Molecular and Recombinational Characterization of *Begomovirus* Infecting an Ornamental Plant *Alternanthera sessilis*: A New Host of Tomato Leaf Curl Kerala Virus Reported in India

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**ABSTRACT**

Begomovirus associated symptoms were observed in *Alternanthera sessilis* ornamentals growing in the gardens of Lakshmangarh, Rajasthan (India). Amplification of a PCR product was found up to the expected size (∼ 550 bp). The PCR product was cloned and sequenced and it was utilized for molecular characterization. Recombination plays a key role in the evolution of begomovirus and may be contributing to the emergence of new species. With the development of computational recombination detection tools and an increasing number of available genome sequences, many studies have reported evidence of recombination. Various recombination events suggested that interspecific recombination has resulted in significant diversity among begomovirus. Sequence analysis showed closed similarity (98%) with Tomato leaf curl Kerala virus, substantiating its new host as *Alternanthera sessilis*.

**Key words:** Begomovirus, *Alternanthera sessilis*, ornamental, recombination, tomato leaf curl Kerala virus, new host


**INTRODUCTION**

*Alternanthera sessilis* is an ornamental plant, also known as dwarf copperleaf. It belongs to the family *Amaranthaceae*. It occurs in both wetlands and uplands and can grow on a variety of soil types. For the propagation of the plant, it is generally spread by rooting at stem nodes and by seeds, which are wind and water-dispersed. The young leaves are very rich in iron, vitamin A and dietary fiber especially the tip portion are eaten as vegetable in the Southeast Asian countries, thus the plant is an immense remedy for the anaemic patients in rural areas. Moreover the carotenoid rich content of the *A. sessilis* plant helps in curing night blindness. The plant enhances the secretion of milk in new mothers and it is used as a remedy against intestinal cramps, diarrhoea and dysentery (intestinal disorder) and externally as a cooling agent to treat fever. Naples also reported that *A. sessilis* is used internally against intestinal inflammation, externally to treat wounds, to treat hepatitis, tight chest, bronchitis, asthma, lung troubles, to stop bleeding and as a hair tonic. *Geminiviruses* are characterized by circular single stranded DNA (ssDNA) genome encapsidated in twinned quasi-isometric particles of about 18×30 nm. The *Geminiviridae* family has been divided into four genera based on genome organization and host range: *Mastrevirus*, *Curtovirus*, *Topocurivirus* and *Begomovirus*. *Begomovirus* is the only genus of the *Geminiviridae* have bipartite genome with virus genes resident on two different circular ssDNA molecules (DNA A, DNA B) each of about 2.6–2.8 kb, or monopartite with all genes resident on one (DNA A-like) ssDNA of about 2.8 kb. *Begomovirus* is one of the largest genus of the family *Geminiviridae* and the vector white fly (*Bemisia tabaci*) is prevalent in the tropical and subtropical regions of the world. Some monopartite *begomoviruses* are associated with betasatellites (DNAβ), which require *begomoviruses* for replication, encapsidation, insect transmission and movement in plants. Increasing knowledge about its epidemiology, sequence diversity and biodiversity is highly important in order to implement preventative strategies.

Recombination has played and continues to play, a pivotal role in geminiviral evolution and may be contributing to the emergence of new forms of *Geminiviruses* because the high frequency of mixed infections of *Begomoviruses* provides an opportunity for the emergence of new viruses arising from recombination among strains and/or species. In some cases, the recombinants exhibited a new pathogenic phenotype which is often more virulent than the parents. Hence the object of this study is molecular, phylogenetic and *in silico* recombinational analysis of *Begomovirus* infecting an ornamental plant *A. sessilis*. 

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MATERIAL AND METHODS

Samples collection and DNA extraction: Survey for the epidemiology of Begomovirus was carried out in 2010-2011. Begomovirus associated symptoms were observed in A. sessilis plants growing in garden fields of Lakshmanagar, Rajasthan (India). To investigate the possibility, Total DNA was extracted from leaves of plants with and without symptoms using CTAB (Cetyl Trimethyl Ammonium Bromide) method1.

PCR amplification: PCR was performed using a pair of primers designed to the coat protein region of begomovirus12. Forward primer sequence was GGRRTTGARCGATGHTATCTG (AC 1048) and reverse primer sequence was GCCYATRTAYAGRAAGCMA (AV 494). A typical PCR reaction contained about 100 ng DNA template, Taq 10x buffers (10 mM Tris-HCl, pH 8.8, 50 mM KCl and 1% [v/v]) 25 mM MgCl2, 200 μM of each dNTPs, 2 units of Taq DNA Polymerase, Nuclease free water and 10 pM of each primer. The PCR thermal profile were pre-PCR denaturation at 94°C for 120 sec followed by 35 cycles of denaturing at 94°C for 45 sec, annealing at 55°C for 60 sec and extension at 72°C for 60 sec and a final extension at 72°C for 5 min9.

Cloning, sequencing and Phylogenetic tree construction: PCR product of ~550 bp from infected A. sessilis samples was cloned and partially sequenced and has been deposited in NCBI GenBank having Accession No: JQ693140. Homology sequence search was carried out through BLASTn using which Phylogenetic and molecular evolution analyses were conducted using MEGA version 4.014.

RDP: To detect the possibility of recombination in Geminivirus isolates by using their sequence information Recombination Detection Program (RDP) was used, which is based on a pair wise scanning approach. It usually runs under Windows 95/98/NT/XP/VISTA/7 and couples a high degree of analysis automation with an interactive and detailed graphical user interface15. Using various recombination detection method the conclusion of recombination studies are evaluated16,17. The recombination breakpoint could be identified by using Recombination detection program [RDP], GENECONV, Maximum-Chi. BOOTSCAN, CHIMAERA and 3SEQ methods. All these methods were implemented in RDP v.3.4418.

RESULTS AND DISCUSSION

Begomovirus associated symptoms such as Leaf curl disease of A. sessilis (Fig. 1) was observed on several plants growing in the gardens of Lakshmanagar (Rajasthan) in Dec 2011. The coordinates where infection found in A. sessilis and from where the plant sample was collected were Latitude: 27N 80° 16.52” and Longitude: 75E 03° 47.76”. Through PCR amplification product of the expected size (approx. 550 bp) was produced from all symptomatic samples of A. sessilis but not from non-symptomatic samples. BLAST analysis was conducted with Geminivirus sequences available in the GenBank database. The alignment process of begomoviral sequence reveals 98% identity with Tomato leaf curl Kerala virus isolate ToLCV (EU910141) and 97% identity with Tomato leaf curl Kerala virus isolate ToLCV-K5 (EU910140). Phylogenetic analysis of coat protein gene isolated from A. sessilis (Fig. 2) was done by using MEGA 4.0 showing the relationship with other closely related viruses.

Recombination is a major mechanism in virus evolution, allowing viruses to evolve more quickly by providing immediate direct access to many more areas of a sequence space than are accessible by mutation alone. The maximum χ2 method is modification of the program MaxChi for identifying recombination breakpoints. Given an alignment MaxChi examines sequence pairs and seeks to identify recombination breakpoints by looking for significant differences in the proportions of variable and non-variable polymorphic alignment positions in adjacent regions of sequence. Although the maximum χ2 method performs best when only two parental sequences and a recombinant sequence are compared, it is possible to use the method to examine alignments of more than 3 sequences. The approximate p-values of two peaks in MaxChi plot were 6.596 × 10^-4 and 5.844 × 10^-4 (Fig. 3).

Beginning breakpoint position was 140 in alignment and ending breakpoint position was 578 in alignment. According to MaxChi the major parent was DQ343284 (Tomato leaf curl virus from soybean coat protein gene, complete cds) and minor parent was DQ376039 (Papaya leaf curl virus isolate Kavittha coat protein gene, complete cds). MaxChi provides information on the

positions of potential breakpoints but does not give information on the extent of recombinant regions. The “scan triplets” setting used RDP3 making attempt to match potential breakpoints and that sequences between match breakpoints within a single recombinant region. Along with CHIMAERA, MaxChi also one of the most accurate breakpoint detection methods implemented in RDP3.
Fig. 3: An example of MaxChi plot for recombinant *Altanthera leaf curl virus* coat protein-like gene, partial sequence (JQ693140). In this case the left and right bounds of the pink region indicate breakpoint positions.

Fig. 4: An example of CHIMAERA plot. The left and right bounds of the pink region indicate breakpoint positions.

CHIMAERA is David Posada’s modification of Maynard Smith’s maximum $\chi^2$ method. The differences between CHIMAERA and MaxChi are (1) the way in which polymorphic sites are chosen and (2) CHIMAERA can only be used to screen triplets. As with MaxChi, CHIMAERA provides information on the positions of potential breakpoints but does not give information on the extent of recombinant regions. RDP3 determines recombinant regions from $\chi^2$ peaks in exactly the same way as it does for MaxChi. Along with MaxChi, CHIMAERA is one of the most accurate breakpoint detection methods implemented in RDP3.

CHIMAERA Plot indicate as major parent as recombinant (DQ343284, green), minor parent as recombinant (DQ376039, blue) and recombinant as recombinant (purple). The approximate p-values of two peaks were $7.389 \times 10^{-08}$ and $7.013 \times 10^{-09}$. Uppermost red bars indicating positions of informative sites and region bounded by estimated breakpoint positions represented by dotted line (Fig. 4). The Chimaera beginning breakpoint position was 156 in alignment and ending breakpoint position was 577 in alignment. Beginning breakpoint probability was 2.594 E-03 and ending breakpoint probability was 2.364 E-04. The region probability (MC Uncorrected) was 5.273 E-09 and region probability (MC corrected) was 7.013 E-06.

Recombination positions in virus *Altanthera leaf curl virus* (JQ693140) infecting *A. sessilis* were identified (Fig. 5). The schematic sequence display is where the results of automated recombination scans are presented and it is the part of the program that is used to drive the manual checking of automated analysis results. The coloured rectangles represent sequence fragments representing the recombinant (JQ693140), major parent (DQ343284) in brown colour and minor parent (DQ376039) in dark green colour.

The construction of Recombinant Region Count Matrix$^{20,31}$ is an overview of the unique events detected in an automated recombination analysis and indicates how often different parts of the analysed sequences are
CONCLUSION

A. sessilis an ornamental plant is found to be infected with begomovirus, which was identified through PCR and BLAST analysis. Phylogenetic analysis of coat protein gene isolated from A. sessilis was done by using MEGA 4.0 showing the relationship with other closely related viruses. Based on the symptoms and sequence information, the Alternanthera leaf curl disease in India is associated with Tomato leaf curl Kerala virus. This result suggested that Alternanthera sessilis is a new host of Tomato leaf curl Kerala virus and is a serious threat to other economically important crops and may contribute to the epidemiology of Tomato leaf curl Kerala virus diseases in India. Uses of computational recombination detection tools such as MaxChi and CHIMAERA implemented in RDP3 have demonstrated the evidence of recombination in a wide range of the available genome sequences of Begomovirus. Geminiviruses cause a variety of symptoms in host plant species and are spreading easily due to a high rate of recombination and pseudorecombination events that contribute in the evolution of new viral species. This study could be used to understand the role of recombination and pseudorecombination in evolution of new begomovirus species and genetic diversity.

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