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The Current Status of New Emerging Begomovirus Diseases on *Jatropha* Species from India

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Abstract

Jatropha species have many excellent characteristics like bio-fuel, high oil content, resistance to drought and other commercial importance values. The several reports have been indicated that *Jatropha* mosaic disease has been drastically affected the *Jatropha* cultivation in India. The association of begomovirus diseases consists of symptoms like mosaic on *Jatropha* species has been reported worldwide including in India. The status of the work used and the results obtained in methodology for identification and characterization of begomovirus isolates associated with symptomatology like mosaic disease of *Jatropha* species grown in India, are presented in this review article. Because mostly *Jatropha* species are being propagated by cuttings, and new species of Begomoviruses are emerging in newly introduced locations for *Jatropha* cultivation in India. The review articles is highlighted the new emerging begomovirus diseases on *Jatropha* species and will be incited to researchers for better improvements of *Jatropha* cultivations for oil productivity in worldwide.

Keywords: *Jatropha* species; Mosaic disease; Begomovirus; Genome organization; Molecular Characterization, Sequence analysis

Introduction

Begomoviruses, of family *Geminiviridae*, are whitefly transmitted viruses which cause diseases in many dicotyledonous crops including important vegetables and weeds in the tropics and subtropics. They consist circular single strand DNA (monopartite: DNA-A, or bipartite: DNA-A and DNA-B) genome. Most of the described begomoviruses are bipartite containing DNA-A and DNA- B molecules, each being approximately 2.6-2.8 kb in size, which are responsible for different functions in the infection process and their life cycle. DNA A encodes a replication-associated protein (Rep) responsible for viral replication, a replication enhancer protein (REn), the coat protein (CP) and transcription activator protein (Trap) that regulate the expression of gene. DNA-B encodes for two proteins, movement protein (MP) and nuclear shuttle protein (NSP) involved in cell to cell movement within the plant, host range and symptom modulation [1]. However, some Old World begomoviruses lack a DNA-B component and only the DNA-A component is required to infect plants systemically. The satellite molecules DNA-β has been find out to be associated with monopartite (DNA-A) and bipartite (DNA-A and DNA-B) begomoviruses and are responsible for the symptom development and systemic infection [2-6].

The genus *Jatropha* of family *Euphorbiaceae* has more than 400 species distributed worldwide and among them *Jatropha curcas*, *J. gossypifolia*, *J. cuneata*, *J. integerrima*, *J. multifida* and *J. podagrica* are recorded from India (Figure 1).

Jatropha curcas L. is commonly known as physic or purging nut. It is a multipurpose and drought resistant crop which is grown in marginal lands with lesser input. Jatropha plants natively occurred in tropical areas of India, Africa, North America and the Caribbean. It is an efficient substitute fuel for diesel engines and forms an essential ingredient in various soaps, dye and wood industries. *Jatropha* extract is also efficacious in dropsy, sciatica and paralysis. Moreover, the plant is very reliable in curing several diseases including rheumatism, leprosy, scabies, eczema, ringworm, chronic dysentery, urinary discharges, abdominal complaints, anaemia, fistula and disease of the heart [7-9].

For *Jatropha* plantation have been identified on the basis of availability of wasteland, drought land and climatic conditions suitable for *Jatropha* plantation in 19 states of India likes: Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Maharashtra, Gujarat, Goa, Rajasthan, Uttar Pradesh, Bihar, Madhya Pradesh, Chhattisgarh, Orissa, Jharkhand, Himachal Pradesh, Haryana, Punjab (Figure 2). In India *Jatropha* is cultivated in large plots of waste land and provide employment to the poor people of rural areas. Government of India has identified 98 million acres of land where *Jatropha* can be grown, and probably will replace ~30% of India's bio diesel consumption.

Unfortunately, the mosaic disease of has been affected drastically the *Jatropha* cultivation in India. The symptoms of diseases is showing on the leaves like mosaic, yellow mosaic, blistering, leaf curl, leaf distortion and stunting of whole *Jatropha* plant (Figure 3). The natural infection of begomovirus has been reported in *Jatropha* species across the world: *Jatropha* mosaic virus on *J. gossypifolia* in Jamaica and *Puerto Rico* [10-14], African cassava mosaic virus reported on *J. multifida* in East and West Africa [15]. Two strains of African cassava mosaic virus on *J. curcas* from Kenya were reported [16,17]. Recently *Jatropha* mosaic Nigeria virus on *J. curcas* from Nigeria [18] and *Jatropha* mosaic virus-[Jamaica: Spanish Town: 2004] on *J. gossypifolia* [19] reported from Jamaika.

The *J. curcas* crop was introduced first time at Southern India in the year of 2002 for biodiesel production. The mosaic disease caused by begomovirus was noticed for the first time on *J. curcas* with a high disease incidence >46% during September, 2004 in Karnataka, southern India [20,21], incidence of a mosaic disease in *J. curcas* reported from Uttar Pradesh [22] and also about 25% incidence was noticed in northern

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Figure 1: *Jatropha* species grown in India: (A) *Jatropha curcas*, (B) *J. multifida*, (C) *J. gossypifolia* Red leaf, (D) *J. gossypifolia* Green leaf, (E) *J. integerrima* and (F) *J. podagrica*.

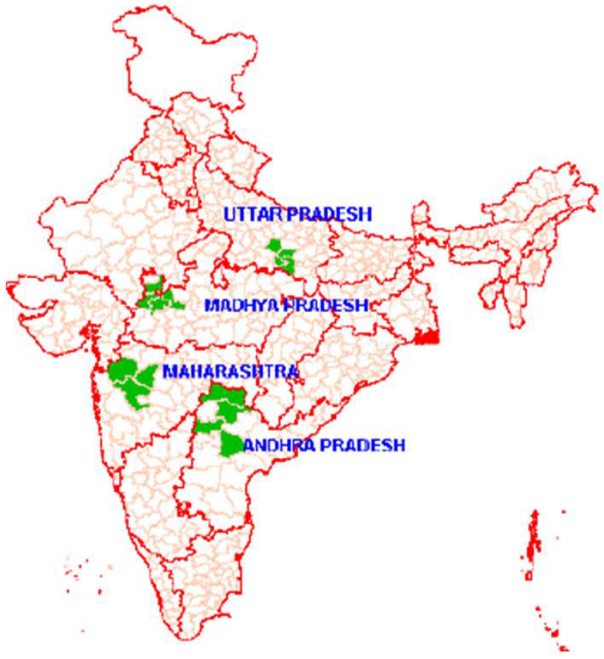


Figure 2: Some of India's ideal growing regions for *Jatropha* species. Source Global energy Network Institute (http://www.ecoworld.com/Home/Articles2.cfm?TID=385).

India during 2006-07 [23]. A strain of Indian cassava mosaic virus has been reported to cause a mosaic disease of *J. curcas* in Dharwad, southern India [24]. The Complete nucleotide sequence of Croton yellow vein mosaic virus and DNA-β associated with yellow vein mosaic disease of *J. gossypifolia* in India [25] and a new begomovirus associated with yellow mosaic disease of *J. gossypifolia* reported in India [26]. Recently, *Jatropha* mosaic India virus [27], *Jatropha* leaf yellow mosaic Katerniaghat virus [28] and *Jatropha* leaf crumple India virus [29] has been reported from Uttar Pradesh, India. Identification and molecular characterization of *Jatropha* mosaic Lucknow virus associated with yellow mosaic disease of three ornamental species of *Jatropha* cultivated in India [30] (Table 1).

Identification and characterization of a begomovirus isolates associated with mosaic disease of *Jatropha curcas* from India

Indian cassava mosaic virus and Sri Lankan cassava mosaic virus: The naturally occurrence of mosaic disease (JMD) was shown on *Jatropha curcas* for the first time in the year of 2004 with the incidence ranged from 12.50 to 46.66%, with the highest incidence in Kolar, followed by Hassan, Tumkur and Bangalore Bangalore districts from Karnataka, South India. The symptoms on naturally infected *J. curcas* plants like mosaic, reduced leaf size, leaf distortion, blistering and stunting. The disease was successfully transmitted from naturally infected *J. curcas* to healthy plants through the grafting, the dodder *Cuscuta subinclusa* and through whiteflies (*Bemisia tabaci*) [20].

In Karnataka, South India, Jatropha mosaic disease caused significant yield losses with the incidences of up to 47% and affecting the growth of the infected plant [21]. The begomovirus was detected by polymerase chain reaction using two sets of coat protein (CP) gene specific primes and obtained ~575 bases CP were from two isolates of *J. curcas* collected at Bangalore and Dharwad, South India. On the basis of highest nucleotide sequence identities and close (90-95%) phylogenetic analysis of the core CP sequences with the selected begomoviruses it was identified as Indian cassava mosaic virus and Sri Lankan cassava mosaic virus. The two JMIV isolates were 94% similar to each other. These results further confirm that JMD in India was caused by begomoviruses and they were most closely related to cassava mosaic viruses from the Indian sub-continent [21].

Figure 3: A field view showing natural infection and showing various type symptoms on *Jatropha* species (a) *J. curcas* (b) *J. gossopifolia (Red species)* (c) *J. gossopifolia (Green species)* (d) *J. multifida* (e) *J. Podagrica* (f) *J. integrrima.*

Virus	Acronym	Disease	Jatropha Species	Country	Reference
Begomovirus	—	*Jatropha* mosaic disease	*Jatropha gossypifolia*	Puerto Rico (USA)	[10]
Jatropha mosaic begomovirus	JMB	Mosaic disease	*J. multifida*	Puerto Rico (USA)	[11]
Begomovirus	—	*Jatropha* mosaic disease	*J. gossypifolia*	Jamaica	[13]
Jatropha mosaic virus	JMV	Yellow mosaic	*J. gossypifolia*	Jamaica	[14]
Begomovirus related to cassava mosaic viruses	ICMV	*Jatropha* mosaic disease	*J. curcas*	Karnataka, India	[21]
Indian cassava / Sri Lankan cassava mosaic virus	ICMV/ SrLCMV	*Jatropha* mosaic disease	*J. curcas*	Lucknow, India	[23]
Indian cassava mosaic virus	ICMV	*Jatropha* curcas mosaic disease	*J. curcas*	Dharwad, India	[24]
Croton Yellow vein mosaic virus	CYVMV	Yelloe vein mosaic disease	*J. gossypifolia*	Lucknow, India	[25]
Jatropha Yellow mosaic India Virus	JYMIV	Yellow mosaic	*J. gossypifolia*	Kathupahadi, India	[26]
Jatropha mosaic India virus	JMIV	Mosaic	*J. curcas*	Lucknow, India	[27]
African cassava mosaic virus	ACMV	Reduced leaf size, severe Dwarfing	*J. curcas*	Kenya	[17]
Jatropha mosaic Nigeria virus	JMNV	Severe mosaic, mottling and blistering of leaves	*J. curcas*	Nigeria	[18]
Jatropha mosaic virus-[Jamaica: Spanish Town:2004]	(JMV-[JM:ST:04])	Yellow mosaic	*J. gossypifolia*	America	[19]
Jatropha leaf yellow mosaic Katerniaghat virus	JLYMKV	Severe leaf yellow mosaic	*J. curcas*	India	[28]
Jatropha leaf crumple India virus	JLCrIV	Leaf crumple	*J. curcas*	India	[29]
Jatropha mosaic Lucknow virus	JLCV	Yellow mosaic	*J. integerrima, J. podagrica* and *J. multifida*	India	[30]

Table 1: Begomoviruses affecting *Jatropha species* and their worldwide distribution.

The second another report was reported related to JMD on of *J. curcas* in Balrampur district, Uttar Pradesh, north India, in the year of 2005 [22]. They mention same symptoms reported to South India symptoms including mild to severe mosaic, marked reduction in leaf size, rolling of leaf margins, puckering of the leaf surface and Chlorotic areas of irregular shapes were present between the secondary veins. The disease was successfully transmitted by only sap transmission test from infected/diseased *J. curcas* to healthy seedlings resulted in the development of mild mosaic after 20 days post inoculations. The viral disease was considered distinct from mosaic diseases previously recorded on *J. curcas* on the basis of symptomatology and transmission study [22].

The association of a begomovirus with Jatropha mosaic disease was found in NBRI field Lucknow, north India with the incidence was significant ~25% in 2006 and 2007. The disease transmitted by whitefly (*Bemisia tabaci*) in a persistent manner but could not be transmitted through sap inoculations. The begomovirus showed highest identities 94.7% sequence identities in BLASTn and showed closest relationships with Indian cassava mosaic virus and Sri Lankan cassava mosaic virus isolates [23].

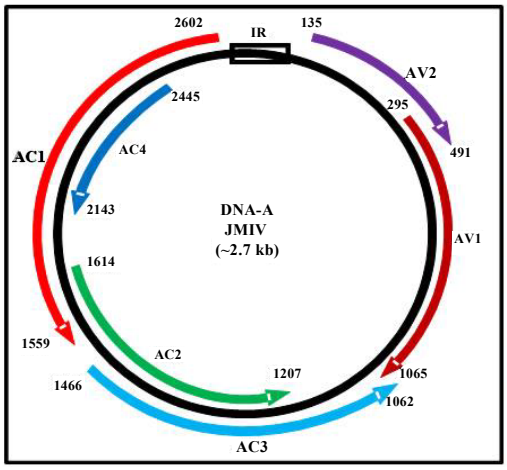
***Jatropha* mosaic India virus:** The natural occurrence of mosaic disease was observed on *J. curcas* growing in experimental plots of the CSIR-NBRI, Lucknow, northern India. in the years 2008 and 2009 and disease incidence was significant. In the year 2008-2009 the disease incidence was about 30% which increases up-to to 45-55% during 2009-2011.

The naturally infected *J. curcas* plants exhibited severe mosaic, curling, blistering, thickening/swelling of the veins, yellow-green patches and malformation (Figure 4) were observed. Infected plants also showed stunted growth, bearing small and deformed flower heads, and bear less fruit set as compared to the healthy ones. The severely infected plants did not bear any flowers or fruits resulting in about 40-60% of total yield loss in field. The disease was successfully transmitted from naturally infected *J. curcas* to healthy *J. curcas* and other experimental plant species by graft and whiteflies (*Bemisia tabaci*) transmission test which developed severe and mild leaf curl symptoms by 30 days post inoculations. The begomovirus was detected by PCR using total DNA as template from naturally infected *J. curcas* leaf samples and four sets of begomovirus specific primers to DNA-A.

Further, the full length viral genome were amplified from the DNA isolated from infected *J. curcas* associated with mosaic disease using Rolling Circle Amplification (RCA) based TempliPhi™ DNA amplification kit. RCA reaction was performed using Ø29 DNA polymerase as per manufacturer's instructions. The RCA product was monomerized by digestion with *Xba*I, *Xho*I and *Bam*HI restriction enzymes and the digested products were electrophorezed on 1% agarose gel. Three positive clones were got sequenced by primer walking. The consensus sequence data of three identical sequences were analyzed and complete DNA-A genome of 2740 nucleotides was deposited in the GenBank database under the accession HM230683. The genome of the virus isolate under study (HM230683 and JN692494) contained six ORFs, (Figure 5). BLASTn analysis of the DNA-A (HM230683) of begomovirus isolated from *J. curcas* revealed highest 88-89% sequence identity with Jatropha curcas mosaic virus (JCMV) isolated from *J. curcas* from Dharwad (GQ924760) and Jalgaon (JF496657). The virus isolate also shared 86-88% identities with isolates of Indian cassava mosaic virus and Sri Lankan cassava mosaic virus isolate *Manihot esculenta* from India and aboard. Phylogenetic analysis of the Begomovirus Jatropha isolate also showed distinct relationship with Indian cassava mosaic virus and Sri Lankan cassava mosaic virus and other reported Begomoviruses isolates including from *Jatropha curcas*.

Based on highest 85% sequence similarities and distinct phylogenetic analysis of DNA-A component of the virus isolate (HM230683) under study associated with mosaic disease of *J. curcas* has been considered as a new begomovirus species (sequence identity<89% of complete DNA-A genome) and its name "*Jatropha* mosaic India virus (JMIV)" is proposed according to the guidelines of the International Committee on Taxonomy of Viruses (ICTV-2008).

Figure 4: Different type of symptoms on infected *J. curcas* leaves (a) Initiation of mosaic (b) Mild mosaic (c) Mottle-mosaic (d) Severe mosaic and distortion of leaf (e) Crinkling and vein swelling (f) Yellow patches.



ORFs	Predicted ORF size (nucleotides)	Translation product (amino acids/kDa)
AV2	357 (135-491)	115/13.35
AV1	771 (295-1065)	256/29.62
AC3	405 (1062-1466)	134/15.88
AC2	408 (1207-1614)	134/15.13
AC1	1044 (1559-2602)	361/40.85
AC4	303 (2143-2445)	85/9.43
IR*	272 (2603-2740)	-

Figure 5: Genome organization and ORFs of Jatropha mosaic India virus (HM230683) associated with the mosaic disease of *J. curcas* Lucknow, U.P., India isolate.

Jatropha leaf crumple India virus: A new monopartite begomovirus isolate associated with leaf crumple disease of *Jatropha curcas was* identified and characterized on the basis of sequence analysis of cloned viral DNA-A genome (~2.7-kb) from three samples amplified by RCA using phi-29 DNA polymerase collected from Jodhpur, Rajasthan, India. Sequence analysis of virus isolates SKJ2: KM189818 (2735 bp) and SKJ3: KM189819 (2738) have seven ORFs: V3, V2 and V1 in virion sense and C3, C2, C1 and C4 in the complementary sense. Based on Pairwise alignment nucleotide sequence similarities (99%) to each other shared less than 81% nucleotide identity to other begomovirus isolates reported worldwide. Based on the ICTV, 2008 demarcation criteria for a new species (sequence identity<89%) of begomovirus, the *Jatropha* isolates were identified as the members of a new Begomovirus species and designated as Jatropha leaf crumple India virus (JLCrIV) [28].

Jatropha leaf yellow mosaic Katerniaghat virus: A survey was conducted in June, 20011 and observed severe leaf yellow mosaic disease with incidence about 45% on *Jatropha curcas* plant growing in the Katerniaghat wildlife sanctuary in India. Begomovirus disease was detected in symptomatic leaf samples (15-20 no.) by PCR using begomovirus genus-specific primers. The Koch's postulates proved by generated Agroinfectious clones of the DNA molecule of the begomovirus isolate in *J. curcas* plants. The full length viral genome was amplified using total DNA (five representative samples) with Phi-29 DNA polymerase by RCA kit and then digested with Bam HI. The RCA products were cloned and sequenced by primer walking and submitted in GenBank database under accession numbers JN698954 (SKRK1) and JN135236 (SKRK2). BLASTn analysis of both the begomovirus isolates of DNA-A revealed highest 86% nucleotide sequence identities to *Jatropha* mosaic India virus (JMIV: HM230683) and other begomoviruses reported worldwide. The sequences of the two begomovirus isolates (SKRK1 and SKRK2) were showed 97% identical to each other. In phylogenetic analysis SKRK1 and SKRK2 shared close relationships and clustered together and showed distant relationships to Jatropha mosaic India virus, Jatropha curcas mosaic virus, Indian cassava mosaic virus, Sri Lankan cassava mosaic virus and other begomoviruses isolates.

Based on 86% nucleotide sequence identities and distant

phylogenetic relationships to Jatropha mosaic India virus and other related begomovirus isolates and the begomovirus species demarcation criteria of the ICTV (<89% sequence identity), the begomovirus isolates were identified as a new begomovirus species and designated as Jatropha leaf yellow mosaic Katerniaghat virus (JLYMKV) [29].

Identification and characterization of a begomovirus isolates associated with yellow mosaic disease of *J. gossypifolia from India*

Croton yellow vein mosaic virus and its DNA-β molecule: During surveys in February 2008, the natural infection of severe yellow vein mosaic disease was observed on a large number of *J. gossypifolia* plants growing nearby agriculture sites in Lucknow, Uttar Pradesh, India. Naturally infected plants exhibited yellow vein mosaic symptoms accompanied with reduction in leaf size and height of plants. PCR was performed using three sets of begomovirus DNA-A and DNA beta specific primers specific primers. As expected, bands of ~800 bp, ~1.2, ~1.2 kb and ~1.3 kb were consistently amplified from (3/3) symptomatic asymptomatic sample. PCR amplicons of DNA-A and DNA beta were purified and cloned. Three independent clones for each amplicons were sequenced in both orientations. The consensus sequence data of three identical sequences were deposited in the GenBank database under accession numbers: EU727086 (DNA-A) and EU604296 (DNA- *β)*, respectively.

During BLASTn analysis of the complete of DNA-A (EU727086) genome sequence of virus isolate showed highest 95% identity with Croton yellow vein mosaic virus (CYVMV, AJ507777) isolated from *Croton bonplandianum*. The phylogenetic analysis of the virus isolate (EU727086) based on complete DNA-A showed closest relationship with Croton yellow vein mosaic virus (CYVMV: AJ507777) isolate from *Croton bonplandianum* in India. Virus isolate also shared close relationships with other isolates of CYVMV (FN645915, FJ593629, FN645901, FN645898, FN645926, and FN645902) reported, but showed distinct relationship with other begomoviruses including Jatropha begomovirus isolates.

The DNA-β (EU604296) showed the highest 96% nucleotide sequence identity in BLASTn and closest phylogenetic relationship with Croton yellow vein mosaic virus-associated DNA-β (AM410551) isolated from *Croton sp.* in Pakistan.

On the basis of highest sequence identities and closest phylogenetic relationships of the DNA-A genome (EU727086) and DNA-β (EU604296) of the virus isolate *J. gossypifolia*, Lucknow, Uttar Pradesh was identified as isolates of Croton yellow vein mosaic virus, associated with yellow vein mosaic disease of *J. gossypifolia* [25].

Jatropha yellow mosaic India virus: A surveys conducted in February 2008, natural infections resulting in yellow mosaic disease of *J. gossypifolia*, with about 60% incidence recorded and diseased plant were collected at nearby agriculture sites in Kathaupahadi, Madhya Pradesh, India. Infected plants exhibited yellow mosaic, leaf deformation, vein swelling symptoms and stunting of whole plant. The disease incidence was about 40% in the area surveyed. The disease was successfully transmitted from *J. gossypifolia* to test seedlings of *J. gossypifolia* (3/4) and *Nicotiana tabaccum* cv. White Burley (4/4). The similar yellow mosaic symptoms were developed on *J. gossypifolia* after 30 days of post inoculation, indicting the positive Koch's postulates.

The full length viral genome was amplified from the DNA isolated from infected *J. gossypifolia* using RCA based TempliPhi™ DNA amplification kit. The RCA product was monomerized by digestion with *Xba*I, *Xho*I and *Bam*HI restriction enzymes. The ~2.7 kb band obtained was cloned and sequenced by primer walking. The consensus sequence data of two identical sequences were analyzed and complete viral genome of 2757 nucleotides was deposited in the GenBank database (Acc. FJ177030).

BLASTn search was carried out for preliminary analysis of the relationship between the begomovirus isolate from *J. gossypifolia* under study (FJ177030) and other begomoviruses which revealed 85% sequence identities with DNA-A of Tomato leaf curl virus-Bangalore II (U38239) fallowed by 84-82% with Tomato leaf curl Karnataka virus (ToLCKV); 81-80% with Tomato leaf curl Pakistan virus (ToLCPKV) and also 81-80% identities with Pedilenthus leaf curl virus (PeLCV), Tomato leaf curl Kerala virus (ToLCKeV) and Ageratum enation virus (AgEV) from India and Pakistan. Phylogenetic analyses of the viral genome (FJ177030) formed a separate cluster and showed distinct relationships with ToLCKV, AgEV, ToLCKeV, PeLCV and ToLCPKV.

Based on 85% identities with all other begomoviruses known to date and ICTV species demarcating criteria (less than 89% identities) and distinct relationships with other begomovirus, the name Jatropha yellow mosaic India virus (JYMIV) has been proposed. JYMIV was considered to be monopartite as neither DNA-B nor DNA-β components associated with begomovirus species were detected [26].

Molecular identification and characterization of begomoviruses infecting ornamental *Jatropha* species from India

Jatropha mosaic Lucknow virus: Three ornamental Jatropha species viz. *J. integerrima, J. podagrica and J. multifida* was affected by the severe yellow mosaic disease grown in gardens at Lucknow, Uttar Pradesh, India, during the year, 2013. The disease was successfully transmitted from symptomatc to healthy Jatropha ornamental plants species by whitefly (*Bemisia tabaci*). The associated begomovirus was detected in symptomatic Jatropha ornamental plant samples by PCR using begomovirus gene specific primers.

The full length viral genome (~2.7 kb) was amplified from the DNA isolated from infected *J. integerrima, J. podagrica and J. multifida* by rolling circle amplification using Phi-29 DNA polymerase and ~2.9 kb DNA-A genome was cloned and sequenced. The DNA-A genome of the begomovirus isolates: JI (KC513823), JP (KF652078) and JM (KF652077) shared highest 94-95% nucleotide sequences identities each other and also showed 93-95% sequence identities with an uncharacterized begomovirus isolated from *J. curcas* (Jatropha leaf curl virus) and also shared highest identity of 61% nucleotide sequence ideates with other begomoviruses. In phylogenetic analysis of JI, JP and JM virus isolate shared distant relationships with other begomovirus isolates reported worldwide.

Based on 61% sequence identities (less than 89%) the species demarcation criteria ICTV for a new begomovirus the isolates under study (*J. integerrima, J. podagrica and J. multifida*) were identified as members of a new begomovirus species and name proposed as "Jatropha mosaic Lucknow virus (JMLV)" [30].

During surveys in three subsequent years 2009-2011, severe mosaic disease like symptoms were also observed on other species of Jatropha viz. *J. podagrica*, *J. integerrima and J. multifida* grown for their ornamental values in CSIR-NBRI Lucknow, India. The naturally infected *J. podagrica* exhibited severe yellow mosaic and vein yellowing symptoms, *J. multifida* showed mosaic and leaf curl symptoms and *J. integerrima* showed mosaic and yellow mosaic symptoms. The disease incidence was about ~30-35% in *Jatropha* species growing field.

The begomovirus was detected by PCR using begomovirus specific degenerate primers. The resulting amplicons of ~1.2 kb of in all the *Jatropha* species samples were sequenced and sequence data was analyzed. The consensus sequence data of three identical sequences were combined to partial DNA-A genome and were deposited in the GenBank database under accession number *J. podagrica:* HQ848382 (1177 bp); *J. multifida:* JQ043440 (1288 bp) and *J. integerrima:* HQ848381 (1201 bp).

Based on BLASTn highest nucleotide sequence identities of partial DNA-A genome (~1.2 kb) and close phylogenetic relationships begomovirus isolates was identified as Jatropha mosaic India virus on *J. Podagrica*; Tomato leaf curl Patna on *J. multifida* and Papaya leaf curl virus on *J. integerrima* . The DNA-β satellite molecule was also detected in *J. integerrima* (JQ178364) and Cotton leaf curl virus betasatellite was identified based on sequence and close phylogenetic analysis [31].

Genetic diversity among begomoviruses associated with mosaic disease of Jatropha species grown in India

The sequence analysis of genomic DNA resulted in identification of nine begomovirus species based on completed DNA-A (~2.7 kb): Indian cassava mosaic virus and Sri Lankan cassava mosaic virus, Jatropha mosaic India Virus (JMIV: HM230683) and Jatropha curcas mosaic virus (JCMV: JN692494) on *J. curcas* from northern India [27]. The Jatropha curcas mosaic virus (JCMV: GQ924760) has reported from Dharwad, India [24] on *J. curcas*. Based on begomovirus species demarcation criteria for a new species (sequence identity<89%), the begomovirus isolates were identified as the members of a new Begomovirus species and provisionally designated as Jatropha leaf crumple India virus (JLCrIV: KM189818, KM189819, KM023146) [28] and Jatropha leaf yellow mosaic Katerniaghat virus (JLYMKV: JN698954, JN135236) [29] on *J. curcas* from Uttar Pradesh, India.

Croton yellow vein mosaic virus (CYVMV: EU727086) and Jatropha yellow mosaic India virus (JYMIV: FJ177030) have been identified and characterized associated with yellow vein net and yellow mosaic disease on *J. gossypifolia* first time in India [25,26].

Identification and Characterization of Jatropha mosaic Lucknow virus (JMLV) associated with yellow mosaic disease of *J. integerrima* (KC513823), *J. podagrica* (KF652078) and *J. multifida* (KF652077) from Lucknow, Uttar Pradesh, India [30]. The partial sequence analysis of ~1.2 kb partial DNA-A amplicons also revealed presence of three begomovirus species: Jatropha mosaic India virus on *J. podagrica* (HQ848382), Tomato leaf curl Patna virus on *J. multifida* (HQ848381) and Papaya leaf curl virus on *J. integerrima* (JQ043440) in Lucknow, India [31].

In during phylogenetic analysis using MEGA v. 7.0 programs of complete DNA-A of Jatropha virus isolates under study JMIV (HM230683); JCMV (JN692494), JCMV (GQ924760, JF496657); JLCV (EU798996, GU451249); JLCrIV (KM1898118, KM189819); JLYMKV (JN698954, JN135236) on *J. curcas;* CYVMV (EU727086) and JYMIV (FJ177030) from *J. gossypifolia* and JMLV (KC513823, KF652077, KF652078) on *J. integerrima, J. multifida* and *J. podagrica,* respectively with other abroad Jatropha begomovirus isolates like East African cassava mosaic virus (ACMV: JN053421, JN053447); Jatropha curcas Nigeria virus (JCNigV: JX025358, JX025359); Jatropha mosaic virus-[Spanish town:2004] (JMV: KF723259, KF723258) reported worldwide formed separate clusters, therefore, they were considered as eight distinct begomovirus species in India and three distinct species in abroad (Figure 6).

These results indicated that the genetic diversity exists among the begomovirus isolates infecting Jatropha species grown in India as well as in worldwide.

Management strategies

The disease is spreading from one district to other districts/ states in India may be due to the migration of the infected p*ropagative* plant materials of Jatropha as well as insect vector like whiteflies. Therefore, molecular studies of virus/es causing severe mosaic disease in *Jatropha* species cultivated in India and development of their management strategies seems to be very essential so that viruses affecting *Jatropha* plants may be identified and *Jatropha* cultivars may be screened to search virus-free prerogative materials to be used for breeding programme, mass propagation or large scale Jatropha cultivation in India.

The virus vectors: leaf sucking insects, whitefly, thirps, leafhoppers and aphids by which viruses spread in nature may be controlled by regular insecticide spraying but viruses present in the plants can not be destroyed. Since Jatropha are being propagated by cutting, virus present in the propagating materials also propagates and as a result of which viral diseases are being maintained from one to several generations through propagations of infected materials.

Whitefly (*Bemisia tabaci*) is the efficient vector of the begomoviruses, a common insecticide 'Malathion' was used to minimize the populations of insect-vector (*B. tabaci*). The three round foliar spraying of 0.2% insecticide after 21 days interval was found promising for the control of whitefly in *J. curcas* crop. Improvement in plant growth and appreciable enhancement in fruit yields was also recorded in sprayed plants of three *J. curcas* as compared to the unsprayed plants. Therefore, three sprayings of Malathion insecticide (0.2%) at 21 days intervals is recommended for possible management of mosaic disease of *J. curcas*. These findings may be utilized for designing the efficient control measures of the mosaic disease for better production of *J. curcas* in India [32].

The recent developments in biotechnology and emergence of genetic engineering have offered the powerful techniques for molecular breeding and incorporation of genes from taxonomically unrelated species in developing verities of plant species with novel, useful agronomical and value added traits. In recent years, genetic engineering technique using virus gene elements have been employed as an alternative strategy to produce virus resistant plants. Therefore, the coat protein and replicase protein genes of begomovirus characterized in this study may be utilized in future for the development of transgenic *J. curcas* for development of inbuilt resistance against begomovirus.

Recently the transgenic of *J. curcus* plant have been generated through tissue culture resistant to expressing a hairpin, double-stranded (ds) RNA with sequences homologous to five key genes of Indian cassava mosaic virus-Dharwad (ICMV-Dha) strain DNA-A, which silences sequence-related viral genes. They have also checked the resistant on transgenic line of T1 progeny by Quantitative PCR analysis and showed that resistant transgenic lines had no detectable virus [33].

Since nucleotide sequence data of the CP gene region has been considered as a major characterizing factor for a number of RNA and DNA viruses by the ICTV. The PCR reactions were optimized for begomovirus for indexing of Jatropha species/samples. Indexing of germplasm by sensitive detection method would be utilized for search of virus-free *Jatropha* materials for mass propagation.

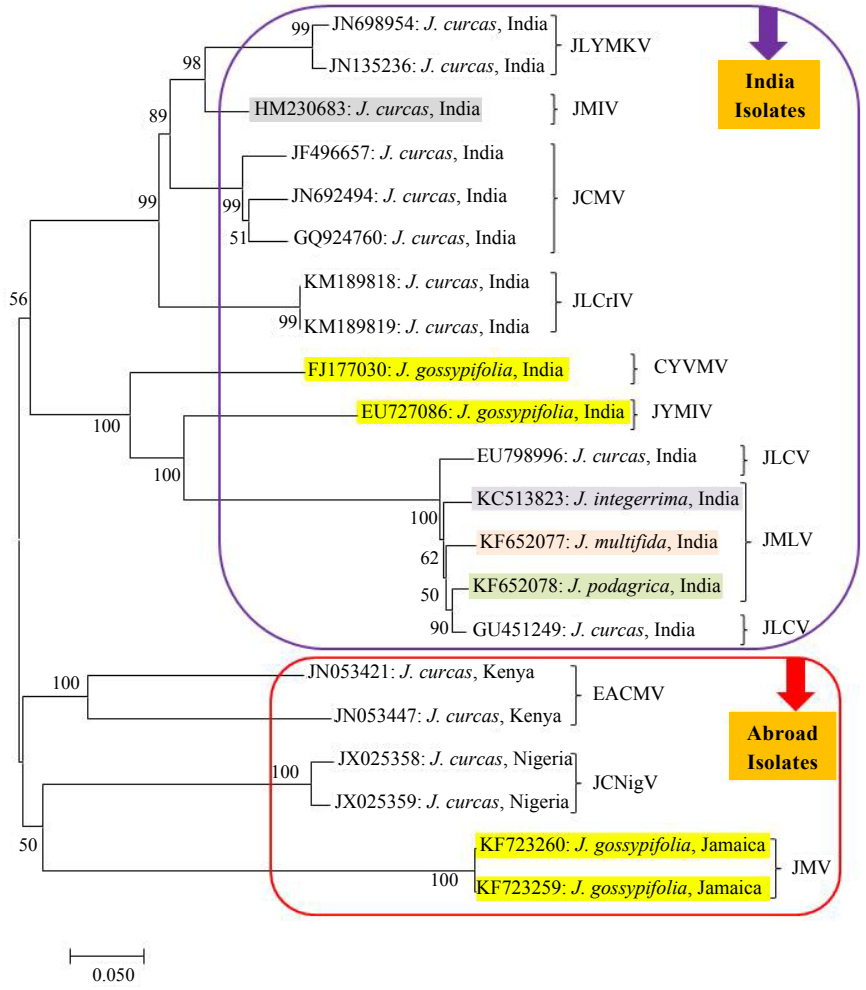


Figure 6: Phylogenetic analysis of complete DNA-A of begomovirus isolates infecting Jatropha species all over the world including India. The under study four begomoviruses showed distinct relationships with other begomovirus isolate reported from *Jatopha species*. Phylogenetic analyses tree generated by Molecular Evolutionary Genetics Analysis tool (MEGA v. 7.0) with 1000 replicates bootstrapping, and the tree were generated with the Neighbour joining method and viewed by the NJ plot program.

Conclusions

During the study total nine begomovirus species viz. Indian cassava mosaic virus, Sri Lankan cassava mosaic virus, Jatropha mosaic India Virus, Jatropha curcas mosaic virus, Jatropha leaf crumple India virus, Jatropha leaf yellow mosaic Katerniaghat virus on *J. curcas*, Croton yellow vein mosaic virus and Jatropha yellow mosaic India virus on *J. gossypifolia*. Jatropha mosaic Lucknow virus *on J. integerrima, J. podagrica* and *J. multifida* have been identified in India. These results indicated that genetic diversity exists among the begomoviruses infecting *Jatropha* species grown in India.

Molecular studies of virus/es causing severe mosaic disease in *Jatropha* species cultivated in India and development of their management strategies seems to be very essential so that viruses affecting *Jatropha* plants may be identified and *Jatropha* cultivars may be screened to search virus-free prerogative materials to be used for breeding programme, mass propagation or large scale *Jatropha* cultivation in India.

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