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Molecular Detection and Identification of *Begomovirus* Isolates Associated with Mosaic Disease of Ornamental Jatropha species from India

Sunil Kumar Snehi[1*], Shri Krishana Raj[2],Vivek Prasad[3] and Vinod Singh[1]

[1]Department of Microbiology, Barkatullah University, Bhopal-462026, MP, India
[2]Plant Molecular Virology Lab, CSIR-National Botanical Research Institute, Lucknow-226001, UP, India
[3]Molecular Plant Virology Lab, Department of Botany, University of Lucknow,Lucknow-226007, UP, India

***Corresponding Author:**Sunil Kumar Snehi, Department of Microbiology, Barkatullah University, Bhopal-462026, MP, India, E-mail: sunilsnehi@gmail.com

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Abstract

During surveys severe mosaic disease like symptoms were observed on ornamental species of Jatropha viz. *J. podagrica*, *J. multifida* and *J. integerrima* grown in CSIR-NBRI garden, Lucknow in the year of 2009-2011. For molecular detection of begomovirus associated with mosaic disease of *J. podagrica*, *J. integerrima and J. Multifida* were detected by polymerase chain reaction (PCR) using begomovirus specific primers. The resulting amplicons of ~1.2 kb of in all the ornamental species of Jatropha samples were sequenced and sequence data was analyzed and submitted in GenBank database. Based on highest sequence identities of partial DNA-A genome (~1.2 kb) and close phylogenetic relationships Jatropha mosaic India virus in *J. podagrica* (HQ848382)*;Tomato leaf curl Patna* in *J. multifida* (HQ848381) and *Papaya leaf curl virus* in *J. integerrima* (JQ043440) were identified. Present study we report here, molecular detection identification of the begomovirus isolates associated with mosaic disease of ornamental Jatropha species is a new report from India.

Keywords: Jatropha species; Mosaic disease; Begomovirus; PCR; Molecular identification; Sequence analysis.

Introduction

Begomoviruses of the family Geminiviridae are whitefly transmitted which cause diseases of important crops in the tropics and subtropics [1]. Their genome consists of one or two circular single-stranded DNA components, referred to as DNA-A and DNA-B, each about 2.6-2.8 kb in size [1, 2]. 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 has been shown to produce typical symptoms, confirming that single genomic component is solely responsible for the disease [1, 3]. A satellite molecule called DNA-β has also been found to be associated with mono-partite and bi-partite begomoviruses and is required for the systemic infection and symptom development [4-7].

The genus Jatropha (family *Euphorbiaceae*) and has 476 species distributed throughout the world, among them *Jatropha curcas*, *J. gossypifolia*, *J. integerrima*, *J. multifida*, *J.Podagrica* and *J. cuneata*are grown in India. Many excellent characteristics, including high yield, resistance to drought as well as content and good quality of the plant oil have generated the interest of many researchers to *J. curcas*, *w*hile other species are of ornamental value or traditionally used for their medicinal values. *J. podagrica*, *J. multifida* and *J. integerrima* are important for their ornamental values [8].

The natural infection of begomoviruses has been reported in *Jatropha* species across the world such as *Jatropha mosaic virus* on *J. gossypifolia*in Jamaica [9-12], *African cassava mosaic virus* on *J. multifida*in East and West Africa and India [13] and a begomovirus closely related to *Indian cassava mosaic virus* and *Sri Lankan cassava mosaic virus* on *J.curcas*in India [14-18] and *African cassava mosaic virus* on *J. curcas* in Kenya [19,20] and new begomovirus species associated with mosaic disease of *J. curcas* reported from India and Nigeria [21, 22]. The Complete nucleotide sequence of *Croton yellow vein mosaic* virus and DNA-β associated with yellow vein mosaic disease of *J. gossypifolia* in India [23] and a new begomovirus associated with yellow mosaic disease of *J. gossypifolia* reported in India [24]. Therefore, begomoviruses have been considered as a main threat to Jatropha cultivation.

During surveys in three subsequent years 2009 to 2011, severe mosaic disease like symptoms were observed on ornamental Jatropha species viz. *J. podagrica*, *J. multifida* and *J. integerrima* grown in CSIR-NBRI, garden Lucknow, India. The population of whiteflies (*Bemisia tabaci*) on these ornamental Jatropha species was also noticed; therefore, Begomovirus infection was suspected. In the present study we report here, molecular detection identification of the begomovirus isolates associated with mosaic disease of ornamental Jatropha species from India.

Materials and Methods

To find out how many begomoviruses/species are associated with the mosaic disease of these ornamental Jatropha species, the total DNA were isolated from 100 mg from newly emerging infected leaves of *J. podagrica*, *J. multifida* and *J. integerrima* by the Dellaporta,et al. [25] method and PCRs were performed using a pair of begomovirus specific primers PALIv 722/PALIc 1960 [26]. PCRs were set up in a 50µl reaction mixture containing: template DNA (100 ng), dNTPs (10mM each), primers (each 25 pM), $Mgcl_2$ (25 mM), *Taq*DNA polymerase (3.0 U, Bangalore GeneiPvt. Ltd), *Taq* buffer (1X, Bangalore GeneiPvt. Ltd) in a Peltier thermal cycler PTC200 engine (MJ Research, Waltham, MA, USA) with the PCR conditions: initial denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 1 min; specific annealing at 52°C temperatures for 1 min and extension at 72°C for 1.5 min. The final extension cycle was for 5 min at 72°C.The PCR products obtained were analysed by 1% agarose gel electrophoresis.

To detect DNA-B genome, the PCR was also performed using DNA-B specific primers [27] with the conditions: 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, extension at 72°C for 1.5 min and a final extension at 72°C for 5 min.

For the molecular identification of the begomovirus, ~1.2 kb amplicons obtained from the symptomatic samples were eluted using PCR Clean-up System (Promega, USA) and got sequenced in both orientations (Bangalore GeneiPvt. Ltd). The sequence data obtained through sequencing results was analysed for consensus data of two identical sequences and submitted in National Centre for Biotechnology Information GenBank database (NCBI, http://www.ncbi.nlm.nih.gov/Bankit). The open reading frames (ORFs) in sequenced data were predicted by bioinformatics tool: ORF finder (www.ncbi.nlm.nih.gov/projects/gorf/) to find out in frame AUG (ATG)-start and UAG (TAG)-termination codon. ORFs were translated in to amino acid using Expasy tool (www.expasy.org/tools/dna.html).

To observe the nucleotide identity within and with other reported strains of begomoviruses, Basic Local Alignment Search Tool (BLAST) searches were performed with all available databases using the NCBI-BLAST server (www.ncbi.nlm.nih.gov). The data were compared within and with other reported strains of begomovirus sequences obtained with the Entrez program using the BLAST (NCBI, Bethesda, USA, http://www.ncbi.nlm.nih.gov/blast). Multiple nucleotide and amino acid sequence alignments of selected strains reported from India and abroad were performed using *GenomatixDiAlign* program (www.genomatix.de/cgi-bin/dialign/dialign.pl).Phylogenetic analyses were perused using Clustal W version 1.8 and Molecular Evolutionary Genetics Analysis (MEGA version 4.0)[28] program with 100 replicates bootstrapping and phylogram were generated with Neighbour-joining method. Dendrograms were viewed by the NJ plot program.

Results and Discussions

The surveys were conducted in years 2009 to 2011 at CSIR-NBRI, garden Lucknow and observed the natural infection of virus disease in ornamental Jatropha species with the ~30 - 35% disease incidence. The naturally infected *J. podagrica* exhibited severe yellow mosaic and vein yellowing symptoms (Figure 1A), *J. multifida* showed yellow mosaic & mild leaf curl symptoms (Figure 1B) and *J. integerrima* showed severe mosaic and yellow mosaic symptoms (Figure 1C).

Fig. 1

Figure 1. Naturally infected *Jatropha podagrica* exhibited severe yellow mosaic and vein yellowing symptoms (Figure A), *J. multifida* showed yellow mosaic & mild leaf curl symptoms (Figure B) and *J. integerrima* showed mosaic and yellow mosaic symptoms (Figure C).

For molecular detection and identification of begomovirus associated with the mosaic disease of *J. podagrica*, *J. multifida* and *J. integerrima*, the ~1.2 kb amplicon samplified by PCR using begomovirus specific primers from 3/3 symptomatic samples but not from healthy one collected from the same location on 1% agarose gel electrophoresis (Figure 2). Three independent sequence of each amplicons (~1.2 kb) were sequenced in both orientations and the consensus sequence data were combined to partial DNA-A genome. The sequence resulted in the presence of 1177 bp (*J. podagrica*), 1288 bp (*J. multifida)* and 1201 bp (*J. integerrima)* DNA-A, which were deposited in the GenBank database under accession number (*J. podagrica:* HQ848382; *J. multifida*: HQ848381 and *J. integerrima*: JQ043440).

The analysis of partial DNA-A of *J.* podagrica (HQ848382), *J. multifida* (HQ848381) and *J. integerrima* (JQ043440) *virus* isolates contained four ORFs; in the virionsens and complementary sense are summarized in Table 1.However, our several attempts failed to amplify DNA-B genome in *J. podagrica*, *J.multifida* and *J. integerrima* samples.

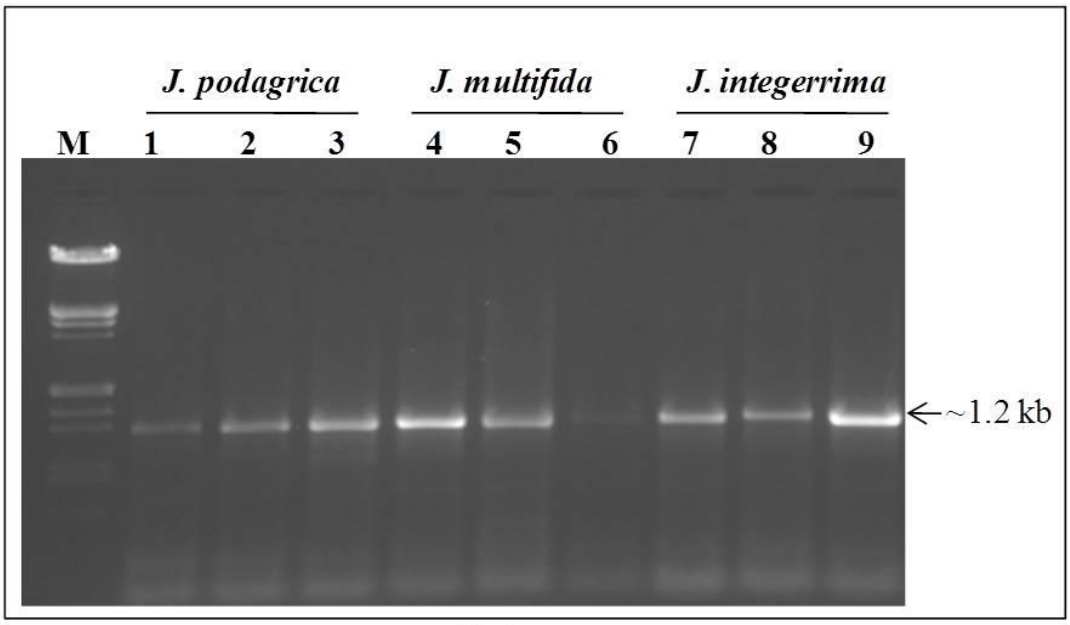

Fig. 2

Figure 2. PCR amplification of partial DNA-A genome of virus isolate. The positive amplicons of ~1.2 kb were obtained from all symptomatic samples (Lane 1-3: *J. podagrica,* Lane 4-6*: J. Multifida* and Lane 7-9: *J. integerrima*) by begomovirus specific primers. Lane M: Lambda DNA digested with *Eco*RI and *Hind*IIIas marker.

BLASTn analysis *J. podagrica* virus isolate under study (HQ848382) showed highest 99-96% sequence identity with several isolates of Jatropha mosaic India virus (JMIV: JN807768, GU906292, GU574210, JN807767, FJ346232, JN698951, HQ910408, GQ847545 and JN698953) of *J. curcas* and *Withania somnifera* from India, 95% with Papaver enation virus (PaEV: HM149260) of *Papaver sominiferum* from India, 91% with Tomato leaf curl Lucknow virus (JN135234) of tomato from India, 87% *Sri Lankan cassava mosaic virus* (AJ579307, AJ890225, AJ607394, AJ890229 and (AJ314737) in cassava from India, 86% sequence identities with *Indian cassava mosaic virus* (ICMV: AY738105, AY730035, AJ314739) in Cassava from India, 85% identity with Jatropha curcas mosaic virus (JCMV: GQ924760) in *J. curcas* from India and other begomovirus isolates reported worldwide. The sequence similarities analyzed by GenomatixDiAlign analysis of *J. podagrica* virus isolate (HQ848382) revealed highest 99% with Jatropha mosaic India virus (JN807768, GU906292 and GU574210), 98-94% similarities with other Jatropha mosaic India virus isolates (JN807767, FJ346232 and HM149260) and virus isolate shared less than 87% sequence similarities with other begomovirus isolates.

During phylogenetic analysis of *J. podagrica* virus isolate (HQ848382) under study shared close relationships with several isolates of JMIV(JN807767, JN807768, GU574210, HM149260, GU906292, FJ346232) in *J. curcas*, *W. somnifera* and *P. sominiferum* from India. The virus isolate also showed distinct relationships with ToLCLuV (JN135234), SrLCMV (AJ579307, AJ890225), ICMV (AY738105, AY730035) and JCMV (GQ924760) isolates reported from India (Figure 3).

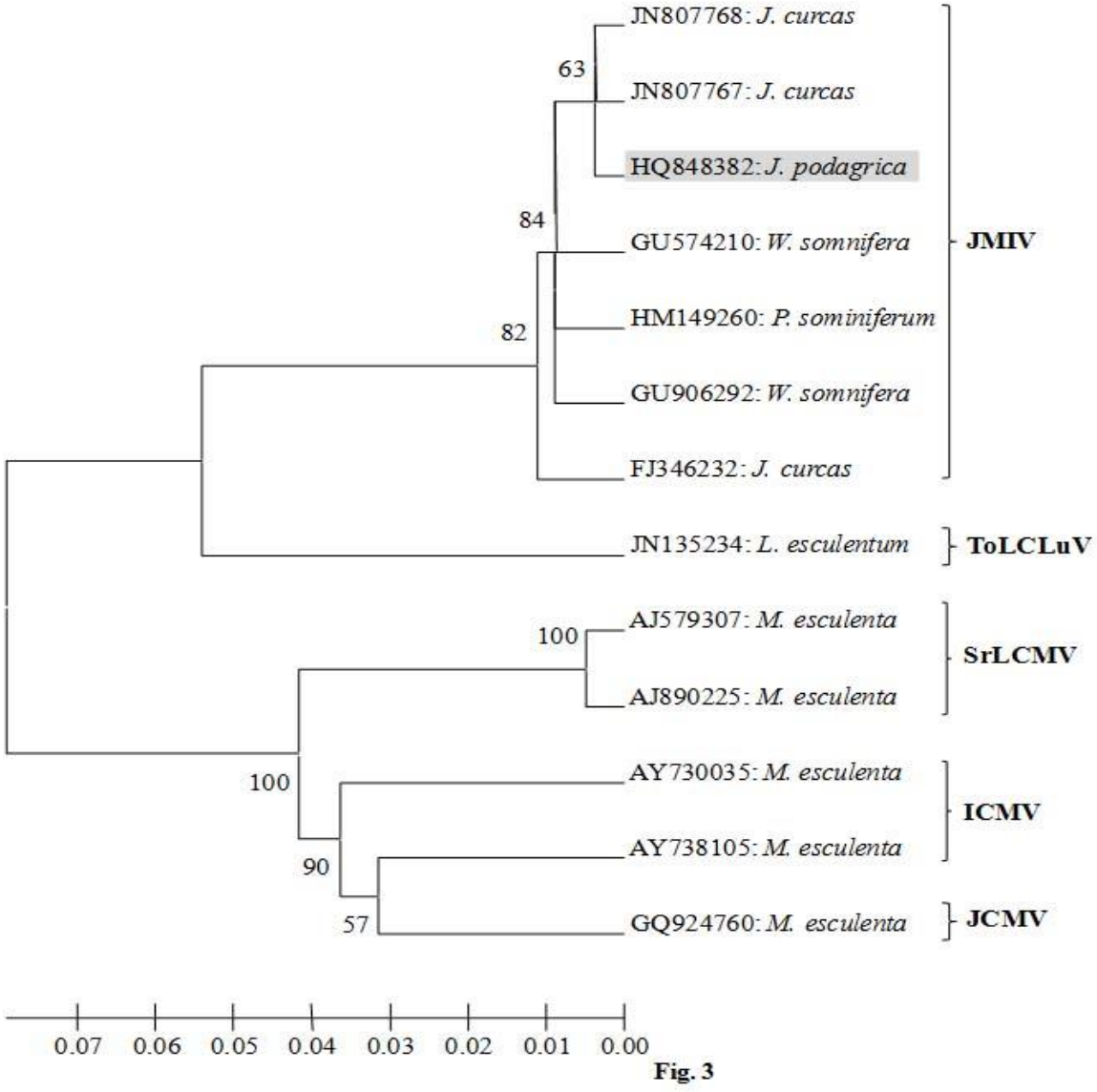

Fig. 3

Figure 3. Phylogenetic analysis of the Partial DNA-A genome of *J. podogrica* virus isolate under study (HQ848382)showing close relationships with Jatropha mosaic India virus isolates and showed distinct relationships with other begomovirus isolates. Phylogenetic analyses were perused using the MEGA v. 4.0 programs with selected begomovirus isolates with 100 replicates bootstrapping and phylogram were generated with Neighbour-joining method. Dendrograms were viewed by the NJplot program. Abbreviations used for study: JMIV: Jatropha mosaic India virus, ToLCLuV: Tomato leaf curl Lucknow virus, SrLCMV: *Sri Lankan cassava mosaic virus*, ICMV: *India cassava mosaic virus* and JCMV: *Jatrophacurcas mosaic virus*.

Based on highest sequence identities of partial DNA-A genome and its closest phylogenetic relationships with *Jatropha mosaic India* virus, the virus isolates associated with mosaic disease of *J. multifida was* identified as isolates of *Jatropha mosaic India* virus.

BLASTn analysis *J. multifida* virus isolate (HQ848381) shared 94% sequence identities with *Tomato leaf curl Patna virus* (ToLCPatV: EU862323, GU253915) in Tomato and Tobacco from India, 94% with *Tobacco leaf curl Pusa virus* (TobLCPuV: HQ180391) in Tobacco from India and 93% identity with *Cotton leaf curl virus* (CoLCV: GU440580) in Cotton from India. Less than 89% sequence identity with *Tomato leaf curl Laos virus* (ToLCLaV: AF195782), *Tomato leaf curl Malaysia virus* (ToLCMalV: AF327436), *Tomato yellow leaf curl Vietnam virus* (ToLCMinV: EU189150), *Tomato leaf curl Mindanao virus* (ToYLCVeiV: EU487046), Ageratum yellow vein virus (AgYVV: JN809821) and other begomovirus isolates reported worldwide.

The percent nucleotide pairwise similarities at partial DNA-A of *J. multifida* virus isolate (HQ848381) through the GenomatixDiAlign revealed the highest 92% sequence similarity with *Tomato leaf curl Patna* (EU862323, GU253915); *Tobacco leaf curl Pusa virus* (HQ180391) and *Cotton leaf curl virus* (GU440580). Less than 85% sequence similarities with other begomo viruses isolates reported worldwide.

During phylogenetic analysis of *J. multifida* (HQ848381) virus isolate under study shared closest relationship with *Tobacco leaf curl Pusa virus* (HQ180391) reported in Tobacco from Bihar, India. The virus isolate under study showed close relationships with *Tomato leaf curl Patna* (GU253915, EU862323), *Cotton leaf curl virus* (GU440580) and also showed distinct relationships with *Tomato leaf curl Laos virus* (AF195782), *Tomato leaf curl Malaysia virus* (AF327436), *Tomato leaf curl Mindanao virus* (EU487046), *Ageratum yellow vein virus* (JN809821) and *Tomato yellow leaf curl Vietnam virus* (EU189150) (Figure 4).

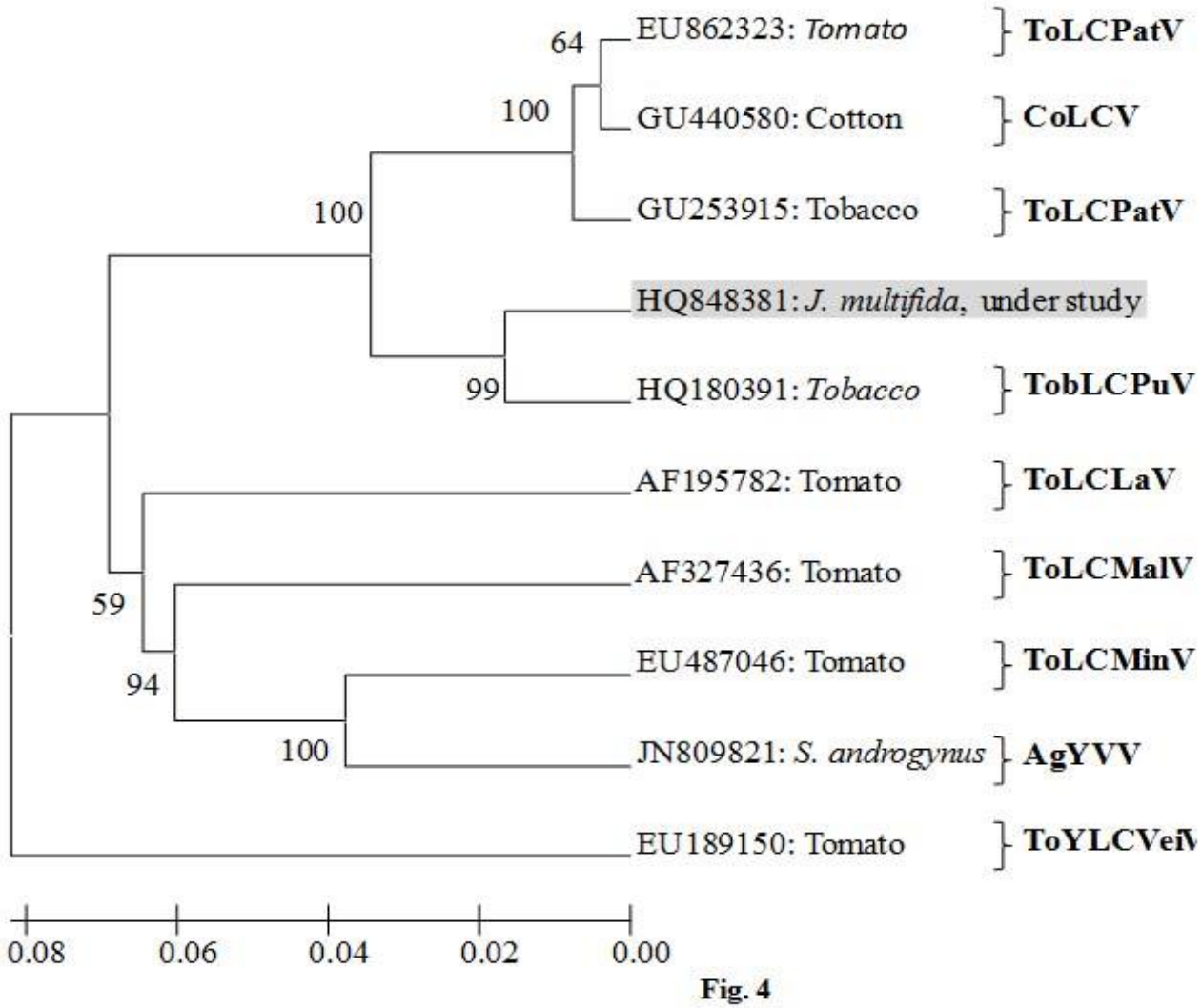

Fig. 4

Figure 4. Phylogenetic analysis of the Partial DNA-A genome of virus isolate under study (HQ848381) associated with mosaic disease of *Jatropha multifida* showing close relationships with isolates of *Tobacco leaf curl Pusa virus* and distinct relationships with other begomoviruses. Abbreviation used for study:ToLCPatV: *Tomato leaf curl Patna virus*, ToLCPuV: *Tobacco leaf curl Pusa virus*, CoLCV: *Cotton leaf curl virus*, ToLCLaV: *Tomato leaf curl Laos virus*, ToLCMalV: Tomato leaf curl Malaysia virus, ToYLCVeiV: *Tomato yellow leaf curl Vietnam virus*, ToLCMinV: *Tomato leaf curl Mindanao virus*, AgYVV: *Ageratum yellow vein virus*.

Based on highest sequence identities and closest/close phylogenetic relationships with *Tobacco leaf curl Pusa virus* and *Tomato leaf curl Patna virus*, the begomovirus isolates associated with mosaic disease of *J. multifida* was identified as isolates of *Tobacco leaf curl Pusa virus* and *Tomato leaf curl Patna virus*.

BLASTn analysis of partial DNA-A of the virus isolate *J. integerrima* (JQ043440) under study showed highest 98% sequence identity with Papaya leaf curl virus (PLCV: HM143914); 93% with *Papaya leaf curl virus* (Y15934), *Tomato leaf curl Karnataka virus* (ToLCKV: AY754812), *Radish leaf curl virus* (RaLCV: EU194914); 92% *Tomato leaf curl Karnataka virus* (ToLCKV: HM851186, HM803118, AY738101), *Tomato leaf curl virus-Bangalore II* (ToLCV-BII: U38239), *Tomato leaf curl New Delhi virus* (ToLCNDV:DQ629102) and *Papaya leaf curl virus* (AY738092, JN807765).

The *J. integerrima* (JQ043440) under study virus isolate also revealed highest 97% sequence similarities with PLCV (HM143914), 92% with ToLCKV (AY754812), 91% with RaLCV (EU194914) and ToLCKV (HM851186, HM803118, U38239) and 90% with PLCV (Y15934, AY738092), ToLCKV (AY738101), ToLCNDV (DQ629102) and 89% similarities with PLCV (JN807765).

Phylogenetic analyses of partial DNA-A genome of *J. integerrima* virus isolate (JQ043440) under study shared close relationship with several isolates of PLCV (HM143914, Y15934, EU194914, AY738092, and JN807765). The virus isolate also shared distinct relationships with ToLCNDV (DQ629102) and ToLCKV (AY754812, HM803118, HM851186, AY738101, and U38239) (Figure5).

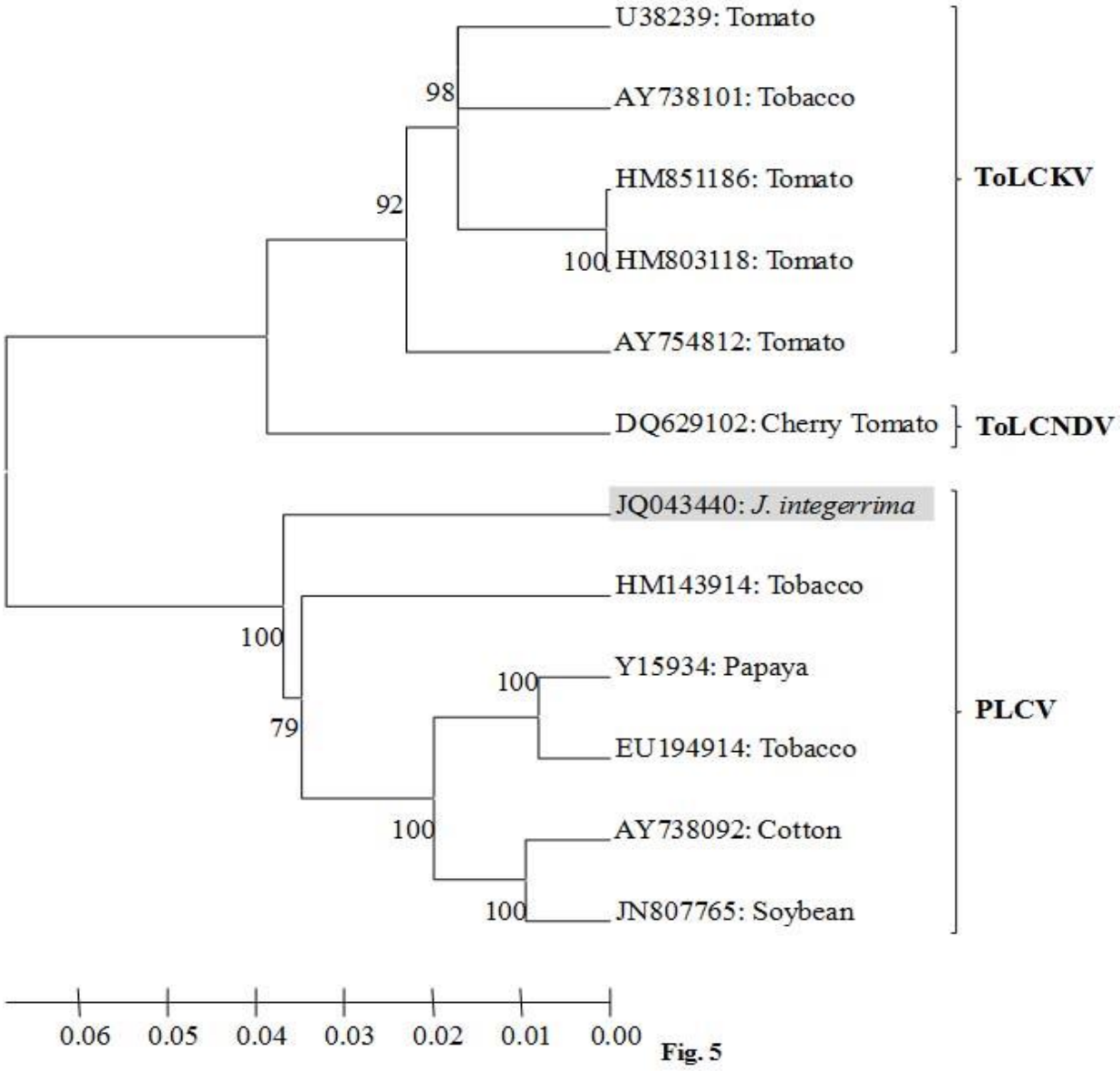

Figure 5. Phylogenetic analysis of the partial DNA-A of *Jatropha integerrima*(JQ043440) (highlighted by grey) showing close relationships with *Papaya leaf curl virus* and distinct relationships with other begomovirus isolates. Abbreviation used for study: PLCV: *Papaya leaf curl virus*, To LCKV: *Tomato leaf curl Karnataka virus*, To LCNDV: *Tomato leaf curl New Delhi virus.*

During this study a total of three begomovirus species viz. Jatropha mosaic India virus (HQ848382) on *J. podagrica*; *Tomato leaf curl Patna virus* (HQ848381) on *J. multifida* and *Papaya leaf curl virus* (JQ043440) on *J. integerrima* have been identified on the basis of ~1.2 kb sequence of partial DNA-A. These results indicated that genetic diversity exists among the begomoviruses infecting ornamental Jatropha species grown in India.

The prevalence of Jatropha mosaic disease has been noticed which drastically affected the Jatropha cultivation in India. The diseases consisted symptoms of severe mosaic, leaf distortion and stunting of the whole plant. The survey also indicated that disease is spreading from one to other districts/provinces in India, which may be due to the migration of the infected preparative materials of Jatropha as well as whiteflies, the known vector of begomovirus.

Aswatha Narayana, et al. [14, 15] reported the natural occurrence of Jatropha mosaic virus disease for the first time in southern India and they identified the causal organism as a distinct begomovirus closely related to *Indian cassava mosaic viruses*. Then after, Raj et al. [17] reported the association of a begomovirus with Jatropha mosaic disease in north India which possessed highest identities and closest relationships with *Indian* and *Sri Lankan cassava mosaic virus* isolates. Gao, et al. [18] reported a new strain of *Indian cassava mosaic virus* based on analysis of complete nucleotide sequences of DNA-A and DNA-B bipartite genome of *Jatropha curcas* isolated from Dharwad, southern India. The monopartite *Croton yellow vein mosaic* virus and its DNA-β satellite molecules associated with yellow vein mosaic disease of *J. gossypifolia* in India [23] and a new begomovirus associated with yellow mosaic disease of *J. gossypifolia* reported in India [24]. Recently a new begomovirus possible strain of *Indian cassava mosaic virus* associated with mosaic disease of *J. curcas* reported from India [21], however, in the literature there are no reports are available related to begomovirus infection on ornamental Jatropha species form India.

Therefore, our studies molecular detection and identification of begomovirusis essential for proper characterization and its PCR products/nucleic acid probe may be used for virus indexing of Jatropha species and searching of virus free propagating materials and to improve the mass cultivation of Jatropha species in India. However, we report here, molecular detection and identification of the begomovirus isolates associated with mosaic disease of ornamental Jatropha species is a new report from India.

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