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Characterization of a novel begomovirus associated with yellow mosaic disease of three ornamental species of *Jatropha* grown in India

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ABSTRACT

Severe yellow mosaic disease was observed in three ornamental species of *Jatropha*: *J. integerrima*, *J. podagrica* and *J. multifida* grown in gardens at Lucknow, India, during a survey in 2013. The causal pathogen was successfully transmitted from diseased to healthy plants of these species by whitefly (*Bemisia tabaci*). The infection of begomovirus was initially detected in naturally infected plant samples by PCR using begomovirus universal primers. The begomovirus was characterized having a monopartite genome based on sequence analyses of the cloned ~2.9 kb DNA-A genome amplified by rolling circle amplification using Phi-29 DNA polymerase. The genome contained 2844 nucleotides that could be translated into seven potential open reading frames. The nucleotide sequences of DNA-A genome of the begomovirus isolates: JI (KC513823), JP (KF652078) and JM (KF652077) shared 94–95% identities together and 93–95% identities with an uncharacterized begomovirus isolated from *J. curcas* (the only sequences available in GenBank database as GU451249 and EU798996 under the name jatropha leaf curl virus). These shared highest identity of 61% and highly distant phylogenetic relationships with other begomoviruses reported worldwide. Based on 61% sequence identities (much less than 89%, the species demarcation criteria for a new begomovirus) the isolates under study were identified as members of a new *Begomovirus* species for which the name was proposed as "Jatropha mosaic Lucknow virus (JMLV)". The recombination analysis also suggested that JMLV was not a recombinant species, hence considered as unidentified *Begomovirus* species. Koch's postulates were also established by agroinfiltration assay of agroinfectious clone of JMLV. Characterization of JMLV associated with yellow mosaic disease of *J. integerrima*, *J. podagrica* and *J. multifida* is being reported for the first time.

1. Introduction

Jatropha, a genus of perennial flowering plants of the family *Euphorbiaceae*, is native to Central America and distributed in Africa and Asia (Speight and Singh, 2014). The genus *Jatropha* includes various species such as *Jatropha curcas*, *J. gossypifolia*, *J. integerrima*, *J. podagrica* and *J. multifida*. Amongst them, *J. curcas* is widely cultivated as a major source of bio-fuel (Openshaw, 2000; Pramanik, 2003) while *J. gossypifolia* grows as a weed along road sides (Snehi et al., 2011a). The species *J. integerrima*, *J. podagrica* and *J. multifida* are grown as ornamental plants in Indian gardens. *J. integerrima* is an ornamental shrub that grows commonly in south parts of India (Sharma and Singh, 2013). *J. podagrica* is an ornamental plant is commonly grown in Australia, the Hawaiian Islands, Southern Africa, Mozambique, Zambia and warmer parts of Asia (Hooker et al., 1848; Ojewole and Odebiyi, 1980). *J. multifida* is a very attractive and widely cultivated species throughout the tropics, in north Australia and South east Africa (Dehgan, 1982; Nayak and Patel, 2009). Besides this, *J. integerrima* is traditionally used as purgative, styptic, emetic, in treatment of warts, tumors, rheumatism, herpes, pruritis, toothaches, scabies, eczema and ringworm (Kirtikar and Basu, 2002).

Begomoviruses of the family *Geminiviridae* are whitefly transmitted and cause diseases of important crops in the tropics and subtropics (Tiwari et al., 2013). Their genome consists of one or two circular single stranded DNA components, referred to as DNA-A and DNA-B, each approximately of 2.6–2.8 kb in size (King et al., 2011; Fauquet et al., 2008). DNA-A encodes replication-associated protein (AC1) essential for viral replication; replication enhancer protein (AC3); transcriptional activator protein (AC2) that transactivates expression of AV1 and BV1 ORFs; AC4 protein; coat protein (AV1) for encapsidation and insect transmission, and precoat protein (AV2) for virus accumulation and symptom development, while

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AV2 ORF is missing in New World begomoviruses. The functions of AC4 protein are not proven for all the begomoviruses. However, some functions of C4 protein were shown only in case of monopartite begomoviruses (Hanley-Bowdoin et al., 2013). The DNA-B encodes the nuclear shuttle protein (NSP; BV1) and movement protein (MP; BC1), both of which determine viral pathogenicity, but the coat protein in case of a defective NSP can rescue the function of NSP (Hanley-Bowdoin et al., 2013; Jeske, 2009).

A number of begomoviruses occurring in the Old World (Eastern Hemisphere, Europe, Africa, Asia) are monopartite and have only a single component equivalent to DNA-A. The cloned genomic component of some of these monopartite begomoviruses have been shown to produce typical symptoms, confirming that single genomic component was solely responsible for the disease (Zhang et al., 2010; Kon and Gilbertson, 2012). A satellite molecule (betasatellite) has also been shown to be associated with mono- and bi-partite begomoviruses, and may be required for systemic infection and symptom development (Saunders et al., 2000; Briddon et al., 2003; Srivastava et al., 2013, 2014a; Simmonds-Gordon et al., 2014).

The occurrence of begomoviruses has been noted on *J. curcas* and *J. gossypifolia* species worldwide (Aswatha-Narayana et al., 2006, 2007; Tewari et al., 2007; Raj et al., 2008; Gao et al., 2010; Ramkat et al., 2011a,b; Snehi et al., 2011a,b, 2012; Appiah et al., 2012; Kashina et al., 2013; Srivastava et al., 2014b). African cassava mosaic virus (syn. Cassava latent virus) from Kenya (Bock et al., 1981) and *Jatropha mosaic virus* from Florida (Polston et al., 2014) have also been reported on *J. multifida*. However, no virus on *J. integerrima* and *J. podagrica* has been reported hitherto. In this communication, the association of an unidentified begomovirus with yellow mosaic disease of *J. integerrima*, *J. podagrica* and *J. multifida* has been investigated for the first time based on the sequence analyses of complete DNA-A genome.

2. Materials and methods

2.1. Surveys and collection of plant material

The surveys were conducted in January and May of 2013 on *J. integerrima*, *J. podagrica* and *J. multifida* species growing in the gardens at Lucknow (Lat. 26° 55′ N Lon. 80° 59′ E), India. The leaf samples from severely diseased plants (three samples from each species) were collected for experiments.

2.2. Virus transmission through whitefly

Whitefly transmission tests were performed using the non-viruliferous *B. tabaci* maintained on healthy *Nicotiana tabaccum* cv. White Burley plants. The adult *B. tabaci* were collected, starved for 2 h and allowed to feed separately on naturally infected *J. integerrima*, *J. podagrica* and *J. multifida* plants showing yellow mosaic symptoms for an acquisition access period of 24 h. The viruliferous *B. tabaci* were transferred onto healthy seedlings (10–12 per plant) of *J. integerrima*, *J. podagrica*, *J. multifida*, *Solanum lycopersicum* and *N. glutinosa*, and were allowed an inoculation access period of 24 h. Then the whiteflies were killed by spraying with insecticide (Confidor, Bayer CropScience Ltd., Mumbai, India) and the inoculated plants were maintained in an insect proof glasshouse for two months to monitor for symptoms.

2.3. Molecular detection of begomovirus in Jatropha species

For initial detection of the begomovirus, the total DNAs were isolated from newly emerging leaves of infected plants (*J. integerrima*, *J. podagrica* and *J. multifida*) by the method of Dellaporta et al. (1983). PCRs were performed using a pair of begomovirus DNA-A specific universal primers: PALIv 722/PALIc 1960 (Reddy et al., 2005). PCR was set up in a 50 μl reaction mixture containing: template DNA (100 ng), dNTPs (200 μM each), primers (each 0.25 μM), $MgCl_2$ (1.5 mM), *Taq* DNA polymerase (1.0 U, Bangalore Genei Pvt. Ltd.), *Taq* buffer (1X, Genei Pvt. Ltd., Bangalore, India) in a Peltier thermal cycler PTC200 engine (MJ Research, Waltham, MA, USA). PCR conditions included initial denaturation at 94 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 1 min; annealing at 52 °C for 1 min and extension at 72 °C for 1 min 30 s and a final extension at 72 °C for 5 min.

The PCR was also performed using DNA-B specific primers (BC1F and BC1R; Padidam et al., 1995) to detect DNA-B genome. The PCR conditions were same as described for DNA-A amplification. For detection of betasatellite association with begomovirus, PCRs were carried out with betasatellite specific primers (β-01 and β-02; Briddon et al., 2003) using the total DNA from same samples. The PCR conditions were again same as described earlier. All PCR products were analyzed by electrophoresis on 1.0% agarose gel using a 1 kb DNA ladder (Thermo Fisher Scientific Inc., Pittsburgh, United States) as size markers.

2.4. Rolling circle amplification, cloning and sequencing of complete begomoviral genome

For molecular characterization of the detected begomovirus, the complete begomoviral genomes were amplified from the DNA isolated from infected *J. integerrima*, *J. podagrica* and *J. multifida* samples by rolling circle amplification (RCA) using Phi-29 DNA polymerase enzyme as per the manufacturer's instructions (TempliPhi™ DNA amplification kit, GE Healthcare, USA). The reaction was stopped by incubating the mixture at 65 °C for 10 min and the RCA products were monomerized by digestion with *Bam*HI restriction enzymes (New England BioLabs, USA) and the digested products were electrophoresed on 1% agarose gel. The expected amplicons obtained were purified using Wizard SV Gel & PCR Clean-Up System (Promega Corporation, Madison, USA) and ligated into pCAMBIA1300 vector at *Bam*HI site. Competent cells of DH5α strain of *Escherichia coli* were transformed using the ligated mixture by the heat shock method (Sambrook and Russel, 1989). Three positive clones of each *Jatropha* species were sequenced by primer walking (Genei Pvt. Ltd., Bangalore, India). Consensus sequence data were deposited in the GenBank database.

2.5. Analysis of sequence data of begomoviral genome

The sequence data from the begomovirus isolates were analyzed by BLASTN (http://www.ncbi.nlm.nih.gov/BLAST) and compared with existing sequences of begomovirus strains available in the GenBank database. The open reading frames (ORFs) were checked by ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and translated into amino acids using the *Expasy* tool (http://www.expasy.org/tools/dna.html). The matrix for pairwise alignment of selected begomovirus isolates was obtained using Clustal-W method of the *Genomatix DiAlign 2* program (Morgenstern et al., 1998). The 45 amino acids at the C-terminus of the coat protein of begomovirus were also aligned using *Genomatix DiAlign 2* program (Morgenstern et al., 1998).

Phylogenetic analyses were performed using the Molecular Evolutionary Genetics Analysis tool, MEGA v6.0 (Tamura et al., 2013) with 1000 replicates bootstrapping, and the dendrograms were generated with the Neighbour joining (NJ) method and viewed by the NJ plot program. The phylogenetic trees of coat proteins (CP) and replication associated proteins (Rep) of begomovirus and other genera of the family *Geminiviridae* obtained from BLASTP database