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# Study of betasatellite molecule from leaf curl disease of sunn hemp (*Crotalaria juncea*) in India

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**Abstract** Leaves of sunn hemp (*Crotalaria juncea*) showing geminiviral symptoms were collected from Lucknow, India during rainy season in 2008. DNA template isolated from the symptomatic leaf tissues were subjected to polymerase chain reaction (PCR) using specific primers to amplify coat protein (CP) gene of DNA-A as well as betasatellite DNA associated with the leaf curl disease. CP gene showed 97% sequence identity with that of *Cotton leaf curl Burewala virus* (CLCuBwV). Further, the betasatellite DNA molecule revealed sequence similarity with previously characterized betasatellite DNA of begomoviruses affecting malvaceous crops from different regions of India and Pakistan. Maximum similarity (>90%) of betasatellite DNA under study was observed with Cotton leaf curl Multan betasatellite (CLCuMB-[Pak: Mul17:08) and other betasatellite DNA from Pakistan thus confirming possible infection of *C. juncea* with begomovirus. A complementary sense open reading frame (ORF) $\beta$C1 is present at nucleotide position 194–550. Sequence comparison of this ORF with other members of begomoviruses further confirmed association of a begomovirus with *C. juncea*. The betasatellite DNA when expressed under the control of CaMV35S promoter *Nicotiana tabacum*, showed leaf deformities. Our results demonstrated that a malvaceous betasatellite is adapted by a nonmalvaceous host and causes similar disease symptoms.

**Keywords** Begomovirus · Betasatellite DNA · PCR · Recombination

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## Introduction

Geminivirus group (family *Geminiviridae*) is divided into four genera viz. *Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus* on the basis of host range, genome organization and insect vector [1]. Begomovirus is the largest subgroup among geminiviruses having more than 100 species. The number of plants affected by begomoviruses is increasing every year. Transmission of begomoviruses takes place by whitefly (*Bemicia tabaci*) vector and can cause significant yield losses to many economically important crops such as bean, cassava, tomato, cotton in tropical and subtropical regions of the world [2, 3].

The begomoviruses possess bipartite or monopartite genomes depending upon the presence of one or two genomic components. Bipartite begomoviruses comprising of two genomic components are designated as DNA-A and DNA-B. Begomovirus genes required for virus replication and encapsidation by coat protein (CP) are encoded by DNA-A component and those for virus movement (both intra and intercellular) by DNA-B [4]. In monopartite begomoviruses, genes for replication, encapsidation and movement are present in a single component (DNA-A).

In recent years, another component designated as betasatellite (single stranded DNA) has been found to be associated with monopartite begomoviruses. Co-inoculation of betasatellite DNA along with *Cotton leaf curl Multan virus* (CLCuMV) DNA-A induces symptoms in cotton, which is typical to cotton leaf curl disease (CLCuD) [5]. Generally satellites are a common feature of a number of RNA viruses. These satellite molecules are viruses or nucleic acids that depend on a helper virus for their replication and generally lack sequence homology to the helper virus genome [6]. The betasatellite DNA requires DNA-A component for their replication and encapsidation, hence it is also known as satellite DNA. Analysis of betasatellite molecules revealed that the genome size of betasatellite is approximately half that of DNA-A (2.5–3.0 kb). These molecules share little sequence similarity to their helper virus except for a conserved hairpin structure and loop sequence (TAATATTAC). In satellite molecules, A-rich sequences are predominant. The A-rich region of betasatellite DNA acts as a stuffer that increases the molecule size to that required for encapsidation and/or spread by the helper begomovirus [7]. Many experiments have demonstrated that betasatellite molecule contains a complementary ORF designate as $\beta$C1, which is responsible for symptom induction. [8, 9]. Occurrence of the betasatellite DNA molecule associated with monopartite begomoviruses is widespread in the Old World but apparently absent in the New World.

In addition to betasatellite DNA, one single stranded circular DNA component (ca. 1.4 kb), termed as alphasatellite has been found to be associated with diseases such as Agretum yellow vein disease (AYVD), CLCuD, and Okra leaf curl disease. Alphasatellites share sequence homology to genomic components of nanoviruses which encode a replication associated protein (Rep) [7, 10, 11]. Function of alphasatellite DNA in symptom induction is still unknown.

Sunn hemp (*Crotalaria juncea*) with about 690 species belongs to family Fabaceae. This species grows mainly in tropical and subtropical areas. In India, this plant is used as fodder and green manures, and is considered as a good source of fiber for manufacturing twine and cord. Sunn hemp is infected by several pathogens such as fungi, nematodes and viruses. It is reportedly infected by several strains of viruses [12–14], including Geminiviruses [15]. A severe leaf curling was observed on sunn hemp (*C. juncea*) plants in Lucknow region (north India) just after rainy season. PCR and Southern based assays confirmed the association of a begomovirus with the diseased plant.

In this article, we report a recombinant betasatellite isolated from leaf curl disease of *C. juncea*. It is for the first time in India that a betasatellite DNA associated with leaf curl disease of *C. juncea* in India has been characterized based on sequence and phylogenetic analysis. Further, expression of the betasatellite DNA in *Nicotiana tabacum* showed typical leaf curling; vein thickening and downward curling of the leaves.

## Materials and methods

### Virus source

Leaves of naturally infected plants of sunn hemp (*C. juncea*) were collected in and around Lucknow, India. The severity of disease incidence was about 20–40% (visual observation).

### Isolation of total DNA and confirmation of begomoviral complex

Total DNA was isolated from the infected leaves of *C. juncea* using DNAeasy plant mini kit column as per the manufacturer's instruction (QIAGEN, Germany). Total genomic DNA was subjected to PCR amplification using CLCuV CP gene specific primers for CP gene P1/P2 (5′-GGGATTTGATTTCAGTAATAAGG-3′/5′-GAGCATGT TGTATATGTAGACCA-3′) [16]. A final volume of 50 µl PCR mix containing 5 µl DNA template, *Taq* DNA polymerase (3 U $\mu l^{-1}$ Bangalore Genei, 1 µl), dNTPs (10 mM each, 1 µl), and 50 pmol of each primer was taken in a PCR tube. PCR condition used was 25 cycles with 94°C for 1 min (denaturation), 48°C for 1 min (annealing) and extension for 1 min at 72°C. The PCR amplicons were visualized on agarose gel and subjected to Southern hybridization by primer extension method [17]. CP gene of previously characterized begomovirus (accession no. GQ247893) labeled with $\alpha^{32}$ P dCTP was employed as a probe in Southern hybridization.

### Isolation of viral DNA and PCR amplification of betasatellite DNA

Viral DNA was isolated from infected leaves by using Viral DNA from serum/plants (Chromous biotech Pvt. Ltd. Bangalore, India) as per the manufacturer's instruction and used as template for amplification of betasatellite DNA in PCR. Primer pair $\beta$01 (5′-GAAACCACTACGCTACG CAGCAGCC-3′) and $\beta$04 (5′-ACCCTCCCAGGGGTA CACACCGCCG-3′) [18] were used and PCR was performed at 94°C/5 min for one cycle as initial denaturation, 94°C/45 s, 50°C/60 s, and 72°C/90 s for 30 cycles and a final amplification 72°C/5 min. PCR yielded a DNA fragment of ca. 1.3 kb from DNA of infected leaves while DNA isolated from healthy plants showed no amplification.

### Cloning and sequencing

PCR amplified product was run on agarose gel and visualized in UV illumination. DNA fragments representing CP gene and betasatellite DNA were excised from the gel and purified using HiPura gel extraction kit (Hi Media Pvt. Ltd., India) following manufacturers instruction. The DNA fragments were cloned into pDrive vector (QIAGEN, Germany). The recombinant plasmid was then transformed into *Escherichia coli* DH5α cells and positive clones containing desired DNA fragments were sequenced using automated sequencer (3730 XL DNA Analyzer, Applied Biosystems, USA).

### Sequence analysis

Nucleotide sequences of CP gene and betasatellite gene under study were aligned with those of other begomovirus sequences from Gen Bank using CLUSTAL W [19] alignment program. Complete nucleotide sequences of both the genes (CP as well as betasatellite DNA) were initially taken into account for similarity search by using BLASTn search program (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences which showed high scores were kept for further analysis. Following multiple sequence alignments, phylogenetic analysis was done using MEGA software version 4.0 [20]. Default parameters used were character-based algorithm (Maximum Parsimony), and distance-based algorithms (Minimum Evolution and Neighbor-Joining). A consensus dendrogram was generated using bootstrap value of 1000 replicates for these algorithms. The expasy proteomic server tool was used to translate set of protein encoding genes. Gen Bank accession numbers of different begomoviruses used for comparison and phylogenetic analysis are given in Tables 1 and 2.

### Construction of infectious clone

Betasatellite DNA cloned in pDRIVE vector was digested with *Eco*RI and the released DNA fragment was ligated under the influence of *Cauliflower mosaic virus* (CaMV) 35S promoter and nopaline synthase terminator (nos). Presence of DNA insert was checked through restriction digestion and PCR. Another construct was developed following amplification of only βC1 gene. Primer pair used to amplify βC1 were c1f (5′-CAGAAATGACAACGAG CGGAAC-3′) and c1r (5′-CGGTTCGTTTACATCCATTC CC-3′), and ligated into plant expression vector pCAMBIA 1301 same manner as described above. Two constructs namely pCAM-β and pCAM-βC1 were thus produced.

### Production of transgenic tobacco plant

The expression vectors were introduced into *Agrobacterium tumefaciens* GV3101 by freeze thaw method [21].Transgenic plants were developed by tobacco leaf disc (*Nicotiana tabacum* cv. Petit Havana) by co-cultivation method [22]. The callus tissues were allowed to grow on MS medium supplemented with antibiotic kanamycin (100 mg/l). After 2 weeks, the callus tissue was differentiated by transferring them into differentiating medium (shooting medium) with supplementary light corresponding to 16 h of day length.

### Analysis of transgenic tobacco plant

Total DNA isolated from transgenic tobacco plants were subjected to PCR using primer pair clf and clr. The

**Table 1** List of betasatellite DNA and their accession numbers used in this study

| Betasatellite name | Beta abbreviation | Accession # |
|---|---|---|
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:Sri:09] | GQ259599 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[PK:Bur1:02] | AM084379 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:Sriabh2:09] | GQ249185 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:ND1:03] | AY438562 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:Bha:05] | DQ191161 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:Dab2:95] | AJ316038 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[Pak:Mul4:07] | EU384588 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[CN:Ok06:09] | FJ770371 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:Har05:Ken:06] | EF614158 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:Nar01:Ken:06] | EF620564 |
| *Sida yellow vein betasatellite* | SYvVB-[IN:Bar:07] | EU188921 |
| *Ludwigia leaf distortion betasatellite* | LuLDB-[IN:BG:04] | AY817151 |
| *Kenaf leaf curl betasatellite* | KLCuB-[IN:Ban:Bar:04] | AY705381 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[Pak:Fai:08] | AM490309 |
| *Cotton leaf curl Multan betasatellite* | CLCuMB-[IN:Kai04:Ken:06] | EU825205 |

**Table 2** The name and acronym of viruses, accession number of sequences taken in this study

| Virus name | Virus acronym | Accession # |
|---|---|---|
| *Cotton leaf curl Burewala virus* [India:Vehari:2006] | CLCuBwV | AM421522 |
| *Cotton leaf curl Burewala virus* [India:Vehari koT:2006] | CLCuBwV | AM774305 |
| *Cotton leaf curl Burewala virus* [Pakistan:Arifwala LA33:2007] | CLCuBwV | AM774303 |
| *Cotton leaf curl kokhran virus* [India:Sirsa:2003] | CLCuKV | AY456683 |
| *Cotton leaf curl Rajasthan virus* [India:Sri Ganganagar:2009] | CLCuRV | GQ247893 |
| *Gossypium hirsutum leaf curl virus* [Pakistan:Multan 18:2007] | GoHiLCuV | EU384571 |
| *Gossypium hirsutum leaf curl virus* [Pakistan:Multan 20:2007] | GoHiLCuV | EU384572 |
| *Cotton leaf curl Multan virus-Hisar* [Pakistan:Multan 311:Okra:1996] | CLCuMVHis | AJ002459 |
| *Cotton leaf curl virus*-Shadadpur [Pakistan:Sindh:2009] | CLCuVSha | FN552003 |
| *Cotton leaf curlBurewala virus* [Pakistan:Multan:2007] | CLCuBwV | EU384570 |
| *Cotton leaf curlBurewala virus* [Pakistan:Octa-9:2007] | CLCuBwV | EU365620 |
| *Cotton leaf curlBurewala virus* [*Pakistan:Octa-5:2007*] | CLCuBwV | EU365619 |
| *Cotton leaf curlBurewala virus* [Pakistan:Octa-2:2007] | CLCuBwV | EU365618 |
| *Papaya leaf curl virus* [Pakistan:2002] | PaLCuV | AJ436992 |
| *Cotton leaf curl kokhran virus* [India:Lucknow:2006] | CLCuKV | DQ343283 |
| *Papaya leaf curl virus* [Pakistan:Kundian:2008] | PaLCuV | FM955602 |
| *Radish leaf curl virus* [India:Varanasi:2006] | RaLCuV | EF175733 |
| *Ageratum enation virus* [India:Kangra:2009] | AEV | FN543099 |
| *Crotalaria juncea leaf curl virus* [India:Lucknow:2006] | CrJnLCuV | EF119337 |
| *Tomato leaf curl virus* [India:Patna:2004] | ToLCuV | AJ810358 |
| *Cotton leaf curl virus*-Shadadpur [Pakistan:Sindh LS4:2009] | CLCuVSha | FN552004 |
| *Cotton leaf curl virus*-Shadadpur [Pakistan:Sindh Sha:2009] | CLCuVSha | FN552001 |
| *Papaya leaf curl Bihar virus* [India:Bihar CPT:2009] | PalCuV | GQ139516 |

amplified products (ca. 350 bp) were run on agarose gel and transferred by capillary action to Hybond N membrane (Amersham Bioscience), and then UV cross-linked for 7 min. The membrane was prehybridized at 42°C (1 h) followed by hybridization with the radiolabeled probe prepared from $\beta$C1 gene at 65°C overnight. It was washed twice with 2× SSC, 0.1% SDS and 1× SSC, 0.1% SDS for 5 and 15 min, respectively, at room temperature followed by another wash with 1× SSC, 0.1% SDS at 65°C for 15 min. The blot was exposed to Fuji film for autoradiography.

### Recombination analysis

Recombination analysis was done using RDP3 (Recombination Detection Program Version 3.0) a window-based program which detects and analyzes recombination signals in a set of aligned DNA sequence. Betasatellites were used for recombination analyses (Table 1). RDP3 identifies possible recombinants and also parental sequences in a number of sequences. It uses six different automated methods namely RDP, GENECONV, MAXIMUM$\chi^2$, BOOTSCAN, CHIMERA, and SISTER SCANNING. Highest acceptable probability value $P = 0.05$ was used.

## Results

### PCR amplification of CP gene and confirmation of begomoviral complex

The PCR amplicons obtained from the diseased plant samples were expected size of (ca. 850 bp) confirming the presence of begomovirus in the diseased plant. This was further confirmed by Southern hybridization assay which gave a strong signal with homologous probes. The healthy plants used as a negative control showed no amplification.

### PCR amplification of betasatellite DNA molecule

Using betasatellite DNA specific primers viz. $\beta$01 and $\beta$04 PCR amplified DNA fragments of expected size (ca. $\sim$1.3 kb) were obtained from the diseased *C. juncea* leaf samples showing association of a betasatellite DNA with the disease. There was no PCR amplification when DNA isolated from the healthy plants was used as negative control.

## Cloning and sequence characterization of betasatellite DNA molecule

PCR-amplified DNA fragment (ca. 1.3 kb) obtained from diseased *C. juncea* samples was cloned and sequenced. The nucleotide sequence was deposited in the Gene Bank under accession no. GQ369731.

The betasatellite DNA molecule under study showed highest nucleotide sequence identity of 94% with betasatellite molecule of *Cotton leaf curl Multan virus* (CLCuMB) indicating possible infection of *C. juncea* with begomoviral betasatellite. Further analysis showed its highest homology with a number of betasatellite DNA isolates associated with *Cotton leaf curl virus* species from the Indian subcontinent. As the species demarcation cut-off value for betasatellite molecules currently is 78% [23], the betasatellite associated with *C. juncea* leaf curl disease appeared to be an isolate of CLCuMB. Presence of the nonanucleotide sequence TAATATTAC in the betasatellite molecule under study further confirms it to be a typical geminiviral beta molecule. This is the motif, which betasatellite molecule shares with the helper virus and forms the characteristic loop of stem loop structure, containing nick site for initiation of virion sense DNA replication [24]. Nucleotide sequence identity of $\beta$C1 gene showed its maximum identity with $\beta$C1 gene of different CLCuV species reported from the Indian subcontinent.

The $\beta$C1 ORF of the betasatellite molecule under study encoded a protein with 118 amino acids. A conserved TATA box was found to be present upstream of $\beta$C1 gene. This conserved TATA box is predicted as the putative transcription factor binding site.

A dendrogram using Neighbor-Joining method was drawn to analyze the phylogenetic relationship of the betasatellite DNA with a number of betasatellite DNA obtained from begomovirus species (Fig. 1). The betasatellite molecules used in this study are listed in Table 1. The betasatellite under study thus clustered with Cotton leaf curl Multan betasatellite and other betasatellite mostly from Pakistan and India (western region, near to Pakistan).

## Sequence analysis of CP gene

Three different clones representing CP genes showed a sequence homology of more than 99% among themselves. Nucleotide sequence of one clone was submitted to the Gen Bank (accession no. GU385766). The putative product of the gene was 258 amino acids long with 28.6 KDa as the molecular weight.

The CP gene showed highest nucleotide sequence homology with *Cotton leaf curl Burewala virus* (AM4215 22). Other isolates which shared maximum similarity are CLCuBwV (AM774305), CLCuKV (AY456683), and CLCuMV (GQ247893). Viruses used to draw phylogenetic tree are listed in Table 2. Interestingly CP gene under study showed highest homology with *Papaya leaf curl virus* at the amino acid level (Fig. 2a, b).

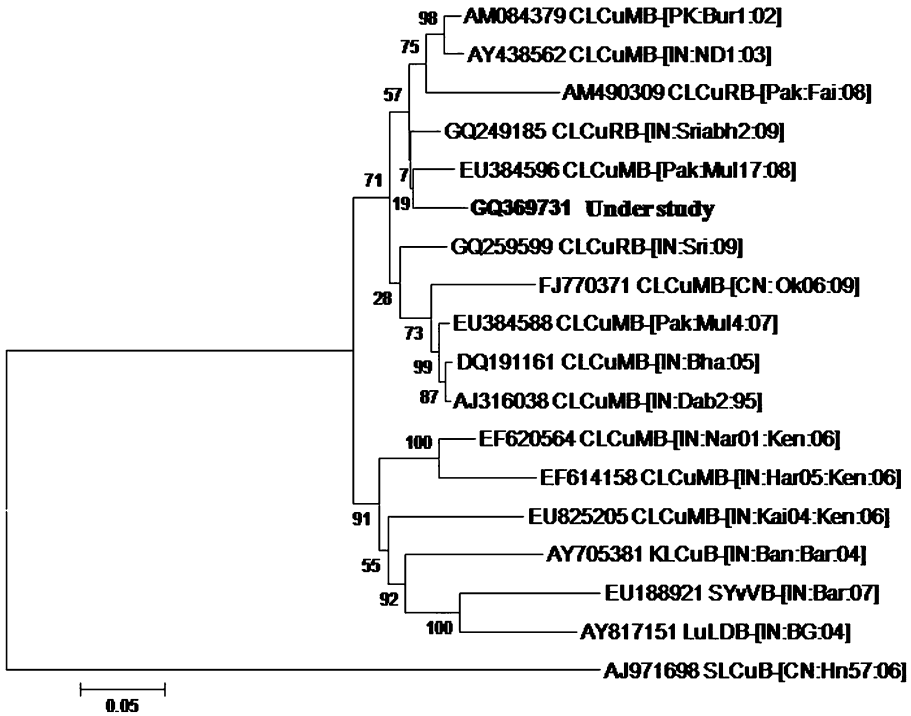


**Fig. 1** Dendrogram showing a relationship of betasatellite (this study) with other betasatellite associated with begomoviruses (listed in Table 1). The optimal tree with the sum of branch length = 1.41882335 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method, and the evolutionary history was inferred using Neighbor-Joining method in MEGA 4 software

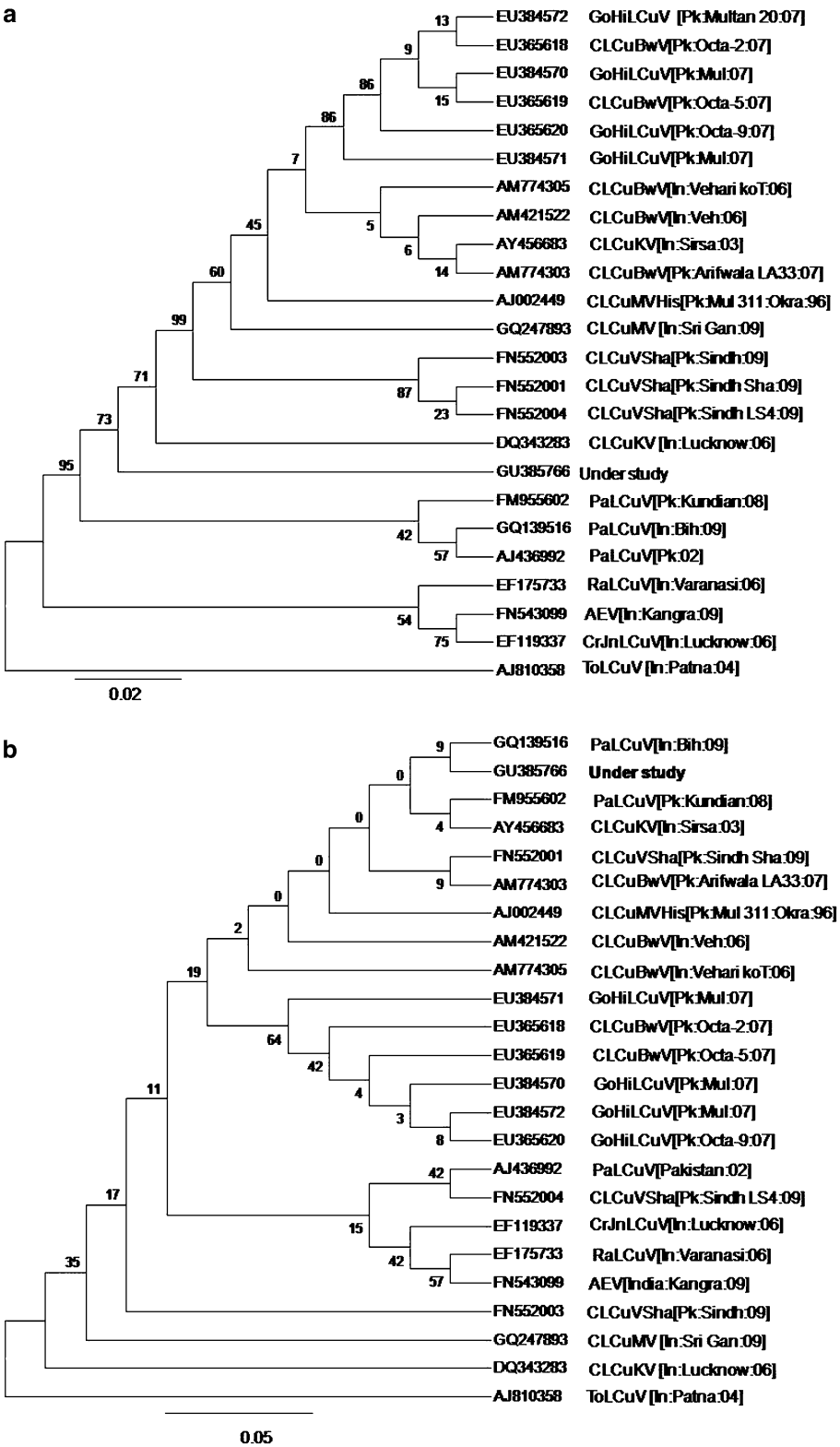


**Fig. 2** **a**, **b** Phylogenetic analysis of nucleotide and amino acid sequences of coat protein gene of begomoviruses (listed in Table 2). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method, and the evolutionary history was inferred using the Neighbor-Joining method in MEGA 4.0

## Infectivity of betasatellite DNA and analysis of transgenic lines

To investigate the functional and biological role of betasatellite DNA and $\beta$C1, *N. tabacum* plants were transformed with *A. tumefaciens* containing clones pCAM-$\beta$C1 and pCAM-$\beta$. About 60% lines of $T_0$ transgenic *N. tabacum* exhibited abnormal phenotype. Typical upward leaf curling, thickening of veins and leaflets were observed (Fig. 3a, b). To check the presence of betasatellite DNA

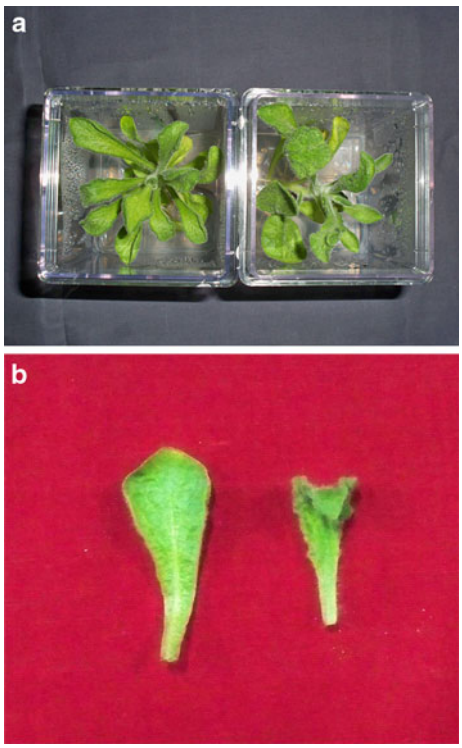


**Fig. 3** **a**, **b** Phenotype associated with transgenic *Nicotiana tabacum* plant showing leaf distortion and curling of the leaves

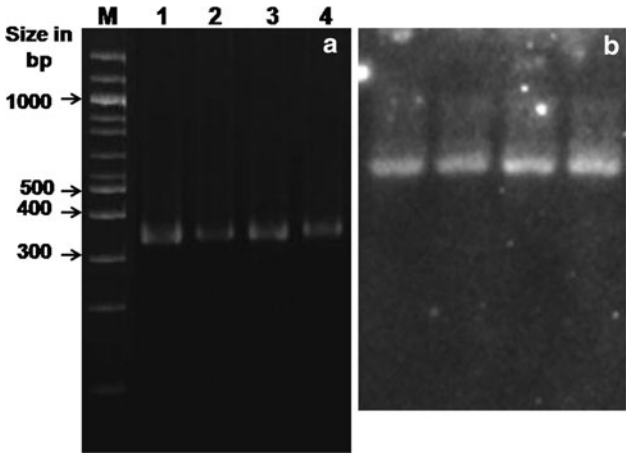


**Fig. 4** **a**, **b** Polymerase chain reaction and Southern blot analysis of transgenic *Nicotiana tabacum*. Total DNA was isolated from the transgenic plants and PCR was performed employing primers viz. clf and clr. PCR amplified DNA fragment derived from transgenic tobacco transformed with betasatellite (lanes 1 and 2) and $\beta$C1 (lanes 3 and 4). Expected size of DNA fragments (ca. 350 bp) were visualized on the gel, which was hybridized with $\alpha$-$P^{32}$-labeled DNA probe representing $\beta$C1. The *dark bands* on the blot indicates strong hybridization signal between the PCR products and radiolabeled probe

and $\beta$C1, DNA from both the transgenic plants ($T_0$) were isolated and subjected to PCR amplification. It yielded a DNA fragment of expected size (ca. 350 bp) which gave strong signal in Southern hybridization (Fig. 4a, b). The betasatellite DNA was capable of inducing symptoms, irrespective of the presence of main virus in the transformed plants.

Recombination analysis

Chances of recombination hypothesis were tested using specially designed software RDP version 3.0 [25]. Results showed a significant probability of recombination events. The betasatellite DNA under this study demonstrated the following recombination events, i.e. between nucleotide (nt) 25–111 probability ($P = 5.1 \times 10^{-23}$) and having major and minor parents are AY438562 and EU384596. Further between nts 112–703, 704–843, and 942–1224 probabilities ($P = 7.396 \times 10^{-59}$, $P = 2.885 \times 10^{-22}$, and $P = 1.289 \times 10^{-03}$) and recombination are between AY705381 and FJ770371, AY705381 and DQ191161 and finally between GQ249185 and AY705381. The recombination events were mostly observed between cotton leaf curl virus from Multan and Bangalore. One recombination event was detected between *Tomato leaf curl New Delhi virus* and *Cotton leaf curl Multan virus*.

## Discussion

*Crotalaria juncea* has been infected with a number of pathogens including fungi, nematodes and viruses. However, involvement of a begomovirus in leaf curl disease of *C. juncea* in this region has been identified only recently [15]. Leaf curl disease of *C. juncea* is a serious threat to its cultivation in north India.

Association of a betasatellite with the leaf curl disease of *C. juncea* has not been reported in the surrounding regions of Lucknow (north India). Earlier studies on this disease in suburbs of Lucknow only demonstrated the involvement of a geminiviral complex [15]. Present study was undertaken with an aim to characterize the betasatellite associated with leaf curl disease of *C. juncea*. Further expression of the betasatellite into tobacco plant demonstrated the functional role of this molecule.

PCR coupled with Southern hybridization based detection indicated the existence of a begomoviral complex in the leaf samples collected from Lucknow. The nucleotide sequence of CP gene obtained from different locations of Lucknow showed significant similarity. Sequence analysis of CP gene showed its homology with cotton leaf curl virus species reported from different regions of India and Pakistan. Interestingly, amino acid analysis of the CP gene under study with a number of other begomoviruses from Indian subcontinent showed highest homology to *Papaya leaf curl virus* reported from Bihar, India. Other isolates which shared maximum homology with CP gene are *Papaya leaf curl*

*virus*, PaLCuV [Pk:Kundian:2008] and *Cotton leaf curl kokhran virus* [In:Sirsa:2008]. Dendrograms drawn to analyze the phylogenetic relationship of CP gene with other begomovirus isolates from the Indian subcontinent placed it into a clad between begomoviruses reported from cotton (a member of *Malvaceae* family), and begomovirus reported from non-malvaceous plants such as Papaya, Radish, and Ageratum. Amino acid sequences based dendrogram placed it close to *Papaya leaf curl virus* (Fig. 2a, b).

The betasatellite obtained from the diseased *C. juncea* plants showed a significant sequence homology to betasatellite reported from different areas of Pakistan and India. The betasatellites which share sequence homology to betasatellite under this study were, however, reported from malvaceous plants. The phylogenetic analysis thus indicates the possibility of adaptation of a malvaceous betasatellite to nonmalvaceous crop. There are several reports of betasatellite from nonmalvaceous plants in several parts of the world including the Indian subcontinent [26]. The involvement of this molecule in symptom induction has been demonstrated by several experiments [8, 27]. Study on betasatellite molecule shows that several putative genes are located on either the virion-sense or complementary-sense strand, but it is the $\beta$C1 gene which is located on the complementary-sense strand, and is conserved in both size and position in almost all betasatellite molecules [28, 29].

The betasatellite under study showed common characteristics of betasatellite reported from other crops across the globe. Its size is approximately half to that of its main virus component, thus suggest a stringent size selection for encapsidation and that smaller size DNA molecule have adopted their size to allow encapsidation within geminate particles [30]. Presence of A-rich region is characteristic of all betasatellite. This region is supposed to increase the encapsidation and systemic movement by CP or movement protein encoded by begomoviruses [7]. It also plays a role in complementary-sense DNA replication [27]. About 300 beta molecules are reported across the world, and the number is increasing every year. A number of new betasatellite molecule reported are of recombinant in origin, same as the betasatellite molecule isolated from *C. juncea* under study. Recombination detection program gave a good indication of recombination between different viruses. The complete nucleotide sequence of present betasatellite molecule is 1359 nt, and its different segments showed possible recombination events between different *Cotton leaf curl viruses* species. These findings are in connection with [31] who advocate recombination between geminiviruses.

Previous studies on *Tomato leaf curl china virus* showed that replication of TYLCCNV-Y10 betasatellite DNA depends upon TYLCCNV-Y10 [32]. In this study, *C. juncea* betasatellite induced symptoms even in the absence of main virus, although whitefly mediated transmission experiment to induce similar symptom in tobacco plants failed (data not shown). The results thus showed inability of a betasatellite to replicate itself. We have also shown that expression of $\beta$C1 gene under the control of 35S caused leaf deformity in *N. tabacum*. Previous studies on the localization of $\beta$C1 protein suggested its expression and accumulation in the nucleus of transgenic *Nicotiana* plants [32].

Under natural condition, betasatellite can be detected only in association with begomoviruses (DNA-A). $\beta$C1 protein is suggested to play a role in symptom induction and not in the replication of betasatellite. Thus, the $\beta$C1 can be replaced by a foreign gene and can be converted into an expression vector. The technological application of this modified betasatellite vector is to study functional genomics in plants [33]. Recently virus vector system based on modified betasatellite associated with a monopartite begomovirus (TYLCCNV) was found effective in virus induced gene silencing analysis in *Solanaceae* species including Tomato [34, 35]. Begomovirus which constitutes largest sub-group among *Geminiviridae* family are whitefly transmitted, and reports of this virus to infect various crops are increasing day by day. Betasatellite DNA is responsible for the induction of leaf curl symptoms in plants, and reporting of this molecule from a nonmalvaceous plant is interesting. It is likely that these local crops act as a reservoir of begomoviruses. The present study also indicates that recombination may play a major role in infecting begomoviruses to a new host, however, other factors cannot be ruled out. Different begomoviruses and their associated satellites undergo recombination and result in infecting new hosts. The betasatellite molecule under study is from north India, whereas recombination events showed parents from Rajasthan (India) and Pakistan. In India there is a great diversity in the various groups of plants and economically important crops. The white fly transmitted geminiviruses (WTGs) are needed to be controlled; otherwise they have potential to damage the economy of countries like India.

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