Archives Of Phytopathology And Plant Protection

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gapp20

Molecular evidence for association of Cotton leaf curl Alabad virus with yellow vein mosaic disease of okra in North India

V. Venkataravanappa [a a c], C.N. Lakshminarayana Reddy [c], P. Swarnalatha [a], Devaraju [a], Salil Jalali [a] & M. Krishna Reddy [a]

[a] Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bangalore - 560089, India

[b] Indian Vegetable Research Institute, Varanasi - 221305, Uttar Pradesh, India

[c] Department of Plant Pathology, Agriculture College, GKVK Campus, University of Agricultural Sciences (B), Bangalore, 560065, India

[d] Department of Plant Pathology, College of Sericulture, University of Agricultural Sciences (B), Chintamani, 563125, India

[e] Department of Phytopathology, Nunhems Pvt. Ltd, Bangalore, India

Version of record first published: 01 Oct 2012.

To cite this article: V. Venkataravanappa, C.N. Lakshminarayana Reddy, P. Swarnalatha, Devaraju , Salil Jalali & M. Krishna Reddy (2012): Molecular evidence for association of Cotton leaf curl Alabad virus with yellow vein mosaic disease of okra in North India, Archives Of Phytopathology And Plant Protection, DOI:10.1080/03235408.2012.721682

To link to this article: http://dx.doi.org/10.1080/03235408.2012.721682

Archives of Phytopathology and Plant Protection
i*First* article 2012, 1–19

Taylor & Francis
Taylor & Francis Group

Molecular evidence for association of *Cotton leaf curl Alabad virus* with yellow vein mosaic disease of okra in North India

V. Venkataravanappa[a,b,c], C.N. Lakshminarayana Reddy[d], P. Swarnalatha[a], Devaraju[a,e], Salil Jalali[a] and M. Krishna Reddy[a]*

[a]*Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bangalore – 560089, India;* [b]*Indian Vegetable Research Institute, Varanasi – 221305, Uttar Pradesh, India;* [c]*Department of Plant Pathology, Agriculture College, GKVK Campus, University of Agricultural Sciences (B), Bangalore – 560065, India;* [d]*Department of Plant Pathology, College of Sericulture, University of Agricultural Sciences (B), Chintamani 563125, India;* [e]*Department of Phytopathology, Nunhems Pvt. Ltd, Bangalore, India*

(*Received 8 August 2012; final version received 11 August 2012*)

Two virus isolates (OY77 and OY81B) from okra plants showing yellow vein mosaic, downward curling and vein twisting symptoms were collected from different farmer's fields in Karnal, Haryana state, India. The genomes of the two isolates were amplified, cloned, sequenced and analysed. The analysis indicated that the isolates are similar with 89.2% nucleotide sequence identity. Based on the current threshold cut-off value for taxonomy distinguishing the genus begomoviruses species from strains, the two isolates are designated as strains of *Cotton leaf curl Alabad virus* (CLCuAV) which shared nucleotide sequence identity of >90% with CLCuAV infecting cotton in Pakistan. Phylogenetic and recombination analyses of the major genome component of OY77 and OY81B is derived from different begomviruses (CLCuAV, BYVMV, CLCuMuV) as the foremost parents for evolution of these new recombinant strains.

Keywords: *Bhendi yellow vein mosaic virus* (BYVMV); *Cotton leaf curl Alabad virus* (CLCuAV); PCR; whitefly; phylogentic analysis; recombination

Introduction

Geminiviruses are a group of plant viruses that possess circular ssDNA genomes encapsidated in small (18 × 30 nm) twinned icosahedral virions (Lazarowitz 1992). Begomoviruses, one of the rapidly emerging groups of plant viruses belonging to the family Geminiviridae, are transmitted by whitefly in a persistent circulative manner. Currently, begomovirus contains 132 species (Fauquet and Stanley 2005; Fauquet et al. 2008) that have either mono or bipartite genomes. Monopartite begomoviruses genome contain the homologous DNA-A of bipartite virus with approximate size of 2.6 kb. DNA component of bipartite begomoviruses are referred as DNA-A and DNA-B which are having approximate size of 2.6 kb each. The DNA-A component encodes five to six proteins required for viral DNA replication [the replication-associated protein (Rep) that initiates replication and the replication-enhancer protein (REn)], the control of gene expression (Transcription activator protein,

*Corresponding author. Email: mkreddyiihr@gmail.com

ISSN 0323-5408 print/ISSN 1477-2906 online

http://dx.doi.org/10.1080/03235408.2012.721682
http://www.tandfonline.com

TrAP) required for initiating transcription of the virion-sense genes and also involved in the suppression of post-transcriptional gene silencing-mediated host defence (Voinnet et al. 1999; Vanitharani et al. 2005) and insect transmission [the coat protein (CP) that is vital for plant–plant transmission by the whitefly (*Bemisia tabaci*)]. The functions of two other DNA-A encoded proteins (AV2 and AC4) remain unclear, although possible roles in the movement and pathogenicity/suppression of post-transcriptional gene silencing, respectively, have been demonstrated. The DNA-B component encodes the nuclear shuttle protein (NSP) and movement protein (MP) that act co-operatively to move the virus both within and between cells in host plants (Hanley-Bowdoin et al. 2000). The two DNA components share no sequence similarity except 200-nucleotide (nt) non-coding region referred to as the common region (CR), which contains the viral replication origin (Lazarowitz 1992; Rojas et al. 2001).

A majority of the monopartite begomoviruses in the Old World have been found to be associated with betasatellites, and alpha-satellites molecules which have are circular ssDNA with approximately half the size of the genomic DNA-A and are having little sequence similarity to either DNA-A or DNA-B components of begomoviruses, except for a conserved hairpin structure and a TAATATTAC loop sequence (Saunders et al. 2000; Briddon et al. 2001; Jose and Usha 2003).

Recombination is a major driving force in the evolution of geminiviruses (Seal et al. 2006a, 2000b). This may occur through mutations, recombination (exchange of DNA between like DNA components) and pseudorecombination (is the exchange of DNA components) between the variants of the same virus, between species and even between genera, which has resulted in a rapid diversification and emergence of novel begomoviruses (Polston and Anderson 1997). These events have been well demonstrated under *in vivo* (Garrido-Ramirez et al. 2000) and *in vitro* conditions (Pita et al. 2001) in begomoviruses infecting cassava and tomato.

Okra (*Abelmoschus esculentus* (L.) Moench) commonly known as bhendi is one of important vegetable crop due to its nutritional value. The major constraint for the production of okra is yellow vein mosaic disease (Usha 2008). The economic loss caused by this disease is 50–90%, depending on the stage of the crop growth at which infection occurs (Sastry and Singh 1974; Sinha and Chakrabarthi 1978). The field survey on begomovirus associated diseases of okra in India during 2005–2009 reported that several fields of okra are infected with yellow vein mosaic disease showing highly variable symptoms. This variation in symptoms may indicate the occurrence of different begomoviruses which are causing yellow vein mosaic disease-like symptoms, but the information on the diversity of okra infecting begomoviruses in India is not available with the exception of two reports from India (Jose and Usha 2003; Venkataravanappa et al. 2012) and one from the Indian subcontinent (Zhou et al. 1998). In the present study, the *Cotton leaf curl Alabad virus* (CLCuAV) strains infecting okra in India has been characterised.

Materials and methods

Virus source

The virus isolates OY77 and OY81B were collected from the okra plants exhibiting downward leaf curling, vein thickening and yellowing symptoms in the major okra growing areas in the Karnal of Haryana state in India (Figure 1(a–c)).

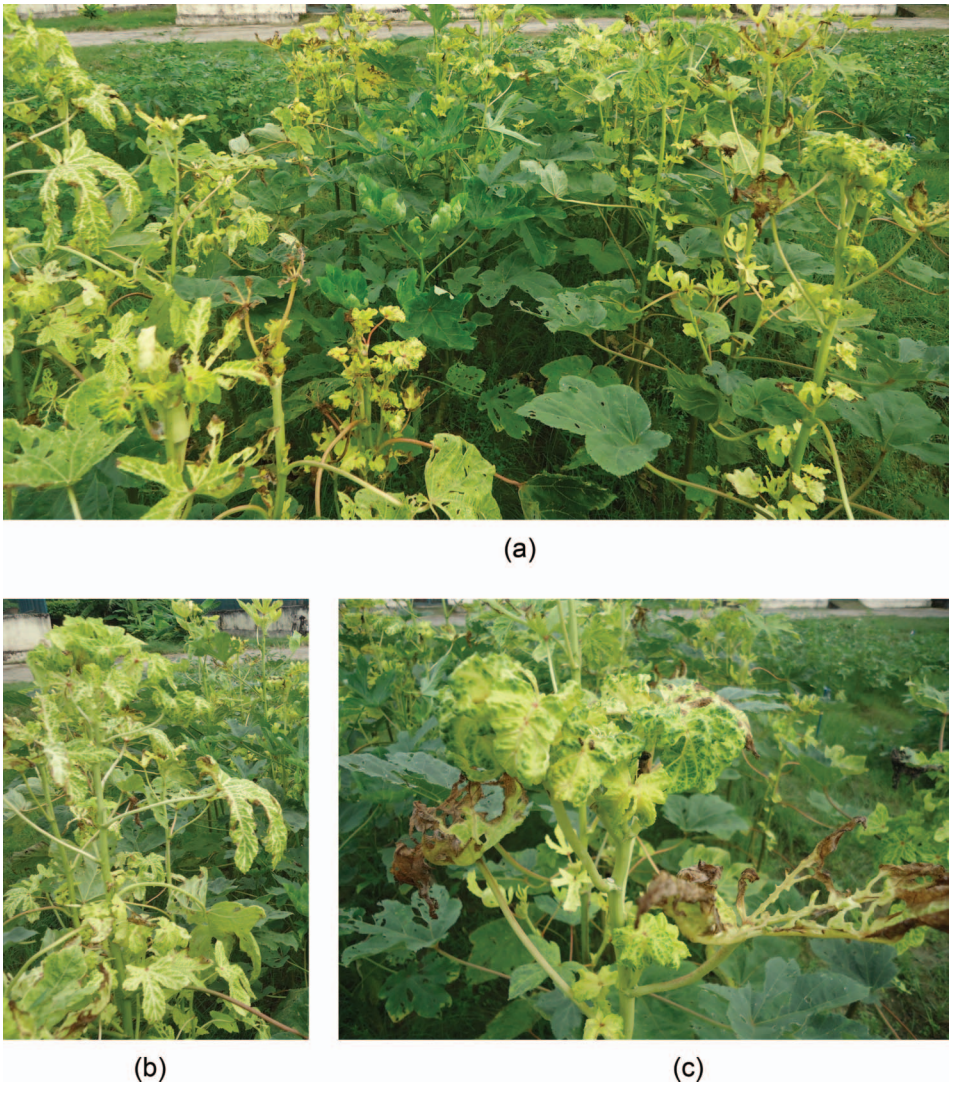
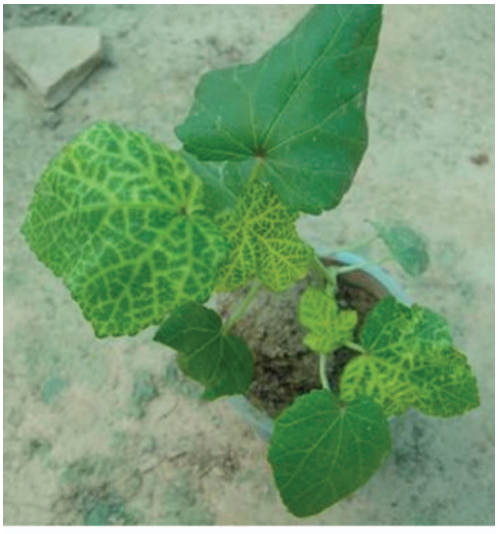


Figure 1. (a) Overview of okra field infected with YVMD, (b) Virus infected okra plants showing yellow vein symptoms, (c) Okra plants showing mottling and downward curling symptoms under natural conditions, (d) Whitefly transmitted Okra cv. 1685 showing yellow vein symptoms, and (e) Okra plants showing the yellow vein mosaic symptoms upon graft transmission.

Transmission

The leaf samples collected from the field may be contaminated with more than one viruses in the natural conditions. To rule out the mixed infection, repeated whitefly transmissions were carried out using okra cv. 1685, which produced the same symptoms which were recorded during the sample collection. The virus inoculum was maintained under greenhouse conditions on healthy okra plants (*Abelmoschus esculentus* (L.) Moench), cv. 1685) through whitefly transmission (10 whiteflies per plant) with acquisition and inoculation access periods of 12 h each. The infected



(d)

(e)

Figure 1. (*Continued*).

sample was tested through DAC-ELISA (by using ACMV polyclonal antibodies from DSMZ, Germany) and nucleic acid hybridisation using specific probe developed for Coat protein region of CLCuAV-okra.

Graft transmission

The infected plants maintained in the greenhouse were used as a source of virus for grafting. Twenty non-symptomatic okra plants were used as rootstocks, and wedge grafting was carried out with scions taken from infected plant of the same variety (cv. 1685). The grafted portion was tied with a polythene strip, and scion was

covered with a polythene bag. The grafted plants were kept in a cool place in the greenhouse for symptom production.

DNA isolation

Total nucleic acids were extracted from symptomatic and non-symptomatic tissues of both field and plants maintained in greenhouse conditions by Cetyl trimethyl ammonium bromide method (Doyle and Doyle 1990). The extracted DNA was diluted 10-fold in sterile distilled deionised water before being subjected to PCR amplification. The quality of DNA was checked on 0.8% agarose gel.

PCR amplification

Total nucleic acids isolated from symptomatic and non-symptomatic okra leaves were amplified by PCR described by Venkataravanappa et al. (2012). Further, to confirm the samples having second component (DNA-B) and betasatellites, the primers specific to DNA-B (Rojas et al. 1993; Venkataravanappa et al. 2012) and betasatellite (Briddon et al. 2002) were used. Potentially, full-length amplified PCR products were purified from agarose gels by using standard protocols, ligated to the plasmid vector PTZ57R vector (Fermentas) according to manufacturer's instructions and transformed into *Escherichia coli* DH5α competent cells (Novagen). The complete nucleotide sequence of the three clones from each sample was determined by automated DNA sequencer ABI PRISM 3730 (Applied Biosystems) at Anshul Biotechnologies DNA Sequencing Facility, Hyderabad, Andhra Pradesh, India.

Comparison of DNA sequence

The similarity of genomic sequence of DNA-A was initially analysed by using the BLAST program available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) (Table 1). The sequences showing highest scores with the present isolates were obtained from database and multiplealigned using the CLUSTAL-X program (Thompson et al. 1994), sequence identity matrixes for the begomoviruses were generated using Bioedit Sequence Alignment Editor (version 5.0.9) (Hall 1999) and phylogenetic tree was generated by MEGA 5.0 software (Tamura et al. 2011) using the neighbour-joining method with 1000 bootstrapped replications.

Detection of recombination events

The phylogenic evidence for recombination was detected by the alignment of selected begomoviruses sequences reported from India which is available in the database along with okra isolate with Splits-Tree version 4.3 using the neighbour-net method (Huson and Bryant 2006). The method depicts the conflicting phylogenetic signals caused by recombination as cycles within unrooted bifurcating tree. Recombination analysis was carried out using Recombination detection program (RDP), GENECOV, Bootscan, Max Chi, Chimara, Si Scan and 3Seq which are integrated in RDP 3 to detect the recombination break points (Martin et al. 2010). Default RDP settings with 0.05 *p*-value

Table 1. GenBank accession numbers of selected begomovirus sequences from Asia used in this study for analysis.

Sl. No.	Begomoviruses	Accession no.	Abbreviation
1	Okra enation leaf curl virus-[India:Sonipet-EL10:06]	GU111996	OELCuV [IN:Soni-EL10: 06]
2	Okra enation leaf curl virus-[India:Sonipet-EL14:06]	GU111997	OELCuV [IN:Soni-EL14: 06]
3	Okra enation leaf curl virus-[India:Sonipet-EL32:06]	GU112000	OELCuV[IN: Soni-EL32: 06]
4	Okra enation leaf curl virus-[India:Munthal-EL37:06]	GU111998	OELCuV[IN: Munt-EL37: 06]
5	Okra enation leaf curl virus-[India:Munthal-EL38:06]	GU112002	OELCuV[IN: Munt-EL38: 06]
6	Okra enation leaf curl virus-[India:Munthal-EL39:06]	GU112001	OELCuV-[IN: Munt-EL39: 06]
7	Okra enation leaf curl virus-[India: Munthal EL41:06]	GU111999	OELCuV-[IN: Munt-EL41: 06]
8	Mesta yellow vein mosaic virus-[India:Kaisargunj:08]	FJ159267	MeYVMV-[India:Kai:08]
9	Mesta yellow vein mosaic virus-[India:Raigunj:08]	FJ159264	MeYVMV-[IN:Rai:08]
10	Mesta yellow vein mosaic virus-[India:Haringhata:08]	FJ159263	MeYVMV-[IN:Hari:08]
11	Mesta yellow vein mosaic virus-[India:Bongaon:08]	FJ159262	MeYVMV-[IN:Bong:08]
12	Mesta yellow vein mosaic virus-[India:Balurghat:08]	FJ159266	MeYVMV-[IN:Balur:08]
13	Mesta yellow vein mosaic virus-[India:Barackpore2:06]	EF428256	MeYVMV-[IN:Barra2:06]
14	Mesta yellow vein mosaic virus-[India Coochbehar:08]	FJ159265	MeYVMV-[IN:Cooch:08]
15	Mesta yellow vein mosaic virus-[India:Bongaon:07]	FJ345400	MeYVMV-[IN:Bong:07]
16	Mesta yellow vein mosaic virus-[India:Barrackpore:07]	FJ345398	MeYVMV-[IN:Barr:07]
17	Mesta yellow vein mosaic virus-[India:Haringhata:07]	FJ345399	MeYVMV-[IN:Hari:07]
18	Mesta yellow vein mosaic virus-[India:Bahraich:07]	EU360303	MeYVMV-[IN:Bah:07]
19	Mesta yellow vein mosaic virus-[India:Barrackpore2:08]	EF432372	MeYVMV-[IN:Barra2:08]
20	Mesta yellow vein mosaic virus-[India:Haryana:2010]	FN645922	MeYVMV-[IN:Har:10]
21	Bhendi yellow vein mosaic virus-[India:Phalaghat:OY014:05]	GU112055	BYVMV [IN:Phal:OY014:05]
22	Bhendi yellow vein mosaic virus-[India:Kaivara:OYKaivara1:06]	GU112058	BYVMV [IN:Kai:OYKaivara1:06]
23	Bhendi yellow vein mosaic virus-[India: New Delhi:OY134:05]	GU112063	BYVMV [IN: ND:OY134:05]
24	Bhendi yellow vein mosaic virus-[India:Phalaghat:OY07:05]	GU112062	BYVMV [IN :Phal:OY07:05]
25	Bhendi yellow vein mosaic virus-[India:Delhi:OY133:06]	GU112078	BYVMV[IN:ND: OY133:06]

(*continued*)

Table 1. (*Continued*).

Sl. No.	Begomoviruses	Accession no.	Abbreviation
26	Bhendi yellow vein mosaic virus-[India:Pandarahalli: OY167:06]	GU112079	BYVMV[IN:Pand: OY167:06]
27	Bhendi yellow vein mosaic virus-[India:Raichur:OY54A:05]	GU112067	BYVMV[IN:Rai: OY54A:05]
28	Bhendi yellow vein mosaic virus-[India:Raichur:OY54B:05]	GU112068	BYVMV[IN:Rai: OY54B:05]
29	Bhendi yellow vein mosaic virus-[India:Bhemarayanagudi:OY45:05]	GU112069	BYVMV[IN:Bhem: OY45:05]
30	Bhendi yellow vein mosaic virus-[India:Raichur:OY59:05]	GU112070	BYVMV[IN:Rai: OY59:05]
31	Bhendi yellow vein mosaic virus-[India:Tirupathi:OY99:05]	GU112071	BYVMV[IN:Tiru: OY99:05]
32	Bhendi yellow vein mosaic virus-[India:Phalaghat:OY138A:06]	GU112072	BYVMV[IN:Phal: OY138A:06]
33	Bhendi yellow vein mosaic virus-[India:Raichur:OY56:05]	GU112074	BYVMV[IN:Rai: OY56:05]
34	Bhendi yellow vein mosaic virus-[India:Sonipat:OY83:05]	GU112075	BYVMV[IN:Soni: OY83:05]
35	Bhendi yellow vein mosaic virus-India[India: Madurai]	AF241479	BYVMV-IN[IN: Mad]
36	Bhendi yellow vein mosaic virus-Pakistan[Pakistan:Multan301:96]	AJ002453	BYVMVPK[PK:M301:96]
37	Bhendi yellow vein mosaic virus-[India:Bangalore:OY34:05]	GU112064	BYVMV[IN:Bang: OY34:05]
38	Bhendi yellow vein mosaic virus-[India:Chintamani:OYCHINT:06]	GU112065	BYVMV[I IN:Chint: OYCHINT:06]
39	Bhendi yellow vein mosaic virus-[India: Sonipat OY92B:2005]	GU112006	BYVMV-[India: Soni: OY92B:05]
40	Bhendi yellow vein mosaic virus-[India: Guntur OY112:05]	GU112005	BYVMV[IN: Gun: OY112:05]
41	Bhendi yellow vein mosaic virus-[India:Karnal:OY79A:05]	GU112076	BYVMV[IN:Kar: OY79A:05]
42	Bhendi yellow vein mosaic virus-[India:Karnal:OY80B:06]	GU112077	BYVMV[IN:Kar: OY80B:06]
43	Bhendi yellow vein mosaic virus-[India:Kaivara:OYKaivara:06]	GU112057	BYVMV[IN:Kai: OYKaivara:06]
44	Bhendi yellow vein mosaic virus-[India:Raichur:OY49:05]	GU112066	BYVMV[IN:Rai: OY49:05]
45	Bhendi yellow vein mosaic virus-[India:Kerala:OYG6AG:05]	GU112060	BYVMV[IN:Ker: OYG6AG:05]
46	Bhendi yellow vein mosaic virus-[India:Chelur:OYCN6:06]	GU112059	BYVMV[IN:Chel: OYCN6:06]
47	Bhendi yellow vein mosaic virus-[India:Sonipat:OY93:05]	GU112061	BYVMV[IN:Soni: OY93:05]
48	Bhendi yellow vein mosaic virus-[India:coimbatore:OY93:05]	GU112080	BYVMV[IN:COI:OY93:05]
49	Okra yellow vein mosaic virus-[Pakistan:Faisalabad 201:95]	AJ002451	OYVMV-[PK:Fai201:95]
50	Bhendi yellow vein mosaic virus.NOL751-India[India: Maharashtra]	EU589392	BYVMV NOL751-IN [IN.Maha:08]

(*continued*)

Table 1. (*Continued*).

Sl. No.	Begomoviruses	Accession no.	Abbreviation
51	Cotton leaf curl Alabad virus-[Pakistan:Kohiwala 802a:1996]	AJ002455	CLCuAV-[PK:K802a:96]
52	Cotton leaf curl Alabad virus-[Pakistan:Alabad 804a:1996]	AJ002452	CLCuAV-[PK:A804a:96]
53	Cotton leaf curl Multan virus-[Pakistan:Multan:08]	FJ218487	CLCuMuV-[Pk:Mul:08]
54	Cotton leaf curl Multan virus-[Pakistan Hirs-1:08]	FJ218486	CLCuMuV-[PK:Hirs-1:08]
55	Cotton leaf curl Multan virus-Hisar-[Pakistan:Multan 311:Okra:96]	AJ002459	CLCuMuVHis [PK:M311:Ok:96]
56	Cotton leaf curl Multan virus-[Pakistan;Rajasthan;09]	EU365616	CLCuMuV-A[PK;RAJ;09]
57	Cotton leaf curl Multan virus-Rajasthan[India:Abohar:2003]	AY795606	CLCuMVRaj[IN:Abo:03]
58	Cotton leaf curl Shadadpur virus-[Pk:Sindh:Tjam:09]	FN552002	CLCuShV[PK:Tjam:05]
59	Cotton leaf curl Shadadpur virus-[Pk:Sindh Shadadpur:09]	FN552001	CLCuShV-[PK:Sha:05]
60	Cotton leaf curl Shadadpur virus-[Pk:Sindh Shadadpur:09]	FN552005	CLCuShV-[PK:Sha2:04]
61	Cotton leaf curl Shadadpur virus-[Pk:Sindh Shadadpur:09]	FN552004	CLCuShV-[PK:Sha1:04]
62	Cotton leaf curl Bangalore virus-[India:Bangalore:2004]	AY705380	CLCuBaV-[IN:Ban:04]

cut-offs throughout and standard Bonferroni correction except that the option ''Reference sequence selection'' was set at ''internal references only''.

Results

Virus transmission

A total of 100 okra seedlings (cv. 1685) were inoculated by viruliferous whiteflies (10 insects/plant). All inoculated okra seedlings showed initially 100% yellow vein symptoms, with a minimum incubation period of 10–12 days and later the infected leaves become downward curling, which were similar to those observed on the field-infected plants (Figure 1(d)).

Totally, 20 plants were grafted (cv. 1685), of which 13 plants showed the yellow vein mosaic symptoms with a minimum incubation period of 25–30 days and a transmission efficiency of 65% (Figure 1(e)).

Molecular characterisation

The complete DNA-A component of two begomovirus isolates from okra was amplified as overlapping fragments and sequenced to yield the complete genome sequence for each isolate. Attempts to amplify second genomic component (DNA B) by PCR were unsuccessful, but exhibited a positive amplification for the satellite component with a universal abutting primer pair beta0l/beta02 (Data not shown). This result indicated that the virus isolates under the current study are monopartite in nature.

Genome organisation and sequence analysis of DNA-A

The complete nucleotide sequences of DNA-A component of OY77 and OY81B were determined to be 2744 and 2745 nt in length and are available in the nucleotide sequence databases under the accession numbers (GU112081 and GU112004). The alignment of the two begomovirus isolates obtained here showed to have 89.2% nucleotide sequence identity between indicative of them being isolates of a single species (based on the 89% species demarcation threshold for begomoviruses; Fauquet et al. 2008). The isolates showed the typical genome organisation similar to that of other monopartite begomoviruses with two open reading frames (ORFs) [AV1 (CP), AV2] in virion-sense DNA-A and four ORFs [AC1 (Rep), AC2, AC3, AC4, AC5] in complementary-sense DNA-A, separated by an intergenic region (IR) (Table 2).

The alignment of complete DNA-A nt sequences with those from the DNA-A sequences of the begmoviruses retrieved from the databases indicated that OY77 and OY81B showed a higher degree of sequence similarity with begomoviruses found in Pakistan than those found in the India. For instance, it shared $>90\%$ with CLCuAV (AJ002452) infecting cotton and $<89\%$ with begomoviruses infecting okra and other crops in India and Indian subcontinent (Table 3). This result is also supported by a phylogenetic analysis which shows that OY77 and OY81B are closely grouped with CLCuAV infecting cotton in Pakistan for which full-length sequences are available in the databases (Figure 2).

When individual encoded proteins of virus isolates were compared, OY77 shared the highest amino acid sequence similarity of 98.8–99.2% with CLCuAV, OYVMV and BYVMV-IN for Coat protein gene and 94.5–95.5% (OY77) with CLCuAV for ORFAC1-encoded Rep protein. While, OY81B share highest similarity with BYVMV-IN (91.7–98.5%) for Coat protein gene and BYVMV (69.1–86.5%) for ORFAC1-encoded Rep protein. The ORFC4 gene has 57–100% similarity with CLCuAV in both isolates. In case of the pre-coat protein the highest sequence similarity of 92.5–97.5% (OY77) with BYVMV-IN and 61.1–93.6% (OY81B) with Okra enation leaf curl virus (OELCuV-IN) was observed.

Further, ORFAC2 (transcriptional activator protein) shared highest amino acid sequence similarity of 82.0–93.3% (OY77) with BYVMV-IN and 100% (OY81B) with BYVMV-Pak301. However, for ORFAC3 (replication enhancer), the highest amino acid sequence similarity of 86.5–98.5% with BYVMV and for ORFC5, 95.7% with OYVMV-201 and BYVMV-IN in both the isolates (Table 3).

The intergenic region (IR) in theses were ranged from 273–275nt in length and similar to that of CLCuAV and OELCuV-IN. The intergenic region (IR) of OY77B and OY81B shared nucleotide similarity 94.1–96.3% with CLCuAV and 57.7–58.0% with OELCuV-IN respectively and less than 68% with other begomoviruses in both the cases infecting okra, cotton and Mesta (Table 3). The IR contains a predicted stem-loop sequence with conserved nonanucleotide sequence (TAATATTAC) in the loop which can be found in the majority of the geminiviruses characterized to date and marks the origin of virion-strand DNA replication (Heyraud et al. 1993).

Neighbour-net and recombination analysis of DNA-A

The neighbour-net analysis of aligned sequences using Split-Tree program revealed extensive networked evolution in okra isolates (OY77 and OY81B) with other begomoviruses under the present study is indicative of recombination (Figure 3). The split decomposition analysis showed a "rectangular" network structure in which

Table 2. Features of the begomoviruses (CLCuAV-okra) isolated from okra plant.

	OY77				OY81B			
Components DNA-A	Start codon (nucleotide coordinates)	Stop codon (nucleotide coordinates)	Predicted size of ORFs (nt)	Predicted size of protein (no. of amino acids) and Mr	Start codon (nucleotide coordinates)	Stop codon (nucleotide coordinates)	Predicted size of ORFs (nt)	Predicted size of protein (no. of amino acids)
CP*	281	1051	771	256** (28.16 kD)#	281	1051	771	256 (28.16 kD)
V2	121	486	336	121 (13.3 kD)	121	486	336	121 (13.3 kD)
Rep	2591	1500	1092	363 (40.3 kD)	2591	1501	1092	363 (40.32 kD)
TrAP	1603	1151	453	150 (16.5 kD)	1603	1151	453	150 (16.5 kD)
REn	1458	1054	405	134 (14.74 kD)	1458	1054	405	134 (15 kD)
C4	2434	2177	258	85 (9.35 kD)	2434	2132	303	100 (11 kD)
C5	985	629	357	118 (12.98)	985	629	357	118 (12.98)

*Genes are indicated as coat protein (CP), replication-associated protein (Rep), transcriptional activator protein (TrAP), and replication enhancer (REn). The products encoded by ORFs V2, C4 and C5 have yet to be named. **Total number amino acids encoded by gene. # Predicted molecular mass.

Table 3. Pair wise percent of nucleotide identities, amino acid sequence identities of encoded genes from the begomoviruses components isolated from okra and with other closely related begomoviruses selected from the databases.

	Genome		IR		CP(AV1)		V2		Rep(AC1)		TrAP (C2)		REn (C3)		C4		C5	
Begomoviruses[#]	OY77	OY81B	OY77	OY81B	OY77	OY81B	OY77	OY81B	OY77	OY81B	OY77	OY81B	OY77	OY81B	OY77	OY81B	OY77	OY81B
BYVMV (28)*	81.4–84.5	82.9–89.0	50.3–69.5	48.9–59.4	92.5–99.0	91.7–98.5	92.5–97.5	79.3–84.2	70.2–76.5	69.1–86.5	82.0–93.3	86.0–98.0	86.5–97.0	88.0–98.5	15.9–38.0	31.961.7	11.0–90.6	11.0–95.7
CLCuAV (2)	94.594.7	91.791.9	94.196.3	46.447.7	98.899.2	97.698.0	94.295.0	85.085.1	94.595.5	84.084.8	86.687.3	91.392.0	89.091.0	96.098.5	99.0–100	56.0–57.0	–	–
CLCuMuV (5)	81.581.9	79.880.9	48.565.8	40.357.7	83.998.4	82.898.0	82.694.2	71.082.6	71.676.5	71.075.4	83.384.6	78.680.0	31.387.3	32.087.3	19.7–38.0	19.2–42.0	10.1–93.0	10.1–93.0
CLCuShV (4)	76.576.7	74.574.7	49.350.0	53.053.6	92.193.3	90.292.5	65.270.2	53.759.5	76.576.8	74.375.7	84.085.3	78.679.3	83.584.3	85.086.5	38.0–40.0	38.0–39.0	–	–
OELCuV (7)	74.276.9	75.380.5	65.065.3	57.758.0	74.076.1	74.475.7	71.073.5	61.193.6	69.675.4	73.884.5	84.087.3	83.398.0	82.888.8	88.897.0	28.0–35.0	37.0–59.2	–	–
MeYVMV (13)	74.080.5	73.183.8	46.859.5	47.453.1	78.579.2	78.178.9	58.690.9	51.279.3	71.976.3	74.978.7	80.684.6	77.394.6	68.491.0	71.997.0	35.7–36.2	33.9–37.2	10.9–76.0	11.7–83.0
OYVMV-[201] (1)	84.8	85.9	68.8	54.3	99.2	98.0	95.0	85.1	73.5	76.5	92.0	87.3	95.5	89.5	39.0	41.0	95.7	88.1
BYVMV-Pak (1)	82.1	85.5	64.0	56.0	97.6	97.2	93.3	83.4	71.3	75.2	84.6	100.0	91.7	99.2	36.4	35.0	86.4	88.9
CLCuBaV (1)	77.5	76.2	51.8	57.0	92.9	92.1	73.5	64.4	77.4	75.2	86.0	84.6	79.8	85.8	37.0	40.0	60.1	63.5
CLCuRaV (1)	78.0	78.0	55.9	52.0	92.5	91.4	93.3	81.8	76.5	75.4	83.3	77.3	81.3	80.5	40.0	40.0	–	–

*Numbers of sequences from the databases used in the comparisons. #The species are indicated as *Bhendi yellow vein mosaic virus* (BYVMV), *Cotton leaf curl Alabad virus* (CLCuAV), *Cotton leaf curl Multan virus* (CLCuMuV), *Cotton leaf curl Shahdadpur virus* (CLCuShV), Okra enation leaf curl virus (OELCuV), *Mesta yellow vein mosaic virus* (MeYVMV), *Okra yellow vein mosaic virus* (OYVMV), *Cotton leaf curl Bangalore virus* (CLCuBaV), *Cotton leaf curl Bangalore virus* (CLCuRaV). For each column, the highest value is underlined.

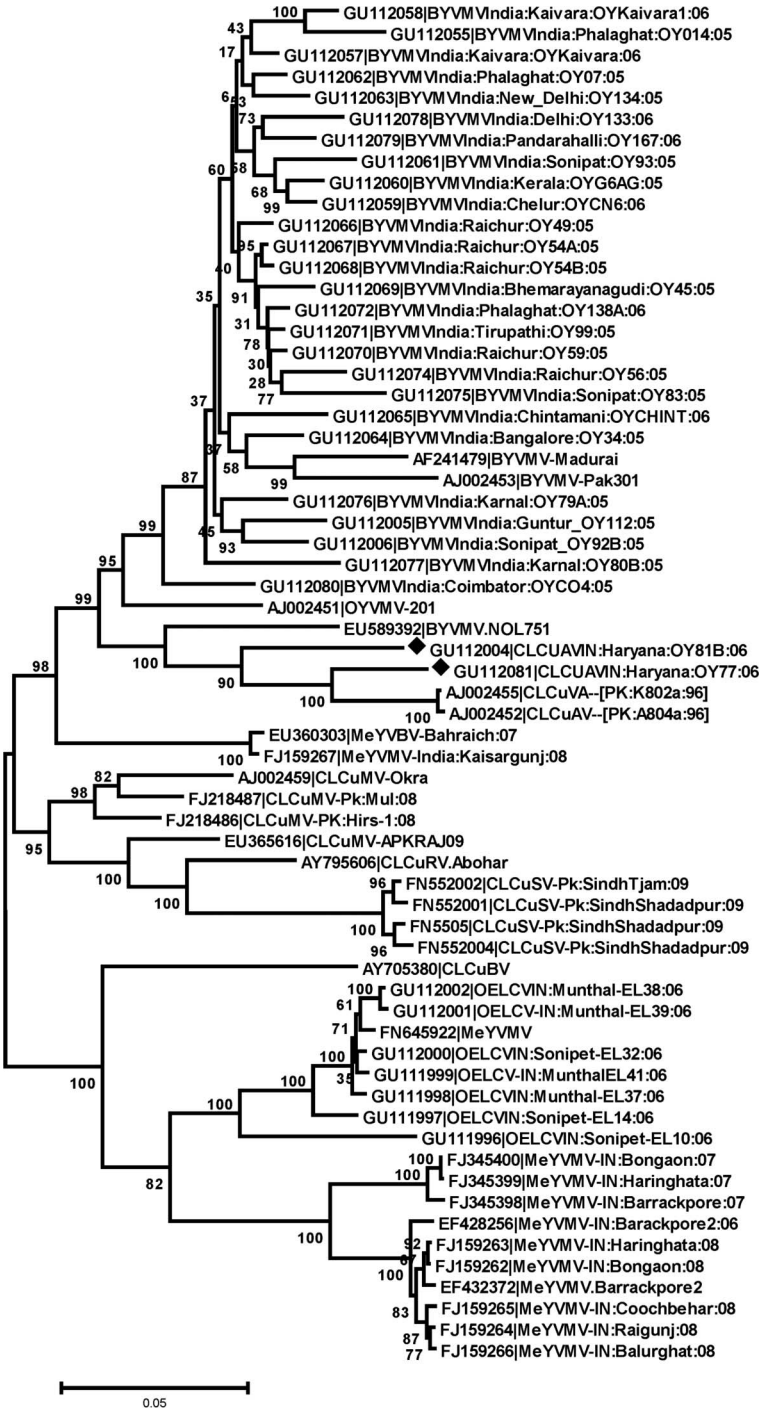


Figure 2. Phylogenetic trees constructed from aligned complete nucleotide sequences of DNA-A components (OY77 and OY81B) with other begomoviruses using a neighbour-joining algorithm. Horizontal distances are proportional to sequence distances and vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed, and the bootstrap percent values more than 50 are numbered along branches.

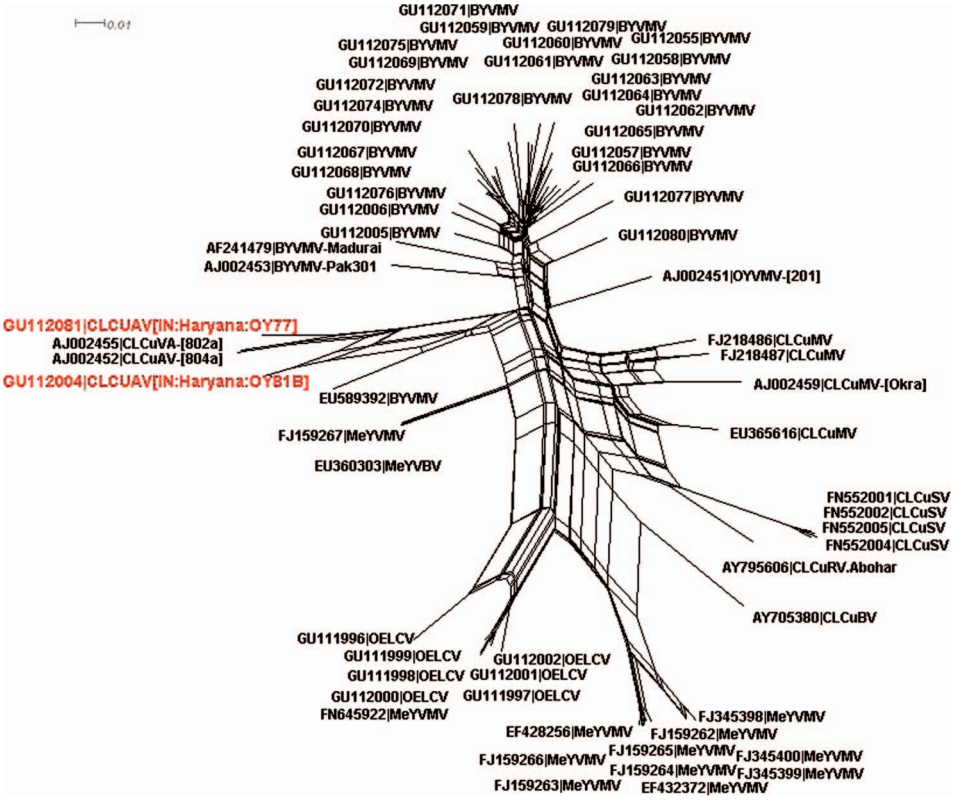


Figure 3. Neighbour-net generated for the DNA-A component of okra isolate (OY77 and OY81B) with other begomoviruses showing significant signals for phylogenic conflict indicating as recombinant virus.

okra-infecting begomoviruses and all other selected begomoviruses were clearly distant. However, a separation between okra isolates (OY77 and OY81B) and other begomoviruses, which was clearly highlighted in the phylogenetic analysis, resulted in the split graph.

A comprehensive analysis for recombination using RDP3, based on the alignment of sequences of okra isolates (OY77 and OY81B) and other selected begomviruses from database, indicates evidence of recombination in okra isolates (OY77 and OY81B). The most part of the DNA fragments is derived from BYVMV, OYVMV, OELCuV, CLCuAV, MeYVMV and CLCuMuV to emerge as new strains of CLCuAV infecting okra (Figure 4 and Table 4). There was a non-random distribution of recombination sites along the genome which are detected in the ORF's AV1, AV2, AC1, AC4, AC3, AC5 and IR regions. Therefore, these results suggest that the virus strains under present study are a recombinants. However, they are a derivative of known virus.

Discussion

Begomoviruses are one of the most economically important groups of plant viruses due to their high incidence and disease severity in vegetable and field crops grown in tropical and subtropical regions of the world (Moriones and Navas-Castillo 2000;

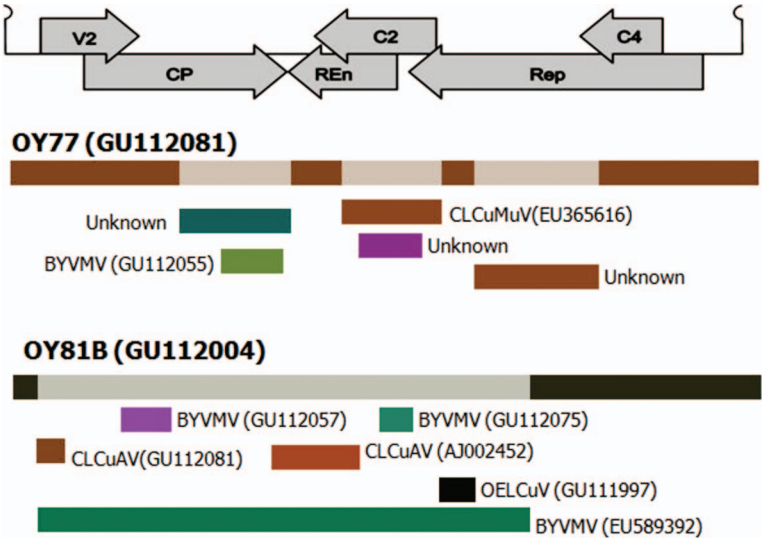


Figure 4. Analysis of recombination of DNA-A isolated from okra. The begomoviruses acronyms given are *Bhendi yellow vein mosaic virus* (BYVMV), *Cotton leaf curl Multan virus* (CLCuMuV), *Okra enation leaf curl virus* (OELCuV) and *Cotton leaf curl Alabad virus* (CLCuAV). The sequence of indeterminate 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.

Morales and Anderson 2001; Briddon et al. 2003). In India, begomoviruses are major limiting factors for okra production since 2003 (Jose and Usha 2003) and increasing in incidence every year. The successful management of any viral diseases needs thorough understanding of biological and molecular variability of the genomes of different isolates of a particular virus. For begomoviruses, vector transmission may give some clue in variation among different species, but for more accurate diagnosis, amplification of viral genome or genomic component(s) by PCR followed by sequencing has proved to be more accurate and easy for understanding the genomic variability among different isolates of begomoviruses (Briddon and Markham 1994; Sharma et al. 2005; Paul et al. 2009).

The present investigation together with the previous study on the variability of betasatellites has revealed the variability of the begomovirus complexes associated with yellow vein mosaic and enation leaf curl disease of okra in India (Venkataravanappa et al. 2011). PCR detection, vector transmission, molecular characteristics and phylogenetic relationship of the two isolates in the current study indicated that yellow vein mosaic disease of okra is associated with new strains of begomovirus (Figure 2), which are designated as CLCuAV-IN[IN:Haryana:okra] according to the guidelines of ICTV Geminivirus Study Group (Fauquet et al. 2008). As per the current literature, this is the first report of characterization of new strains of *cotton leaf curl alabad virus* infecting okra resulting in yellow mosaic disease in northern India.

Cotton and okra belonging to the malvaceous family are grown in all most all states of India and are affected by whitefly-transmitted begomoviruses. In India, the first record of CLCuD was from Rajasthan in 1993 (Ajmera 1994). In subsequent

Table 4. Breakpoint analysis of CLCuAV-okra and their putative parental sequences.

DNA-A	Break point begin-end	Major parent	Minor parent	RDP	GENECOV	Max Chi	Chimera	Si Scan	3Seq
OY77	729–1250	BYVMV[India: ND:OY134: 05][GU112063]	BYVMV[India:Kar: OY80B:05] [GU112077]	2.415×10^{-4}	2.724×10^{-6}	3.484×10^{-6}	1.838×10^{-4}	1.521×10^{-9}	1.751×10^{-5}
	1035–1353	BYVMV[India: Chinti: OYCHINT: 06][GU112065]	BYVMV[India:Phal: OY014:05] [GU112055]	NS	NS	NS	NS	1.589×10^{-2}	4.993×10^{-2}
	1463–1912	BYVMV-Madurai [AF241479]	CLCuMV-A[PK; RAJ;09] [EU365616]	5.659×10^{-37}	9.465×10^{-38}	3.855×10^{-9}	8.969×10^{-10}	4.709×10^{-20}	6.532×10^{-13}
	1558–1857	BYVMV[India:Soni: OY83:05] [GU112075]	BYVMV[India:Rai: OY56:05] [GU112074]	NS	NS	NS	NS	1.077×10^{-7}	3.067×10^{-1}
	2090–2655	BYVMV[India:Phal: OY138A:06] [GU112072]	CLCuMV-A[PK; RAJ;09] [EU365616]	2.047×10^{-8}	2.02×10^{-15}	5.670×10^{-7}	3.191×10^{-9}	1.05×10^{-18}	4.07×10^{-9}
OY81B	24–2298	CLCUAV[IN:Hary: OY77:2006] [GU112081]	BYVMV.NOL751 [EU589392]	1.089×10^{-17}	2.204×10^{-29}	3.926×10^{-13}	2.093×10^{-15}	1.1919×10^{-12}	9.464×10^{-28}
	24–151	CLCuMV- [Pk:Mul:08] [FJ218487]	CLCUAV[IN:Har: OY77:06] [GU112081]	NS	NS	1.432×10^{-3}	NS	NS	2.093×10^{-1}
	397–642	BYVMV[India:Karl: OY80B:05] [GU112077]	BYVMV[India:Kai: OYKaivara:06] [GU112057]	6.111×10^{-5}	1.195×10^{-3}	NS	NS	NS	2.59×10^{-3}

(*continued*)

Table 4. (*Continued*).

DNA-A	Break point begin-end	Major parent	Minor parent	RDP	GENECOV	Max Chi	Chimera	Si Scan	3Seq
	1093–1510	BYVMV[India:Coi:OYCO4:05] [GU112080]	CLCuAV-[804a] [AJ002452]	4.048×10^{-6}	5.459×10^{-6}	1.429×10^{-5}	7.843×10^{-7}	NS	7.409×10^{-6}
	1593–1758	MeYVMV-[IN:Cooch:08] [FJ159265]	BYVMV[India:Soni:OY83:05] [GU112075]	NS	1.943×10^{-4}	NS	NS	NS	4.406×10^{-3}
	1868–2047	BYVMV[India:Chinti:OYCHINT:06] [GU112065]	OELCV[IN:Soni-EL14:06] [GU111997]	2.647×10^{-3}	2.591×10^{-3}	9.953×10^{-7}	4.055×10^{-3}	NS	8.907×10^{-4}

NS, non-significance.

years, this disease continued to spread into all states of India wherever cotton is grown. Moreover, the diversity of begomoviruses infecting cotton was well studied both in India and Indian subcontinents (Mansoor et al. 2003a, 2003b; Kirthi et al. 2004), but the study of diversity of begomoviruses infecting okra lacking in India with exception of two reports (Jose and Usha 2003; Venkataravanappa et al. 2012) and one from Indian subcontinent (Zhou et al. 1998). Recently, cotton viruses, in addition to infecting the cotton, are also spread to the other vegetable and fruit crops were reported (Mansoor et al. 2000, 2003a; Hussain et al. 2003). All the previously characterised isolates of CLCuD were identified from India are infecting cotton and tomato. The infection of both malvaceous and non-malvaceous hosts by cotton leaf curl viruses are likely due to occurrence of high inoculum pressure cotton viruses and their strains, growing of okra and cotton in side by side and existing of new whitefly biotypes vector complex in north India. Since, in and around Haryana cotton-growing extensively, there is all possibility of whitefly vector to carry the virus and transmit to okra, which might have been later adapted to okra.

The recombination analysis indicates that major segment of genome of these strains of CLCuAV has probably descended from a sequence that arose through a recombination between CLCuAV, BYVMV, CLCuMV and MeYVMV-like ancestors and evolved as a new recombinant begomovirus strains infecting okra. The recombination is a major contributor to the richness of currently observed begomovirus species diversity and it is a very frequent and widespread phenomenon that occurs between species as well as within and across genera and playing a significant role in begomovirus evolution. The important contribution of recombination to geminivirus evolution is now well established (Padidam et al. 1999; Unseld et al. 2000; Jeske et al. 2001) and it is suspected that it is directly responsible for the emergence of many of the most agriculturally damaging begomovirus species complexes (Monci et al. 2002; Garcia-Andres et al. 2006).

The diseases caused by begomoviruses on okra have become one of the major hindrances for the cultivation and production of okra in India. Further survey, detection, identification and characterisation of hitherto unknown viruses are required to develop a clearer picture of the diversity and geographical distribution of these viruses. Understanding host-pathogen interactions at molecular level could also be an important future area of research.

Acknowledgements

The research was supported by ICAR NETWORK project on development of diagnostics to emerging plant viruses, Indian Council of Agricultural Research, Government of India, New Delhi.

References

Ajmera BD. 1994. Occurrence of leaf curl virus on American Cotton (*G. hirsutum*) in north Rajasthan. Paper presented at: National seminar on cotton production challenges in 21st century; April 18–20; Hisar, India.

Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID, Dhawan P, Rishi N, Siwatch, SS, Abdel-Salam AM, et al. 2003. Diversity of DNA beta: 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 K, Markham, PG. 2001. Identification of DNA components required for induction of cotton leaf curl disease. Virology. 285:234–243.

Briddon RW, Markham PG. 1994. Universal primers for the PCR amplification of dicot-infecting geminiviruses. Mol Biotechnol. 1:202–205.

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

Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X. 2008. Geminivirus strain demarcation and nomenclature. Arch Virol. 153:783–821.

Fauquet CM, Stanley J. 2005. Revising the way we conceive and name viruses below the species level: a review of geminivirus taxonomy calls for new standardized isolate descriptors. Arch Virol. 150:2151–2179.

Garcia-Andres S, Monci F, Navas-Castillo J, Morions E. 2006. Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: evidence for the presence of a new virus species of recombinant nature. Virology. 350:433–442.

Garrido-Ramirez ER, Sudarshana MR, Gilbertson RL. 2000. Bean golden yellow mosaic virus from Chiapas, Mexico: characterization, pseudorecombination with other bean infecting geminiviruses and germ plasm screening. Phytopathology. 90:1224–1232.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids. 41:95–98.

Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, Robertson D. 2000. Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. Crit Rev Biochem Mol Biol. 35(2):105–140.

Heyraud F, Matzeit V, Kammann M, Schafer S, Schell J, Gronenborn B. 1993. Identification of the initiation sequence for viral-strand DNA synthesis of wheat dwarf virus. EMBO J. 12:4445–4452.

Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. Mol Biol Evol. 23:254–267.

Hussain M, Mansoor S, Amin I, Iram S, Zafar Y, Malik KA, Briddon RW. 2003. First report of cotton leaf curl disease affecting chili peppers. Plant Pathol. 53:794.

Jeske H, Luetgemeier M, Preiss W 2001. DNA forms indicate rolling circle and recombination dependent replication of *Abutilon mosaic virus*. EMBO J. 20:6158–6167.

Jose J, Usha R. 2003. Bhendi yellow vein mosaic disease in India is caused by association of a DNAβ satellite with a begomovirus. Virology. 305:310–317.

Kirthi N, Priyadarshini CGP, Sharma P, Maiya SP, Hemalatha V, Sivaraman P, Dhawan P, Rishi N, Savithri HS. 2004. Genetic variability of begomoviruses associated with cotton leaf curl disease originating from India. Arch Virol. 149:2047–2057.

Lazarowitz SG. 1992. Geminiviruses: genome structure and gene function. Crit Rev Plant Sci. 11:327–349.

Mansoor S, Amin I, Iram S, Hussain M, Zafar Y, Malik KA, Briddon RW. 2003a. Breakdown of resistance in cotton to cotton leaf curl disease in Pakistan. Plant Pathol. 52:784.

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

Mansoor S, Mukhtar S, Hussain M, Amin I, Zafar Y, Malik K, Markham PG. 2000. Widespread occurrence of Cotton leaf curl virus on radish in Pakistan. Plant Dis. 84:809.

Martin DP, Lemey P, Lott M, Vincent M, Posada D, Lefeuvre, P. 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics. 26(19): 2462–2463.

Monci F, Sanchez-Campos S, Navas-Castillo J, Moriones E. 2002. A natural recombinant between the geminiviruses Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. Virology. 303:317–326.

Morales FJ, Anderson PK. 2001.The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. Arch Virol. 146:415–441.

Moriones E, Navas-Castillo J. 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res. 71:123–134.

Padidam M, Sawyer SY, Fauquet CM. 1999. Possible emergence of new geminiviruses by frequent recombination. Virology. 265:218–225.
Paul S, Ghosh R, Chaudhuri S, Ghosh SK, Roy A. 2009. Biological and molecular variability of the begomoviruses associated with leaf curl disease of kenaf in India. J Plant Pathol. 91(3):637–647.
Pita JS, Fondong VN, Sangare A, Otim-Nape GW, Ogwal S, Fauquet CM. 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J Gen Virol. 82:655–665.
Polston JE, Anderson PK. 1997. The emergence of whitefly-transmitted geminiviruses of tomato in the Western Hemisphere. Plant Dis. 81:1358–1369.
Rojas MR, Gilbertson RL, Russel DR, Maxwell DP. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis. 77:340–347.
Rojas MR, Jiang H, Salati R, Xoconostle-Cazares B, Sudarshana MR, Lucas WJ, Gilbertson RL. 2001. Functional analysis of proteins involved in movement of the monopartite begomovirus, Tomato yellow leaf curl virus. Virology. 291(1): 110–125.
Sastry KSM, Singh SJ. 1974. Effect of yellow vein mosaic virus infection on growth and yield of okra crop. Indian Phytopath. 27:294–297.
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.
Seal SE, Jeger MJ, Van den Bosch F, Maramorosch K, Shatkin AJ, Thresh JM. 2006a. Begomovirus evolution and disease management, Adv Virus Res. 67:297–316.
Seal SE, van den Bosch F, Jeger MJ. 2006b. Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. Crit Rev Plant Sci. 25:23–46.
Sharma P, Rishi N, Malathi VG. 2005. Molecular cloning of coat protein gene of an Indian *Cotton leaf curl virus* (CLCuV-hs2) isolate and its phylogenetic relationship with others members of *Geminiviridae*. Virus Genes. 30:85–91.
Sinha SN, Chakrabarthi AK. 1978. Effect of yellow vein mosaic virus infection on okra seed production. Seed Res. 6:67–70.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 10:1093.
Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.
Unseld S, Ringel M, Konrad A, Lauster S, Frischmuth T. 2000. Virus-specific adaptations for the production of a pseudorecombinant virus formed by two distinct bipartite geminiviruses from Central America. Virology. 274:179–188.
Usha R. 2008. Bhendi yellow vein mosaic virus. In: Rao GP, Kumar PL, Holguin-Pena RJ, editors. Characterization, diagnosis and management of plant viruses, vol 3. Houston (TX): Studium Press. p. 387–392.
Vanitharani R, Chellappan P, Fauquet CM. 2005. Geminiviruses and RNA silencing. Trends Plant Sci. 10:144–151.
Venkataravanappa V, Reddy CNL, Jalali S, Krishna Reddy M. 2012. Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. Virus Genes. 44(3): 522–535.
Venkataravanappa V, Reddy CNL, Swaranalatha P, Jalali S, Briddon RW, Krishna Reddy M. 2011. Diversity and phylogeography of begomovirus-associated beta satellites of okra in India. Virol J. 8:555.
Voinnet O, Pinto YM, Baulcombe DC. 1999. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proc Natl Acad Sci USA 96:14147–14152.
Zhou X, Liu Y, Robinson D J, Harrison BD. 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. J Gen Virol. 79:915–923.