap initiation cost of 10 and gap extension cost of 0.5, for protein sequences. Multiple sequence alignments (MSAs) were conducted using the CLUSTAL X software version 1.81 [15] and phylogenetic trees were generated by the neighbor-joining (NJ) method with bootstrap analysis of 10,000 replicates using Mega version 4.0 [14].

Electron microscopy of the purified virus preparation showed flexuous filamentous particles with dimensions of $\sim 720 \text{ nm} \times 13 \text{ nm}$ (magnification $75,000\times$, Scale bar = 0.4 microns) (Fig. 1c). Denatured purified virus resolved into a single major polypeptide corresponding to molecular weight of 32 kDa (Fig. 1d). The virion morphology and coat protein molecular weight indicates that the

present virus is probably a potyvirus [10]. However, it failed to react with 10 tested polyclonal antisera of potyviruses viz. Sorghum mosaic virus (SrMV), Maize dwarf mosaic virus (MDMV), Peanut green mosaic virus (PGMV), Sugarcane streak mosaic virus (SCSMV-AP), Potato virus Y (PVY), Black eye cowpea mosaic virus (BICMV), Pepper vein banding virus (PVBV), Papaya ring spot virus (PRSV), Watermelon mosaic virus (WMV), Chilli vein mottle virus (ChiVMV) in electroblot immunoassay (data not shown).

To confirm the initial tentative identification of present virus isolate, its partial genome sequence was analyzed. Alignment of two amplicons ($\sim 700 \text{ bp}$ and $\sim 1 \text{ kb}$) obtained by RT-PCR resulted in 3' terminal 1678 nucleotides of the viral genome, and its analysis indicates that 3' UTR is 199 nucleotides followed by poly A tail, CP gene is 840 nucleotides and partial NIB gene is 621 nucleotides in length (Ac. No. FJ543110). The deduced amino acid sequence indicated a partial polyprotein with 487 residues i.e. 207 residues from the C-terminus of NIB and 280 residues of the CP.

Comparison of 1678 nucleotides of the present virus isolate with reported potyviruses in GenBank showed maximum identity of 82 % with Jasmine potyvirus Taiwan isolate (JaVT, EF535842; 1289 nts covering 3' UTR and complete CP gene; Lin et al. 2004), 87 % identity with Jasmine potyvirus mogra isolate from India (JN807771; 611 nts covering 3' UTR and partial CP regions; Kaur et al. 2013)

and less than 70 % with the other potyviruses. The 3' UTR sequence of the present virus isolate shared 87.5 % identity with that of JaVT and 83.6 % with jasmine potyvirus mogra isolate. Highest relatedness of CP nucleotide and amino acid sequence was found to be 86.1 and 92.7 %, respectively with JaVT and 71.3 % identity with *Japanese yam mosaic* (AM158908) and *Wild tomato mosaic viruses* (DQ851495) followed by other viruses with less than 70 % identity. Further, sequence analysis indicated a putative Nib/CP cleavage site (VHFQ/S), characteristic of potyviruses [2]. The putative CP showed the conserved motifs: DAG, AFDF and QMKAAL at amino acid positions 8–10, 211–214 and 231–237, respectively, from the Q/S cleavage site [2]. The conserved sequence motif of potyviral polymerase, GDD was also found in the putative Nib sequence of the present virus isolate. Phylogenetic analysis of the deduced CP amino acid sequence and 3' UTR nucleotide sequence revealed clustering of present virus isolate as a separate branch with JaVT and thus confirms its correct identity as an isolate of JaVT (Fig. 2a, b).

The availability of CP gene and full genome sequences has significantly clarified the taxonomic status of several potyviruses and suggested the criteria to distinguish closely related virus species from strains of same species [1]. Based on these criteria, the present virus is considered as an isolate of JaVT. Further, the present isolate is also showing yellow mosaic symptoms on jasmine similar to the symptoms induced by JaVT [9], but it failed to produce chlorotic local lesions in the tested *Chenopodium* species like JaVT in a limited host range studies (data not shown). Recently, Kaur et al. [7] reported the detection of jasmine potyvirus mogra isolate in jasmine using potyvirus degenerate primers by RT-PCR from Uttar Pradesh, India and sequence analysis of PCR amplicon (611 nts) revealed 85 and 98 % identity at nt and aa levels, respectively with the present virus isolate sequence deposited in the GenBank (FJ543110) prior to their publication. The present virus isolate is having 87.5 and 86.1 % at 3' UTR and complete CP levels, respectively with JaVT, but mogra isolate is having 96 and 92 % homology at 3' UTR and partial CP region, respectively with JaVT. This study indicates that the jasmine yellow mosaic virus isolates that are prevalent in different states of India are having varied sequence homology. In conclusion, we propose the name Jasmine yellow mosaic virus-Andhra Pradesh with the acronym JaYMV-AP for the present virus isolate.

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