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GENOME REPORTS

Genome of a known but distinct begomovirus associated with a novel satellite molecule infecting a new host bitter gourd (*Momordica charantia*)

Karthikeyan Muthupandi[1] · Avinash Marwal[2] · Jebasingh Tennyson[1]

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Abstract
Coccinia mosaic Virdhunagar virus (KY860899), *Tomato leaf curl New Delhi virus* (KY860898) and *Tomato leaf curl Virdhunagar alphasatellite* (KY848691) were found to be associated with leaf curl disease in *Momordica charantia* (bitter gourd). The complete nucleotide sequence of *Coccinia mosaic Virdhunagar virus* showed 82% identity with *Coccinia mosaic Tamil Nadu virus* (KM244719), whereas *Tomato leaf curl New Delhi virus* was 96% identical to *Tomato leaf curl New Delhi virus* (KP868764) and *Tomato leaf curl Virdhunagar alphasatellite* illustrated 81% similarity with *Tomato leaf curl New Delhi alphasatellite* (JQ041697). Phylogenetic and RDP analysis revealed the proximity of these begomoviruses with other monopartite begomoviruses and alphasatellites already reported from India. As per the threshold criteria laid down by International Committee on Taxonomy of Viruses for species demarcation in begomoviruses and satellite molecules, the identified virus isolates, *Coccinia mosaic Virdhunagar virus* and *Tomato leaf curl Virdhunagar alphasatellite* are proposed as new species. To the best of our knowledge, this is the first ever account of mixed infection of begomoviruses in *Momordica charantia*, a vegetable crop commonly cultivated throughout India.

Keywords *Momordica charantia* · Leaf curl disease · Begomovirus · Alphasatellite · Mixed infection

Bitter gourd (*Momordica charantia*), a cucurbitaceous vegetable of Asian origin which is used as a medicinal herb in India, and is known for its anti-diabetic, anti-helminthic, antiviral, anti-malarial and anti-cancer properties (Alam et al. 2015). The cultivation of *Momordica charantia* is mainly affected by begomoviruses namely *Bitter gourd yellow vein virus* (Muhammad et al. 2010) and *Indian cassava mosaic virus* (Rajinimala and Rabindran 2007). Begomovirus is the largest genus of the family *Geminiviridae*, comprising the whitefly-transmitted geminiviruses that infect dicotyledonous plants. Begomoviruses have either monopartite (DNA-A) or bipartite (DNA-A and DNA-B) genomes (Stanley 1985; Marwal et al. 2013; Prajapat et al. 2014). DNA-A encodes proteins that are required for the replication, transcription and encapsidation; whereas, DNA-B encodes for proteins required for the virus movement. Begomoviruses are divided into New World and Old World groups according to their geographic origins. All the members of the New World begomoviruses are mostly bipartite, and Old World begomoviruses have either monopartite or bipartite genomes that interact with single-stranded DNA (ssDNA) satellites namely alpha- and beta-satellite. Both the satellite molecules depend upon their helper virus for replication and/or movement (Rojas et al. 2001; Marwal et al. 2018). Mixed infections with two or more virus species have been observed in several hosts, and symptoms are more rigorous than those observed in single viral infections in some occasions; however, mixed infections with begomoviruses have not been studied comprehensively in India (Rentería-Canett et al. 2011a, b). Mixed virus contamination is an important phenomenon in relation to virus evolution, as it is considered as a prerequisite for the recombination that may contribute to the generation of new begomovirus species seeking appropriate diagnosis and detection (Khurana and Marwal 2016). In this study, we report the presence of

✉ Jebasingh Tennyson
jebasinghs@gmail.com

[1] Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu 625021, India

[2] Department of Biotechnology, Mohanlal Sukhadia University, Udaipur, Rajasthan 313001, India

genome of *Coccinia mosaic Virdhunagar virus*, *Tomato leaf curl New Delhi virus,* and *Tomato leaf curl Virdhunagar alphasatellite* in *Momordica charantia* by analyzing their sequence identity, phylogenetic relationship, and recombination events in above-mentioned DNA segments.

Momordica charantia leaves showing mild curly symptoms were collected randomly from 5 selected plants from Varalotti village situated at the Virudhunagar district, Tamil Nadu. Total DNA was extracted from 500 mg of leaf sample (Porebski et al. 1997), and PCR-based detection was carried out with RUGEMF (5′TGTGARGGNCCHTGTAARGTHC3′) and RUGEMR (5′GCATGAGTACATGCCATRTAC3′) primers (Packialakshmi et al. 2010). This resulted in the amplification of ~ 500-bp DNA fragment, which confirmed the presence of begomovirus. Further, DNA was subjected for rolling circle amplification (RCA), followed by endonuclease digestions (*Bam*HI, *Xho*I, *Hin*dIII, *Kpn*I and *Sal*I), which confirmed the presence of two DNA molecules of sizes 2.7 and 1.3 kb, respectively (Sahu et al. 2015). Fragments released from RCA using *Bam*HI digestion were cloned in pUC18 vector. PCR, restriction analysis and sequencing of the clones revealed the presence of two types of DNA-A (clone 1 and 2) and one type of satellite DNA molecule. Using sequence demarcation tool, it was found out that the complete sequence of clone 1 showed 82% identity with *Coccinia mosaic Tamil Nadu virus* (KM244719). Based on DNA-A sequence comparison and ICTV species demarcation for begomoviruses at < 91% nucleotide sequence identity (Fauquet et al. 2008), the virus found in this study belongs to a new species of begomovirus. The complete sequence of clone 1 was deposited in the GenBank and named as *Coccinia mosaic Virdhunagar virus* (Accession number: KY860899) (Table 1). DNA of *Coccinia mosaic Virdhunagar virus* showed the presence of seven ORFs, two (AV1 and AV2) in the virion-sense and five (C1, C2, C3, C4 and C5) in the complementary sense strand. The ORFs were separated by an intergenic region (IR) of 451 bp, and the sequence was identified as TAATATTAC in the stem loop structure (Fig. 1a). The complete sequence of clone 2 showed 96% identity with *Tomato leaf curl New Delhi virus* (KP868764) and was deposited in the GenBank as *Tomato leaf curl New Delhi virus* (Accession number: KY860898). The sequence of *Tomato leaf curl New Delhi virus* showed the presence of eight open reading frames (ORFs), two (V1 and V2) in the virion-sense and six (C1, C2, C3, C4, C5 and C6) in the complementary sense strand. IR between the ORFs of 311 bp showed a sequence of TAATATTAC in the stem loop structure and some of the predicted proteins are unusually small/large than expected (Table 2).

Table 1 MIxS table highlighting the various parameters of two novel virus components (*Coccinia mosaic Virudhunagar virus* and *Tomato leaf curl Virudhunagar alphasatellite*) considered for molecular characterization and sequence submission

Features	*Coccinia mosaic Virudhunagar virus*	*Tomato leaf curl Virudhunagar alphasatellite*
Definition	*Coccinia mosaic Virudhunagar virus* DNA-A, complete sequence	*Tomato leaf curl Virudhunagar alphasatellite* strain severe, complete sequence
Particle shape	Enveloped, twin icosahedral	Enveloped, twin icosahedral
Investigation type	Virus	Virus
Accession number	KY860899.1	KY848691.1
Source of isolation	Leaves of *Momordica charantia*	Leaves of *Momordica charantia*
Classification	Viruses; ssDNA viruses; *Geminiviridae*; Begomovirus	Viruses; satellites; satellite nucleic acids; single-stranded DNA satellites; alphasatellites; begomovirus-associated alphasatellites
Molecular type	Genomic DNA	Genomic DNA
Isolate	Clone 1	–
Environment (material)	Plant material	Plant material
Isolation source	Leaves	Leaves
Host	*Momordica charantia*	*Momordica charantia*
Height	1.6 m above ground level	1.6 m above ground level
db_xref	Taxon: 2048880	Taxon: 2048879
Segment	DNA	Alphasatellite
Country	India	India
Geographical location	Madurai, Tamil Nadu	Madurai, Tamil Nadu
Latitude and longitude	9°56′36.1″N 78°00′23.4″E	9°56′36.1″N 78°00′23.4″E
Collection date	14-Mar-2016	14-Mar-2016
Collected by	Alagu sundaram	Alagu sundaram
Sequencing technology	Sanger dideoxy sequencing	Sanger dideoxy sequencing
Finishing quality	Finish (complete)	Finish (complete)

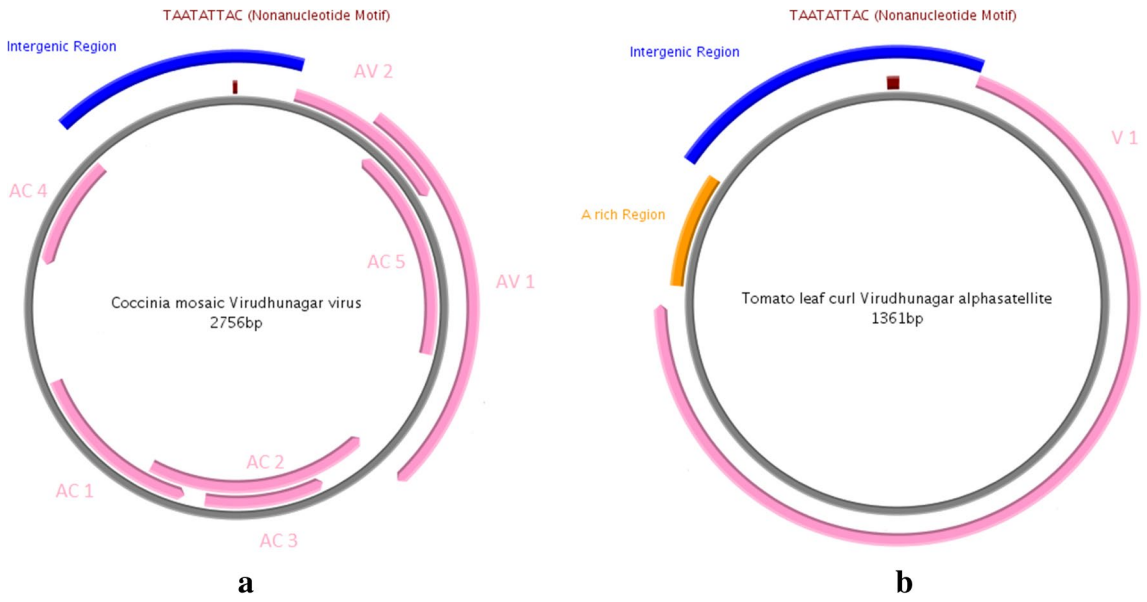

Fig. 1 Genome map of novel *Coccinia mosaic Virdhunagar virus* (KY860899) (**a**) and *Tomato leaf curl Virdhunagar alphasatellite* (KY848691) (**b**) isolated from *Momordica charantia* drawn with the help of PlasMapper http://wishart.biology.ualberta.ca/PlasMapper/. ORFs are marked in the virion-sense and complementary strand. Further information on the ORFs can be found in Table 2

The complete sequence of 1.3 kb clone showed 81% similarity with *Tomato leaf curl New Delhi alphasatellite* (JQ041697). Considering the species demarcation criteria of < 88% nucleotide sequence identity in case of alphasatellite (Mubin et al. 2009), we proposed that the isolated alphasatellite, failed to show this criterion that this was from a different species. The complete sequence of 1.3 kb clone was deposited in the GenBank (Accession number: KY848691) and named as *Tomato leaf curl Virdhunagar alphasatellite* (Table 1). This showed the presence of one ORF in the virion-sense strand, such as Rep protein (Fig. 1b). It showed an IR of 497 bp with the stem loop structure containing the sequence TAGTATTAC (Table 2).

The evolutionary history was inferred using the NJ Tree (Kumar et al. 2016) method, which analyzed the phylogenetic relationship of our isolates obtained from *M. charantia* plant by keeping bootstrap at 1000 using MEGA 7.0. Further, the phylogenetic analysis based on the complete DNA sequence of *Coccinia mosaic Virdhunagar virus* (KY860899) and other selected complete DNA sequences were clustered with this virus *Coccinia mosaic Tamil Nadu virus* (KM244719) [isolated from *Crossandra infundibuliformis*] and *Tomato leaf curl New Delhi virus* (KY97959) [isolated from *Coccinia grandis*] (Fig. 2a). *Tomato leaf curl Virdhunagar alphasatellite* (KY848691) exhibited maximum closeness by clustering with isolates of *Tomato leaf curl New Delhi alphasatellite* (JQ041697) from India and *Papaya leaf curl alphasatellite* (HQ668024) from Pakistan, harboring host tomato and Papaya, respectively (Fig. 2b). *Tomato leaf curl New Delhi virus* is a well-known begomovirus species that has been studied extensively by pioneers; hence, the phylogenetic analysis was omitted.

To determine the existence of recombination events in the reported viral sequences, RDP analysis was conducted using RDP4.2 program (Nehra et al. 2018). The analysis was based on alignments with full length sequences of selected begomoviruses available in the NCBI database. One single recombination breakpoint was observed in *Coccinia mosaic Virdhunagar virus* in the region of AC4 ORF. The major contributing parent in the recombinant sequence was found to be *Tomato leaf curl New Delhi virus* (KX710158), and the minor parent was *Papaya leaf curl virus* (JN135233). The position of the recombination in the sequence was ranging from 2116 bp (Beginning Breakpoint) to 2689 bp (Ending Breakpoint). The statistical significance of the analysis was represented using the *p* value, and values obtained in each of the algorithm were as follows: RDP (1.460×10^{-14}), GENECONV (2.607×10^{-09}), Bootscan (1.780×10^{-12}), MaxChi (2.001×10^{-09}), Chimaera (1.072×10^{-07}), SiScan (6.724×10^{-23}), 3Seq (9.712×10^{-05}), LARD (3.775×10^{-19}), and PhylPro (No Recombination Detected). Similar RDP analysis was performed for the complete sequence of *Tomato leaf curl Virudhunagar alphasatellite*, with a single recombination position from 1051 bp to 1163 bp in the alignment. The major and minor parents contributing to the alphasatellite genome were revealed to be *Tomato leaf curl alphasatellite* (KR612274) and *Croton yellow vein mosaic alphasatellite* (FN658711), respectively. Detection programs such as RDP (2.140×10^{-02}), GENECONV (3.809×10^{-04}), SiScan (1.397×10^{-02}), 3Seq ($6.977 \times$

Table 2 Sequence variability analysis in DNA segments and associated alphasatellite of begomovirus isolates obtained from *Momordica charantia*

DNA component	ORF[a]	Start codon (nucleotide coordinates)	Stop codon (nucleotide coordinates)	Predicted size (no of amino acids)	Predicted molecular weight (kDa)	Predicted highest amino acid identities (%)
Coccinia mosaic Virudhunagar virus (KY860899)	AV2	119	457	112	12.78	84 with *Squash leaf curl China virus* (AGV76861)
	AV1	279	1049	256	29.69	92 with *Chilli leaf curl virus* (ACY72169)
	AC5	309	794	161	17.77	55 with *Tomato leaf curl Palampur virus* (AHB73982)
	AC2	1046	1594	182	20.95	54 with *Tomato leaf curl Bangladesh virus* (AFJ39285)
	AC3	1177	1449	90	10.68	78 with *Coccinia mosaic Tamil Nadu virus* (YP_009056856)
	AC1	1497	1898	131	14.96	85 with *Tomato yellow leaf curl China virus* (AEB40039)
	AC4	2166	2423	85	9.41	87 with *Tomato leaf curl New Delhi virus* (ASU11085)
Tomato leaf curl New Delhi virus (KY860898)	AV2	157	441	95	10.81	99 with *Tomato leaf curl New Delhi virus* (CBJ17648)
	AV1	263	1057	264	30.61	100 with *Tomato leaf curl New Delhi virus* (CAO00517)
	AC5	293	655	120	13.06	94 with *Tomato leaf curl New Delhi virus* (AJE24745)
	AC3	604	774	56	6.47	96 with *Tomato leaf curl New Delhi virus* (AAD14627)
	AC6	941	1099	52	6.31	61 with *Tomato leaf curl Joydebpur virus* (AFJ54085)
	AC2	1303	1569	88	10.03	71 with *Tomato leaf curl New Delhi virus* (ABB52026)
	AC1	1909	2553	214	24.14	98 with *Tomato leaf curl New Delhi virus* (AKM49926)
	AC4	2220	2396	58	6.71	97 with *Tomato leaf curl New Delhi virus* (AKM49927)
Tomato leaf curl Virudhunagar alphasatellite (KY848691)	Rep	76	1020	315	36.44	80 with *Papaya leaf curl virus alphasatellite* (SCN47926)

Coding regions were identified through open reading frame finder and ExPASy translate tool. Amino acid identities were found through protein BLAST. Some segments were overlapping

[a]ORF codes are presented in Fig. 1

10^{-01}), and LARD (2.593×1^{-05}) generated recombination results; whereas, Bootscan, MaxChi, Chimaera, and PhylPro program were negative in detecting any recombination as significant. Likewise, *Tomato leaf curl New Delhi virus* was also excluded in the RDP study.

In the present study, occurrence of mixed infections was accompanied by diverse begomoviruses and their respective satellite DNA as well as appearance of newer viruses via recombination (Marwal et al. 2016) may pose a serious threat to many economical important crops (Varma and Malathi 2003). Previously, a number of cases have been reported where different begomoviruses were found to infect the same host plant. *Tomato severe leaf curl virus* (ToSLCV), *Tomato leaf curl Sinaloa virus* (ToLCSinV) and *Pepper golden mosaic virus* (PepGMV) were found to infect both tomato and pepper in Nicaragua (Ala-Poikelaa

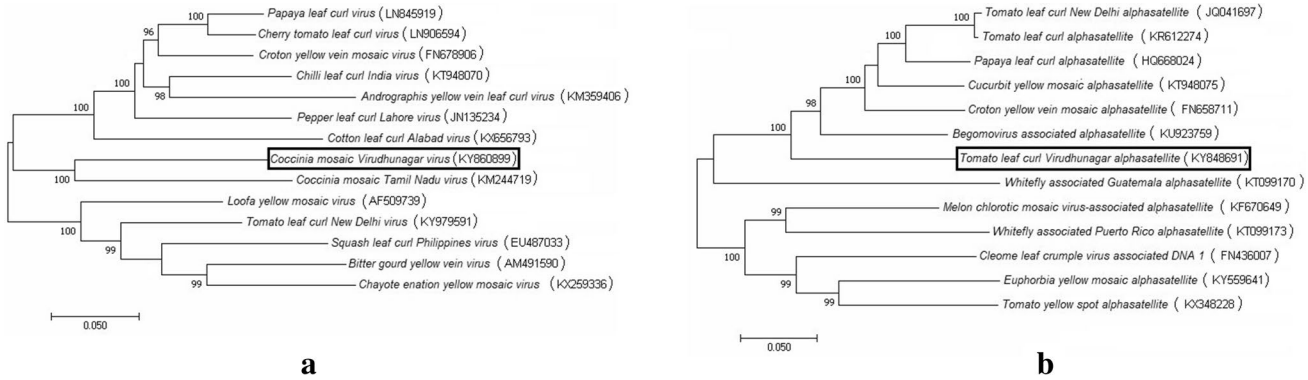

Fig. 2 Neighbour-joining (NJ) phylogenetic tree of aligned complete nucleotide sequences of the begomovirus of bitter gourd (*Momordica charantia*), *Coccinia mosaic Virdhunagar virus* (**a**) and the *Tomato leaf curl Virdhunagar alphasatellite* (**b**), with their close relatives. The accession numbers are shown in brackets. Bootstrap values > 95% are shown at the internodes. The scale bar represents 0.05 substitution per site

et al. 2005). Similarly, *Pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV) were reported in Mexico and Southern US infecting the same pepper plants (Rentería-Canett et al. 2011a, b). A mixed infection of Potyvirus, *Zucchini yellow mosaic virus* (ZYMV), and Geminivirus, *Tomato leaf curl New Delhi virus* (ToLCNDV), in *Momordica charantia* have also been previously reported by Sharma et al. (2015) from northern India. But, so far, no report is available to establish the association of begomoviruses mixed infection with satellite molecules in *Momordica charantia*. The present study for the first time, revealed the presence of two distinct begomoviruses, *Coccinia mosaic Virdhunagar virus* (KY860899) and *Tomato leaf curl New Delhi virus* (KY860898), and their satellite molecule *Tomato leaf curl Virudhunagar alphasatellite* (KY848691) in *Momordica charantia*. The existence of different begomoviruses in the same host plant might be due to the occurrence of two different *B. tabaci* harboring those viruses. This causes a series menace to other plant material signifying a strong possibility for varying and increased pathogenicity to reduced pathogenicity to null effect. Further research is needed to predict the specific virus–plant host–vector combinations in relation to precise interactions in mixed infection.

Compliance with ethical standards

Conflict of interest All the authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

References

Alam MA, Uddin R, Subhan N, Rahman MM, Jain P, Reza HM (2015) Beneficial role of bitter melon supplementation in obesity and related complications in metabolic syndrome. J Lipids 2015:496169

Ala-Poikelaa M, Svenssona E, Rojasa A, Horkod T, Paulind L, Valkonena JPT, Kvarnhedena A (2005) Genetic diversity and mixed infections of begomoviruses infecting tomato, pepper and cucurbit crops in Nicaragua. Plant Pathol 54:448–459

Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X (2008) Geminivirus strain demarcation and nomenclature. Adv Virol 153:783–821

Khurana SMP, Marwal A (2016) Recent developments towards detection and diagnosis for management of plant viruses. Indian Phytopathol 69:30–34

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Marwal A, Sahu AK, Gaur RK (2013) Molecular characterization of begomoviruses and DNA satellites associated with a new host Spanish Flag (*Lantana camara*) in India. ISRN Virol. https://doi.org/10.5402/2013/915703

Marwal A, Prajapat R, Gaur RK (2016) First report of recombination analysis of betasatellite and aplhasatellite sequence isolated from an ornamental plant marigold in India: an in silico approach. Int J Virol 12:10–17

Marwal A, Kumar R, Khurana SMP, Gaur RK (2018) Complete nucleotide sequence of a new geminivirus isolated from *Vitis vinifera* in India: a symptomless host of *Grapevine red blotch virus*. Virus Dis. https://doi.org/10.1007/s13337-018-0477-x

Mubin M, Briddon RW, Mansoor S (2009) Complete nucleotide sequence of *Chili leaf curl virus* and its associated satellites naturally infecting potato in Pakistan. Adv Virol 154:365–368

Muhammad T, Muhammad SH, Rob WB (2010) Complete nucleotide sequences of a distinct bipartite begomovirus, *Bitter gourd yellow vein virus*, infecting *Momordica charantia*. Arch Virol 155:1901–1905

Nehra C, Marwal A, Verma RK, Gaur RK (2018). Molecular characterization of Begomoviruses DNA-A and associated Beta satellites with new host *Ocimum sanctum* in India. In: Proceedings of the National Academy of Sciences, India Section B. https://doi.org/10.1007/s40011-018-1006-9

Packialakshmi RM, Srivastava N, Girish KR, Usha R (2010) A simple and efficient method for agroinfection of *Vernonia cinerea* with infectious clones of *Vernonia yellow vein virus*. Virus Genes 41:135–143

Porebski S, Bailey LG, Baum BR (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep 15:8–15

Prajapat R, Marwal A, Gaur RK (2014) Recognition of errors in the refinement and validation of three-dimensional structures of AC1 proteins of begomovirus strains by using ProSA-web. J Viruses. https://doi.org/10.1155/2014/752656

Rajinimala N, Rabindran R (2007) First report of *Indian Cassava Mosaic Virus* on bitter gourd (*Momordica charantia*) in Tamil Nadu, India. Australas Plant Dis Notes 2:81–82

Rentería-Canett I, Xoconostle-Cázares B, Ruiz-Medrano R, Rivera-Bustamante RF (2011a) Geminivirus mixed infection on pepper plants: synergistic interaction between PHYVV and PepGMV. Virol J 8:104

Rentería-Canett I, Xoconostle-Cázares B, Ruiz-Medrano R, Rivera-Bustamante RF (2011b) Geminivirus mixed infection on pepper plants: synergistic interaction between PHYVV and PepGMV. Virol J 8:104

Rojas MR, Jiang H, Salati R, Xoconostle-Cázares B, Sudarshana MR, Lucas WJ, Gilbertson RL (2001) Functional analysis of proteins involved in movement of the monopartite begomovirus, *Tomato yellow leaf curl virus*. Virology 291:110–125

Sahu AK, Nehra C, Marwal A, Gaur RK (2015) First report of a begomovirus associated with betasatellites infecting new host spinach (*Spinacia oleracea*) in India. J Gen Plant Pathol 81:146–150

Sharma S, Kang SS, Sharma A (2015) First report of mixed infection of *Zucchini yellow mosaic virus* and *Tomato leaf curl New Delhi virus* in bitter gourd in India. J Plant Pathol 97:391–403

Stanley J (1985) The molecular biology of geminiviruses. Adv Virus Res 30:139–177

Varma A, Malathi VG (2003) Emerging geminivirus problems: a serious threat to crop production. Ann Appl Biol 142:145–164