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Research paper

Isolation and sequence characterization of DNA-A genome of a new begomovirus strain associated with severe leaf curling symptoms of *Jatropha curcas* L.

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ABSTRACT

Begomoviruses belong to the family Geminiviridae are associated with several disease symptoms, such as mosaic and leaf curling in *Jatropha curcas*. The molecular characterization of these viral strains will help in developing management strategies to control the disease. In this study, *J. curcas* that was infected with begomovirus and showed acute leaf curling symptoms were identified. DNA-A segment from pathogenic viral strain was isolated and sequenced. The sequenced genome was assembled and characterized in detail. The full-length DNA-A sequence was covered by primer walking. The genome sequence showed the general organization of DNA-A from begomovirus by the distribution of ORFs in both viral and anti-viral strands. The genome size ranged from 2844 bp–2852 bp. Three strains with minor nucleotide variations were identified, and a phylogenetic analysis was performed by comparing the DNA-A segments from other reported begomovirus isolates. The maximum sequence similarity was observed with Euphorbia yellow mosaic virus (FN435995). In the phylogenetic tree, no clustering was observed with previously reported begomovirus strains isolated from *J. curcas* host. The strains isolated in this study belong to new begomoviral strain that elicits symptoms of leaf curling in *J. curcas*. The results indicate that the probable origin of the strains is from Jatropha mosaic virus infecting *J. gassypifolia*. The strains isolated in this study are referred as *Jatropha curcas* leaf curl India virus (JCLCIV) based on the major symptoms exhibited by host *J. curcas*.

1. Introduction

Jatropha curcas L., a member of Euphorbiaceae family, has gained significance as a potential biodiesel crop. Biodiesel obtained from *J. curcas* seed oil showed better performance compared to petrodiesel, making it a prime candidate among other commercially available biofuel sources (Sudheer Pamidiamarri et al., 2009; Pamidimarri et al., 2010). *J. curcas* is promoted as an alternative non-conventional energy source because of the present energy crisis and current environmental issues. *J. curcas* is a promising crop that can grow in marginal areas, including drought-affected lands and semi-arid tropics. Several studies have been conducted on the short-term and long-term performance of *J. curcas* grown in marginal lands (Bressan et al., 2011). As a member of the Euphorbiaceae family, *J. curcas* contains latex and a significant amount of phorbol esters, which makes the plant naturally resistant to the majority of disease pests. However, long-term field studies reported that *J. curcas* is susceptible to viral infections. Most *J. curcas* germplasms showed similar symptoms such as leaf curl and mosaic disease, which are caused by begomoviruses. The manifestation of leaf curl showed the upward curling of leaf, leaf vein clearing, reduced leaf size and necrosis in old leaves. Under severe infected conditions, plants showed stunted growth and resulted in no fruit formation or seed

Abbreviations: JCLCV, *Jatropha curcas* leaf curl India virus; CR, Common region; Rep, replication initiation protein; TrAP, transcriptionactivator protein; CTAB, Cetyl trimethylammonium bromide; EDTA, Ethylenediaminetetraacetic acid; RDP, recombination detection program; ORF, open reading frame

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setting (Nigam et al., 2014).

Begomoviruses (Family: Geminiviridae, genus: Begomovirus) is one among highly pathogenic virus group predominantly distributed in tropical and subtropical regions. The viruses in this family possess either monopartite or bipartite genome (Gutierrez, 1999). Bipartite genome containing viruses contains two circular DNA components, namely DNA-A and DNA-B, which share a common region (CR) of ~200 nucleotides. In begomoviral genome, the genes/ORFs are distributed on both genomes (in the case of bipartite genomes). The genes are aligned in both strands overlapping to one another. DNA-A harbours gene AV1 (i.e. coat protein) on the viral strand; three genes AC1 (replication initiation protein [Rep]), AC2 (transcription activator protein [TrAP]), and AC3 (replication enhancer protein) on complementary strand (Fauquet et al., 2008). DNA-B contains the gene BV1 (nuclear shuttle protein) in the viral strand and the gene BC1 (movement protein) in the complementary strand. Recent studies revealed that begomoviruses tend to co-evolve with host plants, resulting in genetic diversity via the recombination and exchange of genomes among themselves. This phenomenon has led to the emergence of potent pathogenic viral strains with increased virulence and extended host range (Wang et al., 2014; Ramkat et al., 2011a; De Bruyn et al., 2012). Hence, a comprehensive study on the characterization of each viral strain in different crop plants is needed for the protection and improvement of crops. If neglected, begomoviruses could have a huge negative impact on field plantation and crop productivity (Ramkat et al., 2011b).

Since *J. curcas* being a potential biodiesel source, precast measures need to be developed for healthy agricultural practice. Begomovirus infections of *J. curcas* has been reported by several experimental field stations (Raj et al., 2008; Srivastava et al., 2015b; SK et al., 2016) and they were found to be a potential threat. Hence, the detailed characterization of the begomovirus genome is required to prevent crop damage. Srivastava et al. recently reported a begomovirus strain that showed leaf yellow mosaic disease in *J. curcas* (Srivastava et al., 2015b). However, in our field studies (CSIR-CSMCRI, Bhavnagar, Gujarat, India), the primary symptom of leaf curling was observed to have characteristic features of begomoviral infection. Hence, the present study was aimed to isolate viral DNA from infected *J. curcas* and characterize it at the genomic level. Isolated sequences were used to study the phylogeny and evolution by comparing to sequence information available in the database. A comparative study was conducted on the full-length sequence of DNA-A and important structural genes (i.e. coatprotein [AC1] and replication associated proteins [AC2]). Comprehensive conclusions were derived from the results.

2. Materials and methods

2.1. Genomic DNA extraction

Genomic DNA was extracted using the CTAB protocol as described by Sudheer et al., (Sudheer Pamidimarri et al., 2009b) with the following minor modifications. The samples were collected from established *J. curcas* plants in the experimental field (21°75′N, 72°14′E) of Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India. Leaves with severe symptoms of leaf curling were carefully collected. About 0.1 g of leaf tissue was ground in liquid nitrogen and taken into 2 mL microcentrifuge tube. To the ground sample, 0.5 mL of extraction buffer (2% CTAB, 100 mM Tris–HCl, 3.5 M NaCl, 20 mM EDTA, 0.2 M β-mercaptoethanol, 2% PVP, pH 8.0.) was added and mixture was incubated at 65 °C for 90 min. The sample was then extracted with an equal volume of chloroform:isoamyl alcohol (24:1), and the supernatant was transferred to a new tube. The obtained supernatant was precipitated with 80% of ethanol for 10 min and centrifuged at 12,000 rpm for 20 min. The pellet was air dried and dissolved in 100 μL of ultrapure water.

2.2. Amplification of viral DNA-A segment

The DNA-A segment was amplified by primers designed in a conserved region (5′-GGGGCCCAGGATTGCAGAGGAAGATTGTCGGA ATG-3′) in the rep protein region (ACI). Among the seven samples collected, three samples showed the expected amplification size (~2.9 kb), which were purified using the QIAquick Gel Extraction Kit (Qiagen, Germany). The purified fragments were ligated into pTZ57R (Insta) T/A cloning vector (MBI Fermentas, USA) according to the manufacturer's protocol, and the recombinant plasmids were transformed into a competent *E. coli* (DH5α) by the heat shock method (Green and Sambrook, 2012). The cells were then spread on Luria-Bertani (LB) agar selection plates containing appropriate concentrations of ampicillin, IPTG, and X-gal and then incubated at 37 °C for 14 h. The putative clone selection was carried out based on blue/white selection and checked for insert by colony PCR using an M13 universal primer set. Recombinant plasmids were extracted using the GenElute Plasmid Miniprep Kit (Sigma, USA). The sequencing analysis was performed using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) using M13 universal primers (IDT, USA). The full-length sequence was obtained by primer walking via designing primers for every ~600 bp forward. All three sequences showed variations in nucleotide sequence, hence they were named as JCLCIV1, 2 and 3. The annotated sequences were submitted to the NCBI-GenBank database (NCBI accession numbers: JCLCIV1-GU451249, JCLCIV2-KU667096, and JCLCIV3-KU667097).

2.3. DNA-A sequence analysis and annotation

The sequences obtained by primer walking were assembled with the help of BioEdit DNA editing software (Version 7.2). The genome annotation was done by comparing with the begomoviruses reported in the NCBI database. Sequence mining was done from the database and sequences in FASTA format were downloaded for phylogenetic analysis. The sequences were aligned and edited with the help of BioEdit software. The genetic distances among the sequences were obtained with the help of MEGA6 software (Felsenstein, 1985), and the mean genetic distance was calculated by the sum of individual genetic distances divided by the number of samples. Similarities in the sequence of DNA and proteins were obtained by the MUSCLE analysis, which was available in the EMBL-EBI online service facility. Based on the maximum parsimony method including bootstrapping (1000 replicates), the phylogenetic tree was obtained with the help of MEGA6 analysis software (Tamura et al., 2007). The phylogram was rooted using sweet potato leaf curl virus (KJ476509).

2.4. Detection of recombination events

Putative recombinant events were identified using the recombination detection program (RDP4, version 4.0). The strains listed in Table 1 were included in the analysis and analysis was conducted by taking 1000 bootstrap replicates. The same analysis was conducted using the Croton yellow vein mosaic virus (EU727086), which was found to be strongly clustered in the phylogenetic analysis. For analysis, all other options were put at default settings specified in the software.

3. Results and discussion

J. curcas as a biodiesel source, has attracted the attention of several researchers. With the aim of establishing the species as a potential crop for oil production in semi-arid tropical land, CSMCRI India conducted major field studies in various conditions of dry and the semi-arid tropical land (Bressan et al., 2011). The primary symptom of severe leaf curling (Fig. 1a), which was spread quickly by insects, was observed in our field plantation. It was suspected to be caused by begomoviral infection based on the symptoms. Begomoviral infection in *J. curcas* was