Iranian J Biotech. 2019 January; 17(1): e2134 DOI: 10.21859/ijb.2134
Research Article

Association of Tomato Leaf Curl New Delhi Virus, Betasatellite, and Alphasatellite with Mosaic Disease of Spine Gourd (*Momordica dioica Roxb.* Willd) in India

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Background: Spine gourd (Momordica dioica Roxb. Willd) is one of the important cucurbitaceous crops grown across the world for vegetable and medicinal purposes. Diseases caused by the DNA viruses are becoming the limiting factors for the production of spine gourd reducing its potential yield. For the commercial cultivation of the spine gourd, propagation material used by most of the growers is tuberous roots and stem cuttings, which in turn results in an increased occurrence of the mosaic disease. There is a need for understanding the causal agent; through characterization of which will lead to the designing management strategies for the spine gourd mosaic disease control.
Objectives: Characterization of a begomovirus and its satellites associated with mosaic disease on spine gourd.
Materials and Methods: Total DNA was extracted from spine gourd samples exhibiting symptoms typical to the begomoviruses infection (mosaic mottling, leaf curl) and was tested by PCR using begomovirus specific primers. Furthermore, the complete genome of begomo viruses (DNA A, DNA B, alpha satellite, and beta satellite) was amplified by rolling circle amplification (RCA) method.
Results: The full-length sequences of DNA A, DNA B, alpha satellite, and beta satellite isolated from symptomatic spine gourd were determined. The full length genomes (DNA A and DNA B) of the Tomato leaf curl New Delhi Virus (ToLCNDV) infecting spine gourd were compared with the other begomovirus genomes available in the data base. The sequence analysis has revealed that DNA A and DNA B components of the begomovirus infecting spine gourd share 95.4-96.2 and 86.7-91.2% identical sequence (i.e., nucleotide (nt) identity) with that of ToLCNDV infecting potato and cucurbits in the Indian subcontinent isolates reported earlier (available in GenBank), respectively. Further, alpha satellite and beta satellite were also detected in the begomovirus infected spine gourd samples. The recombination analysis of the DNA A, DNA B, beta satellite, and alpha satellite of the begomovirus infecting spine gourd showed the associated begomovirus and satellite DNAs were driven from the different begomoviruses, leading to emergence as a new variant of the begomovirus infecting spine gourd.
Conclusions: The commercial cultivation of the spine gourd by most growers depends on the tuberous roots and stem cutting. The occurrence of begomovirus in spine gourd gives an alarming signal against utilization of such infected plant materials in the crop breeding and improvement programs. Using the clean virus-free vegetative propagation material is considered as one of the most important methods for controlling viral diseases. The study is highly useful for detection of the begomovirus infecting spine gourd in the detection of the virus infection in the clonally propagated planting material.

Keywords: Alphasatellite; Betasatellites; Begomovirus; Recombination

1. Background

Spine gourd (*Momordica dioica* Roxb. Willd) is a perennial, rhizomatous, dioecious, climbing creeper which belongs to the family of *Cucurbitaceae* and commonly is known as Kakrol. It is originated from Indo-Malayan region and distributed throughout India, China, Nepal, Bangladesh, Myanmar, Pakistan, and Sri Lanka (1). In India, the crop is being widely cultivated in Orissa, Maharashtra, Karnataka, Andhra Pradesh, Bihar, and West Bengal for its good taste and high nutritional value. Generally, spine gourd is largely cultivated through vegetative propagation and is less propagated by seeds (2). The edible fruits contain a high amount of protein, carbohydrate, fiber, moisture, ash, iron, calcium, phosphorus, thiamine, riboflavin, and niacin (3). Apart from nutritional value, all parts of spine gourd have medicinal properties to cure various diseases and disorders in human being. It has a huge demand in the market, but still remains underutilized (4, 5) and commercially under-exploited due to its vegetative mode of propagation, dioecious nature, low percentage of the seed germination, and a long period of seed dormancy (4). The commercial propagation of the spine gourd mainly depends on the tuberous roots followed by stem cuttings. The Spine gourd plants showing symptoms typical to the begomoviruses' infection in the cucurbits is emerging as the major constraint for its production and making the availability of the healthy planting material difficult in the country.

Begomoviruses are single-stranded DNA viruses belonging to the family *Geminiviridae* with the morphology of geminate particles and are transmitted by the whitefly (*Bemisia tabaci*). Begomovirus could either have a monopartite or bipartite genome. The bipartite begomoviruses have two genome components referred to as DNA A and DNA B. The DNA A component contains five open reading frames (ORFs) encoding pre-coat protein, coat protein in the virion strand, as well as DNA replication-associated proteins in the complementary strand (6). The DNA B component contains two ORFs and encodes factors required for inter and intra-cellular movement in the host plants (7). Furthermore, based on the genome organization, genetic diversity, and geographical distribution, it has been further divided into two groups; the old world (OW) (Europe, Africa, Asia and Australia) and the new world (NW) (America) begomoviruses. The NW begomovirus is evaluated to be bipartite with lack of AV2 ORF in the DNA A component. Whereas both bipartite and monopartite begomovirus in the OW encodes AV2 ORF. The monopartite begomoviruses have a single genome analogous to the DNA A of the bipartite viruses with the association of additional ssDNA molecules known as betasatellites and/or alphasatellites (DNA1) (8). Betasatellites are approximately half the genome size of their helper begomoviruses and are required for inducing typical disease symptoms in their original hosts (9). Alphasatellites are self-replicating circular ssDNA molecules and depend on the helper virus for movement, encapsidation, vector transmission, and play no role in symptom induction (10).

2. Objective

Spine gourd is consumed by tribal groups living around the natural forest areas, especially at higher altitudes, where the native folks consume it as a daily vegetable. The spine gourd did not gain much popularity until it was discovered to have a high nutritional and medicinal value, which helps the development of body towards natural immunity from many common ailments. The disease caused by the begomovirus is the major constraint for production of the healthy planting material as well as its production in the country. The virus infected plants are exhibiting severe mosaic, mottling, and leaf curl symptoms. The disease incidence is ranged from 50-60% across different farmer's fields at Varanasi, Uttar Pradesh state of India. Therefore, the current study was undertaken to characterize the probable begomovirus and its associated satellites with the mosaic disease of the spine gourd in India.

3. Materials and Methods

3.1. Virus Source

Five leaf samples of spine gourd exhibiting mosaic, mottling, leaf curl, and distortion symptoms (**Fig. 1A-1D**) and two samples from non-symptomatic plants were collected during 2014-15 from different farmer's fields of the Varanasi, Uttar Pradesh state, India.

3.2. DNA Isolation, PCR-Mediated Amplification, and Sequencing

Total DNA was extracted from symptomatic and non-symptomatic leaf samples using CTAB (11). The presence of begomovirus infection in the spine gourd was tested by PCR using begomoviruses specific degenerative primers (12). The full-length genomic DNA components were amplified from the virus-infected samples using a TempliPhi illustra amplification kit (GE Healthcare, Piscataway, NJ). The resulted rolling circle amplification (RCA) product was digested with *BamH1* (DNA-A) and *XbaI* (DNA-B) restriction endonucleases for isolation of the monomeric units of the genome, cloned into pUC19 plasmid (13), and were transformed into the competent *Escherichia coli* DH5α strain. Restriction digestion was performed for confirming recombinant clones. Similarly, to identify the association of the sample with satellite genomes, the total DNA was amplified using betasatellite (DNA-β) (14) and alphasatellite (15) specific primers. The amplified PCR products were ligated into pTZ57R/T vector (INSTA cloning kit, (Thermo Fisher Scientific Inc., PA). Recombinant clones were identified by restriction endonuclease digestion for the presence of the cloned products. The selected clones were sequenced with an automated sequencing ABI PRISM 3730 (Applied Biosystems) at Amnion DNA Sequencing facility, Bengaluru, Karnataka, India.

Figure 1. Spine gourd plant showing (A) a mild mosaic, (B) severe mosaic, (C) and (D) drying of the fruits under natural conditions.

3.3. Sequence Analysis and Detection of Recombination Events

The sequences obtained were initially analyzed using the Vector NTI AdvanceTM 9 software to remove vector sequences. Further, the sequences were verified for the presence of all begomovirus specific Open reading frames (ORFs) using NCBI ORF finder and conserved nonanucleotide sequence. The selected begomovirus species (Table S1), betasatellites (Table S2) and alphasatellites (Table S3) accessions showing highest percentage sequence identity/similarity/ homology with the current sequences in the study were retrieved from the GenBank for analysis. The retrieved sequences were aligned with the present isolate using the MUSCLE method in SDT version 1.2 (16) and percent pair-wise nt identities were generated. The phylogenetic tree was generated by MEGA 7 software (17) using the neighbour-joining method with 1,000 bootstrapped replications to estimate evolutionary distances between all pairs of the sequences. Split-decomposition trees were constructed with 1,000 bootstrap replicates based on parsimony splits as implemented in SplitsTree version 4.11.3 with default settings (18). Recombination analysis was carried out using the recombination detection program (RDP), GENECOV, Bootscan, Max Chi, Chimera, Si Scan, 3Seq which are integrated in the RDP4 (19). The default RDP settings with 0.05 *P*-value cut off throughout and standard Bonferroni correction were used.

4. Results

4.1. Genome Organization of DNA A Component of Begomovirus

The spine gourd leaf samples showing symptoms typical to the begomovirus infection were showed positive PCR amplification to begomovirus specific primers and no amplification was observed from non-symptomatic samples. Further, the full genome components (DNA A and DNA B) were amplified and the resultant amplified products of 2.7 kb were cloned into pUC19 plasmid. The representative ten recombinant plasmids were sequenced and confirmed by BLAST analysis. The DNA A sequences of the five begomovirus isolates characterized from spine gourd showed 99.8-100 percent nt sequence identity among themselves indicating that they belong to a single species, based on species demarcation criteria for the begomoviruses; 91% nt sequence identity for complete genome (20). Therefore, one representative from the begomovirus isolate of SPYG1 was selected for amplification of the DNA A and DNA B as well as alpha and betasatellite of the begomovirus infecting spine gourd.

4.2. Sequence Identities of the DNA A and DNA B Components with Other Begomoviruses

The DNA A and DNA B component of the begomovirus isolate SPYG1were determined to be 2745 nt and 2696 nt in length, respectively and were deposited in the GenBank database (Accession no.: KY780213 and KY780214). The SDT analysis of the DNA A component of the isolateSPYG1 infecting spine gourd showed the highest nt identity of 95.4-96.2 per cent with the isolates of the Tomato Leaf Curl New Delhi irus (ToLCNDV) infecting potato (EF043231, EF043230, AY286316, AM850115) in the India (**Table 1**), which is followed by the isolates of ToLCNDV reported on cucurbits (83.8 -84.2%), chilli (93.7-95.8%), tomato (92.8-94.9%), and eggplant (93.3%) from India (**Table 1**). While DNA B-like sequence showed the highest level of nucleotide identity of 86.7-91.2% with the isolates of ToLCNDV infecting cucurbits crops (AM286435, AB330080, AY939924, HM989846, JN208137, KC545813, DQ020490) in the Indian subcontinent, which are summarized in the database (**Table 2**). Based on the current species demarcation criteria for the begomoviruses (91% nt sequence identity among complete genome) (20), the begomovirus isolate (SPYG1) with more than 95 percent nt sequence identity with ToLCNDV-pot is considered as a variant of the ToLCNDV. This result was well supported by the phylogenetic analyses of the both DNA A and DNA B components of the SPYG1 isolate by grouping with Indian isolates of ToLCNDV infecting tomato, potato, and cucurbits crops in the India and China (**Fig. 2A** and **2B**).

Table 1. The pairwise percent of nucleotide identities between the genomic components and amino acid sequence identities of the encoded genes from the ToLCNDV-(IN: SPYG1: Var: 15) with the components and genes of the selected begomoviruses available in the databases.

Begomovirus#	Genome DNA A	IR	Gene (percentage amino acid sequence identity)						
			AV2	CP	Rep	TrAP	REn	AC4	AC5
ToLCNDV-Cucurbits (14)*	93.6-96.1	80.3-94.9	86.6-95.5	96.0-98.0	85.1-98.0	76.2-96.2	78.6-94.1	77.5-91.3	65.2-90.6
ToLCNDV-Potato (4)*	95.4-96.2	96.3-100	94.6-96.4	97.2-98.4	95.5-96.3	88.4-89.9	35.2-89.7	94.8-100	87.5-92.5
ToLCNDV-Tomato (11)*	92.8-94.9	90.9-96.0	90.1-95.5	95.7-97.6	93.9-95.8	87.7-92.8	82.3-93.3	86.2-91.3	67.0-90.0
ToLCNDV-Chilli (4) *	93.7-95.8	92.7-96.0	92.8-95.5	97.2-98.0	94.1-97.2	90.6-96.4	90.4-94.1	89.6-96.5	85.7-88.8
ToLCNDV-Eggplant (1)*	93.3	93.8	93.7	96.8	94.1	90.6	91.1	86.2	-
ToLCPaV-Tomato (2)*	84.1-85.1	79.2-82.1	66.9-70.4	90.6	87.4-87.7	78.4	79.4-80.1	84.4-86.2	56.5
ToLCPaV-Cucurbits (24)*	83.8 -84.2	77.0-81.4	65.3-71.3	87.1-91.4	84.3-87.1	72.9-78.4	73.7-80.8	73.1-87.9	57.1
SLCCNV-Pumpkin (5)*	87.0-90.4	79.4-82.0	90.1-91.9	94.1-97.6	88.9-91.9	67.6-69.7	61.7-81.1	75.8-79.3	81.9-86.9
MYMIV (2)	66.2-67.2	42.2-42.9	43.3-41.5	73.1-74.3	70.1-70.4	37.6-42.1	37.5	27.2-28.2	11.1

* Numbers of sequences from the databases were used in the comparisons.

IR- Intergenic region

#The species are indicated as, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Palampur virus* (ToLCPaV), *Squash leaf curl China virus* (SLCCNV), *Mungbean yellow Mosaic Indian virus* (MYMIV). For each column, the highest value is underlined.

Table 2. The pairwise percent of the nucleotide identities between the genomic components and amino acid sequence identities of the encoded genes from the ToLCNDV-(IN: SPYG1: Var: 15) with the components and genes of the selected begomoviruses available in the databases.

Begomovirus#	Genomea DNA B[a]	IR[a]	Gene (percentage amino acid sequence identity)	
			BV1[b]	BC1[b]
ToLCNDV-Tomato (13)	82.0-89.8	51.8-86.4	75.7-97.0	87.3-94.3
ToLCNDV-Potato (6)	87.1-87.4	77.2-84.0	93.2-94.4	92.1-92.8
ToLCNDV-Chilli (4)	85.0-90.0	75.3-85.3	93.2-94.7	87.3-92.8
ToLCNDV-Cucurbits (9)	86.7-91.2	64.3-86.2	80.5-98.1	92.1-95.0
ToLCPaV-Tomato (3)	68.5-69.1	52.0-52.9	78.3-79.1	88.2-89.6
ToLCPaV-Cucurbits (12)	68.2-68.9	44.9-54.7	77.9-79.1	86.1-89.3
SLCCNV-Pumpkin (4)	62.1-62.7	47.0-49.1	72.7-72.3	86.1-88.6
ToLCNDV-Okra (1)	886	783	958	928
MYMIV (2)	41.9-42.1	26.8-27.8	25.0-25.2	38.7-39.0

[a]Nucleotide identity; [b] Amino acid identity

BV1=Nuclear shuttle protein gene, BC1=movement protein gene

#The species are indicated as, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Palampur virus* (ToLCPaV), Squash leaf curl China virus (SLCCNV), *Mungbean yellow Mosaic Indian virus* (MYMIV). For each column, the highest value is underlined.

Table 3. The percentages of nucleotide and amino acid sequence identities between betasatellite of the spine gourd (DNAβ) and betasatellites of other begomoviruses

Betasatellites*	Complete sequence of DNAβ (percentage NSI)	Percentage amino acid sequence identity of βC1 gene
ToLCJoB (17)	81.7-87.0	85.7-100
ToLCBDB (14)	61.1-63.5	38.7-61.1
ChiLCB (7)	60.4-62.9	58.7-60.3
ToLCPnB (2)	70.6-71.7	67.4-73.8
ToLCuB (2)	61.6-63.1	57.7-57.1
CroYVMB (3)	53.7-54.5	46.0-49.2
RaLCB (1)	561	523

* Numbers of sequences from the databases used in the comparisons.

#The species are indicated as Tomato leaf curl Joydepur betasatellite (ToLCJoB), Tomato leaf curl Bangladesh betasatellite (ToLCBDB), Tobacco leaf curl Patna betasatellite (ToLCPnB), Tomato leaf curl betasatellite (ToLCB), Croton yellow vein mosaic betasatellite (CroYVMB), Radish leaf curl betasatellite (RaLCuB). For each column, the highest value is underlined.

Table4. Percentages of the nucleotide or amino acid sequence identities between alphasatellite of spine gourd and alphasatellite of the other begomoviruses

Alphasatellites#	Complete sequence of DNAD1 (percentage NSI)	Percentage amino acid sequence identity of Rep gene
NVLP (7)	72.2-76.7	62.5-93.9
CLCuBuVD1 (3)	74.7-75.3	93.2-93.9
AEVD1 (2)	72.6-75.7	88.5-91.7
ToCSVD1 (2)	72.0-72.9	90.0-90.1
SiLCNVD1 (2)	69.9-70.2	88.2-88.5
CyTLCuD1 (3)	69.6-70.0	79.0-89.2
ToLCKVD1 (1)	76.9	93.9
CLCuVD1 (1)	71.2	86.6
OLCuDD1 (1)	74.8	91.1
BYVMVD1 (1)	74.9	91.1
PaLCuVD1 (1)	70.7	86.3
ToLCYCNVD1 (1)	69.8	86.9
SnLCuKVD1 (1)	74.5	84.4
SiLCVD1 (1)	69.6	90.1

The species are indicated as, Nanovirus-like particle (NVLP) , Cotton leaf curl Burewala alphasatellite (CLCuBuVD1), Ageratum enation alphasatellite (AEVD1), Tobacco curly shoot alphasatellite (ToCSVD1), Sida yellow vein China alphasatellite (SiLCNVD1), Cyamopsis tetragonoloba leaf curl alphasatellite (CyTLCuD1), Tomato leaf curl Karnataka alphasatellite (ToLCKVD1), Cotton leaf curl alphasatellite (CLCuVD1), Okra leaf curl virus alphasatellite (OLCuDD1), Bendhi yellow vein mosaic alphasatellite (BYVMVD1), Papaya leaf curl alphasatellite (PaLCuVD1), Tomato yellow leaf curl China virus (ToLCYCNVD1), Sunflower leaf curl Karnataka alphasatellite (SnLCuKVD1), Sida leaf curl alphasatellite (SiLCVD1).For each column, the highest value is underlined.

ORF wise, the amino acid (aa) sequence identities at the protein level showed the highest aa sequence identities with the different isolates of ToLCNDV, the regions *viz.* pre-coat (AV2), coat protein (CP), C4, and C5 shared maximum amino acid identity with the ToLCNDV infecting potato. Whereas, Rep (C1), TrAP (C2), and REn (C3) regions shared the most identity with the ToLCNDV infecting chilli (**Table 1**) in the DNA A component.

In the IR region, the identity of begomovirus (i.e., clone SPYG1) was more than 96% with the intergenic regions (IRs) of the reported ToLCNDV infecting potato isolates (**Table 1**). The length of IR is 275 nt and similar to those of bipartite begomoviruses reported so far for the DNA A component.

Similarly, individually encoded proteins of the DNA B component were compared. The highest amino acid (aa) sequence similarities were found for movement protein (80.5-98.1%) and nuclear shuttle protein (92.1-95.0%) with that of ToLCNDV infecting cucurbitaceous crops (**Table 2**). The length of the IR is 311 nt and similar to those of ToLCNDV isolates available in the database and shared 51.8-86.4 percent identity with the ToLCNDV isolates infecting tomato.

4.3. *Genome Organization of the Alpha and Betasatellite and Sequence Affinities to Other Alpha and Betasatellite*

The alphasatellite (DNA D1) and betasatellite (DNA β1) isolated from infected spine gourd were determined to be 1388 nt (Acc NO. MG571523) and 1360 nt (Acc. NO. MG571522) in length, respectively.

The alphasatellite have a single large ORF with a coding capacity of 315 aa in the virion-sense (coordinates 89-1036) (21). The sequence showed the maximum nt sequence identity (76.9%) with Tomato leaf curl Karnataka alphasatellite (ToLCKVD1, Acc. NO JX570736) (**Table 4**). Since the alphasatellite sequence was showing very low levels of nt sequence identity (less than 77%) with other isolates reported earlier and there is no proposed species demarcation threshold for classification of the alphasatellites, the present alphasatellite will be named as spine gourd mosaic alphasatellite.

The phylogenetic analysis of this studied genome component shown in **Figure 3A** indicated that the alphasatellite isolated from spine gourd segregated separately from all other satellites infecting different crops. Similarly, betasatellite sequence in the current study contains all the features typical to the betasatellites reported so far (12, 14) and showed maximum nt identity of 81.7 to 87 percent with the tomato leaf curl Jodeypur betasatellite (ToLCJoB) isolates originating from the Indian subcontinent infecting different crops (**Table 3**). Based on the recently proposed species demarcation threshold for betasatellites (22), the betasatellite identified here is an isolate of ToLCJoB infecting chilli and tomato, which is well supported by phylogenetic analysis (**Fig. 3B**).

4.5. *Neighbor-Net and Recombination Analysis*

A neighbor-network, Pairwise Homoplasy Index (PHI) test and breakpoint analysis for the recombination was carried out for nt sequences of the different begomoviruses genomic components (DNA A, DNA B, betasatellite, and alphasatellite) along with the sequences of the virus isolated from the spine gourd. This revealed that SPYG1 isolate was evolved through recombination.

The RDP 4 analysis indicated the evidence of recombination in ToLCDNV (SPYG1) infecting spine gourd with most of the DNA A fragments derived from ToLCNDV, Squash leaf curl China virus (SLCCNV) and Tomato leaf curl Palampur virus (ToLCPaV) to emerge as a new strain of ToLCNDV infecting spine gourd (Table S4, **Fig. 4**).

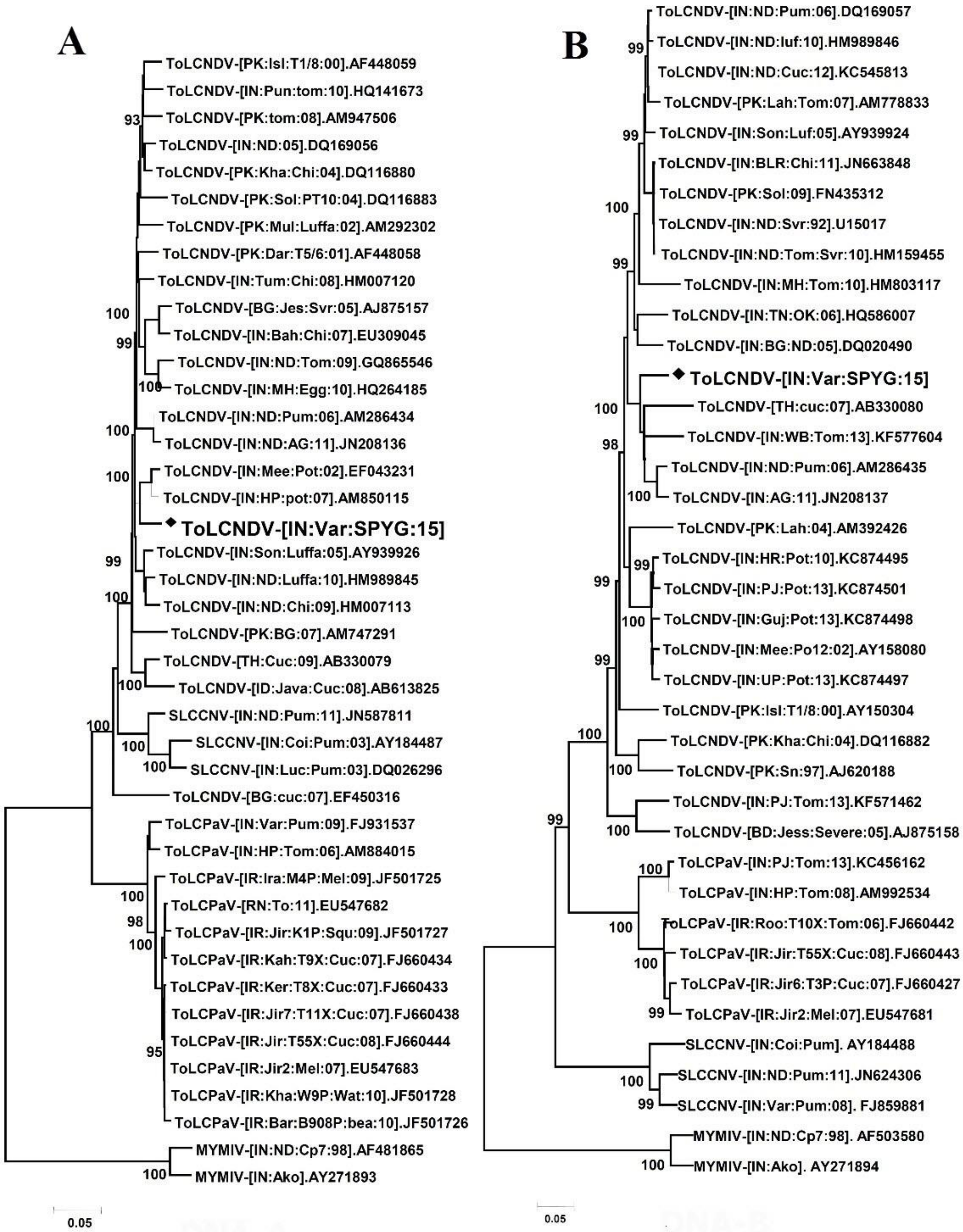

Figure 2. Phylograms were constructed using the neighbour joining (NJ) method. (2A) The DNA-A sequence of the begomovirus (SPYG1 clone) and (2B) the DNA-B sequence of the begomovirus (SPYG1 clone) associated with the severe mosaic disease of the spine gourd in India. Branches corresponding to the partitions reproduced in the less than 50% bootstrap replicates are collapsed. The percentage of the replicate trees in which the associated taxa clustered together in the bootstrap test (1000 Replicates) is shown below the branches.

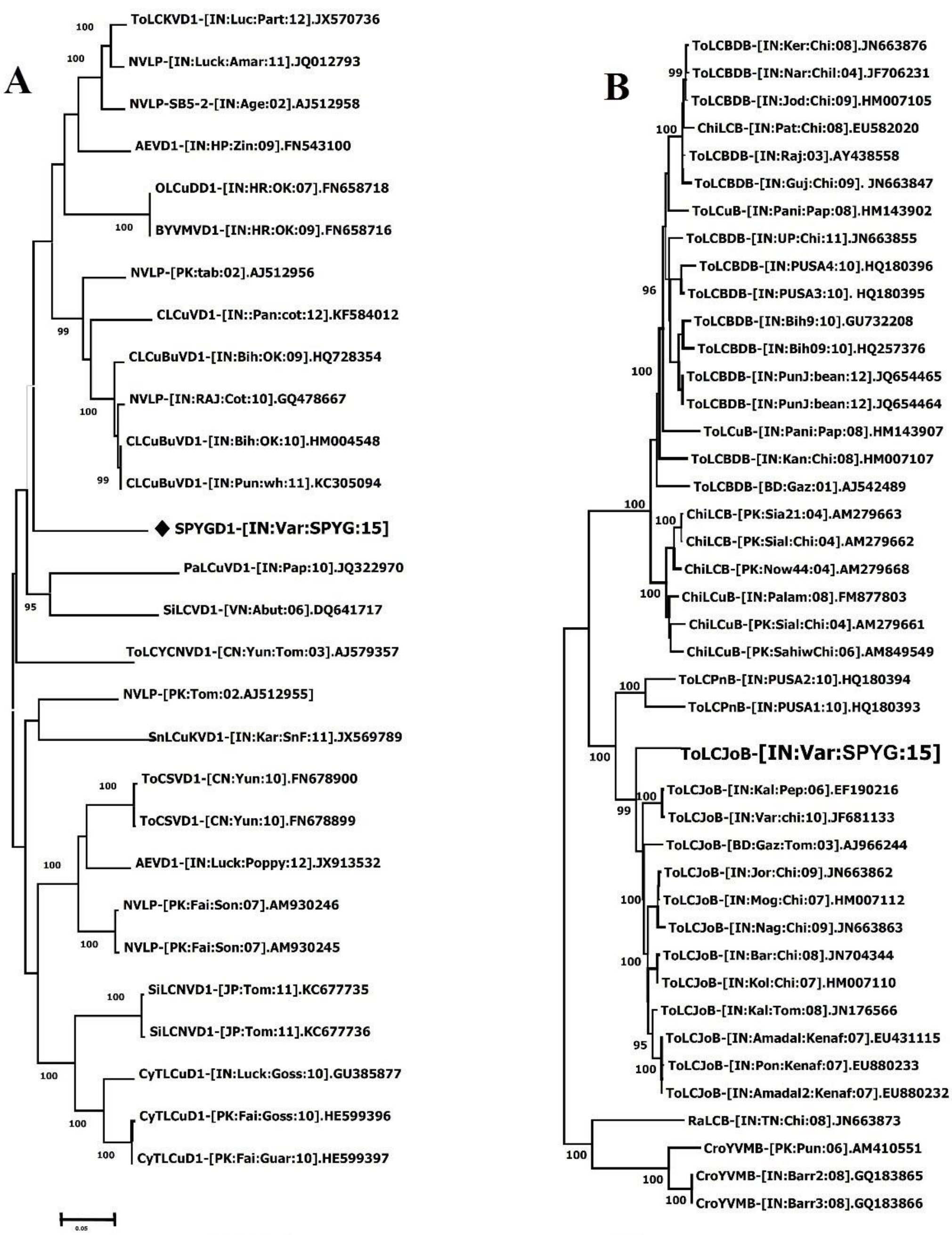

Figure 3. Phylograms were constructed from the aligned complete nucleotide sequences of (3A) Alphasatellite (DNA D1) and (3B) betasatellite (DNAβ) associated with severe mosaic disease of spine gourd in India using the NJ method. The horizontal distances are proportional to the sequence distances; vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.

In case of DNA B sequence, most part of BV1 (movement protein) and BC1 (nuclear shuttle protein) is derived from ToLCNDV and ToLCPaV infecting tomato, okra, and melons.The IR region is derived from the Mungbean yellow mosaic India virus (MYMIV) and ToLCNDV (Table S4, Fig. 4). The RDP analysis indicated that evidence of recombination in betasatellite suggestive of the most parts of betastallite DNA descended from Tobacco leaf curl Patna betasatellite (ToLCPnB), Tomato leaf curl Bangladesh betasatellite (ToLCBDB), and Tomato leaf curl Joydebpur betasatellite (ToLCJoB) infecting different solanaceous, and malvaceous crops in India.

The analysis of the alpha-satellite indicated that most of satellites DNA descended from Cotton leaf curl alphasatellite (CLCuVD1), Cyamopsis tetragonoloba leaf curl alphasatellite (CyTLCuD1), Okra leaf curl alphasatellite (OLCuDD1), and Sida leaf curl alphasatellite (SiLCNVD1) satellites (Table S3) are associated with cotton, guar, okra, and tomato infecting begomoviruses, respectively.

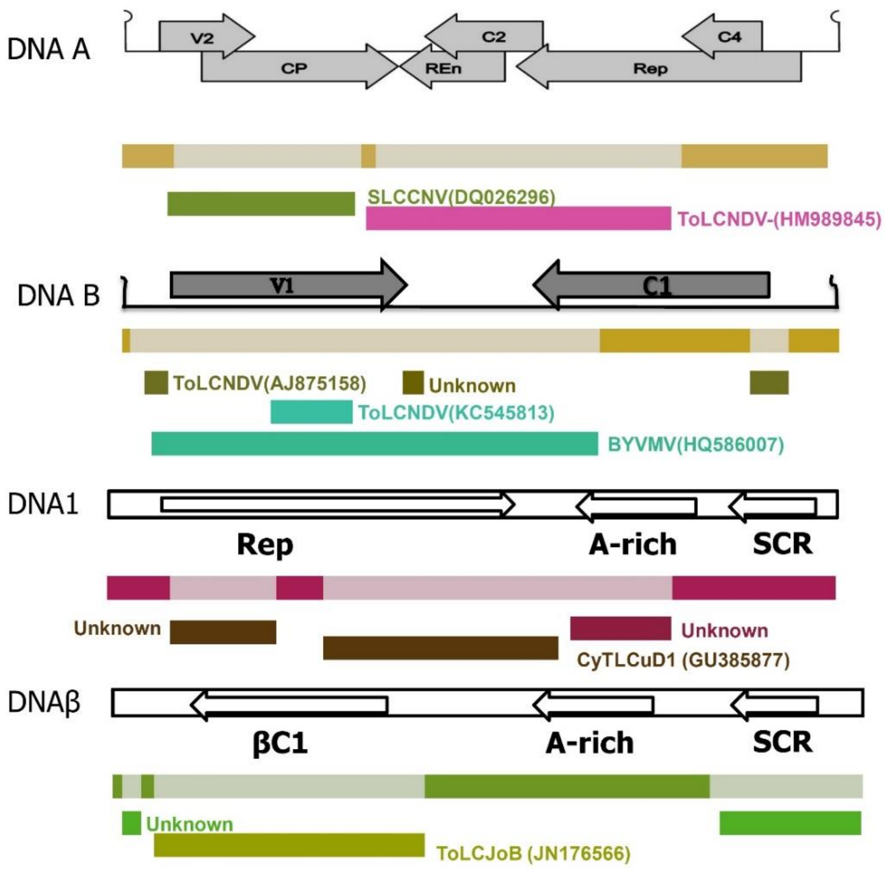

Figure 4. Analysis of recombination for ToLCNDV isolate isolated from spine gourd: The begomoviruses, alphasatllites and betasatllites acronyms given are squash leaf curl China virus (SLCCNV), tomato leaf curl new Delhi virus (ToLCNDV), cyamopsis tetragonoloba leaf curl alphasatellite (CyTLCuD1), Tomato leaf curl joydebpur betasatellite (ToLCJoB), sequence of indeterminate origin is indicated as "unknown". The box below at the top of the diagram indicates the approximate position recombination is occurring in the genome of the begomoviruses.

5. Discussion

Spine gourd is one of the cucurbitaceous crops and mosaic disease associated by begomoviruses is a limiting factor for its production. The ToLCNDV was initially identified on solanaceous crops in India (23), since then it has been reported across many countries and known to infect 43 plant species belong to the different families (24-26). The frequency of the newly emerging strains of the ToLCNDV on several cultivated and non-cultivated plant species are increasing in the recent years, indicating that the virus poses a serious threat to vegetables belonging to the family Cucurbitaceae, Euphorbiaceae, Solanaceae, Malvaceae and Fabaceae throughout the world (26). Generally, the betasatellites are found associated with the old world (OW) monopartite begomoviruses (10) and not in bipartite begomoviruses with the two exceptions, MYMIV (27, 28) and ToLCNDV (29) infecting mung bean and tomato, respectively in India. In the present study, for the first the time, we have characterized ToLCNDV bipartite virus with both betasatellite and alphasatellite associated with the mosaic disease of the spine gourd in India. The tripartite interaction between ToLCNDV and betasatellite in tomato was well proved through agro-inoculation of the cloned DNA A, DNA B, and betasatellites in the *N. benthamiana* and tomato (30). It was evident that the plants co-inoculated with DNA A, DNA B, and betasatellites will enhance the symptom severity in the *N. benthamiana* and tomato directly increases the helper viral DNA A and DNA B replication levels in the host cells.

The betasatellite associated with spine gourd mosaic disease is identified as ToLCJoB, which is also associated with the heterologous begomoviruses infecting chilli (31, 32), tomato (33), and kenaf (34) plants in India. The trans-replication of ToLCNDV and defective betasatellites has been shown for many distinct begomovirus species/betasatellite combinations (30, 34-36). The other bipartite begomoviruses confirmed to trans-replicate betasatellite are Sri Lankan cassava mosaic virus (36), Tomato yellow leaf curl Thailand virus (37), and the New World bipartite Cabbage leaf curl begomovirus (38). These observations clearly indicated that there is no distinct demarcation between monopartite and bipartite begomoviruses for their ability to trans-replicate betasatellites in the genome. The nt sequence of alphasatellite isolated from spine gourd showed less than 77 per cent identity with the previously reported alphasatellites, hence we proposed the name spine gourd mosaic alphasatellite. The exact role of alphasatellites has not been fully understood.

However, alphasatellites attenuate the disease symptoms and involve in the maintenance of the low level of betasatellite accumulation (39). It was also shown that the Rep of alphasatellites can suppress RNAi pathway in monopartite begomovirus disease complexes (40). The PHI value in the present study has strongly supported the presence of recombination in SPYG1 isolate. The different methods used for recombination breakpoint analysis provided strong evidence for the presence of the past recombination events in most of the genome components. The role of such overlapping recombination between different isolates or different species in adaptation to spine gourd may be an interesting aspect that needs to be resolved. Such inter and intra-species recombinations are the predominant feature of begomovirus evolution (8) and have been implicated in the emergence of new begomovirus species and adaptation in new hosts in the agricultural system (40).

The commercial cultivation of spine gourd by most of the growers is depends on the tuberous roots and stem cutting. The occurrence of the mosaic disease on spine gourd gives an alarming signal against utilization of such virus infected planting materials in the crop breeding and improvement program. The technique developed here will be highly useful to detect the virus infection in clonally propagated plants such as spine gourd.

Acknowledgements

This research was supported by the project “Consortium platform on vaccines and diagnostics” funded by the Indian Council of Agricultural Research, Government of India, New Delhi, India.

Conflicts of Interest

The authors declare that they have no competing interests.

Ethical Approval

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

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Supplementary Table 1: Gene Bank accession numbers of selected Begomovirus sequences used in the present study for analysis

Sl.No	Begomoviruses	Accession No.		Abbreviation
		DNA-A	DNA-B	
1	Tomato leaf curl New Delhi virus - [India:New Delhi:Pumpkin 2:2005]	AM286434	AM286435	ToLCNDV-[IN:ND:Pum2:05]
2	Tomato leaf curl New Delhi virus - [India:IARI:Pumpkin:2006]	JN129254	-	ToLCNDV-[IN::ND:Pum:06]
3	Tomato leaf curl New Delhi virus - [India:New Delhi:Pumpkin 1:2005]	AM286433	-	ToLCNDV-[IN:ND:Pum:05]
4	Tomato leaf curl New Delhi virus - [India:Lucknow]	Y16421	-	ToLCNDV-[IN:Luc:98]
5	Tomato leaf curl New Delhi virus - [India:Ash gourd:2011]	JN208136	-	ToLCNDV-[IN:Ag:11]
6	Tomato leaf curl New Delhi virus - [India:Meerut:Potato:2005]	EF043231	-	ToLCNDV-[IN:Mee:Pot:05]
7	Tomato leaf curl New Delhi virus - [India:Happur:Potato:2005]	EF043230	EF043233	ToLCNDV-[IN:Hap:Pot:05]
8	Tomato leaf curl New Delhi virus - [India:Meerut:Potato 12:2002]	AY286316	AY158080	ToLCNDV-[IN:Mee:Po12:02]
9	Tomato leaf curl New Delhi virus - [India:Himachal:Potato:2006]	AM850115	-	ToLCNDV-[IN:HP:Pot:06]
10	Tomato leaf curl New Delhi virus - [Thailand:Cucurbit:2006]	AB330079	AB330080	ToLCNDV-[TH:Cuc:06]
11	Tomato leaf curl New Delhi virus - [Thailand:Cucurbit:2006]	AB368448	-	ToLCNDV-[TH:Cuc:06]
12	Tomato leaf curl New Delhi virus - [Thailand:Bottle gourd:2006]	AB368447	-	ToLCNDV-[TH:Cuc:06]
13	Tomato leaf curl New Delhi virus - [Indonesia:Java:Cucumber:2008]	AB613825	-	ToLCNDV-[ID:Java:Cuc:08]
14	Tomato leaf curl New Delhi virus - [Bangladesh:Cucumber:2006]	EF450316	-	ToLCNDV-[BG:cuc:06]
15	Tomato leaf curl New Delhi virus - [Pakistan:Islamabad:T1/8:2000]	AF448059	AY150304	ToLCNDVIN[PK:Isl:T1/8:00]
16	Tomato leaf curl New Delhi virus - [India:Pune:JID27:2008]	HQ141673	HQ141674	ToLCNDV-[IN:Pun:Tom:08]
17	Tomato leaf curl New Delhi virus - [India:Pune 8:2008]	FJ468356	-	ToLCNDV[IN:Pune:Tom:08]
18	Tomato leaf curl New Delhi virus - [Pakistan:Solanum nigrum:PT10:2004]	DQ116883	-	ToLCNDV-[PK:Sol:PT10:04]
19	Tomato leaf curl New Delhi virus - [Pakistan:tomato:2008]	AM947506	-	ToLCNDV-[PK:Tom:08]
20	Tomato leaf curl New Delhi virus - [India:New Delhi:2005]	DQ169056	DQ169057	ToLCNDVIN[IN:ND:05]
21	Tomato leaf curl New Delhi virus - [Pakistan:Dargai:T5/6:2001]	AF448058	-	ToLCNDV-[PK:Dar:T5/6:01]
22	Tomato leaf curl New Delhi virus - [India:New Delhi:2006]	EF068246	-	ToLCNDV-[IN:ND:Tom:06]
23	Tomato leaf curl New Delhi virus - [Bangladesh:Jessore:Severe:2005]	AJ875157	AJ875158	ToLCNDV-[BG:Jes:Svr:05]
24	Tomato leaf curl New Delhi virus - [India:New Delhi:2009]	GQ865546	-	ToLCNDV[IN:ND:Tom:09]
25	Tomato leaf curl New Delhi virus - [India:Maharastra:Eggplant:2009]	HQ264185	-	ToLCNDV[IN:MH:Egg:09]
26	Tomato leaf curl New Delhi virus - [India:Sonepat:Luffa:2005]	AY939926	AY939924	ToLCNDV-[IN:Son:Luffa:05]
27	Tomato leaf curl New Delhi virus - [India:New Delhi:Lufa acutangula:JLH13:2008]	HM989845	HM989846	ToLCNDV-[IN:ND:Luffa:08]
28	Tomato leaf curl New Delhi virus - [Pakistan:Multan:Luffa:2004]	AM292302	-	ToLCNDV-[PK:Mul:Luffa:04]
29	Tomato leaf curl New Delhi virus - [India:Bahraich:Chilli:2006]	EU309045	-	ToLCNDV-[IN:Bah:Chi:06]
30	Tomato leaf curl New Delhi virus - [India:New Delhi:Chilli:2009]	HM007113	-	ToLCNDV-[IN:ND:Chi:09]
31	Tomato leaf curl New Delhi virus - [India:Tumkur:Chilli:2008]	HM007120	-	ToLCNDV-[IN:Tum:Chi:08]
32	Tomato leaf curl New Delhi virus - [Pakistan:Khalawal:Chilli:2004]	DQ116880	DQ116882	ToLCNDV-[PK:Kha:Chi:04]
33	Tomato leaf curl New Delhi virus - [Pakistan:Multan:Momordica:2007]	AM747291	-	ToLCNDV-[PK:BG:07]
34	Tomato leaf curl Palampur virus - [India:Palampur:Pumpkin:2008]	FJ931537	-	ToLCPalV[IN:Var:Pum:08]
35	Tomato leaf curl Palampur virus - [India:Palampur:2007]	AM884015	AM992534	ToLCPalV[IN:HP:Tom:07]
36	Tomato leaf curl Palampur virus - [Iran:Jiroft 1:T55X:Cucumber:2008]	FJ660444	FJ660443	ToLCPalV[IR:Jir:T55X:Cuc:08]
37	Tomato leaf curl Palampur virus - [Iran:Kahnooj:T9X:Cucumber:2007]	FJ660434	FJ660424	ToLCPalV[IR:Kah:T9X:Cuc:07]
39	Tomato leaf curl Palampur virus - [Iran:Kerman:T8X:Cucumber:2007]	FJ660433	FJ668379	ToLCPalV[IR:Ker:T8X:Cuc:07]
40	Tomato leaf curl Palampur virus - [Iran:Roodan:T7X:2007]	EU547682	FJ660442	ToLCPalV-[IR:Roo:07]
41	Tomato leaf curl Palampur virus - [Iran:Khash:W9P:Citrullus lanatus:2010]	JF501728	-	ToLCPalV[IR:Kha:W9P:Wat:10]
42	Tomato leaf curl Palampur virus - [Iran:Jiroft:T5X:Cucumis sativus:07]	JF501724	-	ToLCPalV[IR:Jir:T5X:Cuc:07]
43	Tomato leaf curl Palampur virus - [Iran:Jiroft:T13X:Cucumis melo:2006]	JF501719	-	ToLCPalV[IR:Jir:T13X:Me:06]
44	Tomato leaf curl Palampur virus - [Iran:Jiroft:T56X:Cucumis sativus:2008]	JF501721	-	ToLCPalV[IR:Jir:T56X:Cuc:08]
45	Tomato leaf curl Palampur virus - [Iran:Jiroft 1:T1X:Cucumber:2007]	FJ660440	-	ToLCPalV[IR:Jir:T1X:Cuc:07]
46	Tomato leaf curl Palampur virus - [Iran:Jiroft 9:T7X:Cucumber:2007]	FJ660437	FJ660435	ToLCPalV[IR:Jir9:T7X:Cuc:07]
47	Tomato leaf curl Palampur virus - [Iran:Jiroft:Melon:2007]	EU547683	EU547681	ToLCPalV[Jiroft:Mel:07]
48	Tomato leaf curl Palampur virus - [Iran:Jiroft 4:T6X:Cucumber:2007]	FJ660436	FJ660429	ToLCPalV[IR:Jir5:T6X:Cuc:07]
49	Tomato leaf curl Palampur virus - [Iran:Jiroft 8:T58P:Cucumber:2007]	FJ660431	FJ660425	ToLCPalV[IR:Jir8:T58P:Cuc:07]
50	Tomato leaf curl Palampur virus - [Iran:Jiroft 3:T4X:Cucumber:2007]	FJ660439	FJ660430	ToLCPalV[IR:Jir3:T4X:Cuc:07]
51	Tomato leaf curl Palampur virus - [Iran:Jiroft 5:T51X:Cucumber:2007]	FJ660432	FJ660428	ToLCPalV[IR:Jir5:T51X:Cuc:08]
52	Tomato leaf curl Palampur virus - [Iran:Iranshahr:M4P:Cucumis melo:2009]	JF501725	-	ToLCPalV[IR:Ira:M4P:Mel:09]
53	Tomato leaf curl Palampur virus - [Iran:Jiroft:T65X:Cucumis sativus:2008]	JF501720	-	ToLCPalV[IR:Jir:T65X:Cuc:08]
54	Tomato leaf curl Palampur virus - [Iran:Jiroft 6:T3X:Cucumber:2007]	FJ660441	FJ660427	ToLCPalV[IR:Jir6:T3X:Cuc:07]
55	Tomato leaf curl Palampur virus - [Iran:Jiroft 7:T11X:Cucumber:2007]	FJ660438	FJ660426	ToLCPalV[IR:Jir7:T11X:Cuc:07]
56	Tomato leaf curl Palampur virus - [Iran:Jiroft:T61X:Cucumis sativus:2008]	JF501723	-	ToLCPalV[IR:Jir:T61X:Cuc:08]
57	Tomato leaf curl Palampur virus - [Iran:Jiroft:T69P:Cucumis sativus:2008]	JQ825226	-	ToLCPalV[IR:Jir:T69P:Cuc:08]
58	Tomato leaf curl Palampur virus - [Iran:Jiroft:T63X:Cucumis sativus:2008]	JF501722	-	ToLCPalV-[IR:Jir:T63X:Cuc:08]
59	Tomato leaf curl Palampur virus - [Iran:Jiroft:K1P:Cucurbita pepo:2009]	JF501727	-	ToLCPalV-[IR:Jir:K1P:Squ:09]
60	Tomato leaf curl Palampur virus - [Iran:Barantin:B908P:Phaseolus vulgaris:2010]	JF501726	-	ToLCPalV-[IR:Bar:B908P:Pv:10]
61	Mungbean yellow mosaic India virus - [India:New Delhi:Cowpea 7:1998]	AF481865	AF503580	MYMIV-[IN:ND:Cp7:98]
62	Mungbean yellow mosaic India virus - [India:Akola]	AY271893	AY271894	MYMIV-[IN:Ako]
63	Squash leaf curl China virus - [India:pumpkin:IARI:2010]	JN587811	-	SLCCNV-[IN:ND:Pum:10]
64	Squash leaf curl China virus - India [India:Varanasi:Pumpkin:2008]	EU573715	FJ859881	SLCCNV-[IN:Var:Pum:08]
65	Squash leaf curl China virus - India [India:Coimbatore:Pumpkin:2003]	AY184487	AY184488	SLCCNV-[IN:Coi:Pum:03]
66	Squash leaf curl China virus - India [India:Lucknow:Pumpkin:2003]	DQ026296	-	SLCCNV-[IN:Luc:Pum:03]
67	Squash leaf curl China virus - India [India:Varanasi:Pumpkin2:2008]	GU967381	GU967382	SLCCNV[IN:Var:Pum:08]
68	Tomato leaf curl New Delhi virus - [India: Ash gourd:2011]	-	JN208137	ToLCNDV[IN:AG:11]
69	Tomato leaf curl New Delhi virus - [India: New Delhi:Cucucmber:2012]	-	KC545813	ToLCNDV-[IN:ND:Cuc:12]
70	Tomato leaf curl New Delhi virus - [India:Bangalore:Chilli::2011]	-	JN663848	ToLCNDV-[IN:BLR:Chi:11]
71	Tomato leaf curl New Delhi virus - [India:Bangalore:Chilli::2011]	-	JN663867	ToLCNDV-[IN:BLR:Chi:11]
72	Tomato leaf curl New Delhi virus - [India:New Delhi:Severe:1992]	-	U15017	ToLCNDVIN[IN:ND:Svr:92]
73	Tomato leaf curl New Delhi virus - [India:Palampur:Chilli::2011]	-	JN663871	ToLCNDV-[IN:Pal:Chi:11]
74	Tomato leaf curl New Delhi virus - [Pakistan:Solanum nigrum:2009]	-	FN435312	ToLCNDV-[PK:Sol:09]
75	Tomato leaf curl New Delhi virus - [India:2009]	-	HM159455	ToLCNDV-[IN:09]
76	Tomato leaf curl New Delhi virus - [India:Tamil Nadu:Okra::2006]	-	HQ586007	ToLNDV-[IN:TN:OK:06]
77	Tomato leaf curl New Delhi virus - [India:West Bengal:Tomato::2013]	-	KF577604	ToLCNDV-[IN:WB:Tom:13]
78	Tomato leaf curl New Delhi virus - [India:Gujarat:Potato:2013]	-	KC874498	ToLCNDV-[IN:Guj:Pot:13]
79	Tomato leaf curl New Delhi virus - [India:Haryana:Potato:2010]	-	KC874495	ToLCNDV-[IN:HR:Pot:10]
80	Tomato leaf curl New Delhi virus - [India:Punjab:Potato:2013]	-	KC874501	ToLCNDV-[IN:PJ:Pot:13]

81	Tomato leaf curl New Delhi virus - [India: Maharashtra :Tomato:2010]	-	HM803117	ToLCNDV-[IN:MH:Tom:10]
82	Tomato leaf curl New Delhi virus - [India: Uttar Pradesh:Potato:2013]	-	KC874497	ToLCNDV-[IN:UP:Pot:13]
83	Tomato leaf curl New Delhi virus - [India: New Delhi: Bitter Gourd:2005]	-	DQ020490	ToLCNDV-[IN:ND: BG:05]
84	Tomato leaf curl New Delhi virus - [Pakistan:Lahore:2004]	-	AM778833	ToLCNDV-[PK:Lah:04]
85	Tomato leaf curl New Delhi virus - [Pakistan:Lahore:2004]	-	AM392426	ToLCNDV-[PK:Lah:04]
86	Tomato leaf curl New Delhi virus - [Pakistan:Solanum nigrum:1997]	-	AJ620188	ToLCNDV-[PK:Sn:97]
87	Tomato leaf curl New Delhi virus - [India:Punjab:Tomato:2013]	-	KF571462	ToLCNDV-[IN:PJ:Tom:13]
88	Tomato leaf curl Palampur virus - [India:Punjab:Tomato:2013]	-	KC456162	ToLCPalV-[IN:PJ:Tom:13]
89	Tomato leaf curl Palampur virus - [Iran:Jiroft:T63X:Cucumis sativus:2008]	-	FJ660423	ToLCPalV-[IR:Jir1:T55P:Cuc:08]
90	Squash leaf curl China virus - India [India: New Delhi:Pumpkin:11]	-	JN624306	SLCCNV-[IN:ND:Pum:11]

Supplementary Table 2: Gene Bank accession Numbers of selected Betasatellites Sequences from Asia used in this Study for Analysis

Sl.No	Betasatellites	Accession numbers	Abbreviation
1	Tomato leaf curl Joydebpur betasatellite -[Bangladesh:Gazipur:2005]	AJ966244	ToLCJoB-[BD:Gaz:05]
2	Tomato leaf curl Joydebpur betasatellite-[India: Jorehat:Chilli:2009]	JN663862	ToLCJoB-[IN:Jor:Chi:09]
3	Tomato leaf curl Joydebpur betasatellite-[India:Mograhat:Chilli:2007]	HM007112	ToLCJoB-[IN:Mog:Chi:07]
4	Tomato leaf curl Joydebpur betasatellite-[India:Amadalavalasa1:Kenaf:2007]	EU431115	ToLCJoB-[IN:Amadal:Kenaf:07]
5	Tomato leaf curl Joydebpur betasatellite-[India:Kolkata:Chilli:2007]	HM007110	ToLCJoB-[IN:Kol:Chi:07]
6	Tomato leaf curl Joydebpur betasatellite-[India:Ponduru:Kenaf:2007]	EU880233	ToLCJoB-[IN:Pon:Kenaf:07]
7	Tomato leaf curl Joydebpur betasatellite-[India: Baruipur:chilli:2008]	JN704344	ToLCJoB-[IN:Bar:Chi:08]
8	Tomato leaf curl Joydebpur betasatellite-[India; Nagpur:Chilli:2009]	JN663863	ToLCJoB[IN:Nag:Chi:09]
9	Tomato leaf curl Joydebpur betasatellite-[India: Kolkata:Tomato:2008]	JN176566	ToLCJoB-[IN:Kal:Tom:08]
10	Tomato leaf curl Joydebpur betasatellite-[India: Kalyani:Pepper:2006]	EF190216	ToLCJoB-[IN:Kal:Pep:06]
11	Tomato leaf curl Joydebpur betasatellite-[India:Varanasi:Chilli:2010]	JF681133	ToLCJoB-[IN:Var:Chi:10]
12	Tomato leaf curl Joydebpur betasatellite-[India:Jodhpur:Chilli:2009]	HM007105	ToLCBB-[IN:Jod:Chi:09]
13	Tomato leaf curl Bangladesh betasatellite [India:Rajasthan:2003]	AY438558	ToLCBDB-[IN:Raj:03]
14	Chilli leaf curl betasatellite [Pakistan:Sialkot21:2004]	AM279663	ChiLCB-[PK:Sia21:04]
15	Tomato leaf curl Bangladesh betasatellite-[India:Uttar Pradesh:Chilli:2011]	JN663855	ToLCBDB-[IN:UP:Chi:11]
16	Tomato leaf curl Bangladesh betasatellite-[India:Kerela:Chilli:2011]	JN663876	ToLCBDB-[IN:Ker:Chi:08]
17	Tomato leaf curl Bangladesh betasatellite-[India:Bihar:2010]	GU732208	ToLCBDB-[IN:Bih9:10]
18	Tobacco leaf curl Patna betasatellite –[India:PUSA2:2010]	HQ180394	ToLCPnB-[IN:PUSA2:10]
19	Chilli leaf curl betasatellite- [Pakistan:Sialkot:Chilli:2004]	AM279662	ChiLCB-[PK:Sial:Chi:04]
20	Tomato leaf curl Bangladesh betasatellite-[India:Gujarat:Chilli:09]	JN663847	ToLCBDB-[IN:Guj:Chi:09]
21	Tomato leaf curl Bangladesh betasatellite[Bangladesh:Gazipur:2001]	AJ542489	ToLCBDB-[BD:Gaz:01]
22	Tomato leaf curl Joydebpur betasatellite-[India:Amadalavalasa2:Kenaf:2007]	EU880232	ToLCJoB-[IN:Amadal2:Kenaf:07]
23	Tomato leaf curl Bangladesh betasatellite -[India: Nar:Chilli:2004]	JF706231	ToLCBDB-[IN:Nar:Chil:04]
24	Chili leaf curl betasatellite-India:Pataudi:Chilli:2008]	EU582020	ChiLCB[IN:Pat:Chi:08]
25	Tomato leaf curl Bangladesh betasatellite –[India:PUSA4:2010]	HQ180396	ToLCBDB-[IN:PUSA4:10]
26	Tomato leaf curl Bangladesh betasatellite –[India:PUSA3:2010]	HQ180395	ToLCBDB-[IN:PUSA3:10
27	Chilli leaf curl betasatellite [Pakistan:Nowshera44:2004]	AM279668	ChiLCB-[PK:Now44:04]
28	Tomato leaf curl Joydebpur betasatellite-[India:Ponduru:Kenaf:2007]	EU880233	ToLCJoB-[IN:Pond:Kenaf:07]
29	Tomato leaf curl Bangladesh betasatellite-[India:Bihar:Bih09:2010]	HQ257376	ToLCBDB-[IN:Bih09:10]
30	Tomato leaf curl betasatellite-[India:Panipat:Papaya:2008]	HM143907	ToLCuB-[IN:Pani:Pap:08]
31	Tomato leaf curl betasatellite-[India:Panipat:Papaya:2008]	HM143902	ToLCuB-[IN:Pani:Pap:08]
32	Tomato leaf curl Bangladesh betasatellite-[India:Punjab:Beans:2012]	JQ654465	ToLCBDB-[IN:PunJ:Bean:12]
33	Tomato leaf curl Bangladesh betasatellite-[India:Punjab:Beans:2012]	JQ654464	ToLCBB-[IN:PunJ:Bean:12]
34	Tobacco leaf curl Patna betasatellite–[India:Pusa 2:2010]	HQ180393	ToLCPnB-[IN:PUSA1:10]
35	Tomato leaf curl Bangladesh betasatellite-[India:Kanpur:Chilli:2008]	HM007107	ToLCBDB-[IN:Kan:Chi:08]
36	Croton yellow vein mosaic betasatellite-[India:Barrackpore2:2008]	GQ183865	CroYVMB-[IN:Bar2:08]
37	Croton yellow vein mosaic betasatellite[India:Barrackpore3:2008]	GQ183866	CroYVMB-[IN:Barrack:08]
38	Chilli leaf curl betasatellite-[India:Palampur:2008]	FM877803	ChiLCuB-[IN:Palam:08]
39	Chili leaf curl betasatellite-[Pakistan:Sialkot:Chilli:2004]	AM279661	ChiLCuB-[PK:Sial:Chi:04]
40	Chili leaf curl betasatellite-[Pakistan:Sahiwal:Chilli:06]	AM849549	ChiLCuB-[PK:Sahiw;Chi:06]
41	Croton yellow vein mosaic betasatellite-[Pakistan:Punjab:2006]	AM410551	CroYVMB-[PK:Pun:06]
42	Radish leaf curl betasatellite-[India:Tamil Nadu:Chilli:2008]	JN663873	RaLCB-[IN:TN:Chi:08]

Supplementary Table 3: Gene Bank Accession Numbers of selected Alphasatellites Sequences from Asia used in this Study for Analysis

Sl.No	Alphasatellites	Accession numbers	Abbreviation
1	Nanovirus-like particle-[India: Ageratum conyzoides:02]	AJ512958	NVLP-SB5-2[IN:Age:02]
2	Tomato leaf curl Karnataka alphasatellite -[India:Lucknow:Parthenium hysterophorus:2012]	JX570736	ToLCKVD1-[IN:Luc:PaR:12]
3	Nanovirus-like particle-[India:Lucknow:Amaranthus cruentus:2011]	JQ012793	NVLP-[IN:Luc:Amar:11]
4	Cotton leaf curl Burewala alphasatellite-[India:Bihar:Okra:2009]	HQ728354	CLCuBuVD1-[IN:Bih:Okra:09]
5	Cotton leaf curl Burewala alphasatellite-[India:Bihar:Okra:2009]	HM004548	CLCuBuVD1-[IN:Bih:Okra:10]
6	Cotton leaf curl Burewala alphasatellite-[India: Punjab:Wheat:2011]	KC305094	CLCuBuVD1-[IN:Pun:Wheat:11]
7	Nanovirus-like article[India:Rajasthan:Cotton:2010]	GQ478667	NVLP-[IN:Raj:Cot:10]
8	Nanovirus-like particle- [Pakistan:Tobacco:2002]	AJ512956	NVLP-[PK:Tob:02]
9	Ageratum enation virus-[India:Himachal Pradesh:Zinnia sp:2009]	FN543100	AEVD1-[IN:HP:Zinnia sp:09]
10	Nanovirus-like particle-[Pakistan:Tomato:2002]	AJ512955	NVLP-[PK:Tom:02]
11	Nanovirus-like particle-[Pakistan:Faisalabd:Sonchus arvensis:07]	AM930246	NVLP-[PK:Fai:Son:07]
12	Nanovirus-like particle-[Pakistan:Faisalabd :Sonchus arvensis:07]	AM930245	NVLP-[PK:Fai:Son:07]
13	Tobacco curly shoot alphasatellite-[China:Yunnan:2010]	FN678900	ToCSVD1-[CN:Yun:10]
14	Tobacco curly shoot alphasatellite-[China:Yunnan:2010]	FN678899	ToCSVD1-[CN:Yun:10]
15	Okra leaf curl virus alphasatellite [India:Haryana:2007]	FN658718	OLCuDD1-[IN:HR:Okra:07]
16	Bendhi Yellow Vein Mosaic alphasatellite[India:Haryana:Okra:2009]	FN658716	BYVMVD1-[IN:HR:Okra:09]
17	Sida yellow vein China alphasatellite-[Japan:Tomato:2011]	KC677735	SiLCNVD1-[Japan:Tom:11]
18	Sida yellow vein China alphasatellite-[Japan:Tomato:2011]	KC677736	SiLCNVD1-[Japan:Tom:11]
19	Cotton leaf curl alphasatellite-[India::Panjab:Cotton:2012]	KF584012	CLCuVD1-[IN::Pan:Cot:12]
20	Guar leaf curl alphasatellite-[Pakistan:Faisalabad:Gossypium sp:2010]	HE599396	CyTLCuD1-[PK:Fai:Guar:10]
21	Guar leaf curl alphasatellite-[Pakistan:Faisalabad:Gossypium sp:2010]	HE599397	CyTLCuD1-[PK:Fai:Guar:10]
22	Ageratum enation alphasatellite-[India:Lucknow:Poppy:2012]	JX913532	AEVD1-[IN:Luck:Poppy:12]
23	Guar leaf curl alphasatellite-[India:Lucknow:Cyamopsis tetragonoloba:2010]	GU385877	CyTLCuD1-[IN:Luck:Guar:10]
24	Papaya leaf curl alphasatellite-[India:Papaya:2010]	JQ322970	PaLCuVD1-[IN:Pap:10]
25	Tomato yellow leaf curl China alphasatellite-[China:Yunnan:Tomato:03]	AJ579357	ToLCYCNVD1-[CN:Yunnan:Tom:03]
26	Sunflower leaf curl Karnataka alphasatellite-[India:Karnataka:Sunflower:2011]	JX569789	SnLCuKVD1-[IN:Kar:SnF:11]
27	Sida leaf curl alphasatellite [Viet Nam:Thanhhoa:Abutilon indicum:2006	DQ641717	SiLCVD1-[V N:Than:Abu:06]

Supplementary Table 4: Details of recombination between ToLCNDV infecting spine gourd and other begomoviruses detected using RDP4.

	Parent-like sequences		P-Values					
Break point begin-end a	**Major Parent**	**Minor parent**	**RDP**	**GENECOV**	**Max Chi**	**Chimera**	**Si Scan**	**3Seq**
		DNA-A						
139-1080 (IR, CP)	ToLCPaV[IN:HP:Tom:06].AM884015	SLCCNV-[IN:Luc:Pum:03].DQ026296	1.636X10-9	1.214X10-11	1.085X10-5	1.187X10-7	2.113X10-18	5.486X10-3
719-2338 (CP, AC3, AC2)	ToLCNDV-[IN:HP:pot:07].AM850155	5 ToLCNDV-[IN:ND:Luffa:10].HM98984	3.625X10-7	NS	NS	NS	8.106X10-9	1.348X10-14
DNA-B								
2819-141 (IR)	MYMIV-[IN:ND:Cp7:98].AF503580	ToLCNDV-[BD:Jess:Severe:05].AJ875158	NS	NS	1.191X10-2	9.49X10-2	NS	NS
1474-1595 (BV1)	ToLCNDV-[IN:WB:Tom:13].KF577604	ToLCPaV-[IR:Jir2:Mel:07].EU547681	1.148X10-10	1.483X10-3	4.589X10-2	9.029X10-4	NS	NS
40-2522 (BV1, BC1)	ToLCPaV-[IR:Roo:T10X:Tom:06].FJ660442	ToLCNDV-[IN:TN:OK:06].HQ586007	NS	NS	6.969X10-1	NS	2.721X10-38	NS
		DNAD1(Alphasatellite)						
33-100 (SCR)	CLCuVD1-[IN::PJ:cot:12].KF584012	CyTLCuD1-[IN:Luck:Guar:10].GU385877	1.071X10-3	2.796X10-3	NS	NS	NS	3.42X10-2
907-1200 (Rep)	OLCuDD1-[IN:HR:Okra:07].FN658718	SiLCNVD1-[Japan:Tom:11].KC677735	1.038X10-3	NS	7.012X10-3	3.499X10-3	2.274X10-6	1.724X10-2
475-1027 (Rep)	OLCuDD1-[IN:HR:Okra:07].FN658718	CyTLCuD1-[IN:Luck:Guar:10].GU385877	4.444X10-4	NS	1.212X10-5	1.974X10-4	5.121X10-4	1.691X10-4
DNAβ (Betasatellite)								
1167-56 (SCR)	ToLCPnB-[IN:PUSA 1:10].HQ180393	ToLCBDB[IN:Bih9:10].GU732208	5.919X10-14	2.521X10-6	3.53X10-7	7.86X10-9	6.542X10-12	1.114X10-12
89-758 (βC1)	ToLCJoB[IN:Kal:Pep:06] .EF190216	ToLCJoB-[IN:Kal:Tom:08].JN176566	8.195X10-3	1.483X10-2	1.189X10-6	NS	4.102X10-8	5.348X10-5

NS- Recombination is non-significance

Definition for acronyms of begomviruses, betasatellite and alphasatellite given in the supplementary Table 1-3

[a] The text in the parenthesis of this column indicates ORF's in which break points are identified