

# **Molecular Identification and Characterization of Alphasatellites Associated with Okra Enation Leaf Curl Virus**

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## **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors GK and AS designed the study, executed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NKS and IK managed the literature searches and analyses of the study. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Symptomatic okra plants showing leaf curling, typical yellowing and thickening of veins were collected and characterized. Full genome of the virus was amplified using phi-29 polymerase exploiting rolling circle amplification (RCA) mechanism. The concatemer was digested with restriction enzyme and cloned in a vector for sequencing. Sequence analysis and nucleotide (ntd) comparison revealed that the viral genome is of 2,741 bp in length and showed the highest nucleotide identities of 91-99% with *Okra enation leaf curl virus* (OELCuV) which has spread in all okra growing region in India. The plants possessing OELCuV has also shown association of alphasatellites. Four out of six alphasatellites characterized showed nucleotide similarity with Sida yellow vein China alphasatellite which has been earlier reported from Nepal and China. Two alphasatellite molecule showed highest identity with *Gossypium darwinii* symptomless alphasatellite (GDarSLA) and *Gossypium mustelinum* symptomless alphasatellite (GMusSLA) each, respectively.

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**Keywords:** *Alphasatellites; begomovirus; disease; phylogenetic analysis.*

## 1. INTRODUCTION

India is the most important okra producing country in the world. Due to high content of tryptophan and lysine, okra plays a significant role in balanced diet. The immature fruits are used as salads, soups and stews [1]. It has been reported that okra is vulnerable to at least 19 plant viruses [2,3] and these viruses pose serious constraints to okra production. Among these viruses yellow vein mosaic disease (YVMD) and okra enation leaf curl disease (OELCuD) causes significant losses in the okra production. Kulkarni reported the occurrence of yellow vein mosaic disease for the first time in 1924 in Bombay province [4] while the okra enation leaf curl disease (OELCuD) was first reported from Bangalore in early 1980's. Based on the stage of developmental stage of plant, about 50 to 90 per cent drop in the yield has been observed [5,6]. The typical symptoms of OELCuD include curling of leaves, thickening of vein and reduction in leaf size [7]. The virus causing the disease belongs to a species of genus *Begomovirus*, family *Geminiviridae* [8]. The genome of viruses in the genus *Begomovirus* is either a bipartite (having two genomic components known as DNA-A and DNA-B) or a monopartite (having a single component DNA-A). The DNA-A and DNA-B have equal size (~2.8 kb). Monopartite *begomoviruses*, in addition to main helper genome DNA-A, contain additional circular ssDNA satellite molecules known as beta satellite and *alphasatellite* having half the genome size of DNA-A. These satellite molecules do not show any similarity to the genome of helper virus but rely on the helper virus for replication [9]. Beta satellites play role in pathogenicity and symptom induction but the role of *alphasatellites* in this aspect is not clear [10,11]. The association of *alphasatellites* with a yellow vein disease in *Ageratum conyzoides* was first reported in 1999 [12]. *Alphasatellite* genomes are approximately 1,375 nts and have an A-rich region and a predicted hairpin structure. It also encodes an ORF (*alpha-Rep*) which translates into amino acid protein having the molecular weight of ~37 kDa. This encoded protein bears resemblance to Rep protein of nanovirus. Although the function of *alphasatellites* is not known but in early stages of infection, they can reduce the disease symptoms caused by *begomovirus*-beta satellite complexes [13]. The aim of this study was to identify and

characterize the *alphasatellite* molecules associated with *Okra enation leaf curl virus* causing okra OELCuD of okra from Punjab (India).

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Leaves samples of six symptomatic okra plants showing leaf curling, typical yellowing and thickening of veins were collected from distinct locations and genomic DNA was isolated using the modified cetyl trimethyl ammonium bromide (CTAB) method [14] and stored in -20°C.

### 2.2 RCA and PCR-mediated Amplification

The samples were initially analysed by PCR with a pair of degenerate primers AV494 and AC1048 corresponding to the coat protein region (CP) [15] showed the presence of a *begomovirus* while for the amplification of the *alphasatellites*, a specific pair of primers DNA101/DNA102 [16] was used. PCR reaction was carried out in a reaction volume of 25 µl. In all PCR reactions 100ng of DNA and 0.2 µM of primers were used alongwith 1X PCR buffer, 0.2 mM dNTPs and 1.5 mM MgCl<sub>2</sub> and 1 unit of Taq DNA polymerase. All PCR amplifications were carried out in a programmable DNA thermocycler (Mastercycler Gradient-ependorf™) with an initial denaturation at 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 58°C for 2 min and 72°C for 3 min followed by final extension at 72°C for 10 min. The PCR amplified fragments were resolved on 1% agarose gel and visualized in a gel documentation system (Alpha Imager HP, USA) after staining with ethidium bromide. The samples which failed to show any amplification were subjected to RCA [17] with φ29 DNA polymerase using the TempliPhi DNA amplification Kit (GE Healthcare, Buckinghamshire, United Kingdom). The resultant RCA product was diluted with distilled water to the ratio 1:5 which was then used as template in PCR.

### 2.3 Cloning

PCR products of both CP (~570 bp) and *alphasatellites* (~1.4 kb) were purified from the gel using the Nucleospin Gel and PCR clean-up kit (Macherey-Nagel GmbH & Co. Germany). The products after the purification of gel were

cloned into the pGEM-T Easy vectors which were then sequenced. For the full genome cloning, the virus genome was amplified by RCA. The RCA product was then digested with EcoRV for 3 h at 37°C with 5 U of the enzyme. The resultant product was analysed on a 1% agarose gel, which indicated the presence of ~2.7 kb fragment which was then purified and cloned into pJET1.2 vector. Restriction digestion was done to confirm the clone. Three clones for each fragment were subjected to sequencing.

## 2.4 Sequence Analysis

The sequences (both forward and reverse) were assembled using BioEdit Sequence Alignment Editor software (version 5.09) [18]. The similarity of nucleotide sequences was initially depicted by using the BLAST program available at the National Centre for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/BLAST/>). The representative sequences were downloaded from GenBank database so that the comparisons could be made between the sequences found in this study with similar sequences from different hosts and geographical regions. To calculate the pairwise nucleotide sequence identity percentage, the Clustal W algorithm [19] available in MegAlign (Lasergene, DNASTAR, Madison, WI, USA) was used. For the phylogenetic analysis, the best model that fit the data was tested for full genome and alphasatellite sequences using Mega 7 software [20]. To describe the best substitution pattern, out of maximum likelihood fits of 24 different nucleotide substitution models, the model with lowest BIS score (Bayesian Information Criterion) was adopted and by using one thousand bootstrap iterations phylogram was developed. The best base substitution model selected for full genome was HKY (Hasegawa-Kishino-Yano) and for the alphasatellites TN93 (Tamura-Nei) was used. The ORFfinder tool available in NCBI was used to find ORF in the sequences (<http://www.ncbi.nlm.nih.gov/orffinder/>).

## 3. RESULTS

### 3.1 PCR Detection and Sequencing

PCR with specific primers (AV494 and AC1048) in all the six plants resulted in the amplification of fragments of size ~570 bp which were cloned and sequenced. The partial sequencing indicated that all the sequences were identical (results not shown). Therefore, only one sample was selected for the cloning of full genome. The full

genome was cloned and sequenced. The size of the genome was determined to be 2,741 ntd and is submitted in the databases under the accession number KP208672.

### 3.2 Full Genome Analysis

The nucleotide comparisons showed that the full genome is 91-99% identical to all the OELCuV nucleotide sequences present in the NCBI database with the highest similarity to an OELCuV isolate emerged from Gandhinagar, India (KC019308). The organization of the genome has characteristic features of a begomovirus. The 2.7 kb genome consists of six open reading frames (ORFs). It encodes two ORFs (AV1 and AV2) in virion sense and four ORFs (AC1, AC2, AC3 and AC4) in complementary sense orientation. The coat protein (CP) and V2 proteins are encoded by virion-sense ORFs whereas replication enhancer protein, transcription activator protein, Rep and C4 protein are encoded by complementary sense strand. An intergenic region (IR) which contains a nonnucleotide sequence "TAATATTAC" that form a stem-loop structure is present between the virion- and complementary-sense ORFs. This non-coding nonnucleotide motif plays role in replication of the genome [21]. The full nucleotide sequence of the clone produced in this study was aligned with selected nucleotide sequences of DNA-A of begomoviruses available in the databases (Table 1). The pairwise nucleotide identity percentage matrix (Fig. 1) and a phylogenetic tree constructed from the alignment shows that the virus obtained from the okra segregates with the OELCuV sequence used in alignment, justifying it as an isolate of OELCuV (Fig. 2).

### 3.3 Alphasatellites Analysis

The six alphasatellites obtained from okra infected with OELCuV, were determined to be 1,319-1,374 ntd, in length. The four alphasatellite sequences [KJ614231, KJ843304, KJ843305 and KM108329] showed 94.1% to 98.1% nucleotide sequence identity with isolates of Sida yellow vein China alphasatellite (SiYVCNA) (FN806782) originating from China and SiYVCNA originating from Nepal [22]. These alphasatellites shared 98.5%-100% sequence identity with themselves. This inferred that the alphasatellites are isolates of SiYVCNA. These sequences show the typical organization of alphasatellites, having a single ORF which encodes Rep protein (315 amino acids) in the virion-sense, an A-rich region with 51% adenine

composition and a hairpin loop having nonanucleotide sequence “TAGTATTAC” characteristic of nanoviruses [23]. The alphasatellite KJ843306 showed 89% similarity with *Gossypium mustelinum*, the symptomless alphasatellites from Pakistan. For the alphasatellite sequence KJ843307, a nucleotide identity of 91.4% was observed with an isolate of *Gossypium darwinii*, the symptomless alphasatellite (GDarSLA) (KM103525), from

Pakistan. The Rep of this alphasatellite (295 AA) contained 20 amino acid residues less than the other alphasatellites. The pairwise nucleotide identity percentage matrix (Fig. 3) and a phylogenetic tree (Fig. 4) constructed from the alignment of the alphasatellite sequences produced in this study with alphasatellite sequences from the database (Table 2) confirmed that these molecules are the isolates of previously identified alphasatellites.

		Percent Identity																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Divergence	1	■	80.6	81.1	80.1	80.5	82.2	82.3	81.7	82.0	83.9	87.6	88.1	93.0	57.7	71.1	71.0	71.1	59.9	1	KP208672
	2	23.3	■	76.7	78.4	73.9	80.6	79.8	77.5	79.2	78.6	80.6	82.4	80.2	59.9	71.0	70.9	71.1	60.9	2	FJ589571
	3	22.0	28.4	■	87.6	90.1	89.4	90.3	93.6	87.5	78.9	79.3	79.3	81.4	67.2	71.1	71.2	71.4	59.2	3	AF241479
	4	24.4	27.4	14.0	■	85.3	88.6	87.0	88.7	88.9	79.1	78.5	78.4	86.4	56.8	70.7	70.4	71.0	56.7	4	EU589392
	5	20.6	28.0	8.5	15.7	■	88.7	92.7	91.4	84.3	76.6	77.7	77.1	79.5	57.5	70.7	70.8	70.2	57.8	5	FN645923
	6	20.8	24.5	11.6	13.3	10.6	■	92.4	90.3	88.9	81.2	82.2	80.9	82.2	58.6	72.1	71.9	71.7	59.2	6	AJ002451
	7	20.3	24.2	10.3	15.0	5.5	8.3	■	92.8	89.1	80.7	80.7	81.2	82.4	60.2	71.8	71.6	71.0	60.0	7	FJ179372
	8	21.7	27.6	6.7	12.8	7.6	10.9	7.7	■	87.4	79.7	79.1	80.1	83.0	57.3	70.7	70.7	70.7	59.3	8	FJ176235
	9	21.1	25.3	13.6	12.9	15.3	12.8	11.9	14.0	■	84.3	79.2	81.4	83.5	58.5	70.4	70.4	70.1	57.5	9	EU360303
	10	19.2	26.1	25.4	26.1	25.8	22.8	22.9	24.4	18.0	■	83.2	87.8	81.2	57.7	69.1	69.3	68.9	58.2	10	FJ159269
	11	14.1	24.2	25.1	27.5	24.0	21.2	23.0	25.7	25.6	19.9	■	87.7	84.4	58.8	70.2	70.2	70.8	60.0	11	FR715681
	12	13.6	20.7	24.9	26.5	24.9	23.2	22.5	24.0	22.4	13.8	13.9	■	85.2	57.5	69.7	70.3	68.5	58.5	12	FJ345400
	13	7.7	24.3	22.0	16.0	21.9	21.1	20.6	19.9	19.2	23.1	18.7	17.5	■	57.3	71.1	70.9	71.0	58.7	13	GU111996
	14	47.2	48.4	45.8	48.9	46.9	47.8	45.9	46.7	48.4	50.5	47.5	47.8	48.4	■	60.5	60.8	58.1	77.1	14	EU914817
	15	36.8	36.7	35.5	37.3	36.2	36.3	35.5	36.6	36.8	39.5	37.0	38.4	37.2	48.9	■	98.9	94.6	59.5	15	FM210275
	16	36.7	36.8	35.6	37.7	36.2	36.6	35.8	36.9	37.0	39.3	36.8	38.1	37.2	48.1	1.1	■	95.0	59.5	16	HE793424
	17	35.9	36.6	35.1	37.0	36.3	36.1	35.9	36.5	36.8	39.2	37.9	39.2	36.3	48.9	5.5	5.1	■	57.4	17	EU024119
	18	46.6	45.9	48.7	50.7	48.1	48.2	46.6	48.6	50.3	50.1	46.9	47.3	48.3	24.8	49.2	48.9	48.7	■	18	DQ022611
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			

Fig. 1. The pairwise nucleotide identity percentage matrix of the cloned DNA-A sequence (KP208672) produced in this study and selected begomovirus sequences obtained from the database

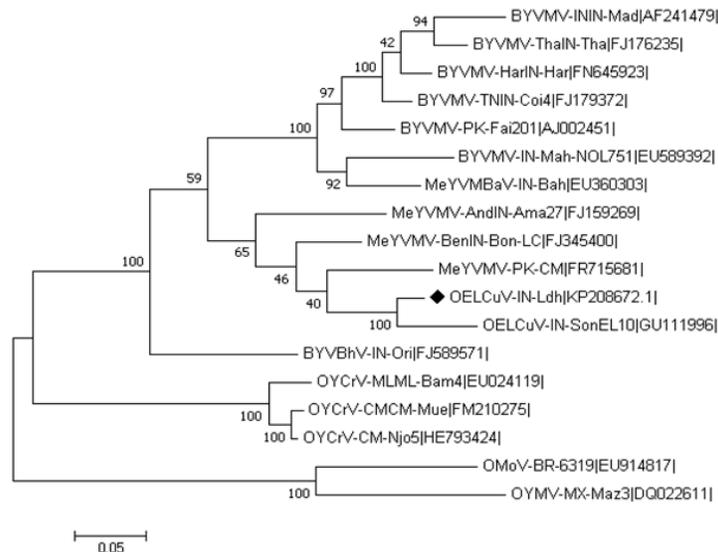
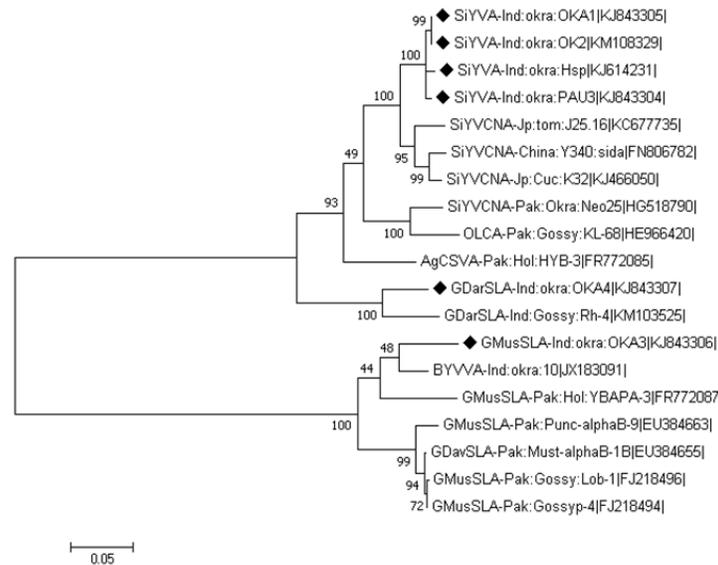


Fig. 2. Maximum likelihood tree was depicted from cloned DNA-A sequence produced in this study (labeled with black diamond) and selected begomovirus sequences obtained from the database under HKY model (BIC = 40821.280; INL = -20207.59; AIC = 40491.247; freqA = 0.263; freqT = 0.301; freqC = 0.201; freqG = 0.233; R = 1.093). The tree is unrooted and the number on nodes represent the percentage bootstrap values (1000 replicates)

		Percent Identity																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Divergence	1	■	98.5	98.5	53.3	79.0	93.4	93.5	93.2	88.2	87.5	54.1	50.6	53.6	51.8	51.8	51.4	79.9	98.5	1	KJ614231
	2	1.5	■	98.2	53.2	78.9	94.3	94.0	93.9	88.6	88.2	54.0	50.8	53.2	51.4	51.7	51.2	80.6	98.2	2	KJ843304
	3	1.6	1.4	■	56.6	75.3	93.3	93.0	92.6	88.2	87.6	58.2	54.3	54.5	52.6	52.5	54.7	79.7	100.0	3	KJ843305
	4	59.3	58.5	58.2	■	50.1	53.3	56.3	53.0	49.2	50.9	92.4	89.3	87.8	88.4	88.6	87.1	50.9	56.4	4	KJ843306
	5	20.7	20.4	20.2	62.1	■	75.2	78.1	78.1	79.9	80.4	51.8	48.0	48.7	47.0	47.2	49.1	91.4	75.2	5	KJ843307
	6	6.8	5.8	6.6	58.2	21.8	■	94.2	96.6	87.9	87.6	54.0	50.1	54.0	52.1	52.3	51.7	80.8	93.6	6	FN806782
	7	6.9	6.2	6.7	59.5	21.2	5.4	■	96.0	89.0	87.9	54.3	54.9	57.3	55.5	55.4	54.6	79.8	93.2	7	KC677735
	8	7.0	6.0	6.8	58.2	21.6	2.8	4.2	■	88.1	87.8	53.8	54.9	56.7	54.9	54.9	55.0	79.7	92.6	8	KJ466050
	9	12.9	12.4	12.7	61.0	20.2	13.9	12.0	13.8	■	87.7	53.3	50.7	52.2	50.4	50.7	52.1	80.3	88.2	9	HE966420
	10	13.2	12.8	13.3	58.5	21.2	14.0	13.7	13.8	14.1	■	52.2	52.6	49.8	48.8	48.6	54.5	79.9	87.7	10	FR772085
	11	57.7	57.4	57.4	7.6	61.3	57.2	56.3	56.9	59.2	57.0	■	90.8	89.4	89.9	90.1	88.8	52.8	57.8	11	JX183091
	12	61.1	60.8	61.3	11.8	63.6	59.8	60.4	60.1	61.4	61.3	9.7	■	86.7	87.6	87.6	86.0	48.4	53.5	12	FR772087
	13	61.8	61.3	62.1	12.6	64.1	61.2	61.7	61.2	63.4	60.4	10.8	14.3	■	99.0	99.1	97.0	53.3	54.4	13	EU384655
	14	62.2	61.6	62.6	12.4	65.8	61.9	62.1	61.6	64.1	60.8	10.7	13.4	0.8	■	99.8	96.9	51.5	52.6	14	FJ218496
	15	62.4	61.9	62.9	12.5	66.1	62.2	62.4	61.9	64.4	61.4	10.8	13.4	0.7	0.2	■	97.0	48.6	52.8	15	FJ218494
	16	60.9	60.2	61.0	13.4	64.4	60.3	60.8	60.5	62.1	59.5	11.9	15.6	2.8	3.4	3.3	■	53.2	55.0	16	EU384663
	17	21.2	20.3	20.6	62.0	8.5	21.2	20.8	21.6	20.5	20.7	60.4	63.1	63.0	64.8	65.1	64.0	■	80.0	17	KM103525
	18	1.6	1.4	0.0	58.2	20.2	8.6	6.7	6.8	12.7	13.3	57.4	61.3	62.1	62.6	62.9	61.0	20.6	■	18	KM108329
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		

**Fig. 3. The pairwise nucleotide identity percentage matrix of the cloned alphasatellite sequences (KJ614231, KJ843304, KJ843305, KJ843306, KJ843307 and KM108329) produced in this study and selected alphasatellite sequences obtained from the database**



**Fig. 4. Maximum likelihood tree was depicted from cloned alphasatellite sequences produced in this study (labeled with black diamond) and selected alphasatellite sequences obtained from the database under TN93 model (BIC = 14638.95; INL = -7124.56; AIC = 1427.27; freqA = 0.296; freqT = 0.284; freqC = 0.195; freqG = 0.223; R = 1.235). The tree is unrooted and the number on nodes represent percentage bootstrap values (1000 replicates).**

**4. DISCUSSION**

Okra is an important vegetable crop in India. It has high commercial value due to its nutritional properties. The productivity of this crop is susceptible to several biotic and abiotic stresses. The *Okra enation leaf curl virus* is one of the biotic stresses effecting cultivation of okra

worldwide. The OELCuV is forming complexes with other satellite molecules which alter the symptoms. According to a study by Chandran [24] the association of Okra enation leaf curl alphasatellite (OELCA) with OELCuV was discovered. On the other hand, the alphasatellites identified in this study shows the association of alphasatellites other than OELCA

with OELCuV which causes typical enation, reduction in size and leaf curling in okra plants. Previously Hameed and coworkers [25] found the association of OELCuV with Cotton leaf curl Multan alphasatellite infecting cotton in Pakistan (FN658728). Similarly SiYVCNA has been found associated with *Tomato yellow leaf curl virus* and *Ageratum yellow vein virus* in Japan [26]. The function of the alphasatellites is not clear till date but in some cases these satellites have been shown to decrease the symptoms produced by virus infection by reducing the level of beta satellites. The beta satellites help the helper virus in the symptom induction while alphasatellites down-regulate the activity of the beta satellites by decreasing their replication activity. Thus allows the virus infected plant to live for long time and in turn provides more time for vector-mediated spread [27]. In another study it has been

observed that the Rep protein involved in the post-transcriptional gene silencing, which overcome the plant host defence mechanism. Recently, the Rep of *Gossypium darwinii* symptomless alphasatellite (GDarSLA) and *Gossypium mustelinum* symptomless alphasatellite (GMusSLA), have been shown to suppress RNA silencing [13]. These two alphasatellites were previously found associated with cotton leaf curl virus. It is therefore difficult to say what would be the effect of the introduction of an alphasatellite into the agroecosystem of Punjab (India). The identification of alphasatellites produced in this study will be helpful in different approaches being used for the development of strategies against the begomoviruses and their associated satellite molecules.

**Table 1. The accession number of the begomovirus sequences obtained from GenBank database used for analysis in this study**

Begomoviruses	Location	Abbreviation	Accession number
Bhendi yellow vein Bhubhaneswar virus	Orissa, India	BYVBhV-[IN-Ori-03]	FJ589571
Bhendi yellow vein mosaic virus	Madurai, India	BYVMV-IN[IN-Mad]	AF241479
Bhendi yellow vein mosaic virus	Maharashtra, India	BYVMV-[IN-Mah- NOL751]	EU589392
Bhendi yellow vein mosaic virus	Haryana, India	BYVMV-Har[IN-Har-07]	FN645923
Bhendi yellow vein mosaic virus	Faisalabad, Pakistan	BYVMV-[PK-Fai201-95]	AJ002451
Bhendi yellow vein mosaic virus	Coimbatore, Tamil Nadu, India	BYVMV-TN[IN-Coi4-04]	FJ179372
Bhendi yellow vein mosaic virus	Thanagan, India	BYVMV-Tha[IN-Tha-05]	FJ176235
Mesta yellow vein mosaic Bahaich virus	Bahaich, India	MeYVMBaV-[IN-Bah-07]	EU360303
Mesta yellow vein mosaic virus	Amadalavalasa, India	MeYVMV-And[IN- Ama27-08]	FJ159269
Mesta yellow vein mosaic virus	Changa Manga, Pakistan	MeYVMV-[PK-CM-09]	FR715681
Mesta yellow vein mosaic virus	Bongaon, Bengal, India	MeYVMV-Ben[IN-Bon- LC-07]	FJ345400
Okra enation leaf curl virus	Sonipat, India	OELCuV-[IN-SonEL10-06]	GU111996
Okra mottle virus	Brazil	OMoV-[BR-6319-08]	EU914817
Okra yellow crinkle virus	Mue, Cameroon	OYCrV-CM[CM-Mue-08]	FM210275
Okra yellow crinkle virus	Njombe, Cameroon	OYCrV-[CM-Njo5-07]	HE793424
Okra yellow crinkle virus	Bamako, Mali	OYCrV-ML[ML-Bam4-06]	EU024119
Okra yellow mosaic Mexico virus	Mazatepec, Mexico	OYMV-[MX-Maz3-04]	DQ022611

**Table 2. GenBank accession numbers of selected alphasatellite sequences used in this study for analysis**

Alphasatellite	Abbreviation	Accession number
Sida yellow vein disease associated DNA 1 complete sequence	SiYVCNA-[China:Y340:sida]	FN806782
Sida yellow vein China alphasatellite clone J25.16, complete	SiYVCNA-[Jp:tom:J25.16]	KC677735
Sida yellow vein China alphasatellite isolate K32	SiYVCNA-[Jp:Cuc:K32]	KJ466050
Ageratum conyzoides symptomless alphasatellite complete sequence	SiYVCNA-[Pak:Okra:Neo25]	HG518790
Okra leaf curl alphasatellite	OLCA-[Pak:Gossy:KL-68]	HE966420
Ageratum conyzoides associated symptomless virus alphasatellite	AgCSVA-[Pak:Hol:HYB-3]	FR772085
Bhendi yellow vein mosaic virus-associated alphasatellite	BYVVA-[Ind:okra:10]	JX183091
Gossypium mustelinum symptomless alphasatellite	GMusSLA-[Pak:Hol:YBAPA-3]	FR772087
Gossypium davidsonii symptomless alphasatellite	GDavSLA-[Pak:Must- alphaB-1B]	EU384655
Gossypium mustelinum symptomless alphasatellite[Gossypium lobatum]	GMusSLA-[Pak:Gossy:Lob-1]	FJ218496
Gossypium mustelinum symptomless alphasatellite [Gossypium gossypoides]	GMusSLA-[Pak:Gossyp-4]	FJ218494
Gossypium mustelinum symptomless alphasatellite isolate	GMusSLA-[Pak:Punc- alphaB-9]	EU384663
Gossypium darwinii symptomless alphasatellite isolate Rh-4	GDarSLA[Ind:Gossy:Rh-4]	KM103525

## 5. CONCLUSION

In summary, we report for the occurrence of Okra enation leaf curl virus associated with alphasatellite virus molecules existed in cotton vegetable agroecosystem of Punjab. The prevalence of diverse alphasatellite molecules of sida and gossypium origin showed that there is large diversity among the helper virus and their associated satellite molecules. This association of helper virus is helping them to generate new complex disease symptoms.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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