

Molecular Detection of a Begomovirus Species on Chaya (*Cnidoscolus acontifolia*) from Madhya Pradesh, India which is Distantly Related to Sri Lankan Cassava Mosaic Virus

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Abstract

The natural occurrence of a Begomovirus associated with severe mosaic disease on Chaya (*Cnidoscolus acontifolia*) has been detected by PCR from Madhya Pradesh, India. The Begomovirus showed highest nucleotide sequence identities and distinct phylogenetic relationships of coat protein gene (CP) with several isolates of Sri Lankan Cassava Mosaic Virus (SrLCMV). To the best of our knowledge, this is the first report of SrLCMV infecting *Cnidoscolus acontifolia*, and it is a new host of Begomovirus from India.

Keywords: Chaya; Begomovirus; Mosaic disease; Coat protein; PCR; Sequence identities

Abbreviations: SrLCMV: Sri Lankan Cassava Mosaic Virus; ICMV: Indian Cassava Mosaic Virus; JMIV: Jatropha Mosaic India Virus; JCMV: Jatropha Curcas Mosaic Virus; AYMV: Acalypha Yellow Mosaic Virus; ToLCJV: Tomato Leaf Curl Joydebpur Virus; ToLCKV: Tomato Leaf Curl Karnataka Virus; ToLCV: Tomato Leaf Curl Virus; ChLCIV: Chilli Leaf Curl India Virus; CLCPV: Chilli Leaf Curl Pakistan Virus.

Introduction

Cnidoscolus acontifolius (Family Euphorbiaceae) commonly known as chaya or tree spinach is a large, fast growing leafy perennial shrub native to Mexico that produces lots of attractive, large, dark green leaves. It can grow well on a wide range of soils in both hot, rainy climates and areas with occasional drought [1]. Chaya has been introduced to Central and South America for potential uses as a leafy vegetable and medicinal plant. Chaya has been used for food since pre Columbian times, and is still eaten regularly, especially in Central America and southern Mexico. Chaya is a good source of protein, vitamins, calcium, and iron; and is also a rich source of antioxidants [2].

Only few reports are reported of virus diseases on chaya plant. The Cassava common mosaic virus (CCMV) isolates Genus *Potexvirus* were detected by serologically from chaya associated with mosaic disease in Florida [3] and identified by transmission electron microscopy (TEM), host range (biological) and RT-PCR in Venezuela [4].

The genus Begomovirus is a member of family Geminiviridae, which is the second largest group of plant viruses in all over the world. Begomoviruses are transmitted by whitefly (*Bemisia tabaci*) and cause important diseases in many dicotyledonous crops including important agricultural crop plants [5].

During a survey in February 2016, the Begomovirus-like symptoms including severe mosaic, leaf distortion, leaf curl, yellowing and stunting of whole plant were observed on chaya plant with the ~35-40% disease incidence in Bhojpur, Bhopal, Madhya Pradesh of India (Figure 1) grown by farmers as a hedge to protect crops. Three symptomatic plant samples were collected for detection and identification of associated Begomovirus on these plant species.



Fig. 1

Figure 1: Chaya (*Cnidoscolus acontifolia*) showing severe mosaic, leaf distortion, leaf curl, yellowing and stunting of whole plant (A) symptoms compared with healthy one (B).

To find the Begomovirus species is associated with the severe mosaic disease of chaya plant, the total DNA were isolated from 100 mg from newly emerging symptomatic leaves by the Dellaporta method [6] method and Polymerase chain reaction (PCR) was performed using a pair of Begomovirus coat protein (CP) gene specific primers CPIT-T/CPIT-T [7]. PCRs was set up in a 50 µl reaction mixture containing: template DNA (100 ng), dNTPs (10 mM each),

primers (each 25 pmol), Taq DNA polymerase (1.5 U), assay buffer (5 mL 10 Meark Pvt. Ltd) and were cycled 30 times (denaturation: 94°C for 5 min; 47°C; annealing temperatures: for 1 min; extension: 72°C for 1.5 min; and The final extension was 7 min) at 72°C.

The PCR products were analysed by 1% agarose gel electrophoresis. The PCR products showed the expected size ~800 bp amplicons in symptomatic plant samples (3/3) but no such amplicons were obtained in healthy one sample (Figure 2). Three positive PCR products were purified by using PCR Clean-up System Kit (Promega, USA) and the consensus sequence data of three identical sequences were analyzed and complete coat protein gene of 771 nucleotides of chaya isolate was submitted to NCBI GenBank database under Accession no. (KX343908).

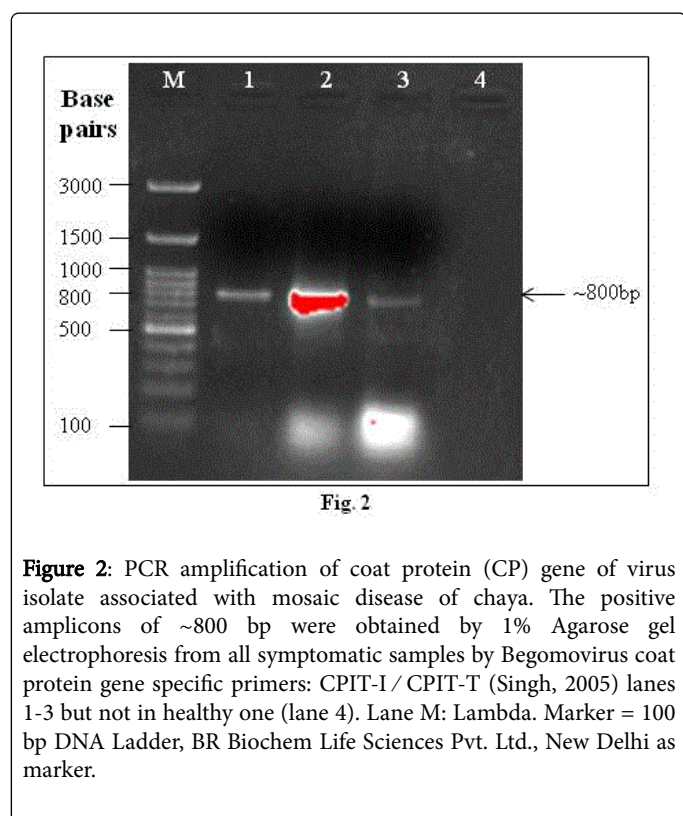


Figure 2: PCR amplification of coat protein (CP) gene of virus isolate associated with mosaic disease of chaya. The positive amplicons of ~800 bp were obtained by 1% Agarose gel electrophoresis from all symptomatic samples by Begomovirus coat protein gene specific primers: CPIT-I / CPIT-T (Singh, 2005) lanes 1-3 but not in healthy one (lane 4). Lane M: Lambda. Marker = 100 bp DNA Ladder, BR Biochem Life Sciences Pvt. Ltd., New Delhi as marker.

The BLASTn search analysis of coat protein gene based on nucleotide sequence of KX343908 revealed 96–98% sequence identity with various strains of Sri Lankan cassava mosaic virus isolates (SrLCMV: KP455486, KR611579, KC424490, KT861468) and Indian cassava mosaic virus isolates (ICMV: AY312989, KU308385, AF423180, AY998122) on cassava from India.

Nucleotide sequence identity between our isolate with other Begomovirus isolates retrieved from GenBank ranged between 83 and 96% like *Jatropha mosaic* India virus (JMIV: HM230683); *Jatropha curcas* mosaic virus (JCMV: JN692494) on jatropha; Acalypha Yellow Mosaic Virus (AYMV: JN807769, JN807766) on acalypha and croton; Tomato leaf curl Joydebpur virus (ToLCJV: KM383750, AJ875159) on tomato from Bangladesh; Tomato leaf curl Karnataka virus (ToLCKV: HM803118, HM851186) on tomato and Tomato leaf curl virus (ToLCV: EU263016, DQ343284) on menthe and soybean from India.

Pair-wise alignment analyses of nucleotide and amino acid sequences of the CP gene of the virus isolate (KX343908) was also

completed to compare it with CP gene sequences of selected SrLCMV isolates, and other Begomoviruses reported from cassava, jatropha, tomato, acalypha, croton and chilli host species using Genomatix DiAlign Program.

Pair-wise alignment of the virus isolate (KX343908) revealed the 78-97% sequence similarity at the nucleotide level and 89–99% similarity at the amino acid level with various isolates of Begomovirus (Table 1).

Accession number	Abbreviated virus name	Host plant	Location/ Country	% Nucleotide identity	% Amino Acid identity
KP455486	SrLCMV	Cassava	India	97 (0.925)	99 (0.994)
KR611579	SrLCMV	Cassava	India	97 (0.925)	99 (0.994)
KC424490	SrLCMV	Cassava	India	97 (0.925)	99 (0.994)
KT861468	SrLCMV	Cassava	Cambodia	97 (0.920)	99 (0.994)
AY312989	ICMV	Cassava	Trivandrum, India	97 (0.921)	99 (0.992)
AJ579307	SrLCMV	Cassava	Kerala, India	97 (0.919)	98 (0.989)
KU550961	SrLCMV	Cassava	India	97 (0.918)	99 (0.994)
KR611577	SrLCMV	Cassava	India	97 (0.922)	99 (0.994)
KU308385	ICMV	Cassava	India	96 (0.921)	99 (0.994)
AF423180	ICMV	Cassava	India	96 (0.910)	98 (0.989)
AY998122	ICMV	Cassava	Kolli hills, India	96 (0.910)	98 (0.982)
HM230683	JMIV	Jatropha	Lucknow, India	96 (0.888)	97 (0.958)
JN692494	JCMV	Jatropha	Lucknow, India	95 (0.880)	97 (0.969)
JN807766	AYMV	Croton	Lucknow, India	83 (0.560)	92 (0.911)
JN807769	AYMV	Acalypha	Lucknow, India	82 (0.560)	92 (0.916)
KM383750	ToLCJV	Tomato	Bangladesh	82 (0.528)	92 (0.916)
AJ875159	ToLCJV	Tomato	Bangladesh	82 (0.523)	91 (0.913)
HM803118	ToLCKV	Tomato	New Delhi, India	81 (0.505)	92 (0.916)
HM851186	ToLCKV	Tomato	New Delhi, India	81 (0.502)	92 (0.909)
DQ343284	ToLCV	Soybean	Lucknow, India	81 (0.492)	91 (0.900)
EU263016	ToLCV	Mentha	Lucknow, India	78 (0.498)	89 (0.895)
HM587709	ChLCPV	Chilli	Pakistan	80 (0.487)	91 (0.899)
FM877858	ChLCIV	Capsicum	India	79 (0.499)	91 (0.908)

Table 1: Percentage identities in the coat protein gene of the virus isolate from chaya (KX343908) at nucleotide and amino acid levels

with various Begomovirus isolates based on Genomatix DiAlign programme.

During phylogenetic analysis of CP gene of the Begomovirus under study (KX343908) together with isolates of SrLCMV, ICMV, JCMV, AYMV, ToLCJV, ToLCKV, ToLCV, and other Begomoviruses formed seven independent clusters by using MEGA 7.0 V tool. All SrLCMV isolates (KR611577, AJ579307, KT861468, KP455486, KR611579, KU550961, KC424490), ICMV (KU308385, AF423180, AY312989, AY998122) and our isolate under study (KX343908) clustered distinctly together in cluster I, while isolates of JMIV (HM230683) and JCMV (JN692494) clustered together in cluster II.

AJ875159), ToLCV (EU263016) & ChLCIV (FM877858) were clustered in cluster IV, ChLCPV (HM587709), ToLCV (DQ343284) and ToLCKV (HM803118, HM851186), respectively clustered in cluster V (Figure 3).

Although our Begomovirus isolate under study distinctly clustered in cluster I together with SrLCMV & ICMV, it showed a clear-cut divergence with them indicating distinct relationships.

On the basis of positive PCR amplification, sequence analysis and phylogenetic relationships, the Chaya virus isolate under study was identified as a Begomovirus that is distinctly related to the isolates of Sri Lankan cassava mosaic viruses (SrLCMV) and Indian cassava mosaic virus (ICMV).

At the present a few reports of Cassava common mosaic virus (CCMV) Genus Potexvirus have been reported from Florida [4] and Venezuela [5], however, there are no reports have been published in literature from India about any kind of plant viruses including Begomovirus on chaya plant.

Therefore, we report here the natural occurrence of mosaic disease on chaya (*Cnidoscopus acontifolia*) is a new host of Begomovirus species that is distinctly related to Sri Lankan cassava mosaic virus from Madhya Pradesh.

Because chaya plant is being propagated by cuttings, and new species of Begomoviruses are emerging in newly introduced locations in India, the Begomovirus species in future may also migrate from Madhya Pradesh to other states of India and therefore use of healthy planting material is recommended to restrict the Begomovirus disease.

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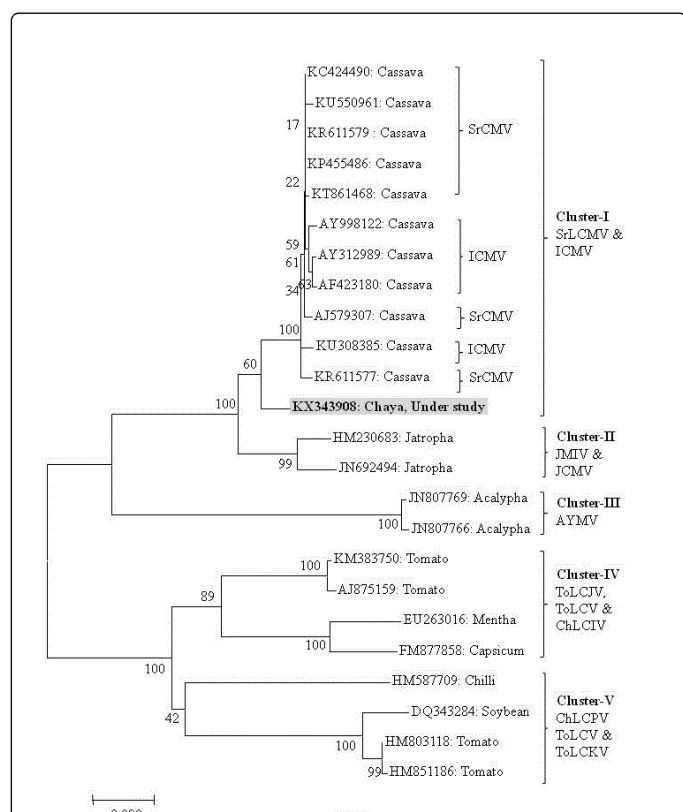


Fig. 3

Figure 3: Phylogenetic analysis of complete coat protein (CP) gene of Begomovirus isolate under study infecting chaya (KX343908) compared with various Begomovirus isolates reported all over the world including g India. The Begomoviruses isolate under study from chaya (KX343908) showed distinct relationships with SrLCMV, ICMV and other Begomovirus isolate reported from cassava, Jatropa, tomato, acalypha, croton, mentha, soybean and other plant 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.

AYMV (JN807769, JN807766) isolates clustered separately into cluster III. The other Begomoviruses like ToLCJV (KM383750,