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Complete genome sequence of an isolate of Clerodendron yellow mosaic virus – a new begomovirus from India

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Summary. – *Clerodendron inerme*, a common hedge plant grown in India, is affected by a yellow mosaic disease caused by a begomovirus. In the present study, the complete genome (DNA A) of this virus was cloned and sequenced. The total size of DNA A is 2760 nucleotides. The genome of this virus contains six open reading frames and a non-coding intergenic region of 293 nucleotides. Nucleotide sequence comparison analysis revealed maximum sequence identity with Papaya leaf curl virus-Pakistan [Pakistan:Cotton:2002] (73.9%). As this virus had less than 89% identity with other begomoviruses, it was identified as a new begomovirus species and tentatively, named as Clerodendron yellow mosaic virus-[India:New Delhi:2007] (ClYMV-[IN:ND:07]).

Keywords: Clerodendron; yellow mosaic disease; begomovirus

Introduction

Geminiviruses are a group of plant viruses causing several diseases in the tropical and subtropical regions of the world (Varma and Malathi, 2003). Species belonging to the genus *Begomovirus* with either mono- or bi-partite genomes, infect dicotyledonous plants and are transmitted by the whitefly, *Bemisia tabaci* (Genn). Bipartite begomoviruses consist of two genomic components designated as DNA A and DNA B. DNA A encodes a replication initiator protein, a coat protein, transcription activator protein and a replication enhancer, while DNA B encodes a movement protein and a nuclear shuttle protein. Both components are mutually dependent and share their gene products. Monopartite begomoviruses have only DNA A, but still have the ability to infect and cause disease in their hosts (Gutierrez *et al.,* 2004). The genome of the virus is a circular, single stranded DNA packed in icosohedral-twinned particle.

Clerodendron inerme, a common hedge plant grown widely in India, is affected by a severe yellow mosaic disease. Infected plants exhibit bright chlorotic spots along the midrib, reduction in leaf lamina, leaf deformation and stunting. The disease is so widespread that symptom-free plants are very rare. Association of a begomovirus with the clerodendron yellow mosaic disease was proved earlier (John *et al.*, 2006). However, complete genome sequence of the causal virus was lacking. Here we report the complete genome sequence of Clerodenron yellow mosaic virus (designated as ClYMV-Del1) from India and its comparison with other begomoviruses.

Materials and Methods

ClYMV-Del1 isolate was collected from a severely affected clerodendron plant from the fields of the Indian Agricultural Research Institute, New Delhi, and stem cuttings from diseased plants were raised in pots and maintained under glasshouse conditions. Total nucleic acid was extracted from infected leaf tissue using Gem-N'-N'-Cetyl trimethyl ammonium bromide (Gem-CTAB) method (Rouhibakhsh *et al.,* 2008). To amplify full length DNA A genome, primers CleroAF- 5′-AGTGCATGCACTCATGCCTCTA-3′ and

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Abbreviations: ICTV = International Committee on Taxonomy of Viruses; ClYMV = Clerodendron yellow mosaic virus

Table 1. Genome organization of ClYMV-[India:New Delhi:2007]

ORFs	Nucleotides	Molecular mass (kDa)	Predicted function
CP/V1	121–477	29.8	Coat protein
V2	281–1051	13.6	Movement
Rep/C1	1494–2588	41.3	Replication initiation
TrAP/C2	1193–1600	15.2	Transcription activation and silencing suppressor
REn/C3	1048–1452	15.9	Replication enhancer
C4	2174–2431	9.8	Silencing suppressor

CleroAR-5′-ATGGCATGCCATATACAACATA-3′ were designed based on coat protein gene sequence described earlier (John *et al.,* 2006) (GenBank Acc. No. AY950580). The PCR reaction mixture consisted of 1x PCR buffer, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTP, 2 ng/µl of each primer (CleroAF & CleroAR), 1 Unit of *Taq* DNA polymerase (MBI Fermentas, USA) and ~ 500 ng of total DNA. Total reaction volume was made up to 50 µl using sterile double distilled water. Following PCR program was used in a thermocycler (Bio-metra); initial denaturation at 94°C for 2 mins, followed by 30 cycles of denaturation (94°C for 1 min), annealing (55°C for 2 mins) and extension (72°C for 3 mins). At the end of 30 cycles, a final extension step at 72°C for 5 mins was set after which the temperature was held at 4°C. PCR products were analyzed by electrophoresis in a 1% agarose gel pre-stained with ethidium bromide (0.5 µg/ml) and photographed on a UV transilluminator. Attempts were made to determine the association of satellite DNA β with the ClYMV-Del1 using universal DNA β primers (β01/ β02) (Briddon *et al.*, 2003) through PCR and the association of DNA B through rolling circle replication and cloning methods (Haible *et al.,* 2006).

An approximately 2.7 kb DNA fragment was amplified and eluted from an agarose gel by using a kit according to the manufacturer's instruction (Qiagen, Genetix Biotech Pvt. Ltd). Eluted DNA fragment was cloned in pGEM-T Easy vector (Promega) and transformed into *Escherichia coli* strain DH5α. After transformation, white colonies were selected and screened by colony PCR. PCR procedure was followed as described earlier. Three colony-PCR positive colonies were selected and sequenced at the DNA sequencing facility available at University of Delhi (South Campus), Delhi. DNA sequence of these three clones was compared using BioEdit sequence alignment editor version 5.09.04 (Hall, 1999) and found identical. Nucleotide and amino acid sequence data were analyzed using BioEdit (Hall, 1999) and GENERUNNER software (Hastings Software Inc. Hastings, NY, USA; http://www.generunner.net). Phylogenetic analysis were done by using ClustalX (Thompson *et al.*, 1997) and TreeView 1.6.5 (Page, 1996).

Results and Discussion

Complete genome of ClYMV-Del1 isolate from India is 2760 nucleotides long and the sequence has been submitted to GenBank (Acc. No. EF408037). The DNA A component

Table 2. Nucleotide sequence identity comparison of ClYMV-Del 1 DNA A component (this study) and other begomoviruses from Asia

Virus	Nucleotide sequence identity (%)
ToLCKV-Jan[IN:Jan:05]	70.0
ToLCGV-[IN:Vad:99]	69.1
AEV-[NP:01]	71.4
TbCSV-[CN:Yn1:99]	71.7
ToLCJoV-BD[BD]	71.8
PaLCuV-PK[PK:Cot:02]	73.9
ToLCBV-B[IN:Ban5]	70.7
CLCuMV-Raj[IN:ND2:03]	69.7
CLCuMV-Bha[IN:Bha]	69.3
CLCuKV-Man[IN:Dab]	70.2
ToLCPKV-[PK:RYK1:04]	70.8
ChiLCV-PK[PK:Sho:05]	73.5
LYMV-[VN]	72.3
ToLCNDV-IN[IN:ND:Svr:92]	67.9
AYVV-TW[TW:Tai:99]	72.3
SLCMV-IN[IN:KerC4]	70.9
ICMV-IN[IN:Mah:88]	70.0
MYMIV-[IN:ND:Bg3:91]	59.1
MYMV-[IN:Vig]	60.8
PepLCBDV-PK[PK:Kha:04]	73.4
CYVMV-[IN]	72.3

ToLCKV-Jan[IN:Jan:05]-Tomato leaf curl Karnataka virus-Janti [India:Janti:2005]; ToLCGV-[IN:Vad:99]-Tomato leaf curl Gujarat virus-[India:Vadodara:1999]; AEV-[NP:01]-Ageratum enation virus-[Nepal:2001]; TbCSV-[CN:Yn1:99]-Tobacco curly shoot virus-[China:Yunnan 1:1999]; ToLCJoV-BD[BD]-Tomato leaf curl Joydebpur virus-Bangladesh [Bangladesh]; PaLCuV-PK[PK:Cot:02]-Papaya leaf curl virus-Pakistan [Pakistan:Cotton:2002]; ToLCBV-B[IN:Ban5]-Tomato leaf curl Bangalore virus-B [India:Bangalore 5]; CLCuMV-Raj[IN:ND2:03]-Cotton leaf curl Multan virus-Rajasthan [India:NewDelhi2:2003]; CLCuMV-Bha[IN:Bha]-Cotton leaf curl Multan virus-Bhatinda [India:Bhatinda]; CLCuKV-Man[IN:Dab]-Cotton leaf curl Kokhran virus-Manisal [India:Dabawali]; ToLCPKV-[PK:RYK1:04]-Tomato leaf curl Pakistan virus-[Pakistan: Rahim Yar Khan1:2004]; ChiLCV-PK[PK:Sho:05]-Chilli leaf curl virus-Pakistan [Pakistan: Shorkot:2005]; LYMV-[VN]- Luffa yellow mosaic virus-[Vietnam]; ToLCNDV-IN[IN:ND:Svr:92]-Tomato leaf curl New Delhi virus-India [India:New Delhi:Severe:1992]; AYVV-TW[TW:Tai:99]-Ageratum yellow vein virus-Taiwan [Taiwan:Tainan:1999]; SLCMV-IN[IN:KerC4]-Sri Lankan cassava mosaic virus-India [India:Kerala C4]; ICMV-IN[IN:Mah:88]-Indian cassava mosaic virus-India [India:Maharashstra:1988]; MYMIV-[IN:ND:Bg3:91]-Mungbean yellow mosaic India virus-[India:New Delhi:Blackgram 3:1991]; MYMV-[IN:Vig]-Mungbean yellow mosaic virus-[India:Vigna]; PepLCBDV-PK[PK:Kha:04]-Pepper leaf curl Bangladesh virus-Pakistan [Pakistan:Khanewal:2004]; CYVMV-[IN]-Croton yellow vein mosaic virus-[India].

contains six ORFs, two in virion sense and four in complementary sense. Details of the coding region, molecular weight of putative proteins and their predicted functions are given in Table 1. The non-coding intergenic region is 293 nucleotides long and contains the invariant nonanucleotide sequence, TAATATTAC. The replication initiation protein binding iteron sequence was identified as TGGGGACAC occurring as tandem repeats and 114 nucleotides upstream of TATA box. We were not able to find any DNA fragments using the universal DNA β primers through PCR. DNA B was not found to be associated with this disease after repeated attempts by employing the rolling circle replication and cloning methods. All the clones were found to be DNA A after sequencing.

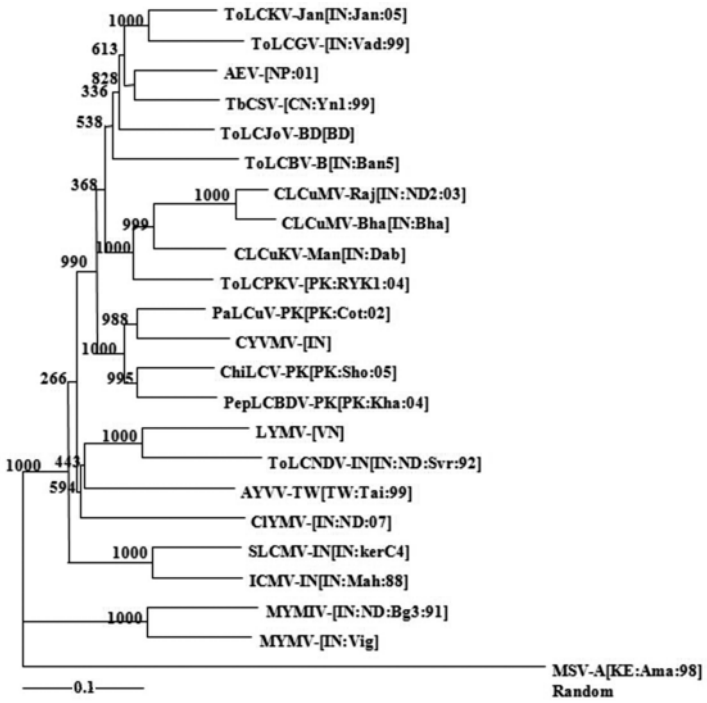

Fig. 1

Dendrogram showing relation between the complete nucleotide sequence of DNA A component of ClYMV-Del1 from India with GenBank begomovirus sequences

Dendrogram was constructed with bootstrapping (1000 replicates) using Clustal X and TREEVIEW software. The number at nodes refers to number of times branching was supported. Acc. Nos of :

ToLCKV-Jan[IN:Jan:05]-Tomato leaf curl Karnataka virus-Janti [India:Janti:2005] AY754812
ToLCGV-[IN:Vad:99]-Tomato leaf curl Gujarat virus-[India:Vadodara:1999] AF413671
AEV-[NP:01]-Ageratum enation virus-[Nepal:2001] AJ437618
TbCSV-[CN:Yn1:99]-Tobacco curly shoot virus-[China:Yunnan 1:1999] AF240675
ToLCJoV-BD[BD]-Tomato leaf curl Joydebpur virus-Bangladesh [Bangladesh] AJ875159
PaLCuV-PK[PK:Cot:02]-Papaya leaf curl virus-Pakistan [Pakistan:Cotton:2002] AJ436992
ToLCBV-B[IN:Ban5]-Tomato leaf curl Bangalore virus-B [India:Bangalore 5] AF295401
CLCuMV-Raj[IN:ND2:03]-Cotton leaf curl Multan virus-Rajasthan [India:NewDelhi2:2003] AY795605
CLCuMV-Bha[IN:Bha]-Cotton leaf curl Multan virus-Bhatinda [India:Bhatinda] DQ191160
CLCuKV-Man[IN:Dab]-Cotton leaf curl Kokhran virus-Manisal [India:Dabawali] AY456683
ToLCPKV-[PK:RYK1:04]-Tomato leaf curl Pakistan virus-[Pakistan: Rahim Yar Khan1:2004] AB116884
ChiLCV-PK[PK:Sho:05]-Chilli leaf curl virus-Pakistan [Pakistan: Shorkot:2005] DQ114477
LYMV-[VN]-Luffa yellow mosaic virus-[Vietnam] AF509739
ToLCNDV-IN[IN:ND:Svr:92]-Tomato leaf curl New Delhi virus-India [India:New Delhi:Severe:1992] U15015
AYVV-TW[TW:Tai:99]-Ageratum yellow vein virus-Taiwan [Taiwan:Tainan:1999] AF307861
SLCMV-IN[IN:KerC4]-Sri Lankan cassava mosaic virus-India [India:Kerala C4] AJ890226
ICMV-IN[IN:Mah:88]-Indian cassava mosaic virus-India [India:Maharashstra:1988] AJ314739
MYMIV-[IN:ND:Bg3:91]-Mungbean yellow mosaic India virus-[India:New Delhi:Blackgram 3:1991] AF126406
MYMV-[IN:Vig]-Mungbean yellow mosaic virus-[India:Vigna] AJ132575
PepLCBDV-PK[PK:Kha:04]-Pepper leaf curl Bangladesh virus-Pakistan [Pakistan:Khanewal:2004] DQ116881
CYVMV-[IN]-Croton yellow vein mosaic virus-[India] AJ507777.

Comparative sequence analyses of DNA A of ClYMV-Del1 with the Indian and other Asian begomoviruses revealed that the ClYMV-Del1 isolate shared with these viruses about 59–74% sequence identity at the nucleotide level (Table 2). Maximum identity was observed with Papaya leaf curl virus-Pakistan [Pakistan:Cotton:2002] (73.9%) followed by Chilli leaf curl virus-Pakistan [Pakistan:Shorkot:2005] (73.5%) and Pepper leaf curl Bangladesh virus-Pakistan [Pakistan:Khanewal:2004] (73.4%). Sequence identity of ClYMV-Del1 with other Asian begomoviruses was less than 70% at the nucleotide level and did not cluster with any of them in the phylogenetic analysis (Fig. 1). Therefore, the virus isolate ClYMV-Del1, is proposed as a new begomovirus species Clerodendron yellow mosaic virus-[India:New Delhi:2007] (ClYMV-[IN:ND:07]) according to ICTV as it shares less than 89% sequence identity with any other species in the genus *Begomovirus* (Fauquet *et al.*, 2008).

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