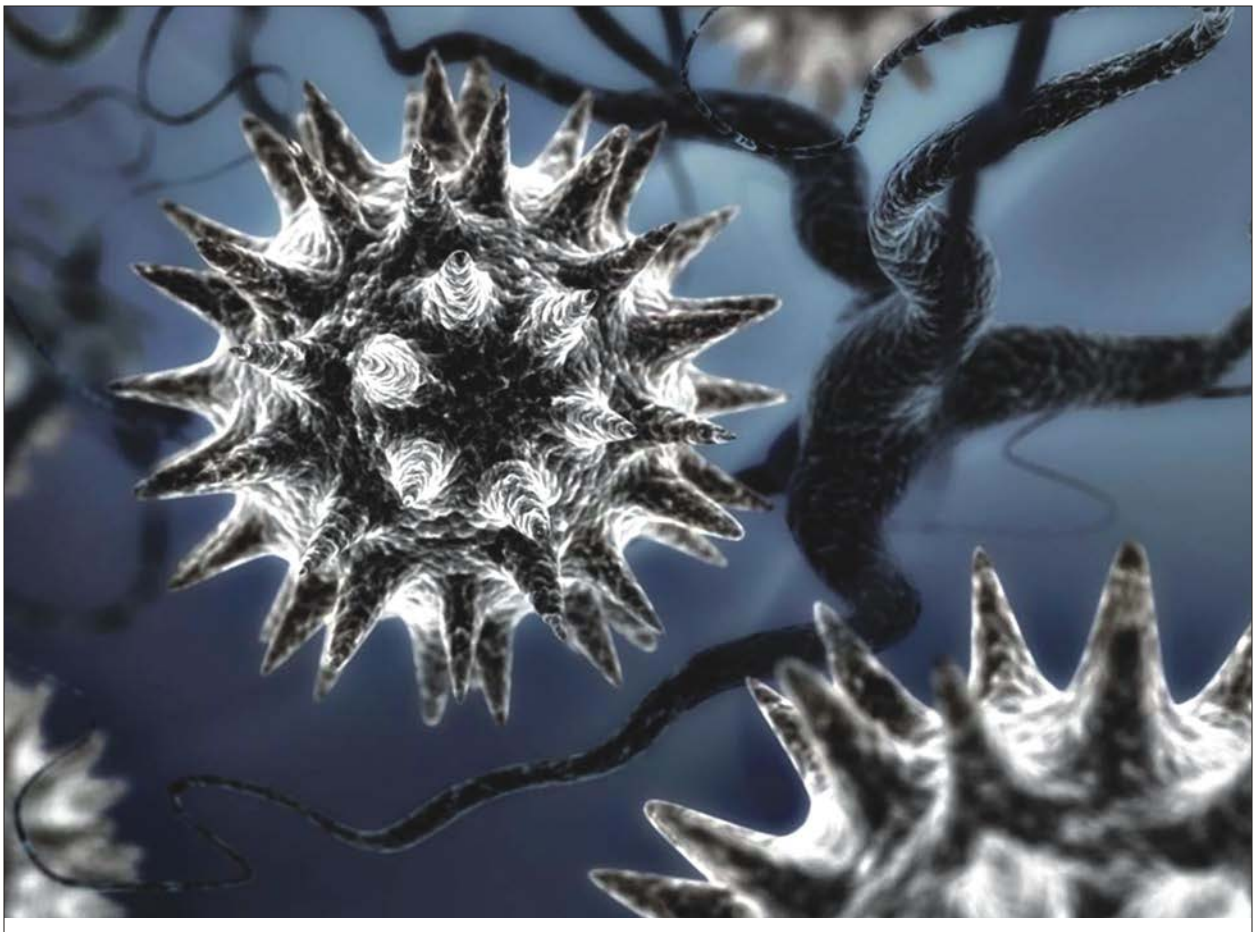

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Distinct Begomoviruses Closely Related to Cassava Mosaic Viruses Cause Indian Jatropha Mosaic Disease

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Abstract: A begomovirus was recently shown to be causing Jatropha mosaic disease (JMD) on Jatropha for the first time in India. A typical begomovirus-like symptoms characterized by chlorotic specks on leaves, curling and malformation of leaves, severe reduction in leaf size, partial or complete sterility were seen on infected plants. In Karnataka state, South India, JMD caused significant yield losses by affecting the growth of the infected plant and by disease incidences of up to 47%. The putative Jatropha mosaic India virus (JMIV) was successfully transmitted through grafting, the dodder *Cuscuta subinclusa* and the whitefly, *Bemisia tabaci*. The JMIV was detected in infected plants and individual *B. tabaci* by polymerase chain reaction tests using two sets of begomovirus-specific degenerate primers. The core coat protein (CP) sequences of ~575 bases were obtained from two isolates collected at Bangalore and Dharwad, South India. Phylogenetic analysis of the core CP sequences with those of selected begomoviruses grouped JMIV in a separate cluster close to *Indian cassava mosaic virus* and *Sri Lankan cassava mosaic virus* and shared highest nucleotide identities (90-95%) with them. The two JMIV isolates were 94% similar to each other. The begomoviruses causing JMD in the Americas grouped separately from JMIV and shared only 72.8-75.2% core CP nucleotide identities thus they are distinct. 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.

Key words: Jatropha mosaic India virus, core coat protein, whitefly, polymerase chain reaction, dodder transmission

Introduction

Jatropha (*Jatropha curcas* L.) also known as physic nut is a drought resistant perennial plant, which is popularly cultivated in the tropics as a living fence (Heller, 1992). The tree is of significant economic importance for its numerous industrial and medicinal uses. The oil extracted from Jatropha seeds is being used as biofuel for diesel engines thus Jatropha has a great potential to contribute to the renewable energy source. In India the area under the cultivation of Jatropha is increasing in recent years with the ever increasing demand for fossil fuels that are exhausting at a rapid rate.

Jatropha suffers from several fungal and bacterial diseases and more recently by the Jatropha mosaic India virus (JMIV), which causes Jatropha mosaic disease (JMD) (Rangaswamy *et al*., 2005; Aswatha Narayana *et al*., 2006). JMD was first reported from Karnataka state, South India in 2004 and was shown to be associated with a begomovirus based on virus transmission by the whitefly,

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Bemisia tabaci (Gennadius) and virus detection by polymerase chain reaction (PCR) tests (Rangaswamy *et al*., 2005; Aswatha Narayana *et al*., 2006). However, the nature of the virus was unknown and its phylogenetic relationship with other begomoviruses was not established. Elsewhere, JMD was first reported on Jatropha from Puerto Rico (Bird, 1957) and subsequently from Cuba and Jamaica. JMD in the Americas was shown to be associated with Jatropha mosaic virus (JMV), a bipartite begomovirus, which was also transmitted by *B. tabaci* in a semi persistent manner.

Viruses of the genus *Begomovirus* (family *Geminiviridae*) typically have bipartite, circular single-stranded DNA (ssDNA) genomes with all functions required for virus replication, control of gene expression and encapsidation encoded on DNA-A and genes involved in intra- and intercellular movement encoded on DNA-B (Hanley-Bowdoin *et al*., 1999). More recently many monopartite begomoviruses that have single DNA molecule are reported from bhendi, cotton and tomato (Navot *et al*., 1991; Briddon and Markham, 2000). These are associated with additional satellite molecules called DNA-β, which in some cases modulate symptom expression (Saunders *et al*., 2000; Briddon *et al*., 2001). All begomoviruses encode a coat protein (CP) in which packages all the genomic and satellite molecules. The CP acts as the coat of the virus particle and is essential for virus transmission from diseased to healthy plants by *B. tabaci*. The CP is highly conserved amongst the begomoviruses originating from the same geographical region and thus been adapted to transmission by local vector populations (McGrath and Harrison, 1995; Maruthi *et al*., 2002). The CP is therefore an essential component of begomovirus survival and has been used widely to characterise and establish the relationships of many begomoviruses (Harrison *et al*., 2002).

The core region of the CP sequences have also been used and shown useful for begomovirus diversity and classification purposes (Wyatt and Brown, 1996; Brown, 2000). In this study, JMIV was detected by PCR tests using two sets of begomovirus-specific degenerate primers (Deng *et al*., 1994; Wyatt and Brown, 1996). The core CP sequences were obtained and the phylogenetic relationship of JMIV with those of the American JMV and other begomoviruses was established. In addition, the JMD incidences, symptomatology, virus transmission by *B. tabaci* and a dodder parasitic plant have been demonstrated.

Materials and Methods

Assessment of JMD Incidence

Surveys were carried out from September 2004 to June 2006 in major Jatropha growing areas at several locations (Table 1) covering 10 administrative districts of Karnataka state, South India to determine JMD severity and incidences (Aswatha Narayana *et al*., 2006). The disease incidence at each location was assessed by counting the number of infected plants out of the total planted in a 25 m long live fence. Stem cuttings of the two isolates of JMD from Bangalore and Dharwad, South India were collected from naturally infected Jatropha plants and planted in earthen pots containing soil and farmyard manure and grown in an insect proof glasshouse at the University of Agricultural Sciences (UAS), Bangalore.

Virus Transmission Tests

Experiments were designed to transmit putative begomovirus from Jatropha (Bangalore isolate) using the whitefly vector *B. tabaci*. Adult *B. tabaci* were collected from a colony of an indigenous *B. tabaci* biotype that has been maintained on cotton plants at the UAS glasshouse. The white flies were released on to the infected Jatropha plants for 24 h for virus acquisition, which were then transferred to healthy Jatropha seedlings at 3-5 leaf stage that were enclosed in polyvinyl cages (5×8 cm). Individual Jatropha seedlings were inoculated by 25 viruliferous white flies for 48 h. Plants were then sprayed with a systemic insecticide (Confidor 0.05%) and maintained in insect proof cages

Table 1: Incidence of Jatropha mosaic virus disease at different locations of Karnataka state, South India recorded from 2004 to 2006

District	Taluk	Place/location	Date of record	No. of plants infected/examined	% disease incidence[b]
Kolar	Chintamani	Chintamani	12-09-2004	14/30	46.7
	Sidlaghatta	Gangigunte	15-06-2006	2/7	28.5
		Thimmanayakanahalli	15-06-2006	2/12	16.6
Bangalore	Bangalore north	Hesarghatta	16-01-2005	4/15	26.6
	Doddaballapura	Marasandra	16-01-2005	30/92	32.6
	Kanakapura	Kanakapura	12-09-2004	23/66	34.8
Tumkur	Kunigal	Yadiure	12-09-2004	11/36	31.1
	Tumkur	Kuchhangi	12-09-2004	11/30	36.6
Mandya	Malavalli	B.G. Pura	24-12-2005	0/15	0.0
		Hosahalli	24-12-2005	0/55	0.0
		Nagegowdanahalli	24-12-2005	23/209	11.0
	Nagamangala	Manthanahalli,	24-12-2005	8/21	38.8
Mysore	Hunsur	Bailukoppa	24-12-2005	2/10	2.0
	Piriapattana	Nagarahole	24-12-2005	3/138	2.1
Hassan	Channarayapattana	Irisave	12-04-2005	14/30	46.1
Chitradurga	Chitradurga	Chitradurga	28-04-2005	26/100	26.0
Bellary	Sandur	Sandur	28-04-2005	30/101	29.7
Uttarkannada	Sirsi	Yekkambi	20-04-2005	21/90	23.3
Coorg	Virajpet	Panjarpet	20-04-2005	20/89	22.5

[a]Disease incidence was calculated by counting the number of infected and healthy plants in a 25 m long (maximum) living fence at each location, [b]Some of the data was extracted from Aswatha Narayana *et al.* (2006) and presented here for comparison purpose

for three months for symptom development. The non-symptomatic plants were tested for the presence of a begomovirus by PCR. Five plants were inoculated for each virus isolate and the experiment was repeated five times.

Virus transmission was also carried out by grafting of the diseased scions on to 15 healthy Jatropha seedlings. Further, the ability of the dodder *Cusuta subinclusa*, a natural phanerogamic parasite on Jatropha, to transmit the putative virus was also tested. The dodder was grown from sowing seeds in plastic bags containing soil and farmyard manure and further maintained on healthy periwinkle plants. Few vines of the dodder were separated from periwinkle plants and allowed to grow on the infected Jatropha plants. The dodder developed on the infected Jatropha plant were then allowed to grow on the 15 healthy Jatropha (3-5 leaf stage) seedlings and the set up was maintained for 30 days. Afterwards the target plants were separated from the dodder and maintained for 3 months in an insect proof glasshouse for symptom development. The presence of the virus in the dodder was confirmed by PCR tests before being used in transmission studies.

Detection of Virus by PCR

Total DNA was extracted from dodder and Jatropha plants expressing mosaic symptoms using the CTAB (cetyl trimethyl ammonium bromide) method (Lodhi *et al.*, 1994). Extraction of total DNA from viruliferous *B. tabaci* was by following the protocol of Cenis *et al.* (1993). *B. tabaci* were allowed to feed on infected Jatropha plants for 24 h, which were then transferred to healthy cotton plants. Ten *B. tabaci* adults were collected from cotton plants at 2 days interval till their death (up to 8 days) to detect virus in them.

PCR reactions were carried out using the Deng A and B primers (Deng *et al.*, 1994) that amplify ~530 bp PCR product from the conserved nonanucleotide TAATATTAC to the amino acid sequence CEGPCKYG within the CP gene of begomovirus DNA-A component. Another set of degenerate primers (AV494 and AC1048; Wyatt and Brown, 1996) that were capable of universally amplifying the core CP region of many begamoviruses were also used for virus detection. The core CP region consist of highly variable 5'-end and the nearly identical 3'-end and is characterized by stretches of identical sequences interspersed by few variable bases thus is the useful marker for virus characterization (Brown, 2000).

Table 2: Details of geminiviruses used in the phylogenetic analyses of core CP nucleotide sequences

Virus species	Abbreviation	EMBL accession No.
African cassava mosaic virus	ACMV	J02057
Bean golden yellow mosaic virus-[Mexico]	BGYMV-[MX]	AF173555
Chilli leaf curl virus-[Pakistan]	ChiLCuv-[Pak]	DQ114477
Cotton leaf curl Multan virus-[Bhatinda]	CLCuMV-[Bha]	DQ191160
Cotton leaf curl Rajasthan virus-[Abohar]	CLCuRV-[Abo]	AY795606
East African cassava mosaic Cameroon virus	EACMCV	AF112354
East African cassava mosaic virus	EACMV	AF126806
East African cassava mosaic Zanzibar virus	EACMZV	AF422174
Indian cassava mosaic virus	ICMV	Z24758
Indian cassava mosaic virus-[Kerala 2]	ICMV-[Ker2]	AJ575819
Indian cassava mosaic virus-[Kolli hills]	ICMV-[KH]	AY998122
Indian cassava mosaic virus-[Maharastra 2]	ICMV-[Mah2]	AY730035
Indian cassava mosaic virus-[Maharastra]	ICMV-[Mah]	AJ314739
Indian cassava mosaic virus-[pCTCRI]	ICMV-[pCTCRI]	AY769966
Indian cassava mosaic virus-[Tri]	ICMV-[Tri]	AF423180
Jatropha mosaic virus-[Puerto Rico]	JMV-[PR]	AF058025
Jatropha mosaic virus-[Jamaica]	JMV-[Jam]	AF324410
Maize streak virus-[South Africa]	MSV-[SA]	Y00514
Papaya leaf curl virus-[AD]	PLCV-[AD]	DQ376037
Papaya leaf curl virus-[Kavitha]	PLCV-[Kav]	DQ376039
Pepper leaf curl Bangladesh virus	PepLCBV	AF314531
South African cassava mosaic virus	SACMV	AF155806
Sri Lankan cassava mosaic virus-[Adivaram]	SLCMV-[Adi]	AJ579307
Sri Lankan cassava mosaic virus-[Colombo]	SLCMV-[Col]	AJ314737
Sri Lankan cassava mosaic virus-[Kerala 15]	SLCMV-[Ker15]	AJ890224
Sri Lankan cassava mosaic virus-[Kerala 17]	SLCMV-[Ker17]	AJ890225
Sri Lankan cassava mosaic virus-[Kerala]	SLCMV-[Ker]	AJ890226
Sri Lankan cassava mosaic virus-[Tamil Nadu 7]	SLCMV-[TN7]	AJ890229
Sri Lankan cassava mosaic virus-[Salem]	SLCMV-[Sal]	AJ607394
Tobacco leaf curl virus-[Karnataka 1]	TbLCV-[Kar1]	AY007615
Tomato leaf curl Bangalore virus-[Ban2]	ToLCBV-[Ban2]	U38239
Tomato leaf curl Joydebpur virus	ToLCJV	AJ875159
Tomato leaf curl New Delhi virus	ToLCNDV	DQ515969
Tomato leaf curl virus-[*Tagetes erecta*]	ToLCV-[TE]	DQ339120

PCR was performed in a 25 μL reaction mix containing 6.0 mM Tris-HCl, pH 8.8, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 20 μM primers, 2.5 U of *Taq* DNA polymerase (Banglore Genei Pvt. Ltd., Bangalore) and 10-15 ng DNA. Reactions were carried out in a thermo cycler according to Wyatt and Brown (1996) and the products were separated by electrophoresis on 1% agarose gels. Agarose gels were stained with 1% ethidium bromide solution and the bands were visualised under the UV light. A 1 kb ladder (MBI Fermentas Life Sciences, Germany, purchased from Genetix, Biotech Asia Pvt. Ltd., Bangalore, India) was included on each gel as molecular weight marker. Experimental controls included DNA extracted from healthy plants and double distilled water.

Cloning and Sequencing of the Core Coat Protein Gene

Total DNA was extracted from the leaves of JMIV-[Bangalore] (JMIV-[Ban]) and JMIV-[Dharwad] (JMIV-[Dha]) as described above. PCR reactions were set up using the primers AV494 and AC1048 (Wyatt and Brown 1996) that were designed to amplify ~575 bp fragment from the core CP gene of begomoviruses. PCR products were separated on 1% agarose gels and purified using the gel extraction kit (QIAGEN, GmbH, Hilden, Germany) and cloned into the pTZ57R/T cloning kit (MBI, Fermentas) following the manufacturer's instructions. The clones were sequenced using the ABI dye terminator kit in an automated sequencer (ABI Prism 377 DNA sequencer) following the dideoxynuleotide chain termination procedure (Sanger *et al*., 1977) at the MWG Biotech Pvt. Ltd., Bangalore, India. Clones were sequenced in both directions using the universal M13 forward and reverse primer to minimise sequencing errors. The core CP sequences of JMIV-[Ban] and JMIV-[Dha]

were compared with those of the reference geminiviruses obtained from the European Molecular Biology Laboratory (EMBL) database (Table 2). The sequences were analysed by parsimony methods (Swofford, 2002) using the software PAUP version 4.0 b10 for Mac. The heuristic method of search with 60% confidence levels was employed for reconstructing phylogenetic trees with 100 bootstrap replicates. The identity of the sequences was confirmed by BLAST search analysis.

After performing the preliminary phylogenetic analysis, putative recombination events were predicted in the core CP sequences, which were detected using the software package Phylpro v1.05 (Weiller, 1998). The pair-wise distances were calculated in a split window size of 40 nucleotides for both upstream and downstream of all aligned sequences with a phylogenetic correlation of +1 (perfectly correlated) to -1 (unrelated). The sequences with a low phylogenetic correlation for particular positions are the likely recombination sites. The ICMV, SLCMV and JMIV isolates were used in the recombination analysis.

Results and Discussion

Disease Incidence

JMD was first observed on naturally grown Jatropha plants during September 2004 in two locations of Karnataka state; Kanakapura, Bangalore district and Sugatur, Kolar district (Aswatha Narayana *et al*., 2006). Typical JMD symptoms were characterized by chlorotic specks on leaves, curling and malformation of leaves, severe reduction in leaf size, partial or complete sterility were seen on infected plants. Early infected plants were severely stunted (Fig. 1). These symptoms were typical of infection by a begomovirus and resembled those of JMV described on *Jatropha gossipiifolia* in Puerto Rico (Bird, 1957).

Surveys were conducted from September 2004 to June 2006 at several locations in 10 districts of Karnataka in order to investigate the extent of JMD spread. JMD was present in all 10 districts and the incidences varied. The highest disease incidence was recorded at Chinthamani (47%), Kolar district while none of the plants examined were infected at two locations in Mandya district (Table 1).

Virus Transmission Tests

The JMIV was successfully transmitted to healthy Jatropha plants through grafting, dodder *C. subinclusa* and using the insect vector, *B. tabaci*. All the graft and dodder inoculated plants (100% transmission) developed symptoms in 6-8 weeks.

The rate of transmission by *B. tabaci* was low. About 25 viruliferous adults that were given 24 h acquisition access period (AAP) and 48 h inoculation access period (IAP) transmitted the virus to an extent of only 40%. Rate of transmission was very low (4%) when five or ten *B. tabaci* were used for inoculation. These results can be comparable to the low transmission rates of some other begomoviruses from South India; *Cotton leaf curl Multan virus*-Hibiscus [Bangalore] (CLCuMV-Hib [Ban]) and *Indian cassava mosaic virus* (ICMV) both required 25 *B. tabaci* adults or more to achieve only 20% transmission rates (Mathew 1988; Rajeshwari *et al*., 2005). However, many other begomoviruses such as cotton leaf curl virus (Nateshan *et al*., 1996), *Tomato leaf curl Bangalore virus*-[Ban4] (ToLCBV-[Ban 4]) (Muniyappa *et al*., 2000) and pumpkin yellow vein mosaic virus (PYVMV) (Muniyappa *et al*., 2003) required only 5-15 *B. tabaci* to achieve 100% transmission efficiency. The whitefly-inoculated plants developed symptoms much quicker in about 4 weeks after virus inoculation.

Fig. 1: Typical symptoms of JMD on Jatropha. A whitefly-inoculated plant showing the symptoms of late infection where only the young leaves developed mosaic symptoms, reduced leaf size and malformed leaves (A). An early infected plant, probably through stem cutting, showing symptoms on all the leaves and abscission of flowers and sterility (B)

Detection of JMIV in Infected Plants and Viruliferous *B. tabaci*

PCR tests using two sets of degenerate primers (Deng *et al*., 1994; Wyatt and Brown, 1996) on Jatropha and dodder samples amplified expected PCR products of size ~530 bp and 575 bp from DNA-A of JMIV (Fig. 2). These and many other primers have been used elsewhere successfully to detect TYLCV and ToMoV, for example, in individual *B. tabaci* adults and infected plants (Mehta *et al*., 1994). The PCR results therefore demonstrate the utility of these two primer sets, in combination or alone, for accurate diagnosis of JMD, which is critical for disease management in a perennial plant such as Jatropha.

PCR was also used to monitor the persistence of JMIV in the adult *B. tabaci* that were fed on a diseased plant and maintained subsequently on cotton plants. The JMIV was detected in individual *B. tabaci* until their death (up to 8 days). However, the number of *B. tabaci* containing the virus and the intensity of the amplified PCR product reduced gradually with the increase in the incubation period, which indicate the decrease in virus concentration in the vector body by continuous feeding on a non-host plant, cotton and may also suggest indirectly the persistent circulative type of virus-vector relationship. Based on PCR and hybridization tests ToLCV-[Ban4] was also shown to share a persistent circulative relationship with *B. tabaci* (Muniyappa *et al*., 2000).

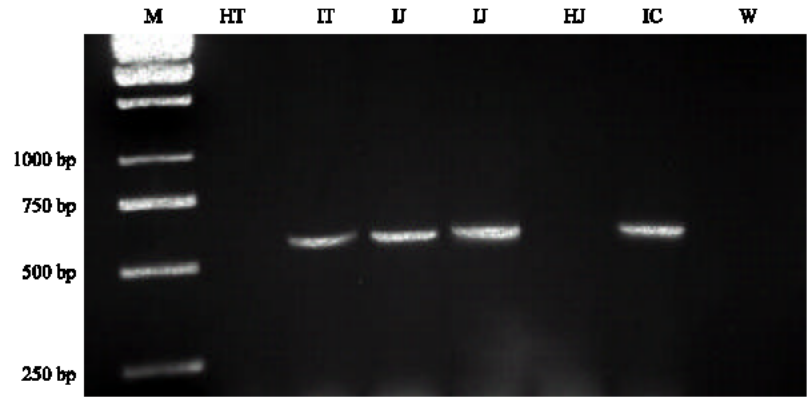

Fig. 2: A photograph of gel electrophoresis showing PCR products (~575 bp) amplified from the DNA-A of JMIV and other begomoviruses used as controls. Sizes of molecular weight markers (250 to 1000 bp) are shown on the left. M = 1 kb molecular weight marker (MBI Fermentas Life Sciences, Germany), HT = Healthy Tomato, IT = Tomato leaf curl virus-infected tomato, IJ = JMIV-infected Jatropha, HJ = Healthy Jatropha, IC = Cassava mosaic virus-infected cassava and W = Water control

Table 3: Comparison of JMIV percentage core CP nucleotide identities with those of selected begomoviruses

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
CLCuMV-[Bha]																							
ACMV	73.4																						
EACMV	73.0	93.9																					
EACMZV	73.7	77.0	81.3																				
SACMV	74.0	76.0	78.3	80.3																			
ICMV	79.5	73.2	74.0	73.0	73.5																		
ICMV-[Mah2]	79.1	72.0	73.0	71.8	72.7	97.2																	
ICMV-[pCTCRI]	80.0	71.3	71.8	72.7	73.2	92.0	91.8																
ICMV-[Ker2]	79.5	72.2	72.3	73.2	73.5	92.2	91.8	96.9															
ICMV-[Tri]	79.8	72.2	72.3	72.9	73.4	92.5	92.7	99.1	97.0														
SLCMV-[Ker]	79.5	72.0	72.6	71.6	73.2	95.5	95.6	95.5	95.5	96.0													
SLCMV-[Adi]	79.8	71.5	71.6	72.9	73.0	92.5	92.3	99.1	97.4	99.3	96.0												
SLCMV-[Col]	79.8	72.5	73.2	72.3	72.7	97.6	97.4	91.8	91.8	92.3	95.7	92.3											
SLCMV-[Sal]	79.3	71.6	71.6	72.7	73.0	91.8	91.5	97.2	99.0	97.4	95.1	97.7	91.5										
SLCMV-[TN7]	79.8	71.5	71.6	72.5	73.0	92.2	92.0	99.1	97.0	99.3	95.7	99.3	92.0	97.6									
JMIV-[Ban]	80.2	73.0	72.8	72.5	72.8	91.7	91.5	93.7	93.2	93.9	93.9	93.7	91.5	92.9	93.6								
JMIV-[Dha]	79.7	73.0	73.0	72.5	73.5	89.9	90.2	92.7	93.0	92.9	92.5	92.9	90.4	92.7	92.5	94.3							
JMV-[Jam]	72.4	65.1	64.6	67.3	68.4	72.1	72.8	71.2	70.7	71.7	72.2	71.3	73.9	70.6	71.3	73.3	75.2						
JMV-[PR]	71.2	65.9	66.5	67.4	70.2	72.9	72.5	72.5	72.2	73.0	72.7	72.4	73.0	72.2	72.4	72.8	73.4	94.9					
PLCV-[Kav]	83.3	72.0	72.1	74.9	73.9	82.3	81.5	81.6	81.7	82.1	82.4	81.9	81.7	81.4	81.7	81.0	81.9	75.5	71.1				
TbLCV-[Kar1]	83.0	73.0	73.5	73.2	72.2	81.2	80.8	81.2	81.2	81.4	81.6	81.2	81.0	80.9	81.0	81.6	81.2	72.3	72.0	88.9			
ToLCBV-[Ban2]	84.7	71.8	72.0	72.5	72.9	82.6	81.7	82.8	82.4	83.0	83.0	82.8	82.4	82.1	82.6	82.6	81.4	73.5	72.0	90.4	96.0		
ToLCNDV	87.1	73.1	73.5	75.5	74.6	81.5	81.7	82.4	82.2	82.9	81.9	82.2	81.8	81.5	82.2	82.9	81.9	71.0	70.8	81.9	83.3	84.7	

1. CLCuMV-[Bha], 2. ACMV, 3. EACMV, 4. EACMZV, 5. SACMV, 6. ICMV, 7. ICMV-[Mah2], 8. ICMV-[pCTCRI], 9. ICMV-[Ker2], 10. ICMV-[Tri], 11. SLCMV-[Ker], 12. SLCMV-[Adi], 13. SLCMV-[Col], 14. SLCMV-[Sal], 15. SLCMV-[TN7], 16. JMIV-[Ban], 17. JMIV-[Dha], 18. JMV-[Jam], 19. JMV-[PR], 20. PLCV-[Kav], 21. TbLCV-[Kar1], 22. ToLCBV-[Ban2], 23. ToLCNDV

Comparison of the Core CP Sequences

The core CP nucleotide sequences of JMIV-[Ban] and JMIV-[Dha] were compared with those of select begomoviruses obtained from the EMBL database. Phylogenetic analysis of the sequences grouped JMIV-[Ban] and JMIV-[Dha] in a separate cluster next to the cassava mosaic viruses from India and Sri Lanka (Fig. 3). They were 94.3% similar to each other and shared the highest nucleotide identity of 93.9% with both ICMV-[Tri] and SLCMV-[Ker] (Table 3). The core CP sequences of both ICMV (91.8-99.1%) and SLCMV (91.5-99.3%) isolates were highly variable and they also shared 91.5-99.3% nucleotide identities between them. JMIV isolates were equally similar in the ranges of 79.7-82.9% to begomoviruses infecting cotton, papaya, tobacco and tomato in India (Table 3).

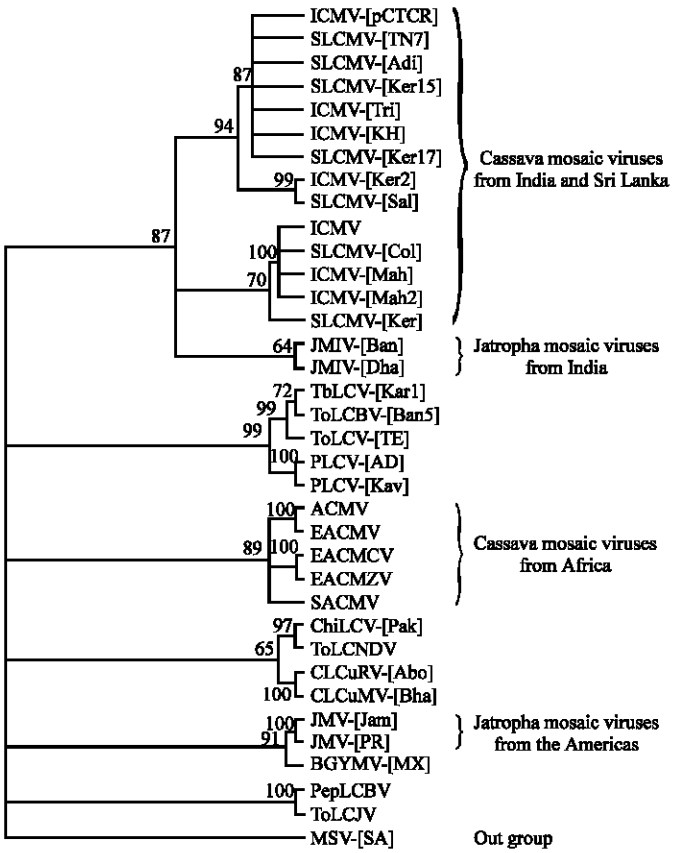

Fig. 3: Most parsimonious tree showing the relationship of JMIV to other selected gomoviruses based on core CP nucleotide sequences. Numbers at nodes indicate percentage bootstrap values. EMBL accession numbers for JMIV-[Ban] and JMIV-[Dha] are AM296493 and AM296494, respectively

The cassava mosaic viruses from Africa grouped separately from JMIV and shared 72.5-73.5% identities. JMV from Puerto Rico and Jamaica also grouped separately from JMIV and shared 72.8-75.2% core CP nucleotide identities, which suggest that JMD in India and the Americas was caused by two distinct begomoviruses.

Although the core CP sequences have been shown useful for begomovirus characterization and taxonomy at the global level, however, their utility in differentiating closely related viruses is questionable because in the parsimony analysis both ICMV and SLCMV were intermixed in two separate clusters supported by high bootstrap scores (Fig. 3). These results were similar to those of Rothenstein *et al.* (2006) where phylogenetic analysis revealed mosaic structures within the DNA-A sequences of ICMV and SLCMV, however, they may also indicate the frequent recombination events that occur between these viruses. In order to investigate this possibility, the ICMV, SLCMV and JMIV core CP sequences were subjected to recombination analysis (Weiller, 1998). The recombination profiles identified at least two separate recombinant viruses (SLCMV-[Ker] and ICMV-[Ker2]), their sequences shown as sharp downward peaks, in cassava from Kerala but not in JMIV (Fig. 4). These results support the grouping of viruses based on parsimony analysis and although collectively seem to suggest that JMIV may be a distinct virus, however, complete DNA-A sequences are required for precise identification.

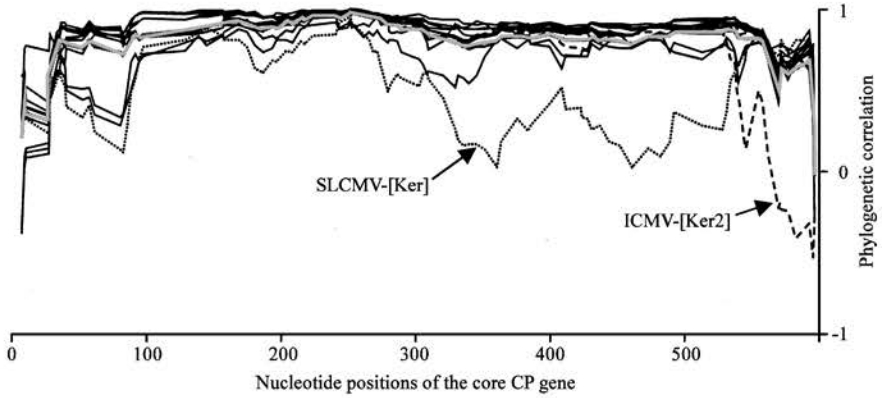

Fig. 4: Phylogenetic profile algorithm diagram revealing recombinant sequences (shown as sharp downward peaks) in the core CP of begomoviruses. Profiles were constructed for core CP sequences of ICMV, SLCMV and JMIV isolates and profiles with significant recombination are shown with dotted lines. The grey line indicates the position of the average algorithm

Conclusions

JMIV is the first non-cassava-infecting begomovirus that is highly related to cassava mosaic viruses and may pose a new additional threat to cassava cultivation by infecting this crop. However, JMIV is an immediate threat to Jatropha because of the proposed large-scale promotion of Jatropha cultivation in India for its oil to use as biofuel. Further, the disease assumes special significance in view of the recent introduction of the aggressive B-biotype *B. tabaci* in to southern India (Banks *et al*., 2001; Rekha *et al*., 2005). The B-biotype is known to transmit hidden begomoviruses from weeds to cultivated plants and has altered the epidemiologies of many begomoviruses worldwide. Whether or not JMIV was transmitted from a weed to Jatropha in India thus the disease emerged suddenly and only after the introduction of B-biotype to South India requires further transmission experiments using the B-biotype. Nonetheless, incidences of JMD increased when the B-biotype was introduced to Puerto Rico (Brown *et al*., 1999), thus it is highly likely that JMD incidences increase also in India, which is a threat to Jatropha and possibly to cassava cultivation. Moreover, Jatropha is perennial plant and serves as continuous source of inoculum for the virus. Further investigations to understand complete nature of the virus, epidemiology and disease control are required urgently in order to develop strategies for effective management of the disease.

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