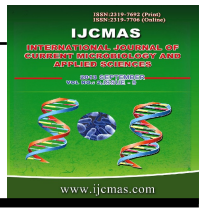
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 9 (2013) pp. 45-48**
http://www.ijcmas.com



Original Research Article

Molecular characterization of a begomovirus infecting a new host Golden Duranta (*Duranta erecta*) in India

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Keywords
Golden Duranta; Begomovirus; PCR; Southern blot; first report

A B S T R A C T

In 2012 leaf curl disease was observed on an ornamental plant Golden Duranta (*Duranta erecta*) in Lakshmangrh, Sikar district of Rajasthan province of India. Affected plants were severely stunted with apical leaf curling and crinkled leaves, symptoms typical of begomovirus infection. An expected PCR product of approximately ~550 bp was amplified, cloned and sequenced. The presence of begomoviruses was also confirmed by Southern blot analysis using cloned DNA-A probe of *Papaya leaf curl virus*. Sequence analysis of virus infecting Golden Duranta showed 98 % identity with *Papaya leaf crumple virus* (HE580236). This is the first report of begomovirus associated with leaf curl disease of an ornamental plant Golden Duranta in India.

Introduction

Golden Duranta is an ornamental plant of the family Verbenaceae often grown as garden hedge in India. During the survey for begomovirus infection nearly 40–50 % of Golden Duranta plants with leaf curling were observed in the gardens of Lakshmangarh, Sikar district of Rajasthan province of India (Figure. 1).

Materials and Methods

To investigate the potential begomoviral infection, infected Golden Duranta samples were collected from the location Latitude: 27N 80’ 16.74 and Longitude: 75E 03’ 55.28” (FASC, Lakshmangarh, Sikar district of Rajasthan province of India). The leaf samples were cleaned, cut, rolled in a piece of tissue paper, and was stored at -20^{o}C until DNA isolation. To begin with the molecular characterization total DNA was extracted from leaves of infected as well as healthy plants using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Manen et al. 2005). PCR was performed using a pair of degenerate primers specific to the coat protein region of begomovirus. The forward primer sequence was GGRTTDGARGCATGHGTACATG (AC 1048) and the reverse primer sequence was GCCYATRTAYAGRAAGCCMAG (AV 494) (Marwal, 2013a).

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 60 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. After amplification, 4 µl aliquot from each sample was electrophoresed in a 1 % agarose gelvisualized by staining ethidium bromide and UV illumination (Marwal, 2013b).

Results and Discussion

Positive PCR reaction confirmed the begomovirus infection in the isolate GD which amplified the coat protein fragment of approx ~550 bp in length from symptomatic, but not from symptomless samples. We haven't found any alphasatellite and betasatellite using universal primer pair (Briddon, 2002; Bull, 2003).

PCR product were suitably cloned into pGEM-T vector and sequenced, having Accession number JN998444. In nucleotide alignments, the begomovirus revealed highest nucleotide sequence identities of 98 % with Papaya leaf crumple virus (HE580236). Further comparison of the isolate with other begomovirus showed moderate sequence identity of 92 – 95 % with Rose leaf curl virus (GQ478342) and Pedilanthus leaf curl virus (HM134231). Whereas lower sequence identity of 87 – 89% matches with Tomato leaf curl virus (GU732204) and Cotton leaf curl Pakistan virus (HF549184).

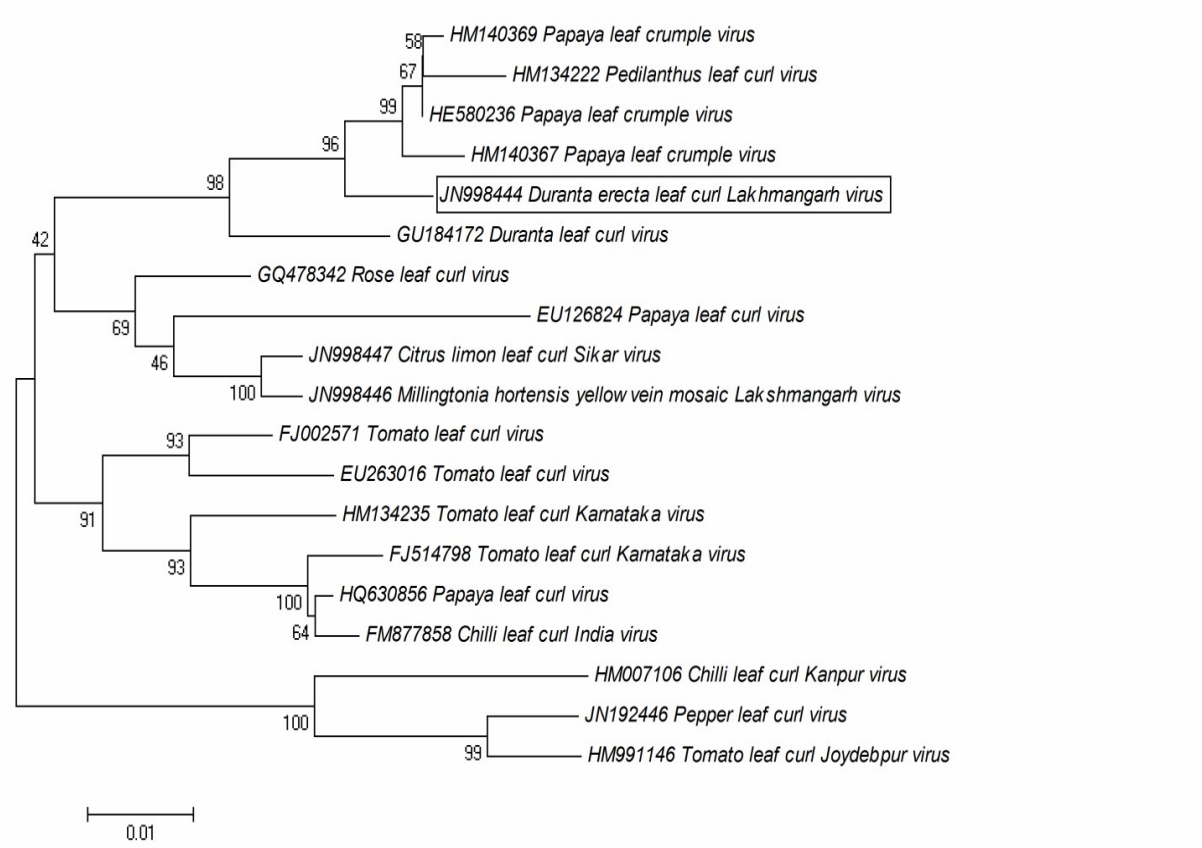
Phylogenetic tree of the obtained sequences was generated by MEGA 4.0 software by using the neighbor-joining method with 1,000 bootstrap replications. Phylogenetic analysis based on the coat protein sequence of begomovirus isolated from Golden Duranta and other selected sequences indicates that isolate GD cluster with the isolates of Papaya leaf crumple virus (Figure. 2) from Pakistan. This evidence enlightens the prevalence of begomovirus from neighboring countries such as Pakistan, into India thus inferring evolutionary history. An expected consequence of this scenario would be recombination which plays an important role for the evolution of new begomovirus strains in India and these new strains are responsible for heavy loss of new host variety.

In southern hybridization technique all samples from symptomatic plants hybridized with the probe, whereas samples extracted from non - symptomatic plants did not show positive results. 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 Golden Duranta had some homology with the Papaya leaf curl virus.

The begomovirus sequenced from isolate GD have a putative conserved domain of the *geminivirus* family which encodes for coat protein of 173 amino acids having a molecular weight of 20.57 kDa (protein id="AEY68276.1"). The ORF is designated as AV1 which resides on the positive sense strand of 2nd frame, having nucleotide coordinates in which start codon begins at position 5 and stop codon at 526. Even with protein alignments, the

Figure.1 Symptoms such as stunting, apical leaf curling and crinkled leaves, typical to that of begomovirus infection exhibited by the Golden Duranta (*D. erecta*) plant from which the begomovirus were isolated.

Figure.2 Neighbor-Joining tree based on the sequence of coat protein gene (JN998444) of the virus isolated from *D. erecta* and other begomovirus sequences available in GenBank. Values at nodes indicate percentage bootstrap values (1000 replicates).



begomovirus isolated from *D. erecta* showed highest amino acid sequence identities of 99 % with Papaya leaf crumple virus (HE580236) and Papaya leaf crumple virus (HM140369).

Thus, this identification represents the possibility of a serious threat to other economically important ornamental and crop plants and there is a need for a more comprehensive study to identify possible further begomoviruses infection in the country and to assess the contribution each makes to losses with a view to devising control strategies. This will form the basis of our future investigations. Results of these techniques effectively applied for disease management, crop protection and development of quarantine strategies at state and national level in India. The possible association of a begomovirus with Golden Duranta had not been investigated previously in India, therefore to the best of our knowledge, this is the first report of begomovirus and its aplhasatellite associated with leaf curl disease of an ornamental plant Golden Duranta (*D. erecta*) in India.

Acknowledgement

The authors would like to acknowledge a vote of thanks to Department of Biotechnology (DBT project No. BT/PR13129/GBD/27/197/2009) and Department of Science and Technology (DST project no SR/FT/LS-042/2009), India for their financial support.

References

Briddon, R. W., S. E. Bull, S. Mansoor, I. Amin and Markham P. G. 2002. Universal primers for the PCR-mediated amplification of DNA α; a molecule associated with some monopartite begomoviruses. Mol. Biotech. 20: 315–318.

Bull, S. E., R.W. Briddon and Markham P.G. 2003. Universal primers for the PCR-mediated amplification of DNA 1: a satellite-like molecule associated with begomovirus-DNA β complexes. Mol. Biotech. 23: 83–86.

Manen, J. F., O. Sinitsyna, L. Aeschbach, A. V. Markov and Sinitsyn A. 2005. A fully automatable enzymatic method for DNA extraction from plant tissues. BMC. Plant. Biol. 5: 3–23.

Marwal, A., A. Sahu, R. Prajapat and Gaur R. K. 2012. First report of association of begomovirus with the leaf curl disease of a common weed, *Datura inoxia*. Indian. J .Virol. 23: 3–84.

Marwal, A., A. Sahu, R. Prajapat and Gaur R. K. 2013a. First report of Begomovirus infecting two ornamental plants: *Jasminum sambac* and *Millingtonia hortensis.* Indian Phytopathol. 66:115 -116.

Marwal, A., A. Sahu, P. Sharma and Gaur R. K. 2013b. Molecular Characterizations of Two Begomoviruses Infecting *Vinca rosea* and *Raphanus sativus* in India. Virol. sinica. 28: 053 – 056.