MOLECULAR CHARACTERIZATION AND SEQUENCE VARIABILITY OF BETASATELLITES ASSOCIATED WITH LEAF CURL DISEASE OF KENAF (*HIBISCUS CANNABINUS* L.) FROM DIFFERENT GEOGRAPHICAL LOCATIONS OF INDIA

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Summary. – Leaf curl disease of kenaf (*Hibiscus cannabinus* L.) in India has been found to be associated with begomoviruses and betasatellites. Here, we report the molecular characterization and phylogenetic relationship of the nine isolates of betasatellites obtained from three geographical locations in India. The betasatellites coming from northern and eastern region of India shared 84.3% nucleotide sequence identity and formed two sub-clusters within the main cluster containing different isolates of Cotton leaf curl Multan betasatellite (CLCuMB) isolated in Indian subcontinent. Betasatellites coming from the southern part of India were identified as the isolates of Tomato leaf curl Joydebpur betasatellite and shared 45.2 and 44.9% sequences identity with their counterparts coming from the eastern and northern India, respectively. The present study represents the first report about the association of the leaf curl disease of kenaf with the betasatellites infecting both malvaceous and non-malvaceous crops in India.

Key words: Begomovirus; betasatellite; kenaf; leaf curl disease; phylogenetic relationship

Introduction

The family *Geminiviridae* is the second largest family of plant viruses and contains four genera *Mastrevirus, Curtovirus, Topocuvirus,* and *Begomovirus* based on the number of genomic components (monopartite or bipartite), type of insect vector (whitefly, leafhopper, or treehopper), and host range (mono- or dicotyledonous hosts) (Fauquet *et al*., 2005). More than 80% of the known geminiviruses that cause devastating diseases of economically important crops in tropical and sub-tropical countries belong to the genus *Begomovirus*. This genus is characterized by their unique particle morphology of twinned incomplete icosahedra and bipartite genome composed of two circular single-stranded DNA each of ~2.7 kb size and designated as DNA A (encodes coat protein and other replication associated proteins) and DNA B (encodes proteins for cell-to-cell movement) (Rybicki *et al*., 2000). Some of the Old World begomoviruses seem to have the ability to dispense with the DNA B component of their genome. This could be due to the presence of ORF AV2 in the DNA A, which encodes a protein that participates in virus movement (Padidam *et al*., 1996). Recently, some single-stranded satellite DNA molecules have been found to be associated with such

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Abbreviations: BLCCNB = Bean leaf curl China betasatellite; BYVB = Bhendi yellow vein betasatellite; CLCuGB = Cotton leaf curl Gezira betasatellite; CLCuMB = Cotton leaf curl Multan betasatellite; EpYVB = Eupatorium yellow vein betasatellite; HYVNB = Honeysuckle yellow vein Nara betasatellite; MaYVB = Malvastrum yellow vein betasatellite; MaYVYnB = Malvastrum yellow vein Yunnan betasatellite; MYMD = Malvastrum yellow vein disease; SiYVB = Sida yellow vein betasatellite; TbLCB = Tobacco leaf curl betasatellite; TbLCJB = Tobacco leaf curl Japan betasatellite; ToLCB = Tomato leaf curl betasatellite; ToLCJoB = Tomato leaf curl Joydebpur betasatellite

monopartite begomoviruses. These DNA satellites are classified into two groups. DNA satellites termed as DNA-1 show similarity with nanoviruses, but are not involved in symptom induction or infectivity of its helper begomovirus (Mansoor *et al*, 1999; Saunders and Stanley, 1999). The second group termed as DNA-ß satellites has approximately half the size of a begomovirus DNA (~1.3 kb), but the sequence is unrelated to their helper viruses except for a conserved hairpin structure and TAATATTAC loop sequence (a begomovirus specific origin of replication). DNA-ß satellites are required for the efficient infection of some hosts (Saunders *et al*., 2000; Briddon *et al*., 2001). Now, they are termed as betasatellites and have been reported to be co-evolved with their cognate helper viruses (Zhou *et al*., 2003) and depend upon their helper viruses for encapsidation, replication, movement, and insect-transmission (Briddon *et al*., 2008). Due to the ease of PCR-based detection and sequencing, these betasatellites have been shown to be associated with an increasing number of diseases caused by the begomoviruses.

Over 260 full-length betasatellite sequences have been deposited in databases indicating the importance and widespread nature of these components at least in the Old World (Briddon *et al*., 2008). Two major groups of betasatellites, one infecting the hosts within the family *Malvaceae* and the second infecting a more diverse group of plants within the families *Solanaceae* and *Compositeae* were classified by phylogenetic analyses (Briddon *et al*., 2003). Within these two clusters, betasatellites showed relatedness among themselves with regard to host and geographic origin what strongly supports co-adaptation of betasatellites with their respective helper begomoviruses.

Hibiscus cannabinus L. is a member of the family *Malvaceae* and is cultivated primarily for the production of bast fibers in different parts of the world as well as in India. It is a potentially valuable industrial crop due to its fiber content, therapeutic value, and use in paper industry (Duke, 1983). In 1960, the USDA selected kenaf from five hundred candidates as the most promising non-wood fiber for pulp and paper production (http://www.ecomall.com/greenshopping/kenafx.htm, http://www.psouth.net/kenaf.php). Kenaf is widely cultivated in different states of India like Andhra Pradesh, Orissa, West Bengal, and Uttar Pradesh. Large number of diseases affecting this crop are possibly caused by different viruses and symptoms of the diseases are observed at different geographical regions in India, but no detailed data has been reported yet. Recently, we found a viral disease on kenaf with prominent "leaf curl" symptom in northern (Paul *et al*., 2006), eastern, and southern part of the country. PCR amplification study confirmed the association of monopartite begomoviruses and betasatellites with this disease.

The aim of the present study was to characterize the betasatellites associated with leaf curl disease of kenaf

Table 1. Isolates of betasatellites associated with leaf curl disease of kenaf

Geographical location	State	Name of betasatellite isolate	Proposed descriptor	Acc. No.	Size (nt)
Eastern India	West Bengal	Cotton leaf curl Multan DNA betasatellite-[India:Barrackpore 01:Kenaf:2006]	CLCuMB-[IN:Bar01:Ken:06]	EF620564	1345
		Cotton leaf curl Multan DNA betasatellite-[India:Haringhata 05:Kenaf:2006]	CLCuMB-[IN:Har05:Ken:06]	EF620565	1347
		Cotton leaf curl Multan DNA betasatellite-[India:Bongaon:Kenaf:2006]	CLCuMB-[IN:Bon:Ken:06]	EU880231	1345
Northern India	Uttar Pradesh	Cotton leaf curl Multan DNA betasatellite-[India:Bahraich 03:Kenaf:2006]	CLCuMB-[IN:Bah03:Ken:06]	EF620566	1343
		Cotton leaf curl Multan DNA betasatellite-[India:Bhangha06:Kenaf:2006]	CLCuMB-[IN:Bhn06:Ken:06]	EU825206	1343
		Cotton leaf curl Multan DNA betasatellite [India:Kaisarganj04:Kenaf:2006]	CLCuMB- [IN:Kai04:Ken:06]	EU825205	1343
Southern India	Andhra Pradesh	Tomato leaf curl Joydebpur DNA betasatellite-[India:Amadalavalasa1:Kenaf:2007]	ToLCJoB-[IN:Ama1:Ken:07]	EU431115	1367
		Tomato leaf curl Joydebpur DNA betasatellite-[India:Amadalavalasa2:Kenaf:2007]	ToLCJoB-[IN:Ama2:Ken:07]	EU880232	1367
		Tomato leaf curl Joydebpur DNA betasatellite-[India:Ponduru:Kenaf:2007]	ToLCJoB-[IN:Pon:Ken:07]	EU880233	1367

isolated from the symptomatic plants in eastern, northern, and southern part of India. The full-length sequences of nine betasatellite isolates and their variability are compared.

Materials and Methods

Collection of samples and whitefly transmission. Kenaf plants showing upward leaf curl symptoms were collected from the farmer's fields in three different locations each from Uttar Pradesh (northern India), Andhra Pradesh (southern India), and West Bengal (eastern India) states (Table 1). The begomovirus complexes of these nine samples were maintained separately on healthy kenaf plants (cv. HC 583) in a glasshouse by whitefly transmission (5 whiteflies per plant) with acquisition and inoculation access periods of 12 hrs in each case. Whitefly transmission was confirmed through successive back-inoculation to new sets of healthy plants.

PCR and DNA isolation. Total nucleic acid was isolated and purified from the symptomatic leaves (Doyle and Doyle, 1990). To amplify the betasatellites, PCR was carried out using universal betasatellite primers (Briddon *et al*., 2002). In all PCR reactions, 100 ng of total plant DNA and 0.2 µmol/l of primers were used. The DNA obtained from healthy plants was used as a control. Each PCR reaction mixture contains 1x PCR buffer, 0.2 mmol/l dNTPs, 1.5 mmol/l $MgCl_2$ and 0.6 U of High Fidelity PCR Enzyme Mix (Fermentas). DNA fragments were amplified with an initial denaturation at 94°C for 2 mins followed by 30 cycles of 94°C for 1 min, 55°C for 2 mins, and 72°C for 3 mins followed by a final extension at 72°C for 10 mins.

Cloning of amplicons and sequencing. Amplified products were cloned in pJET1.2 positive selection vector using GeneJET™ PCR Cloning Kit (Fermentas) following the manufacturer's protocol. One clone of each PCR product was sequenced in both orientations.

Sequence analysis. Sequences were assembled and sequence identity matrix was generated for comparison using Bioedit sequence alignment editor (version 5.0.9). Multiple alignments were performed and phylogenetic tree was constructed using Neighbor-joining algorithm of Clustal X (version 1.8), bootstrapped with 1000 trials and finally displayed, manipulated, and printed using Treeview software (version 1.6.6). Sequences of other betasatellites used for comparison were adopted from the nucleotide sequence database.

Results

Transmission of the disease

After whitefly transmission, the typical symptoms of the disease appeared in healthy plants after a minimum incubation period of 16–20 days under glasshouse conditions. No deviation of the symptoms was recorded even after successive whitefly transmission. Transmission efficiency of the isolates obtained from northern and eastern India was nearly 70% and from the southern India only 30%.

Molecular characterization

Nine symptomatic samples (three from each geographic location) maintained in the glasshouse were examined in PCR and produced a betasatellite specific ~1.3 kb amplicon. No amplification was observed with the DNA from the control healthy plants (Fig. 1). Cloning and sequencing of these amplicons revealed that all these betasatellite isolates shared 99.6–100% sequence identity among themselves with respect to their corresponding geographical locations. The betasatellite sequences were deposited in the database under the Acc. Nos. EF620564, EF620565, EU880231 (eastern India isolates), EF620566, EU825205, EU825206 (northern

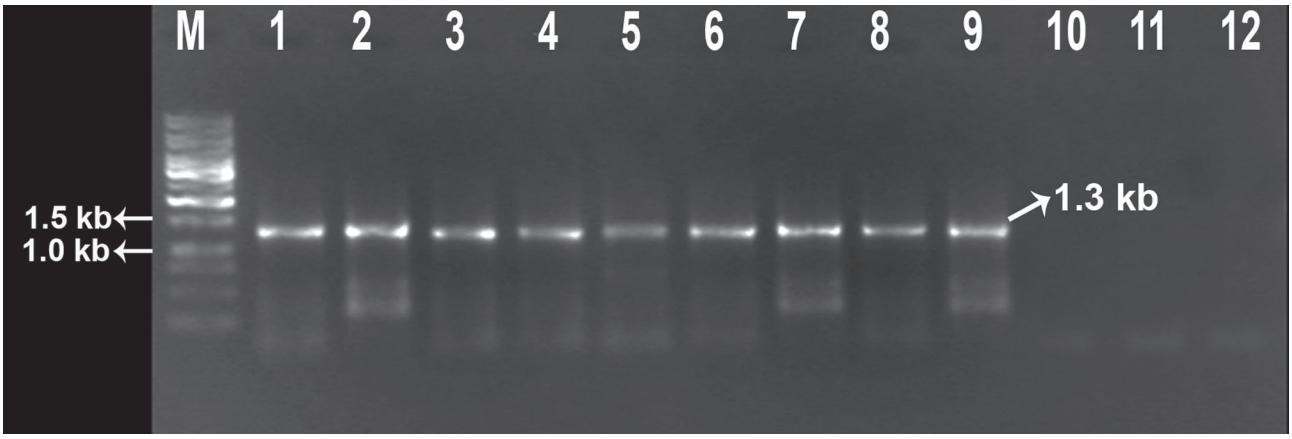

Fig. 1

PCR products of the examined betasatellites detected by electrophoresis

Isolates from eastern (lanes 1, 2, 3), northern (lanes 4, 5, 6), and southern India (lanes 7, 8, 9). Negative control from healthy plants (lanes 10, 11, 12). 1 kb DNA ladder (lane M).

Table 2. Sequence identity of the examined betasatellite isolates with other betasatellites

Name of betasatellite isolates from different crops with their Acc. No.	Sequence identity with examined betasatellite isolates (in %)*		
	Eastern India	Northern India	Southern India
Malvastrum yellow vein betasatellite-[China:Yunnan200]AJ971697	76.3	76.0	44.5
Malvastrum yellow vein Yunnan betasatellite-[China:Yunnan304:2003] AM236776	79.7	79.6	45.1
Ludwigia leaf distortion betasatellite-[India:Bittergourd:2004] AY817151	79.2	77.5	45.1
Cotton leaf curl Multan betasatellite-[India:Dabwali2:1995] AJ316038	86.8	**84.7**	45.9
Cotton leaf curl Multan betasatellite-[India:Bhatinda:2005] DQ191161	86.9	84.6	45.8
Cotton leaf curl Multan betasatellite-[Pakistan:Burewala1:2002] AM084379	85.1	81.5	45.6
Cotton leaf curl Multan betasatellite-[Pakistan:Faisalabad:Hibiscus:1996] AJ297908	86.4	83.6	45.2
Cotton leaf curl Multan betasatellite-[India:Sirsa:2004] AY744380	89.3	82.5	44.6
Cotton leaf curl Multan betasatellite-[India:Basirhat:Hibiscus:2005] DQ298137	**98.0**	83.3	44.7
Sida yellow vein betasatellite-[India:Barrackpore:2007] EU188921	80.1	78.9	45.2
Bhendi yellow vein betasatellite-[India:Muthuppatti:2000] AJ308425	52.5	51.4	44.4
Cotton leaf curl Gezira betasatellite-[Egypt:Fayourn:1996] AJ316039	47.4	46.6	41.2
Tomato leaf curl Joydebpur betasatellite-[Bangladesh:Gazipur:2005] AJ966244	45.2	44.8	**92.7**
Tomato leaf curl betasatellite-[India:NewDelhi:2002] AJ542490	45.5	44.7	66.9
Tobacco leaf curl betasatellite-[Pakistan:Rahim Yar Khan:1998] AJ316033	44.8	45.0	60.9
Ageratum yellow vein Sri Lanka betasatellite-[Sri Lanka:Ageratum:2003] AJ542498	45.7	44.8	61.0
Eupatorium yellow vein betasatellite-[Japan:MNS2:2000] AJ438938	42.4	40.1	44.7
Honeysuckle yellow vein Nara betasatellite-[Japan:Nara:2006] AB287443	41.1	40.9	45.7
Bean leaf curl China betasatellite-[China:Yunnan 295:2004] AM260730	49.6	48.5	63.2
Cotton leaf curl Multan betasatellite-[India:Sri Ganganagar:2002] AY083590	85.6	81.2	52.0
Malvastrum yellow vein Yunnan betasatellite-[China:Yunnan 308:2003] AM236778	80.5	81.1	51.3
Malvastrum yellow vein Yunnan betasatellite-[China:Yunnan 307:2003] AM236777	79.5	79.9	50.8
Cotton leaf curl Gezira betasatellite-[Sudan:Cotton 46:1996] AY669328	48.5	48.8	46.2
Cotton leaf curl Gezira betasatellite-[Sudan:sida32:1996] AY077798	48.1	48.2	46.2

*Highest sequence identities are presented in bold.

India isolates), and EU431115, EU880232, EU880233 (southern India isolates). Sequence analysis revealed that the betasatellite isolates characterized in this study shared only 61% sequence identity among themselves. The isolates obtained from eastern and northern India shared on average 84.3% sequence identity and these two groups of geographical isolates shared only 45.2 and 44.9% sequence identity, respectively, with their counterparts from southern India. Thus, all these isolates appeared to be grouped in two different types. The first type (Type 1) consisted of betasatellite isolates obtained from northern and eastern India, while the second type (Type 2) consisted of isolates obtained from southern India. The Type 1 isolates showed highest average sequence identity (85.4%) with different isolates of Cotton leaf curl Multan betasatellite reported from Indian subcontinent. Within this type, betasatellite isolates obtained separately from eastern and northern India showed highest sequence identity with Cotton leaf curl Multan betasatellite-[India:Basirhat:Hibiscus:05] (98%, DQ298137) and Cotton leaf curl Multan betasatellite-[India:Dabwali2:95] (84.7%, AJ316038), respectively (Table 2). The Type 2 comprised south Indian isolates and shared highest sequence identity (92.7%) with Tomato leaf curl Joydebpur betasatellite-[Bangladesh:Gazipur:05] (AJ966244) (Table 2). Phylogenetic analysis revealed that betasatellite isolates obtained from eastern and northern India formed two distinct sub-clusters within the cluster, where all malvaceous crop-infecting betasatellites were present (Fig. 2). Within this cluster, three different sub-clusters of betasatellites originating from Egypt, China, and India were observed what indicated the geographical relatedness among the betasatellites. The south Indian isolates formed cluster with Tomato leaf curl Joydebpur betasatellite present in the cluster along with other betasatellites reported from solanaceous crops (Fig. 2).

As the species demarcation cut-off for betasatellites rested on 78% (Briddon *et al*., 2008), the betasatellite isolates obtained from eastern and northern India appeared to be the isolates of Cotton leaf curl Multan betasatellite and totally distinct from southern Indian isolates, which were found to be the isolates of Tomato leaf curl Joydebpur betasatellite. According to the latest nomenclature of the betasatellite proposed by Briddon and co-workers (Briddon *et al*., 2008), the descriptors for all these betasatellite isolates under study were proposed and presented in Table 1.

All the presented betasatellite isolates shared similar genome organization pattern. Like the other betasatellites reported earlier, these betasatellites also possessed a sequence of approximately 100 nucleotides that is highly conserved and known as the satellite conserved region (SCR). Besides

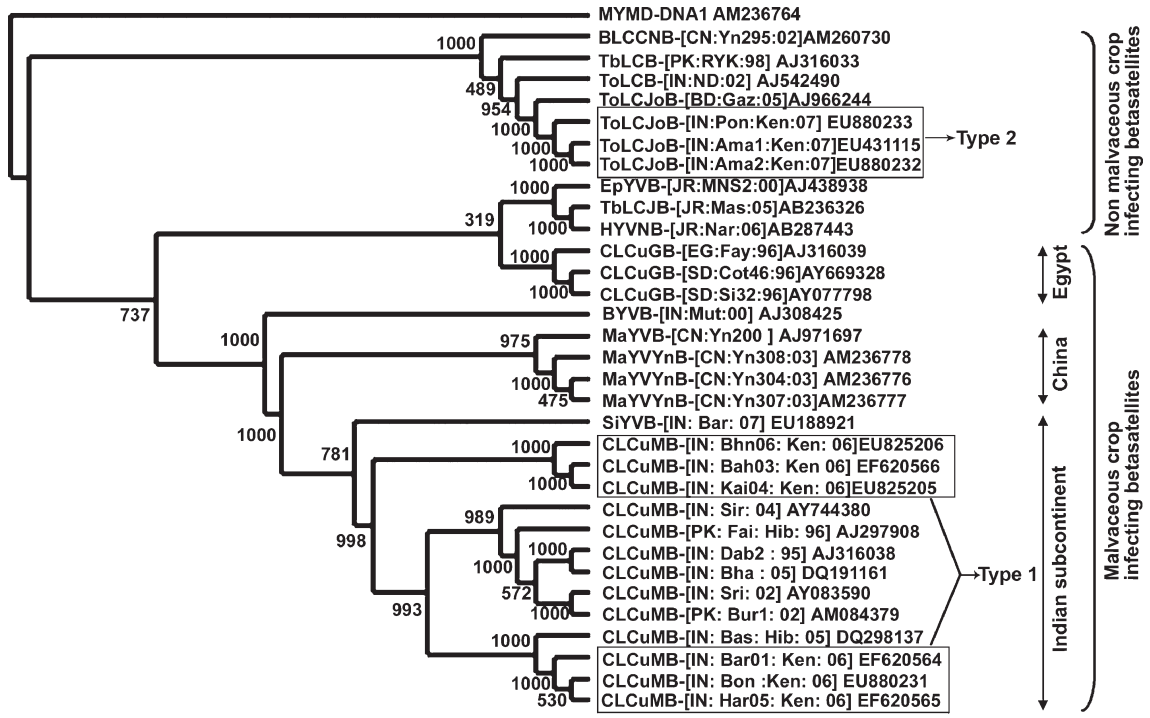

Fig. 2

Phylogenetic tree of the examined betasatellites (in rectangular boxes) with other betasatellites

The tree is arbitrarily rooted in the sequence of DNA1 molecule (AM236764) associated with MYMD, an unrelated sequence of similar size. Numbers at nodes indicate bootstrap scores (1,000 replicates). The full names of the examined Indian betasatellites are presented in Table 1. For abbreviations see the list on the front page.

the SCR, all these betasatellite isolates possess an adenine rich region and a single conserved ORF known as βC1 (Mansoor *et al*., 2003). The βC1 protein of all these isolates is composed of 118 amino acid residues. The βC1 gene of the east, north and south Indian betasatellite isolates showed highest sequence identity of 99, 91, and 87%, respectively, with those of Cotton leaf curl Multan betasatellite-[India:Basirhat:Hibiscus:05], Cotton leaf curl Multan betasatellite-[India:Hisar:04] and Tomato leaf curl Joydebpur betasatellite-[Bangladesh:Gazipur:05]. The adenine-rich region of east and north Indian betasatellite isolates showed highest sequence identity with Cotton leaf curl Multan betasatellite-[India:Basirhat:Hibiscus:05] (98 and 77%, respectively) and the homologous region of those of south Indian isolates showed highest sequence identity (89%) with Tomato leaf curl Joydebpur betasatellite-[Bangladesh:Gazipur:05].

Discussion

Leaf curl disease of kenaf was found to be associated with different begomovirus complexes at different geographical locations in India. Complete sequencing of the associated begomoviruses from northern, eastern, and southern India showed that the north Indian isolate shared highest nucleotide sequence identity (88.2%) with an Yunnan isolate of Malvastrum yellow vein virus (MYVV)-[China:Yunnan206:2004] (AJ744881), the east Indian isolate shared highest nucleotide sequence identity with Mesta yellow vein mosaic virus-[India:Barrackpore2:2007] (98%, EF432372) and the south Indian isolate shared highest nucleotide sequence identity (95%) with Tomato leaf curl Joydebpur virus (ToLCJV, AJ875159) (Paul *et al*., unpublished). These three begomovirus isolates shared 76.06% nucleotide sequence identity among themselves. Analysis on individual pair-wise sequence identity revealed that the east and north Indian isolate shared 81.1%, east and south Indian isolate shared 71.9% and north and south Indian isolate shared 75.2% nucleotide sequence identity (Paul *et al*. unpublished data). Previous studies on the diversity of begomovirus-associated betasatellites focused mainly on the satellites originating from different hosts (Briddon *et al*., 2003; Bull *et al*., 2004). Diversity of the betasatellites with respect to a particular disease was first shown in the case of

cotton leaf curl disease. It was demonstrated that seven different begomoviruses with a single betasatellite species formed the etiological complex at different geographical locations (Mansoor *et al*., 2003). Recently, the diversity of betasatellites associated with MYVV isolated from *Malvastrum coromandelianum* plants from different geographical locations in Yunnan Province, China was examined and the results indicated the presence of one betasatellite species with limited variability (Guo *et al*., 2008). In this study, we tried to characterize the betasatellite molecules that were associated with leaf curl disease of kenaf crop in different geographical locations in India, examined their variability and established phylogenetic relationships with other betasatellites reported earlier from other crops. It was found that the betasatellites associated with leaf curl disease of kenaf in northern and eastern India were isolates of Cotton leaf curl Multan betasatellite, whereas those from southern India were isolates of Tomato leaf curl Joydebpur betasatellite. There are just few reports about infection of malvaceous plants with non-malvaceous betasatellites (Raj *et al*., 2007; Xiong *et al*., 2005). In the present study it is conceivable to assume that at least two distinct betasatellite species are associated with the kenaf leaf curl disease occurring at different geographical locations in India.

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References

Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X, Fauquet CM (2008): Recommendations for the classification and nomenclature of the DNA-b satellites of begomoviruses. *Arch. Virol.* DOI 10.1007/s00705-007-0013-6.

Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID, Dhawan P, Rishi N, Siwatch SS, Abdel-Salam AM, Brown JK, Zafar Y, Markham PG (2003): Diversity of DNA b: a satellite molecule associated with some monopartite begomoviruses. *Virology* **312**, 106–121.

Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG (2002): Universal primers for the PCR mediated amplification of DNA beta – A molecule associated with some monopartite begomoviruses. *Mol. Biotechnol.* **20**, 315–318.

Briddon RW, Mansoor S, Bedford ID, Pinner MS, Saunders K, Stanley J, Zafar Y, Malik KA, Markham PG (2001): Identification of DNA components required for induction of cotton leaf curl disease. *Virology* **285**, 234–243.

Bull SE, Tsai WS, Briddon RW, Markham PG, Stanley J, Green SK (2004): Diversity of begomovirus DNA ß satellites of non-malvaceous plants in east and south east Asia. *Arch. Virol*. **149**, 1193–1200.

Doyle JJ, Doyle JL (1990): Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.

Duke JA (1983): 'Handbook of energy crops.' Available at http://www.hort.purdue.edu/newcrop/duke energy/Hibiscus cannabinus.html.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (2005): *Virus Taxonomy*. *VIIIth Report of the ICTV*. Elsevier/Academic Press, London, pp. 1253.

Guo W, J Tong, Zhang X, Li G, Zhou XP (2008): Molecular Variation of Satellite DNAß Molecules Associated with *Malvastrum yellow vein virus* and Their Role in Pathogenicity. *Appl. Environ. Microbiol*. **74**, 1909–1913.

Mansoor S, Briddon RW, Bull SE, Bedford ID, Bashir A, Hussain M, Saeed, Zafar MY, Malik KA, Fauquet C, Markham PG (2003): Cotton leaf curl disease is associated with multiple monopartite begomoviruses supported by single DNA-β. *Arch. Virol.* **148**, 1969–1986.

Mansoor S, Briddon RW, Zafar Y, Stanley J (2003): Geminivirus disease complexes: an emerging threat. *Trends Plant Sci*. **8**,128–134.

Mansoor S, Khan SH, Bashir A, Saeed M, Zafar Y, Malik KA, Briddon RW, Stanley J, Markham PG (1999): Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology* **259**, 190–199.

Padidam M, Beachy RN, Fauquet CM (1996): The role of AV2 ("precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. *Virology* **224**, 390–404.

Paul S, Ghosh R, Roy A, Mir JI, Ghosh SK (2006): Occurrence of a DNA ß-containing begomovirus associated with leaf curl disease of kenaf (Hibiscus cannabinus L.) in India. *Australasian Plant Dis. Notes* **1**, 29–30.

Raj SK, Khan MS, Snehi SK, Roy RK (2007): Yellow vein netting of Bimili jute (Hibiscus cannabinus L.) in India caused by a strain of Tomato leaf curl New Delhi virus containing DNA ß. *Australas. Plant Dis. Notes* **2**, 45–47.

Rybicki EP, Briddon RW, Brown JE, Fauquet CM, Maxwell DP, Harrison BD, Markham P, Bisaro DM, Robinson D, Stanley J (2000): In van Regenmortel MHV, Fauquet CM, Bishop DIIL (Ed.): *Virus Taxonomy*. *Seventh Report of the International Committee on Taxonomy of Viruses*. Academic Press, San Diego, USA, pp. 285–297.

Saunders K, Stanley J (1999): A nanovirus-like component associated with yellow vein disease of Ageratum conyzoides: evidence for interfamilial recombination between plant DNA viruses. *Virology* **264**, 142–152.

Saunders K, Bedford ID, Briddon RW, Markham PG, Wong SM, Stanley J (2000): A unique virus complex causes ageratum yellow vein disease. *Proc. Natl. Acad. Sci. USA* **97**, 6890–6895.

Xiong Q, Guo XJ, Che HY, Zhou XP (2005): Molecular characterization of a distinct Begomovirus and its associated satellite DNA molecule infecting Sida acuta in China. *J. Phytopathol.* **153**, 264-268.

Zhou X, Xie Y, Tao X, Zhang Z, Li Z, Fauquet CM (2003): Characterization of DNA ß associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. *J. Gen. Virol*. **84**, 237–247.