



Letter

Molecular Characterizations of Two Begomoviruses Infecting *Vinca rosea* and *Raphanus sativus* in India*

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Dear Editor

Samples of *Vinca rosea* and *Raphanus sativus* leaves showing typical leaf curling were collected from gardens and fields of Bhatinda, Punjab (India). An expected product of ~550 bp in size was amplified from total DNA extracts of symptomatic leaf samples with universal primers on the coat protein region of begomoviral DNA-A component. Moreover, DNA β were also detected in both *V. rosea* and *R. sativus* using β satellite universal primers. This is the first report of a β satellite associated with *V. rosea* in India. The presence of begomoviruses was also confirmed by Southern blot analysis using cloned DNA-A probe of Papaya leaf curl virus. Sequence analysis of viruses infecting *V. rosea* (Vinca yellow vein virus) and *R. sativus* (Raphanus sativus leaf curl Bhatinda virus) showed 74% and 84% nucleotide sequence identity with Papaya leaf curl virus, respectively.

Geminiviridae family has been classified into four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus* (Fauquet C M, 2005). *Begomovirus* is the largest genus of this family. The vector is the white fly (*Bemisia tabaci*) that causes begomoviral infections in ornamentals, crops

and weeds and the infection is prevalent in the tropical and subtropical regions of the world (Marwal A, 2012). *Begomovirus* is the only genus of the *Geminiviridae* which has bipartite genome with virus genes resident on two different circular ssDNA molecules (DNA A, DNA B), each of which is about 2.6- 2.8 kb. Some monopartite begomoviruses are associated with β satellite (DNA α or DNA β), each of which is up to 1.3 kb (Markham P, 1994).

In the present report, we identified two begomoviruses and their associated β satellite infecting *Vinca rosea* and *Raphanus sativus*. *V. rosea* belongs to the family *Apocynaceae* and is an herbaceous shrub with glossy dark leaves known as Madagascar periwinkle worldwide (Vadeyar L K, 2010). It is grown mostly as an ornamental plant in perennial tropical regions and cultivated for its alkaloids, which have anticancer activities (Jaleel C A, 2006). The radish, *R. sativus* belonging to the *Cruciferae* family, is an herbaceous vegetable, cultivated especially for its edible roots which in fresh state are used in food (Truta E, 2012).

During the survey of field crops, nearly 40–50 % of *V. rosea* plants and 60–70 % *R. sativus* with leaf curling were observed in Bhatinda, Punjab region of India. Infected *V.rosea* samples were collected from the location Latitude: 30N 20' 82.89" and Longitude: 74E 99' 06.59". For *R. sativus* the infected samples were collected from the location Latitude: 30N 20' 87.53" and Longitude: 74E 99' 06.16".

To investigate the potential begomoviral infection, total DNA was extracted from leaves of infected plants using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Manen J F, 2005). PCR was performed using a pair of degenerate primers specific to the coat protein region of *begomovirus*. The forward primer sequence was GGRITDGARGCATGHGTACATG (AC 1048) and the

Avinash Marwal^{1#}, Anurag Sahu^{1#}, Pradeep Sharma² and Rajarshi Kumar Gaur^{1✉}

1. Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Lakshmangarh, Sikar 332311, Rajasthan, India;

2. Directorate of Wheat Research, PO BOX-158, Agrasain Marg, Karnal 132 001, Haryana, India

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✉ Corresponding author.

Phone: +91-9352911723, Fax: +91-1573225041,
Email: gaurrajarsi@hotmail.com

These authors contributed equally to this work.

reverse primer sequence was GCCYATRTAYAGRAA GCCMAG (AV 494). A typical PCR reaction contained about 100 ng DNA template, Taq 10 x buffers (10 mmol/L Tris-HCl, pH 8.8; 50 mmol/L KCl) 25mmol/L MgCl₂, 200 µmol/L of each dNTPs, 2 units of Taq DNA Polymerase, Nuclease free water and 10 pmol/L of each primer. The PCR thermal profile was pre-PCR denaturation at 94°C for 120 s followed by 35 cycles of denaturing at 94°C for 45 s, annealing at 55°C for 60 s and extension at 72°C for 60 s, and a final extension at 72°C for 5 min. To test whether a satellite molecule was associated with these isolates, a universal primer pair specific for DNA α and DNA β (Bridson R W, 2002; Bull S E, 2003) was also used to amplify the putative DNA.

In order to confirm the Geminivirus infectivity, Southern blotting was performed according to the method of Kon *et al.* (Kon T, 2003). Cloned DNA-A of Papaya leaf curl

virus was used to develop the radio-labeled probes. PCR product was sequenced and has been deposited in NCBI GenBank having Accession No: JQ693139 (Vinca yellow vein virus) and JN998450 (Raphanus sativus leaf curl Bhatinda virus) respectively. Phylogenetic analysis was done using MEGA 4.0 showing the relationship to other closely related viruses (Tamura K, 2006).

Begomovirus might be associated with symptoms such as leaf curling and stunted growth, which were observed in *V. rosea* (Jan 2011) and *R. sativus* (Nov 2011) plants growing in gardens and fields of Bhatinda, Punjab (India). Sequence analysis of the virus infecting *V. rosea* showed 74% nucleotide sequence identity with the Lucknow coat protein (CP) gene (complete cds HQ630856) of the Papaya leaf curl virus, and the MS-6 coat protein (AV1) gene (complete cds FJ002571) of Tomato leaf curl virus.

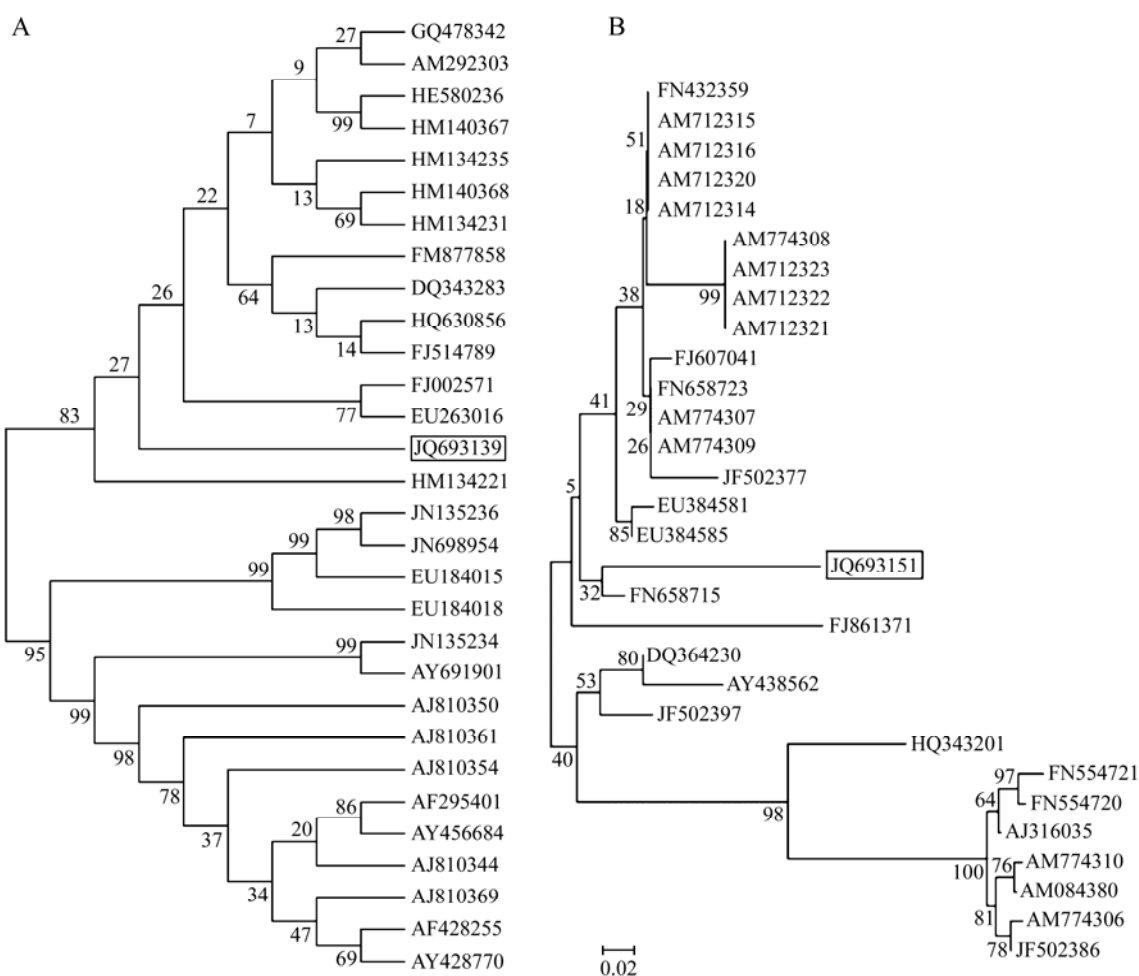


Fig. 1. A: Neighbor-Joining tree based on the sequence of coat protein gene (JQ693139) of the virus isolated from *Vinca rosea* and other begomovirus sequences available in GenBank. Papaya leaf curl virus (HQ630856). B: Neighbor-Joining tree based on the sequence of β satellite (JQ693151), of the virus isolated from *Vinca rosea* and other begomovirus sequences available in GenBank.

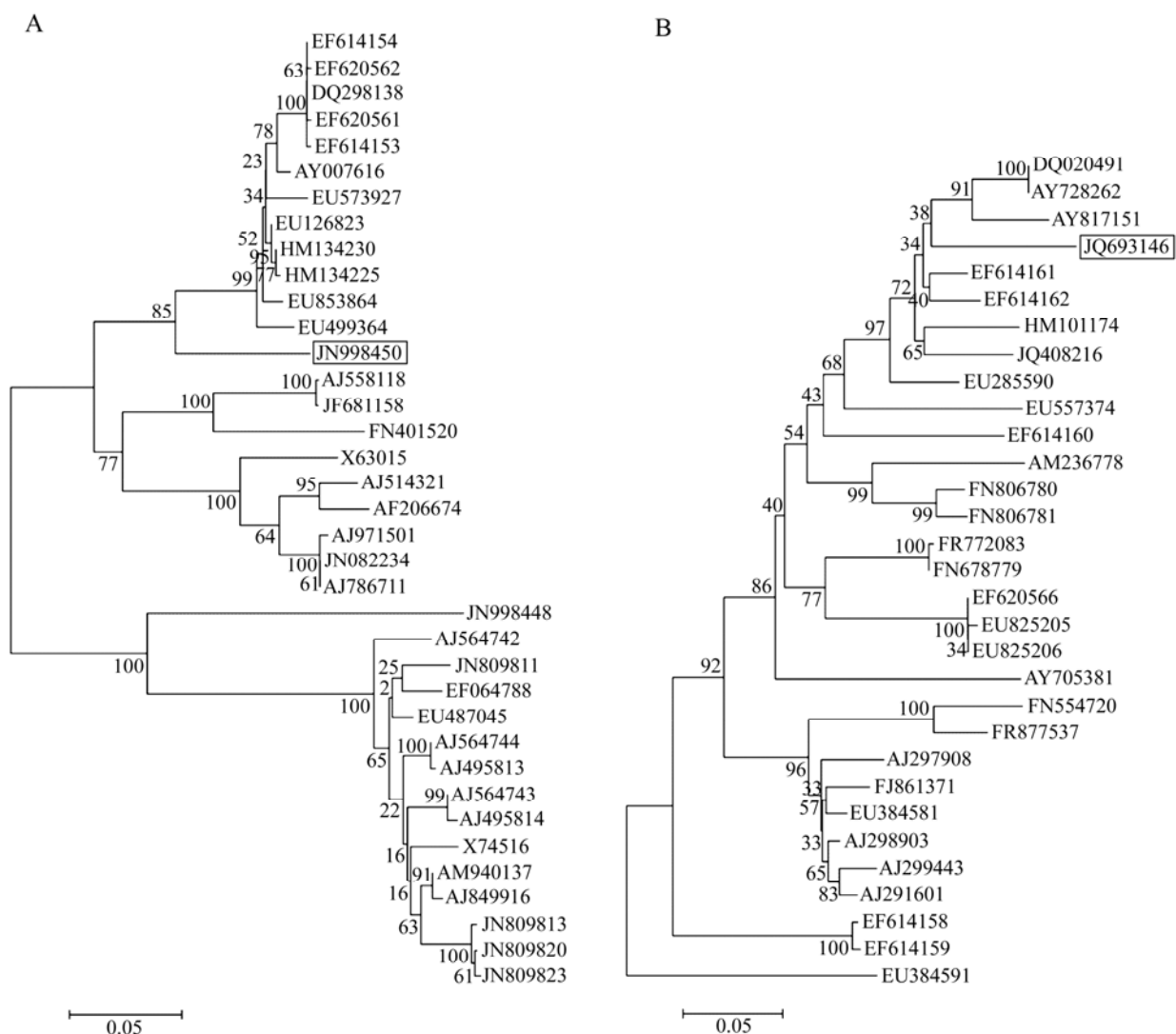


Fig. 2. A: Neighbor-Joining tree based on the sequence of coat protein gene (JN998450) of the virus isolated from *Raphanus sativus* and other begomovirus sequences available in GenBank. B: Neighbor-Joining tree based on the sequence of β satellite (JQ693146) of the virus isolated from *Raphanus sativus* and other begomovirus sequences available in GenBank. Luffa begomovirus associated DNA β (DQ020491).

In the case of *R. sativus*, the alignment process of begomovirus sequence revealed 84% identity each with Papaya leaf curl virus from *Carica papaya* cv. African Dwarf coat protein gene (complete cds EU126823) and Croton yellow vein mosaic virus segment A (complete sequence, clone Ku-RCA-AI-F FN645926). Hybridization of Papaya leaf curl virus probe with the DNA fragment on the filter membrane further indicated that this fragment contained DNA sequence complementary to the probe. The strong signal showed that the virus in *V. rosea* and *R. sativus* had some homology with the Papaya leaf curl virus.

β satellites were also identified in the begomovirus infected plant samples *V. rosea* and *R. sativus*. In *V. rosea* the β satellite (JQ693151) showed nearly 83% identity

both with Cotton leaf curl virus-associated DNA β satellite from India (complete sequence DQ364230) and Cotton leaf curl virus-associated DNA β satellite (complete sequence, clone Lu6 AM774309). While in *R. sativus* (JQ693146), it revealed 92% sequence identity both with Luffa begomovirus associated DNA β satellite (complete sequence DQ020491) and Luffa puckering and leaf distortion-associated DNA β satellite (complete sequence AY728262). In the Neighbor-Joining tree, the coat protein gene, and the β satellite of begomovirus infecting *V. rosea* (Fig.1) and *R. sativus* (Fig.2) respectively, were each placed within the tree, thus inferring evolutionary history. Names of related viruses were summarized in the Supplementary Table 1.

Future studies will be focused on the amplification of the complete genome of the viruses to find out whether these begomoviruses are novel or not. Moreover a recombination analysis and a detailed survey of its distribution and host range in distinct locations of the country will be carried out.

The possible association of a β satellite with *V. rosea* had not been investigated previously, so this is the first report of β satellite associated with *V. rosea*. *Vinca rosea* is a widely used ornamental plant in India whereas *Raphanus sativus* is an important palatable plant species found to be infected with begomovirus. Thus, identification of an associated β satellite represents the possibility of a serious threat to other economically important ornamental and crop plants.

Supplementary Table 1 is linked to the online version of the paper on the website of *Virologica Sinica*. www.virosin.org

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