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First molecular identification of a begomovirus in India that is closely related to *Cassava mosaic virus* and causes mosaic and stunting of *Jatropha curcas* L.

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Abstract. The association of a begomovirus with Jatropha mosaic disease has been found in north India. The begomovirus possessed highest identities and closest relationships with *Indian* and *Sri Lankan cassava mosaic virus* isolates.

Jatropha curcas L. (of family Euphorbiaceae) is grown in India as a major commercial fuel (bio-diesel) crop. The natural occurrence of a mosaic disease was noticed on *J. curcas* growing in experimental plots of the National Botanical Research Institute (NBRI), Lucknow, India in the years 2006 and 2007. The disease incidence was significant (~25%). The symptoms of the disease were severe with leaf mosaic and reduced size (Fig. 1*a*), and stunting of whole plants (Fig. 2). With an acquisition and inoculation period of 24 h, the disease could be transmitted by whitefly (*Bemisia tabaci*) in a persistent manner but could not be transmitted through mechanical inoculations using leaf sap of an infected plant onto *J. curcas* seedlings. The whitefly-inoculated *J. curcas* seedlings developed systemic mosaic and chlorosis at 25–30 days post-inoculation (Fig. 1*b*). Therefore, infection of a whitefly-transmitted begomovirus was suspected.

To confirm the association of a begomovirus, polymerase chain reaction (PCR) was carried out using as template total DNA extracted from naturally infected *J. curcas* leaf tissues. Three sets of begomovirus genus specific primers were employed: Deng A and Deng B (Deng *et al*. 1994), PALIv 1978 and PARIc 496 (Rojas *et al*. 1993), and those designed to amplify the coat protein (CP) gene of *Tomato leaf curl virus* (TLCV, Singh 2005). PCRs were set up in a 50 µL reaction mixture containing: template DNA (100 ng), dNTPs (10 mM each), primers (each 25 pmol), *Pfu* DNA polymerase (1.5 U), assay buffer (5 µL 10× Banglore Genei Pvt. Ltd) and were cycled 30 times (denaturation: 94°C for 5 min; specific annealing temperatures for 1 min according to the primers used; and extension: 72°C for 1.5 min). The annealing temperatures for Deng, Rojas and TLCV-CP primers were 52°C, 50°C and 47°C, respectively. The final extension cycle was 5 min

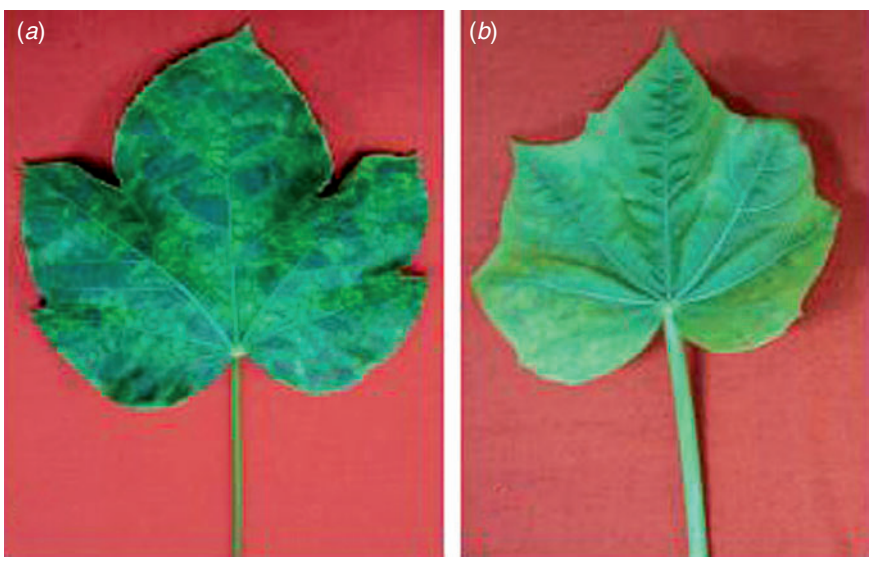

Fig. 1. *Jatropha curcas* showing mosaic symptoms on a naturally infected leaf (*a*) as compared with a whitefly-inoculated leaf (*b*).

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Fig. 2. Mosaic, reduced leaf size and stunting symptoms on infected *Jatropha curcas* (left) as compared with a healthy plant (right).

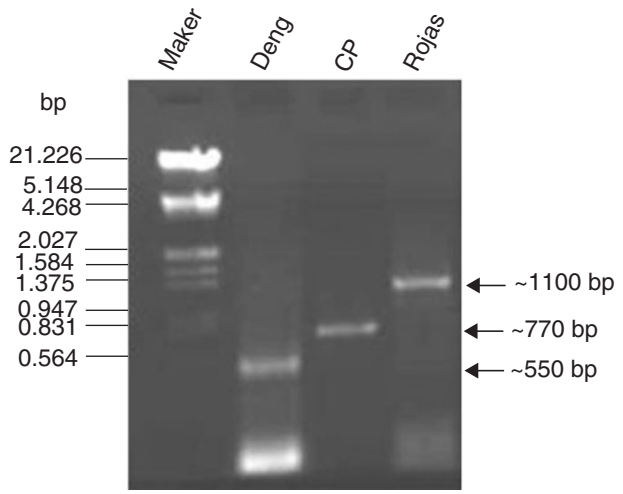

Fig. 3. Amplicons of ~550, 770 and 1100 bp obtained by Deng, TLCV-CP and Rojas primers respectively during PCR using total DNA from naturally infected *J. curcas* leaf tissue. Marker = Lambda DNA digested with *EcoR* I and *Hind*III.

at 72°C. PCR products were analysed by electrophoresis in 1.2% agarose gels. As expected, bands of ~550, 1100, and 770 bp were consistently amplified by Deng, Rojas and TLCV-CP primers, respectively (Fig. 3), which confirmed the association of a begomovirus with the mosaic disease of *J. curcas*.

Table 1. Percentage identities in the coat protein gene region of the virus isolate from *J. curcas* (EU113300) at nucleotide and amino acid levels with various begomoviruses based on Genomatix DiAlign programme

ICMV, *Indian cassava mosaic virus*; SLCMV, *Sri Lankan cassava mosaic virus*; JMIV, *Jatropha mosaic India virus*; JMV, *Jatropha mosaic virus*; ToLCRV, *Tomato leaf curl Rajasthan virus*; ToLCKV, *Tomato leaf curl Karnataka virus*; ToLCJV, *Tomato leaf curl Joydebpur virus*; ToLCNDV, *Tomato leaf curl New Delhi virus*; ToLCPV, *Tomato leaf curl Pune virus*; PLCBV, *Pepper leaf curl Bangladesh virus*; PLCV, *Papaya leaf curl virus*; CLCKV, *Cotton leaf curl Kokhran virus*; –, sequence not available

GenBank accession number	Abbreviation of the virus isolates	Natural host	Location	Identity (%)[A]	
				Nucleotide	Amino acid
AF423180	ICMV-Tri	Cassava	Kerala, India	94 (0.822)	97 (0.968)
AY312989	ICMV-Tri	Cassava	Kerala, India	94 (0.823)	96 (0.962)
AY998122	ICMV-Kolli	Cassava	Kolli hills, India	93 (0.814)	96 (0.960)
AJ575819	ICMV-Ker[2]	Cassava	Kerala, India	93 (0.814)	96 (0.962)
DQ780004	ICMV	Bittergourd	Coimbitore, India	93 (0.807)	–
AJ579307	SLCMV-Adv	Cassava	Adivaram, India	93 (0.814)	96 (0.958)
AJ607394	SLCMV	Cassava	Salem, India	94 (0.810)	96 (0.947)
AJ890225	SLCMV	Cassava	Kerala, India	93 (0.804)	96 (0.949)
AJ890224	SLCMV-Adv	Cassava	Kerala, India	93 (0.800)	95 (0.947)
AM296494	JMIV-Dha	Jatropha spp.	Dharawad, India	94 (0.606)	96 (0.755)
AM296493	JMIV-Ban	Jatropha spp.	Bangalore, India	90 (0.583)	96 (0.758)
AF058025	JMV	*J.gossypifolia*	Puerto Rico, USA	64 (0.194)	79 (0.533)
AF324410	JMV	*J.gossypifolia*	Jamica, West Indies	47 (0.098)	60 (0.272)
AJ875159	ToLCJV	Tomato	Joydebpur, Bangladesh	81 (0.519)	90 (0.889)
DQ339117	ToLCRV	Tomato	Rajasthan, India	79 (0.481)	91 (0.894)
AY754812	ToLCKV	Tomato	Janti, India	79 (0.488)	91 (0.894)
DQ629102	ToLCNDV	Tomato	New Delhi, India	77 (0.457)	89 (0.871)
AY754814	ToLCPV	Tomato	Pune, India	76 (0.433)	87 (0.839)
EF035481	PLCBV	Chilli	Kalyani, India	82 (0.512)	89 (0.874)
AF314531	PLCBV	Chilli	Bogra, Bangladesh	80 (0.491)	90 (0.888)
DQ376039	PLCV	Papaya	Gujarat, India	79 (0.477)	91 (0.900)
DQ376037	PLCV	Papaya	Delhi, India	79 (0.474)	91 (0.897)
AJ002449	CLCKV	Cotton	Pakistan	78 (0.464)	89 (0.877)
AJ496286	CLCKV	Cotton	Faisalabad, Pakistan	78 (0.464)	89 (0.877)

[A]Similarity values are shown in parentheses. 1.000 marks only the two most similar sequences.

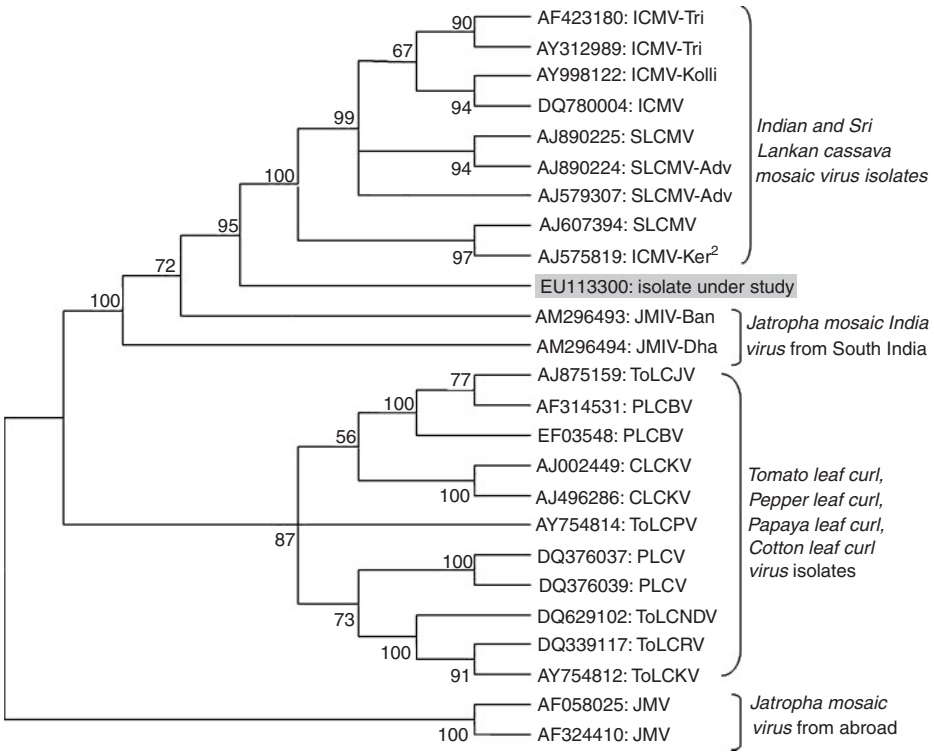

Fig. 4. Phylogenetic relationships of the virus isolate (EU113300) with *Indian* and *Sri Lankan cassava mosaic virus*, *Jatropha mosaic virus and* other begomovirus isolates reported from India and abroad. Phylogenetic tree generated in MEGA 4.0 version using 100 bootstrap values.

Further, the ~770 bp PCR amplicon obtained with TLCV-CP primers was cloned into pGEM-T easy vector system-1 (Promega Corporation, USA). Three clones were sequenced and the consensus data of three identical sequences was deposited in the GenBank database (Accession EU113300). Basic Local Alignment Search Tool (BLAST) analysis of the virus isolate showed 94.7% (730/771) sequence identity with *Indian cassava mosaic virus* sequences (AF423180, AY998122, DQ780004, AJ575819) and 94.4% (728/771) with *Sri Lankan cassava mosaic virus* (AJ579307, AJ607394, AJ890225, AJ890224), which are two begomovirus isolates reported from India (Saunders *et al*. 2002; Dutt *et al*. 2005). The nucleotide and amino acid sequences of the virus isolate also showed maximum 94–93% and 97–96% identities, respectively, with *Indian* and *Sri Lankan cassava mosaic virus* isolates when the Genomatix DiAlign program was used to align this new sequence with selected begomoviruses from a diverse range of host species (Table 1). Phylogenetic analysis of the virus isolate with selected begomovirus isolates using molecular evolutionary genetics analysis (MEGA) 4.0 version (Tamura *et al*. 2007) also revealed closest relationships with *Indian* and *Sri Lankan cassava mosaic virus* but a more distant relationship with *Jatropha mosaic virus* (Fig. 4). On the basis of positive PCR amplification, sequence analysis and phylogenetic relationships, the virus was identified as a begomovirus that is closely related to the isolates of *Indian* and *Sri Lankan cassava mosaic viruses* instead of *Jatropha mosaic virus*.

There are reports on occurrence of *Jatropha mosaic virus* on *J. gossypifolia*, a weed plant from Puerto Rico and Jamaica (Bird 1957; Roye *et al*. 2006) and natural infection of *Jatropha mosaic virus* on *J. curcas* has been reported in south India. However, the *Jatropha mosaic virus* infected *J. curcas* plants exhibited mosaic, leaf distortions and blistering (Narayana *et al*. 2006). Such distortions and blistering were never observed in *J. curcas* in the case of the virus under study. Neither *Indian cassava mosaic virus* nor *Sri Lankan cassava mosaic virus* has been reported earlier on *Jatropha curcas.* However, there is a report of *Indian cassava mosaic virus* on bittergourd (*Momordica charantia*) in Tamil Nadu, India (Rajinimala and Rabindran 2007). We report here the first molecular identification of a begomovirus in India that is closely related to *Indian* and *Sri Lankan cassava mosaic virus* that causes a mosaic and stunting symptoms on *J. curcas*.

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