Virus Genes (2024) 60:568–571
https://doi.org/10.1007/s11262-024-02087-2

BRIEF REPORT

Identification of a novel monopartite begomovirus associated with leaf curl disease of *Citharexylum spinosum* in India

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Received: 14 January 2024 / Accepted: 17 June 2024 / Published online: 27 June 2024

Abstract

The present study reports the complete genome of a novel monopartite begomovirus, named tentatively as "Citharexylum leaf curl virus" (CitLCuV), associated with leaf curl disease of *Citharexylum spinosum* in India. CitLCuV genome (2767 nucleotide) contained the typical genome organization of Old World begomoviruses and shared the maximum nucleotide sequence identity of 89.7% with a papaya leaf crumple virus (PaLCrV) isolate. In addition, two small non-canonical open reading frames (C5 and C6) were determined in the complementary strand of CitLCuV genome. Phylogenetic analysis revealed the relatedness of CitLCuV to PaLCrV and rose leaf curl virus. Recombination analysis detected a possible recombination event in CitLCuV genome. Based on begomovirus species demarcation criteria, CitLCuV can be regarded as a novel begomoviral species.

Keywords *Citharexylum spinosum* · Begomovirus · Novel · Leaf curl · India

Citharexylum spinosum L. (family: Verbenaceae), commonly known as fiddlewood, is a large shrub or small tree with fragrant flowers native to the Caribbean region [1]. The species was introduced to and got naturalized in India where it is generally grown in gardens and occasionally found in wild [1]. The tree is useful in treating various ailments due to its medicinal properties and its wood is used for making stringed musical instruments [2].

Begomoviruses (family: *Geminiviridae*) are single-stranded DNA genome-containing viruses that cause economically important diseases in a wide range of crops worldwide. Broadly, they are categorized as Old World and New World begomoviruses based on their genome organization and geographic distribution. Predominantly, the Old World begomoviruses contain monopartite genome and the New World begomoviruses contain bipartite genome with DNA-A and DNA-B components. Whiteflies efficiently transmit begomoviruses and infected plants typically depict symptoms, like leaf yellowing, leaf curling, and stunted growth [3].

During March 2023, leaf curl disease was observed in *C. spinosum* plants growing in the Telangana State Forest Academy campus, Hyderabad, Telangana, India. The diseased plants exhibited severe leaf curling and leaf mottling symptoms (Fig. 1) and the disease incidence was 91.8% (45 diseased of 49 plants observed). Symptomatic leaf samples were collected from three individuals and total DNA was isolated from 100 mg of collected samples using cetyl trimethylammonium bromide (CTAB) protocol [4]. PCR amplification of isolated DNA using begomovirus coat protein (CP) gene-specific degenerate primers [5] yielded positive bands. However, positive amplification could not be obtained for alpha- [6] and betasatellite-specific universal primers [7]. Further, isolated DNA from one of the samples was subjected to rolling circle amplification (RCA) using phi29 DNA polymerase (Thermo Fisher Scientific). Amplicons obtained after RCA were digested using the restriction endonuclease *Bam*HI to obtain the putative monomers of begomovirus genome (ca. 2.8 kb), which were ligated to

Edited by Seung-Kook Choi.

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Fig. 1 Symptoms of leaf curl disease in *Citharexylum spinosum* growing in Telangana, India. **A** A diseased tree showing severe leaf curl symptom. Close-up view of twigs showing **B** leaf curling and **C.** mottling symptoms

*Bam*HI-digested pUC18 vector and transformed into competent cells of *Escherichia coli* strain DH5α. Plasmids were isolated from positive colonies and the presence of desired insert was confirmed after restriction digestion with *Bam*HI and *Bgl*I. Isolated plasmids from one of the positive colonies with the desired insert was outsourced for sequencing of insert through primer walking strategy (University of Delhi South Campus, New Delhi). The obtained genome sequence along with a few selected begomoviral sequences, including those of closely related viruses identified in BLASTn search, retrieved from National Center for Biotechnology Information (NCBI) were aligned using CLUSTALW tool and subjected to maximum-likelihood (ML) phylogenetic tree construction using the best-fit TN93 + G model with 500 bootstrap replicates in MEGA 7 (v 7.0.26) [8]. Sequence identity matrix was obtained for the MUSCLE aligned sequences using Species Demarcation Tool (v 1.2) [9]. For detection of possible recombination events, the obtained genome sequence along with the top 100 hits resulting from BLASTn analysis of obtained genome were aligned and imported into Recombination Detection Program (RDP) (v 4.101) [10]. Only recombination events detected by more than five methods were considered reliable.

The obtained genome sequence was 2767 nucleotide (nt) long (GenBank accession number: OR437368) and contained two open reading frames (ORFs) (V1 and V2) in the viral strand and four ORFs in the complementary strand (C1–C4), resembling the typical genome organization of Old World begomoviruses [3]. Besides, two small non-canonical ORFs (C5 and C6), similar to the ones detected in a few begomoviruses [11–13], were determined in the viral complementary strand. The 302 nt-long intergenic region (IR) contained two copies of the replication-associated protein (Rep)-binding iteron sequence "GGW CYC" (the corresponding iteron-associated domain in Rep is MPPKRFLIN), identical to tomato leaf curl Kerala virus (ToLCKeV) [14]. Following the iteron are the 'TATA' box and an inverted repeat sequence capable of forming the stem loop structure separated by the conserved nonanucleotide sequence (TAATATTAC). The length of each ORF and predicted motifs in and molecular weight of encoded proteins are provided in Supplementary Table 1. Coat protein sequence-based identity matrix revealed that the coat protein encoded by the obtained genome sequence shared a maximum of 97.7% identity with the corresponding sequence of Duranta leaf curl virus (QJX74439.1) (Supplementary Fig. S1). Genome sequence-based identity matrix revealed the maximum sequence identity (89.7%) of obtained genome sequence with that of a papaya leaf crumple virus (PaLCrV) isolate (KR071789.1) (Fig. 2a; Supplementary Table 2). Based on the < 91% nt sequence identity criterion (of the complete genome sequence in case of monopartite begomoviruses) [15], the identified virus from *C. spinosum* can be regarded as a novel

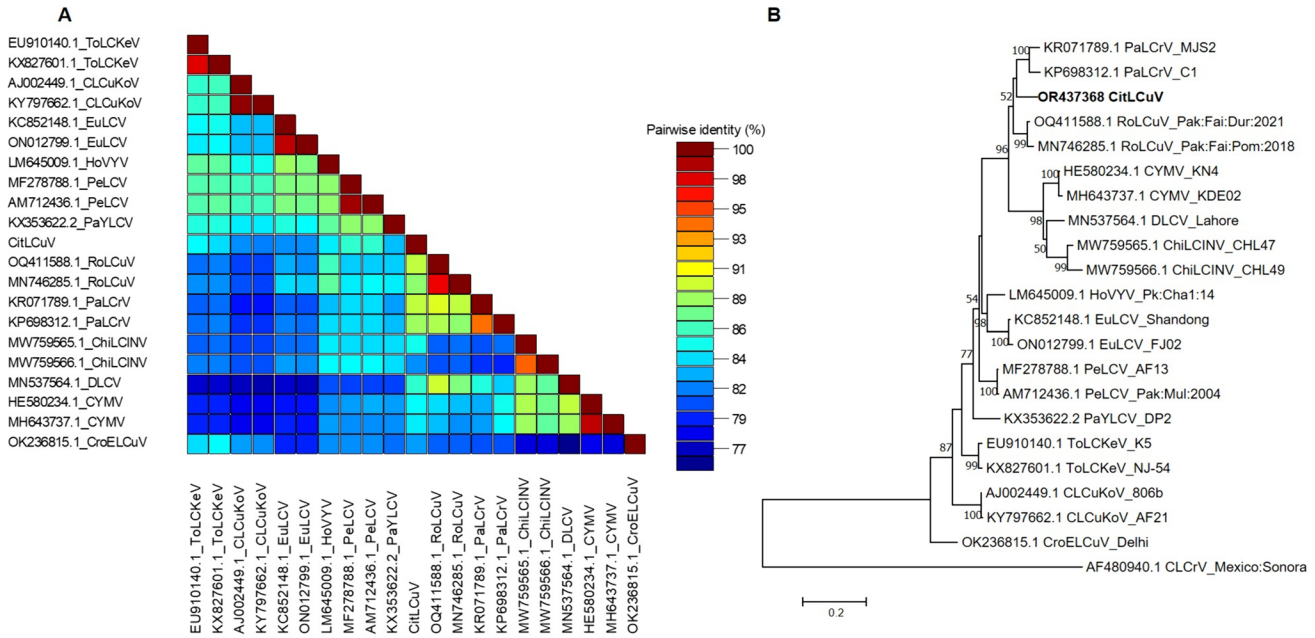

Fig. 2 **A** Identity matrix showing the sequence similarities of Citharexylum leaf curl virus (CitLCuV) with its closely related begomoviruses. **B** Maximum-likelihood tree showing the phylogenetic relationship of CitLCuV with its closely related begomoviruses. The tree was constructed using the best-fit TN93 + G model with 500 bootstrap replicates. Only bootstrap values more than 50% are indicated. DNA-A sequence of cotton leaf crumple virus (CLCrV), a bipartite begomovirus, was used as outgroup during tree construction. Expansions of virus names are provided in Supplementary Table 4. CitLCuV identified in this study is shown in bold

begomoviral species for which the name "Citharexylum leaf curl virus" (CitLCuV) is proposed. Phylogenetic tree placed CitLCuV in a sister clade to PaLCrV isolates and these together with rose leaf curl virus isolates formed a distinct subclade (Fig. 2b). Recombination analysis identified a putative recombination event in CitLCuV genome at nt position 70–2106, with tomato leaf curl Kerala virus (KF551575.1) as major parent and Chenopodium leaf distortion virus (MN423112.1) as minor parent (Supplementary Fig. S2; Supplementary Table 3).

In conclusion, CitLCuV is a novel monopartite Old World begomovirus associated with leaf curl disease of *C. spinosum*, an exotic species to India. Though satellites could not be detected in symptomatic samples through PCR assays, further validation is required to ascertain the same through Next-Generation Sequencing. Further studies are needed to understand the biological properties and geographical distribution of CitLCuV.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11262-024-02087-2.

Acknowledgements The authors are thankful to the Group Coordinator Research and the Director, ICFRE-IFB, Hyderabad and the Director, ICAR-IARI, New Delhi for their support. The authors also thank the Director, TSFA, Hyderabad for granting permission for sample collection and disease estimation. We also thank G. Kiran and Vijay Prakash Reddy, ICFRE-IFB, Hyderabad for their help.

Author contributions Conceptualization: V. Kavi Sidharthan and V.K. Baranwal; Methodology: Damini Diksha and V. Kavi Sidharthan; Formal analysis and investigation: Damini Diksha and V. Kavi Sidharthan; Writing—original draft preparation: V. Kavi Sidharthan; Writing—review and editing: V.K. Baranwal; Resources: V.K. Baranwal; Supervision: V. Kavi Sidharthan and V.K. Baranwal. All authors reviewed and approved the final manuscript.

Funding There is no funding for this study.

Data availability The genome sequence of this study is available in NCBI GenBank with the accession number OR437368.

Declarations

Conflict of interest There is no conflict of interest.

Ethical approval The study does not involve human participants or animals.

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