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### Short Communication

## First report of a betasatellite associated with Radish leaf curl virus infecting new host *Cyamopsis tetragonoloba* (Guar bean) in India

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### Abstract

*Begomovirus* is one of the largest genus of the family *Geminiviridae* causing plant virus diseases (vector *Bemisia tabaci*) in the tropical and subtropical regions of the world. In the present study, we characterized a betasatellite DNA associated with *Cyamopsis tetragonoloba* leaf curl disease which causes leaf curling in Guar bean plants. The full-length satellite DNA comprises 1,332 nucleotides and shows the highest nucleotide sequence identity (99 %) to *Radish leaf curl virus*. This is the first report of association of a betasatellite with *Cyamopsis tetragonoloba* leaf curl disease in Guar bean plants in India.

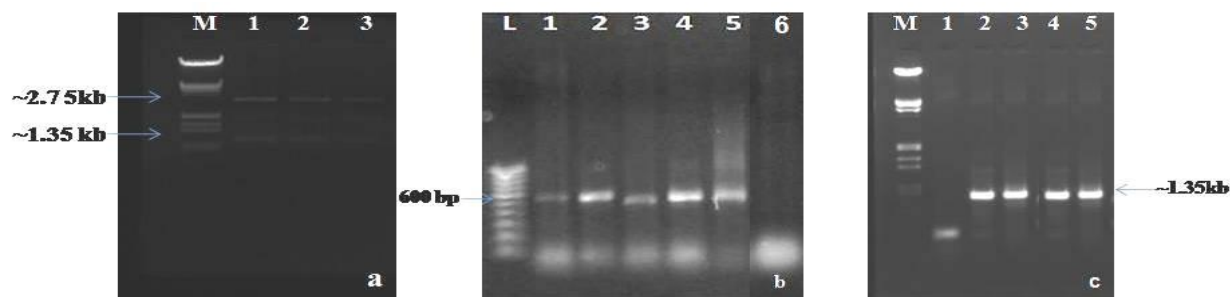
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Geminiviruses (family *Geminiviridae*) have circular single stranded DNA genomes encapsidated in twinned quasi-isometric particles and are responsible for major crop losses worldwide Moffat (1999). The largest genus, *Begomovirus*, comprises viruses transmitted by the whitefly *Bemisia tabaci* infect only dicotyledonous plants. It consists of approximately 1350 nt and depend on their helper begomoviruses for replication and encapsidation. *Cyamopsis tetragonoloba* (guar bean) is cultivated in tropical, subtropical, and warm temperate regions in India (mainly Rajasthan). Guar bean is an annual legume and the source of guar gum of *Fabaceae* family. Guar is not only used for food purpose in developing countries but it is also used in the extraction of oil and shale gas. In the present study we report the presence of a novel betasatellite associated with the new host *Cyamopsis tetragonoloba* leaf curl disease from Rajasthan, India.

In the year 2012 *Cyamopsis tetragonoloba* (guar bean) showing the leaf curling, leaf distortion and dwarfing were collected from guar bean growing fields of Rajasthan, India. To investigate the potential begomoviral infection, total DNA was extracted from the dried samples by using CTAB (Cetyl Tri-methyl Ammonium Bromide) method (Saghai-Marroof et al., 1984). Rolling circle amplification (RCA) which exponentially amplify single- or double-stranded circular DNA templates (Dean et al., 2001) was performed by using TempliPhi DNA amplification kit as per the manufacturer's instruction GE Healthcare, USA. The resulting concatamers were digested with *EcoRI* and *Sall* restriction yielded DNA fragments of ~2.7 and ~1.35 kb (Fig.1a). These fragments were cloned into pGEMT-easy Vector (Chandran et al., 2013) and sequenced (KF218188 and KC854405, respectively). To confirm the Koch's postulate for the virus the infectious clone were constructed into pCAMBIA 2301 (pCAMBIA-A and pCAMBIA-β) and mobilized into *Agrobacterium tumefaciens* by the freeze± thaw method. The construct was further used for agro-inoculation in *Nicotiana benthamiana* plants

For each experiment near about 40 *N. benthamiana* plants grown on insect-free green house were taken. After 20-25 days the plants showed similar kind of symptoms as observed in naturally infected guar bean plants in the fields. Simultaneously, the authenticity of infectivity assay was confirmed by using begomovirus coat protein and satellites specific primers (Bela-ong et al., 1999; Bull et al., 2003) in PCR. Positive PCR reactions were found against coat protein and betasatellites (Fig.1 b & c) established the begomovirus infection. Sequences of the cloned RCA product were aligned with the CLUSTALW program (Larkin et al., 2007) and phylogenetic trees were constructed by using

the neighbor-joining algorithm and viewed with the Tree View program (Page, 1996). Blast analysis was used for selecting the related sequences (Table 1).



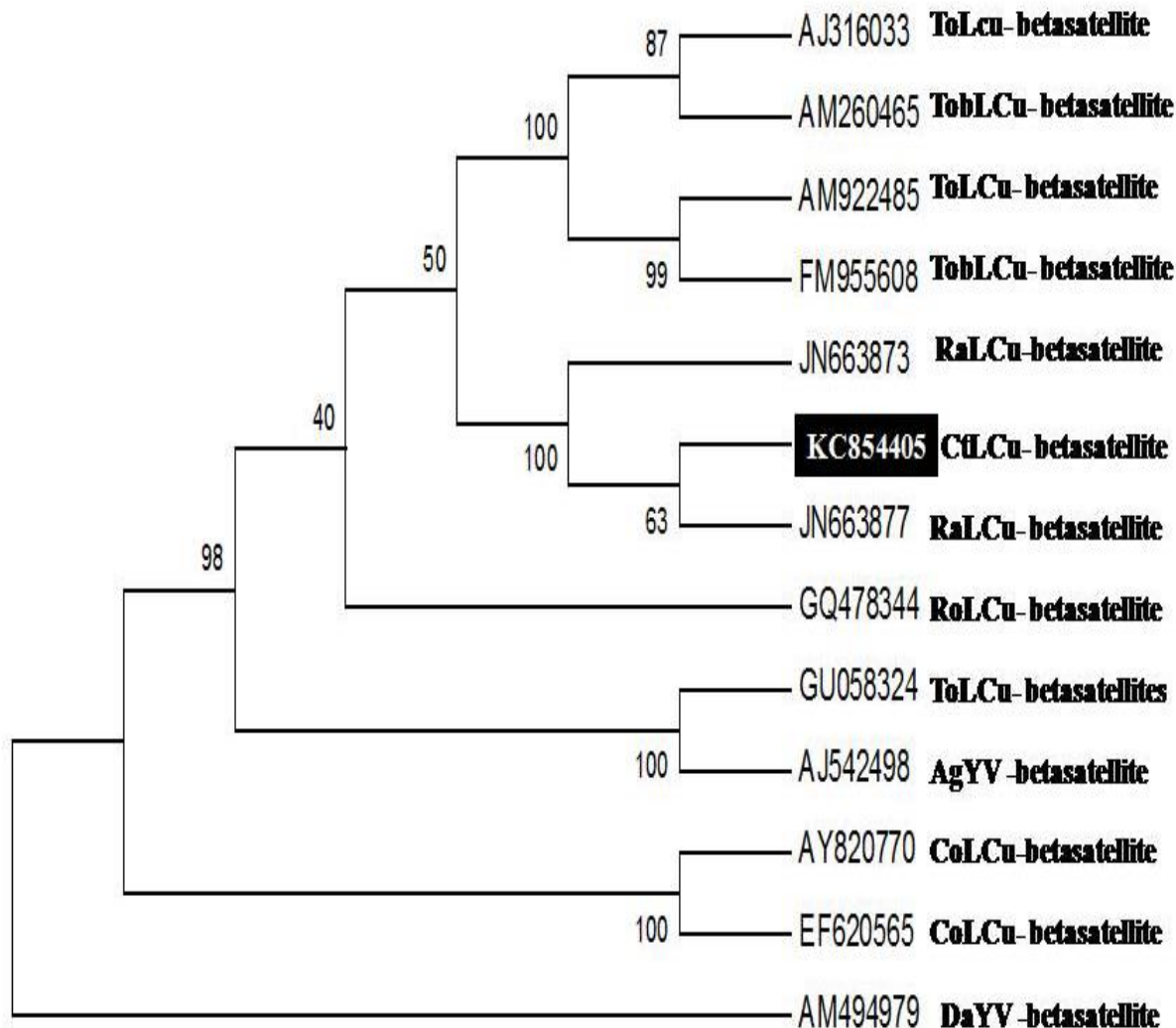
**Figure.1**(a) Agarose gel electrophoresis of the rolling circle amplification digested product (b) Coat protein PCR products, whereas lane 6 stands for healthy plant, L represents the 100 bp Ladder. (c) The presence of ~1.35 kb product by  $\beta$ -satellite specific primer pairs, lane 1 stand for healthy one amplified from DNA extracts prepared from infected plants confirming positive results of begomovirus.

**Table 1:** Nucleotide sequence identity of betasatellites of present begomovirus complex with other associated begomoviruses

Betasatellites under study	Host	Accession no	Abbreviation	Symptoms
1. Radish leaf curl betasatellite	<i>Capsicum annuum</i> [Chili]	JN663873	RaLCuB	LC, VD
2. Radish leaf curl betasatellite	<i>Capsicum annuum</i> [Chili]	JN663877	RaLCuB	LC, VD
3. Tobacco leaf curl disease associated sequence	<i>Nicotiana tabacum</i> [Tobacco]	AJ316033	ToLcuB	LC, VD
4. Tobacco leaf curl betasatellite	<i>Pedilanthus tithymaloides</i>	AM260465	CoLCuB	LC
5. Rose leaf curl betasatellite	<i>Nicotiana benthamiana</i>	GQ478344	RoLCuB	LC
6. Tomato leaf curl Pakistan virus betasatellite	<i>Glycine max</i> [Soya bean]	AM922485	ToLCuB	LC
7. <i>Digera arvensis</i> yellow vein disease-associated DNA betasatellite	<i>Digera arvensis</i>	AM494979	DaYVB	YV
8. Tobacco leaf curl virus-associated DNA betasatellite	<i>Glycine max</i> [Soya bean]	FM955608	TobLCuB	LC
9. Tomato yellow leaf curl Thailand virus satellite DNA betasatellite	<i>Lycopersicon esculentum</i> [Tomato]	GU058324	ToYLCuB	LC
10. Ageratum yellow vein virus-associated DNA betasatellite	<i>Ageratum conyzoides</i>	AJ542498	AgYVB	YV
11. Cotton leaf curl virus-associated DNA betasatellite	<i>Gossypium hirsutum</i> [Cotton]	AY820770	CoLCuB	LC, VD
12. Cotton leaf curl Multan DNA betasatellite	<i>Gossypium hirsutum</i> [Cotton]	EF620565	CoLCuB	LC, VD

**Note.** LC, leaf curl; VD, vein darkening; YV, vein yellowing

DNA-B molecules were not detected from DNA sample using primers specific to DNA-B of begomovirus (Rojas et al., 1993). Both, DNA-A and betasatellites shared the highest sequence identity (99%) with *Radish leaf curl virus* reported from northern part of India. A phylogenetic tree was constructed (Fig. 2) and our isolates were shown to be clustering with *Radish leaf curl virus* (JN663877), is a possible isolate of *Radish leaf curl virus* infect new host Guar bean.



**Figure.2** Phylogenetic tree obtained using Neighbor-joining method in MEGA 4.0 with 1,000 bootstrap replications using complete nucleotide sequences of betasatellites.

This is the first report of association of a betasatellite with *Cyamopsis tetragonoloba* leaf curl disease which causes leaf curling in guar bean plants in India.

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## References

1. Bela-ong, D.B. and Bajet, N.B. (2007): Molecular Detection of Whitefly-Transmissible Geminiviruses (Family Geminiviridae, Genus Begomovirus) in the Philippines. *Philipp J Sci.*, 136: 87–101.
2. Briddon, R.W., Bull, S.E., Amin, I., Idris, A.M., Mansoor, S., Bedford, I.D., Dhawan, P., Narayan, R., Siwatch, S.S., Abdel-Salam, A.M., Brown, J.K., Zafar, Y. and Markham, P.G. (2003): Diversity of DNA  $\beta$ , a satellite molecule associated with some monopartite begomoviruses. *Virology.*, 312: 106-121.
3. Bull, S.E., Briddon, R.W. and Markham, P.G. (2003): Universal primers for the PCR-mediated amplification of DNA 1: A satellite-like molecule associated with begomovirus-DNA  $\beta$  complexes. *Mol Biotechnol.*, 23: 83–86.
4. Chandran, S.A., Packialakshmi, R.M., Subhalakshmi, K., Prakash, C., Poovannan, K., Nixon Prabu, A., Gopal, P. and Usha, R. (2013): First report of an alpha satellite associated with Okra enation leaf curl virus. *Virus Gene.*, DOI 10.1007/s11262-013-0898-y.
5. Dean, F.B., Nelson, J.R., Giesler, T.L. and Lasken, R.S. (2001): Rapid amplification of plasmid and phage DNA using Phi 29 DNA polymerase and multiply-primed rolling circle amplification. *Genome Res.*, 11: 1095-1099.
6. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G. (2007): Clustal W and Clustal X version 2.0. *Bioinformatics.*, 23: 2947–2948.
7. Moffat, A.S. (1999): Geminiviruses emerge as serious crop threat. *Science.*, 286: 18-35.
8. Rojas, M.R., Gilbertson, R.L., Russell, D.R. and Maxwell, D.P. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Disease.*, 77: 340-347.
9. Saghai-Marroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. (1984): Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc Natl Acad Sci USA.*, 81: 8014–8018.