Short Communication
FIRST REPORT OF MOLECULAR CHARACTERIZATION OF BEGOMOVIRUS INFECTING AN ORNAMENTAL PLANT *TECOMA STANS* AND A MEDICINAL PLANT *JUSTICIA ADHATODA*.

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ABSTRACT : *Samples of Justicia adhatoda and Tecoma stans leaves showing typical leaf curling were collected from gardens and fields of Lakshmangarh and Sikar, Rajasthan (India). An expected PCR product of ~600 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. The presence of begomoviruses was also confirmed by Southern blot analysis using cloned DNA-A probe of Cotton leaf curl virus. Sequence analysis of viruses infecting Justicia adhatoda (Justicia leaf curl virus) showed 79 % nucleotide sequence identity with Vinca yellow vein virus (JQ693139) and Tecoma stans (Tecoma leaf curl virus) revealed highest nucleotide sequence identity of 100 % with Lantana yellow vein virus (JQ693142).*

KEY WORDS: *Justicia adhatoda, Tecoma stans, Begomovirus, PCR, Southern blot*

INTRODUCTION

Over the past few decades, there has been more interest in geminiviruses, especially begomoviruses, as many of the diseases they cause have now reached epidemic proportions [1]. Ornamental plants serve as an alternative host for begomovirus in gardens and may allow the transmission of begomovirus to other crop and medicinal plants thus enhances the host range of this virus in different regions of the India [2]. The vector is the white fly (*Bemisia tabaci*) that causes begomoviral infections in ornamentals, crops and weeds and is prevalent in the tropical and subtropical regions of the world [3]. Therefore increasing knowledge about its epidemiology, sequence diversity and biodiversity is highly important in order to implement preventative strategies.

In the present report, we identified two begomovirus associated with *Justicia adhatoda* and *Tecoma stans. Justicia adhatoda* L. (Family Acanthaceae) is a persistent shrub which reaches upto a height of 1.0 m to 2.5 m, widespread throughout the tropical regions of Southeast Asia. It is commonly known as Adulsa or Malabar nut. It is a perennial, evergreen and highly branched shrub having antimicrobial activity with an unpleasant smell and bitter taste [4]. *Tecoma stans* L. (Family Bignoniaceae) is a yellow trumpet bush native to tropical and subtropical regions of Central and South America widely used as an ornamental plants in Indian gardens and are also extensively employed in the Mexican traditional medicine [5].

MATERIAL AND METHODS

Virus Sources and Extraction of Total DNA

The infected and healthy 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 trees using the Cetyl Trimethyl Ammonium Bromide (CTAB) method [6].

Identification of begomovirus components by PCR

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). The primers are validated and have been used previously in screening of variety of begomovirus. Nearly 40 begomovirus were screened earlier such as *Croton yellow vein mosaic virus* (HQ631429), *Croton yellow vein mosaic Hisar virus* (JN000701), *Datura leaf curl virus* (JN000702), *Vinca yellow vein virus* (JQ693139), *Cotton leaf curl virus* (JQ693143) etc [7].

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 by 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 gel and visualized by staining with ethidium bromide and UV illumination [8].

For the detection of any DNA-B component in diseased trees, primer pair PCRc1 and PBLlv2040 were used [9, 10] having the same PCR condition and reaction as used in the case of begomovirus DNA-A. To test whether a satellite molecule was associated with the isolated begomoviruses, a universal primer pair specific for alphasatellite and betasatellite was also used to amplify the putative DNA [11, 12]. The PCR reaction for alphasatellite and betasatellite

were the same as for DNA A, mentioned above in the manuscript. Whereas the PCR thermal profile was pre-PCR by denaturation at 94 °C for 120 s followed by 35 cycles of denaturing at 94 °C for 60 s, annealing at 68 °C for 60 s and extension at 72 °C for 60 s, and a final extension at 72 °C for 5 min.

Cloning and Sequencing

The amplified PCR product was purified and cloned into Promega pGEMT vector system as per the manufacturer's instruction. The clones were sequenced and the details were submitted to NCBI. BLAST analysis was performed to reveal their closeness to other begomovirus sequence in the database.

Southern Hybridization

The presence of a begomovirus was further confirmed by Southern blot hybridization using *Cotton leaf curl virus* as a general probe for begomoviruses [13].

RESULTS

Surveys of different gardens and fields were made during 2010 - 2011. Leaf curl disease of *Justicia adhatoda* (Fig. 1a) was observed on several plants growing near the vicinity of cultivable fields of Sikar (Rajasthan) and similarly *Tecoma stans* (Fig. 1b) were found with leaf curling disease which symbolizes symptoms typical of begomovirus was observed in the gardens of Lakshmangarh (Rajasthan).

An amplification product of the expected size (approx. 600 bp) was produced from all symptomatic samples of both *Justicia adhatoda* and *Tecoma stans* (Fig. 2) but not from non-symptomatic samples. PCR products were suitably cloned into pGEM-T vector and sequenced, having Accession number KC206081 (*Justicia leaf curl virus*) and KC206083 (*Tecoma stans leaf curl virus*) respectively. In nucleotide alignments (BLAST) [14], the begomovirus infecting *Justicia adhatoda* revealed 79 % nucleotide sequence identity with *Vinca yellow vein virus* (JQ693139) whereas the begomovirus component isolated from *Tecoma stans* revealed highest nucleotide sequence identity of 100 % with *Lantana yellow vein virus* (JQ693142).

The positive PCR reaction showed the presence of begomovirus, which was further confirmed by Southern blot hybridization using *Cotton leaf curl virus* as a general probe for begomoviruses. All the samples from symptomatic plants hybridized with the probe, whereas samples extracted from non - symptomatic plants did not show positive results. Moreover we haven't found any DNA – B component and satellites molecules in *Justicia adhatoda* and *Tecoma stans*.

DISCUSSION

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 which will be focused on the amplification of the complete genome of the virus and their recombination analysis that put a light on its origin and to identify possible further begomoviruses infection in the country in order 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.

CONCLUSION

The possible association of a begomovirus with *Tecoma stans* and *Justicia adhatoda* had not been investigated previously, therefore to the best of our knowledge, this is the To the best of our knowledge this is the first report of molecular characterization of begomovirus infecting an ornamental plant *Tecoma stans* and a medicinal plant *Justicia adhatoda*.

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(a)

(b)

Fig. 1: Symptoms such as leaf curl disease exhibited by (a) *Justicia adhatoda* and (b) *Tecoma stans* from which the begomovirus were isolated.

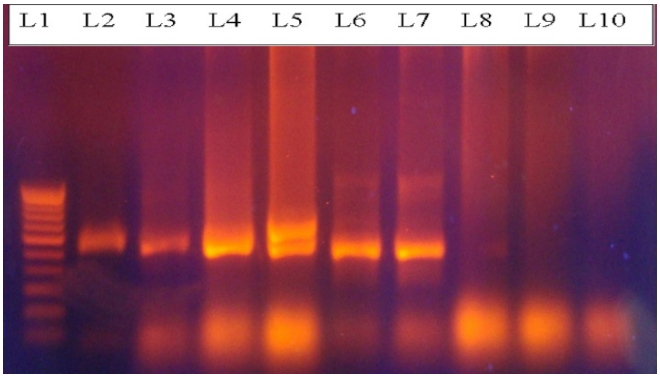

Fig. 2: Gel Electrophoresis showing positive results of begomovirus infecting *Justicia adhatoda* (Lane 4 and 5) and *Tecoma stans* (Lane 6 and 7). Control begomovirus (Lane 2 and 3). Healthy plants (Lane 8, 9 and 10). Lane 1: 1Kb Marker.

REFERENCES

1. Brown JK, Czosnek H. Whitefly transmission of plant viruses. *Advances in Botanical Research* **36**: 65-100, 2002.
2. Marwal A, Sahu A, Prajapat R, Gaur RK. First report of association of begomovirus with the leaf curls disease of a common weed, *Datura inoxia*. *Indian J Virol* **23**: 3–84, 2012.
3. Boulton MI. Geminiviruses: Major threats to world agriculture. *Ann. Applied Biol* **142**:143-143, 2003.
4. Rashmi P, Linu M. Antimicrobial activity of leaf extracts of *Justicia adhatoda* L. in comparison with vasicine. *Asian Pacific Journal of Tropical Biomedicine* S1556-S1560, 2012.
5. Torres AP, Lopez RG. Photosynthetic Daily Light Integral during Propagation of Tecoma stans Influences Seedling Rooting and Growth. *Hortscience* **46**: 282–286, 2011.
6. Manen JF, Sinitsyna, O, Aeschbach L, Markov AV, Sinitsyn A. A fully automatable enzymatic method for DNA extraction from plant tissues. *BMC Plant Biol* **5**: 3–23, 2005.
7. Marwal A, Sahu A, Prajapat R, Gaur RK. First report of Begomovirus infecting two ornamental plants: *Jasminum sambac* and *Millingtonia hortensis. Indian Phytopathology* **66**:115 -116, 2013.
8. Marwal A, Sahu A, Sharma P, Gaur RK. Molecular Characterizations of Two begomoviruses Infecting *Vinca rosea* and *Raphanus sativus* in India. *Virologica sinica* **28**: 053 – 056, 2013.
9. Rojas MR, Gilbertson RL, Russell DR, Maxwell DP. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Disease* **77**: 340-347, 1993.
10. Bela-ong DB, Bajet NB. Molecular Detection of Whitefly-Transmissible Geminiviruses (Family *Geminiviridae*, Genus *begomovirus*) in the Philippines. *Philippine Journal of Science* **136**: 87-101, 2007.
11. Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG. Universal primers for the PCR-mediated amplification of DNA α; a molecule associated with some monopartite begomoviruses. *Mol Biotech* **20**: 315–318, 2002.
12. Bull SE, Briddon RW, Markham PG. 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, 2003.
13. Kon T, Dolores LM, Bajet NB, Hase S, Takahashi H, Ikegami M. Molecular characterization of a strain of *Squash leaf curl China virus* from the Philippines. *J Phytopathol* **151**: 535-539, 2003.
14. Zhang Z, Scott S, Lukas W, Webb M. A greedy algorithm for aligning DNA sequences. *J Comput Biol* **7**:203-14, 2000.