Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2726

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 558-570 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Association of Tomato Leaf Curl New Delhi Virus, A Bipartite Begomovirus with Mosaic Disease of Snake Gourd in India

V. Venkataravanappa[1&2*], C. N. Lakshminarayana Reddy[3], Neha Chauhan[2], D. R. Bhardwaj[2] and M. Krishna Reddy[2]

[1]CHES, Chettalli, Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bangalore, India

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

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

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

*Corresponding Author E-mail: venkatrajani@gmail.com

Received: 20.03.2017 | Revised: 27.04.2017 | Accepted: 5.05.2017

ABSTRACT

Total twenty five snake gourd leaf samples showing severe mosaic symptoms were collected from different farmer's field in Varanasi, Uttar Pradesh State of India. The partial amplified PCR products (1.2kb fragment) were cloned and sequence characterized. On the basis of the determined sequences and sequence analysis, begomovirus associated with symptoms in majority of samples (20) was found to be a member of a bipartite begomovirus species which is closely related to Tomato leaf curl New Delhi virus (ToLCNDV). Therefore one sample was selected for full-length amplification using RCA method. SDT analysis of complete genome of the begomovirus (SnG-1) showed highest nucleotide (nt) identity of 88.5-96.3% (DNA-A) and 82.7-93.3% (DNA-B) with Tomato leaf curl New Delhi virus (ToLCNDV) infecting different cucurbits in Indian subcontinent. An analysis for recombinant origin of genomes (DNA-A and DNA-B-like sequence) showed major part of their genome was likely, originated by recombination of previously reported begomoviruses (ToLCNDV, ToLCPaV and SLCCNV) infecting different cucurbits species resulting in evolution of new recombinant virus. This is the first report of ToLCNDV associated Snake gourd India and significance of these findings is discussed.

Key words: *Snake gourd, Begomovirus, PCR, Recombination, Phylogentic analysis, Tomato leaf curl New Delhi virus (ToLCNDV)*

INTRODUCTION

The family cucurbitaceae includes popular fruits and vegetables grown in various tropical and subtropical regions of the world. However, viral diseases are major limiting factor for cultivation of cucurbits and causing heavy economical yield losses. Among different gourds belongs to the cucurbits, snake gourd is one of the most popular perennial climber grown in different parts of India.

Cite this article: Venkataravanappa, V., Reddy, C.N.L., Chauhan, N., Bhardwaj, D.R. and Reddy, M.K., Association of Tomato Leaf Curl New Delhi Virus, A Bipartite Begomovirus with Mosaic Disease of Snake Gourd in India, *Int. J. Pure App. Biosci.* **5(5):** 558-570 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2726

Snake gourd (*Trichosanthes cucumerina* L.) is a cucurbitaceous plant belongs to the genus *Trichosanthes* comprises about 100 species, of which a wild var. cucumerina and cultivated var. anguina have been domesticated in India, Bangladesh, Sri Lanka, Burma, Malaysia, Australia, Latin America and Africa [1] . It is a perennial climber with an attractive white flower and highly bitter in taste which may be supposed to contain medicinal properties[9] hence it is being used in treatments of various diseases [18]. Snake gourd is a rich source proteins, fat, fibre, carbohydrates, vitamin A and E. The total phenolics and flavonoids content are 46.8% and 78.0% respectively. The fruit is rich source of potassium (121.60mg 100-1g), phosphorus (135.0mg 100-1g), Vitamin C and E. Snake gourd is succulent perennial climber and is susceptible to many viruses from germination of seed to harvest. Among the different virus which hampered the production and cultivation of snake gourd are *Cucumber green mottle mosaic virus* (CGMoMV), *Papaya ring spot virus* (PRSV) [3, 19, 47] and *Zucchini yellow mosaic virus* [14] throughout the world. The report of DNA viruses in India on snake gourd is very scanty. Recently based coat protein gene sequence analysis, it was shown that the begomovirus is associated with mosaic disease of snake gourd in Sri Lanka [4]. However, the exact identity of the begomovirus associated with snake gourd was not confirmed due to unavailability of full length sequence of the begomovirus, which is essential for nomenclature of any begomovirus. So far, only few begomovirus species has been identified on cucurbits are *Squash leaf curl China virus* (SLCCNV) [23, 25] and *tomato leaf curl New Delhi virus* [30, 37, 42] and *tomato leaf curl Palmpur virus* (ToLCPalV) [26], *Indian cassava mosaic virus* [32, 34,] *Ageratum enation virus* [33, 42] which known to affect major cucurbits like Pumpkin, Bottle gourd, Spong gourd, Bottle gourd, Bitter gourd, Ridge gourd, Pointed gourd and Armenian cucumber in India. The roving survey was conducted during 2012-2014 in twenty five different farmer fields in different places of Varanasi and Mirzapur districts, Uttar Pradesh state of India. The plants are showing severe mosaic and mottling type of disease symptoms were predominately observed. This type of diverse morphogenic symptoms makes me difficult to assess the exact virus involved in causing disease in Snake gourd. Therefore the current study was attempted to characterize virus associated with severe mosaic and mottling type symptoms of Snake gourd in India.

MATERIALS AND METHOD

Collection of disease samples

Total twenty five snake gourd leaf samples showing severe mosaic and mottling type of symptoms and symptomless leaves were collected in different farmers' fields and back yard of their houses (kitchen garden) in Varanasi and Mirzapur (82.52 °E longitude; 25.10 °N latitude), Uttar Pradesh state of India. The samples were brought to Indian Institute of Vegetable Research, Plant Pathology laboratory at Varanasi. The field collected samples may have gets infected with more than viruses (RNA and DNA viruses). Therefore the samples were initially tested by DAS-ELISA using different known polyclonal antibodies such as CGMMV, PRSV-W and ZYMV (DSMZ, Germany) to know the possibility of different viruses associated these samples.

DNA isolation, PCR-mediated amplification

To confirm the identity of the virus associated with Snake gourd samples, total nucleic acid was extracted from infected and healthy samples by using CTAB method [10] and amplified by PCR using group specific primers of begomoviruses[44]. The fragments of 1.2kb length amplified using these primers were sequenced. On the basis of the determined sequences, the begomovirus associated with twenty five snake gourd samples was found to be a member of a previously described bipartite begomovirus species ToLCNDV. Therefore one sample was selected for full-length amplification of begomovirus genome (DNA-A and DNA-B) by rolling circle amplification method using an Illustra TempliPhi 100 Amplification kit (Amersham Biosciences, Piscataway, NJ, USA) following

the manufacturer's instructions. The RCA products were digested with *Bam HI* were cloned into *BamHI*-linearized pUC19 plasmid as described by Venkataravanappa *et al* [44]. The ligated products were transformed into competent DH5α strain of *Escherichia coli.* Colony PCR followed by restriction digestion with *BamHI* and *ScaI* was performed for confirmation of recombinant clones. The confirmed clones were sequenced in both orientations from Eurofin Genomic India Pvt. Ltd DNA Sequencing facility, Bangalore, Karnataka, India.

Further these samples were also tested using universal betasatellites (bet01/bet02) [5] and alphasatellites (DNA101/ DNA102) primers [7] to know any subgenomic components is associated with begomovirus.

Sequence analysis and Detection of recombination events

Sequences (DNA-A and DNA-B-like sequence) were assembled and verified for the presence of begomovirus specific ORFs (using NCBI ORF finder). Sequence similarity searches were performed by comparing sequence to all sequences available in the GenBank database using BlastN [1]. Sequences showing the maximum identity scores (Supplementary Table 1) with the present isolate were aligned using the Muscle method implemented in SDT version 1.2 [24], the pair wise identity matrix of identified sequences and the representative sequences from the database were generated. A phylogenetic tree was constructed by MEGA 6.01 software [40] using the Maximum Likelihood method with 1000 bootstrapped replications to estimate evolutionary distances between all pairs of sequences simultaneously. Recombination analysis was carried out using the Recombination detection program (RDP), GENECOV, Bootscan, Max Chi, Chimara, Si Scan, 3Seq which are integrated in RDP4 [22]. Default RDP settings with P- value cut off (0.05) throughout and standard Bonferroni correction were used.

RESULTS

Detection of virus in Snake gourd samples

The snake gourd samples showing the typical mosaic and mottling type's symptoms were tested by DAS-ELISA using polyclonal antibodies of different viruses (Data not shown). None of the plants samples collected from farmer fields and back yard of their houses were reacted with antibodies. Mean while these samples also checked by sap inoculations on ten seedlings each of Cucumber, Tobacco, Snake gourd, Pumpkin, Bottle gourd and Datura metal. None of the plants are showed either local or systemic symptoms on any of the test plants and it was further confirmed by DAS-ELISA as described above, indicating an absence of RNA viruses. Further to confirm the identity of the virus, total nucleic acid was amplified by PCR using degenerate primer as described by Venkataravanappa *et al*. [45]. All samples showed positive amplification with the resulted PCR amplicon of 1.2kb in size. The PCR amplified products were cloned and sequenced. Analysis of partial sequences of 1.2kb fragment showed that the snake gourd was found associated with a member of a previously described bipartite begomovirus species viz; ToLCNDV. Therefore one snake gourd isolate (SnG-1) was selected for full-length amplification of begomovirus genome (DNA-A and DNA-B) by rolling circle amplification.

Genome organization of DNA-A-like sequence of begomovirus

The complete DNA-A-like sequence of snake gourd isolate (SnG-1) was determined to be 2763 nt in length and sequence is available in the database under accession number of KY780214. The analysis of complete nt sequence of DNA-A of SnG-1 showed to be typical of OW bipartite begomovirus with six conserved ORFs: AV2 (precoat protein, 120-458), AV1 (coat protein, 280-1050) in sense orientation; and AC3 (replicase enhancer protein, 1047-1457), AC2 (transcriptional activator protein, 1177-1596), AC1 (replication associated protein, 1499-2585), AC4 (C4 protein, 2252-2428) and AC5 (C5 protein, 310-795) in antisense orientation with the capacity to encode proteins of predicted molecular mass of 11.05 kDa or more.

Comparison complete genome (DNA-A-like sequence) of SnG-1 (KY780214) with the sequences of other begomoviruses available in the database showed highest nt identity with ToLCNDV (88.5-96.3%) infecting different cucurbits in India (Table 1). This result is well supported by a phylogenetic analysis shows, the SnG-1 isolate (ToLCNDV) closely grouped with ToLCNDV isolates infecting different cucurbits in India (Fig.2a). The identities with other ToLCDNV infecting tomato (92.9-95.6%), potato (94.5-94.8%), chilli (93.5-95.8%) and eggplant (93.2%) reported from India. It also showed 82.9-83.5% nt identities with Tomato leaf curl Palampur virus reported on cucurbits and tomato (83.3-84.3%). Based on the current species demarcation criteria for begomoviruses (91% nucleotide sequence identity) [6], the virus isolated from snake gourd is a strain of ToLCDNV infecting cucurbits in India.

ORF wise sequence identities at protein level showed highest with different isolates of ToLCNDV, the regions *viz.* Pre-coat (AV2), coat protein (CP), REn (C3) and C4 shared maximum amino acid identity with ToLCNDV infecting cucurbits and chilli, whereas Rep (C1), TrAP (C2), and C3 region are more homology with an isolates of ToLCNDV infecting cucurbits respectively (Table1). In the IRs region, the sequence identity of isolate (SnG-1) were more than 56.9-62.4 percent with IRs of reported ToLCNDV infecting tomato isolates (Table1).The length of IR is 275 nt in length and is similar to other bipartite begomoviruses reported so for. Within the IR, contains, incomplete direct repeats of an iteron sequence, GGTGTC, present adjacent to the TATA box, which is presumably the Rep promoter [2, 12, 13] and share significant homology with iterons identified in DNA-A so far.

Genomic organization and affinities of the DNA-B-like sequence

The complete DNA-B-like sequence of the snake gourd isolate (SnG-1) was determined in both orientations and it was found to be 2695 nt in length, which is available in the database under accession number of KY780200. Alignment of complete nt sequence of DNA-B-like sequence with other sequences revealed that, the SnG-1 showed maximum nt identity with ToLCNDV (82.7-93.3%) infecting cucurbits in Indian subcontinent (Table 2). This result is well supported by phylogenetic analysis, it has showed that, the SnG-1 isolate (ToLCNDV) is closely grouped with ToLCNDV infecting cucurbits and tomato in India and china (Fig 2b). The IR of SnG-1 isolate share 52.1-89.4 percent identity with ToLCNDV isolates infecting cucurbits. The length of the IR is 310 nt and similar to other ToLCNDV isolates available in the database (Table 2). Further, this region encompasses an absolutely conserved hairpin structure containing nonanucleotide sequence (TAATATTAC) that marks the origin of virion-strand DNA replication and repeated sequences known as "iterons" (GGTGTC) were detected adjacent to the TATA box in SnG-1, that are recognition sequences for binding of the rep promoter [2, 12].

Analysis of complete DNA-B-like sequence showed typical genome organization similar to other OW bipartite begomoviruses having two ORFs, one on the virion strand BV1 (movement protein, 442-1248) and other on the complementary strand BC1 (nuclear shuttle protein, 1306-2151) with the capacity to encode proteins of predicted molecular mass of 30 kDa or more. When individually encoded protein were compared, the highest amino acid sequence similarities (83.5-94.0%) of movement protein and nuclear shuttle protein (NSP, 93.9-99.2%) with ToLCNDV infecting cucurbits crops (Table 2).

Several attempts to amplify a betasatellites and Alphasatellites from symptomatic snake gourd plant samples of using universal primers [5, 7] resulted in no amplified product. These results indicate that the isolates under study are bipartite begomovirus.

Recombination

A comprehensive analysis for recombination using RDP4, based on the alignment of SnG-1isolate sequences and selected begomviruses

from the databases (Supplementary Table 1). The analysis showed evidence for recombination in snake gourd isolate (SnG-1), with most of the sequences in different coding and non-coding region (IR, CP, AC3, AC4) of DNA-A-like sequence originating from ToLCNDV, ToLCPalV and SLCCNV and ToLCNDV and ToLCPalV in DNA-B -like sequence (BC1 region) respectively. The snake gourd isolate (SnG-1) containing origin of replication is originate from ToLCNDV and small fragments of DNA-A of SnG-1 were also derived from the other virus such as ToLCPalV and SLCCNV (Supplementary Table 2).

DISCUSSION

Among the various cultivated cucurbitaceous vegetables grown in India, Snake gourd (*Trichosanthes anguina*), is very popular, which is cultivated both commercially and in kitchen gardens during the spring-summer and rainy season in different parts of the country. The production of snake gourd is limited by many insect pest and diseases from seed germination to harvest, which causes heavy economic losses. Among them whitefly-transmitted begomoviruses are serious impact on agriculture worldwide because of their diversity, ability to recombine, changing in their host range in time to time and the severity of the diseases they caused [43]. In the present study the leaf samples showing severe mosaic and mottling are collected directly from the farmers field were initially tested by DAS-ELISA using different polyclonal antisera (DSMZ, Germany), due to the possibility of different RNA viruses may get infected, particularly potyviruses, cucumoviruses and tobomoviruses which are commonly found and infecting the cucuribits in India [15, 21]. Similar mixed infection different viruses were noticed in different cucurbits under natural conditions [27]. Mean while the samples were also checked by sap inoculation. None of the plants are showed either local or systemic symptoms on any of the test plants. Further the snake gourd samples tested by PCR using begomoviruses specific primers were found to be positive and showed highest nucleotide identity with ToLCNDV infecting cucurbits in India. ToLCNDV is an emerging problem in many agricultural crops and widely distributed in India, Pakistan, Philippines and Thailand. Although, ToLCNDV is a major viral pathogen in solanaceous vegetables[11, 17, 29, 35] and it has and known to infect many cucurbitaceous vegetables such as bottle gourd, bitter gourd, cucumber, ivy gourd, long melon, pumpkin, ridge gourd and watermelon in northern and north-western India[20, 38, 39, 41].

The spread of ToLCNDV to other closely related crops and vice versa in North India may be mainly due to growing of solanaceous crops (Tomato, chilli and brinjal) and cucurbits throughout year in the adjacent fields facilitating the cross movement of viruliferous vectors between these fields. Further, in the absence of main host, whiteflies are feeding on alternate hosts, which are harboring many viruses and also there is possibility of inoculating diverse viruses into new hosts. Thus the transmitted viruses may recombine with already existing viruses and their satellites and adapt to the new host leading to the emergence of novel viruses and their strains.

Recombination is a major mechanism of evolution of begomoviruses and has been shown to have played a part in the evolution of the distinct strains of begomoviruses in different crops in India [31, 46]. The results presented here suggest that recombination has similarly led to the evolution of Snake gourd isolate (SnG-1) in India. The recombination analysis showed that isolate SnG-1 of DNA-A has obtained at least some of its sequence by descend from ToLCNDV, SLCCNV and ToLCPalV and for DNA B is ToLCNDV and ToLCPalV viruses respectively, which are reported from different parts of the India and other parts of the world. Overall the results of the recombination analysis, phylogenetic analysis and the deletions/insertions within genome suggest that isolate SnG-1 evolved from ToLCNDV and ToLCPalV (the major parent) with some contribution of SLCCNV in genomic components.

Table 1: Pairwise percent of nucleotide identities between the genomic component (DNA-A) and amino acid sequence identities of encoded genes from the ToLCNDV-[IN:SnG:Var:14] with the components and genes of selected begomoviruses available in the databases

Begomovirus#	Genome	IR	Gene (percentage amino acid sequence identity)						
			AV2	CP	Rep	TrAP	REn	AC4	AC5
ToLCNDV-Cucurbits (14)	88.5-96.3	52.3-62.2	76.22-100	95.3-96.8	91.4-98.3	79.8-100	75.7-93.3	79.3-98.2	64.5-90.0
ToLCNDV-Tomato (10)	92.9-95.6	56.9-62.4	93.7-100	93.3-96.8	94.4-97.7	79.1-94.2	81.6-91.1	81.0-93.1	64.5-90.0
ToLCNDV-Potato (4)	94.5-94.8	59.7-60.4	96.4-98.2	93.7-94.5	95.5-96.1	92.0-93.2	35.2-90.4	89.6-93.1	86.3-88.1
ToLCNDV-chilli (4)	93.5-95.8	57.9-62.0	97.3-100	96.4-96.8	94.7-97.2	92.8-98.5	88.9-94.8	84.4-98.2	85.0-86.9
ToLCNDV-Egg (1)	93.2	58.7	97.3	96.4	94.4	92.8	89.7	81.0	-
ToLCPaV-Cucurbits (24)	82.9-83.5	50.6-51.1	66.9-73.9	88.6-89.8	84.5-87.4	78.4-79.1	71.7-77.2	77.6-93.1	-
ToLCPaV-Tomato (2)	83.3-84.3	50.6-52.7	68.5-72.1	88.9-89	87.1-87.4	79.0-79.1	77.2-77.9	89.6-91.3	55.9
SLCCNV-Pumpkin (5)	86.4-90.0	52.4-52.6	95.5-97.3	93.3-97.2	86.9-91.9	69.0-72.6	61.0-78.6	72.0-72.4	78.8-83.9
MYMIV (2)	60.0-60.7	24.5-25.0	42.4-44.2	70.0-71.9	69.6-69.8	37.6-428	37.0-37.5	30.3-31.3	40.0

* Numbers of sequences from the databases used in the comparisons.
IR- Intergenic region
#The species are indicated as, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Palampur virus* (ToLCPaV), Squash leaf curl China virus (SLCCNV), *Mungbean yellow Mosaic Indian virus* (MYMIV). For each column the highest value is underlined.

Table 2: Pairwise percent of nucleotide identities between the genomic component (DNA-B) and amino acid sequence identities of encoded genes from the ToLCNDV-[IN:SnG:Var:14] with the components and genes of selected begomoviruses available in the databases.

Begomovirus#	Genome[a]	IR[a]	Gene (percentage amino acid sequence identity)	
			BV1[b]	BC1[b]
ToLCNDV-cucurbits (11)	**82.7-93.3**	**52.1-89.4**	**83.5-94.0**	**93.9-99.2**
ToLCNDV-Tomato (12)	85.6-90.5	70.1-88.7	88.2-93.6	88.6-98.5
ToLCNDV-Potato(6)	87.5-87.9	78.6-85.8	87.5-92.9	96.4-97.1
ToLCNDV-Chilli (4)	85.9-89.6	79.4-88.1	89.6-92.5	88.6-97.5
ToLCPaV-Cucurbits(12)	69.5-69.8	46.6-57.7	76.4-86.4	89.6-92.8
ToLCPaV-Tomato(3)	69.7-70.0	54.5-56.0	76.4-77.2	91.8-93.2
SLCCNV-Pumpkin (4)	64.9-65.9	27.3-50.3	26.3-71.2	88.6-91.1
MYMIV-Mungbean(2)	43.4-43.5	25.5-79.5	26.3-86.4	41.0-41.5
ToLCNDV-okra(1)	88.1	81.0	82.2	96.0

a Nucleotide identity; b Amino acid identity
BV1=Nuclear shuttle protein gene, BC1=movement protein gene
#The species are indicated as, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Palampur virus* (ToLCPaV), Squash leaf curl China virus (SLCCNV), *Bhendi yellow vein mosaic virus* (BYVMV), *Mungbean yellow Mosaic Indian virus* (MYMIV). For each column the highest value is underlined.

Supplementary Table 1:

Gene Bank accession numbers of selected Begomovirus sequences used in this study for analysis

Sl.NO	Begomoviruses	Accession No.		Abbreviation
		DNA-A	DNA-B	
1	Tomato leaf curl New Delhi virus - [India:New Delhi:Pumpkin 2:2005]	AM286434	AM286435	ToLCNDV-[IN:ND:Pum2:05]
2	Tomato leaf curl New Delhi virus - [India:IARI:Pumpkin:2006]	JN129254	-	ToLCNDV-[IN::ND:Pum:06]
3	Tomato leaf curl New Delhi virus - [India:New Delhi:Pumpkin 1:2005]	AM286433	-	ToLCNDV-[IN:ND:Pum:05]
4	Tomato leaf curl New Delhi virus - [India:Lucknow]	Y16421	-	ToLCNDV-[IN:Luc:98]
5	Tomato leaf curl New Delhi virus - [India:Ash gourd:2011]	JN208136	-	ToLCNDV-[IN:Ag:11]
6	Tomato leaf curl New Delhi virus - [India:Meerut:Potato:2005]	EF043231	-	ToLCNDV-[IN:Mee:Pot:05]
7	Tomato leaf curl New Delhi virus - [India:Happur:Potato:2005]	EF043230	EF043233	ToLCNDV-[IN:Hap:Pot:05]
8	Tomato leaf curl New Delhi virus - [India:Meerut:Potato 12:2002]	AY286316	AY158080	ToLCNDV-[IN:Mee:Po12:02]
9	Tomato leaf curl New Delhi virus - [India:Himachal:Potato:2006]	AM850115	-	ToLCNDV-[IN:HP:pot:06]
10	Tomato leaf curl New Delhi virus - [Thailand:Cucurbit:2006]	AB330079	AB330080	ToLCNDV-[TH:cuc:06]
11	Tomato leaf curl New Delhi virus - [Thailand:Cucurbit:2006]	AB368448	-	ToLCNDV-[TH:cuc:06]
12	Tomato leaf curl New Delhi virus - [Thailand:Bottle gourd:2006]	AB368447	-	ToLCNDV-[TH:cuc:06]
13	Tomato leaf curl New Delhi virus - [Indonesia:Java:Cucumber:2008]	AB613825	-	ToLCNDV-[ID:Java:Cuc:08]
14	Tomato leaf curl New Delhi virus - [Bangladesh:Cucumber:2006]	EF450316	-	ToLCNDV-[BG:cuc:06]
15	Tomato leaf curl New Delhi virus - [Pakistan:Islamabad:T1/8:2000]	AF448059	AY150304	ToLCNDVIN[PK:Isl:T1/8:00]
16	Tomato leaf curl New Delhi virus - [India:Pune:JID27:2008]	HQ141673	HQ141674	ToLCNDV-[IN:Pun:tom:08]
17	Tomato leaf curl New Delhi virus - [India:Pune 8:2008]	FJ468356	-	ToLCNDV[IN:Pune:tom:08]
18	Tomato leaf curl New Delhi virus - [Pakistan:Solanum nigrum:PT10:2004]	DQ116883	-	ToLCNDV-[PK:Sol:PT10:04]
19	Tomato leaf curl New Delhi virus - [Pakistan:tomato:2008]	AM947506	-	ToLCNDV-[PK:tom:08]
20	Tomato leaf curl New Delhi virus - [India:New Delhi:2005]	DQ169056	DQ169057	ToLCNDVIN[IN:ND:05]
21	Tomato leaf curl New Delhi virus - [Pakistan:Dargai:T5/6:2001]	AF448058	-	ToLCNDV-[PK:Dar:T5/6:01]
22	Tomato leaf curl New Delhi virus - [India:New Delhi:2006]	EF068246	-	ToLCNDV-[IN:ND:tom:06]
23	Tomato leaf curl New Delhi virus - [Bangladesh:Jessore: Severe:2005]	AJ875157	AJ875158	ToLCNDV-[BG:Jes:Svr:05]
24	Tomato leaf curl New Delhi virus - [India:New Delhi:2009]	GQ865546	-	ToLCNDV[IN:ND:Tom:09]
25	Tomato leaf curl New Delhi virus - [India:Maharastra:Eggplant:2009]	HQ264185	-	ToLCNDV[IN:MH:Egg:09]
26	Tomato leaf curl New Delhi virus - [India:Sonepat:Luffa:2005]	AY939926	AY939924	ToLCNDV-[IN:Son:Luffa:05]
27	Tomato leaf curl New Delhi virus - [India:New Delhi:Lufa acutangula:JLH13:2008]	HM989845	HM989846	ToLCNDV-[IN:ND:Luffa:08]
28	Tomato leaf curl New Delhi virus - [Pakistan:Multan:Luffa:2004]	AM292302	-	ToLCNDV-[PK:Mul:Luffa:04]
29	Tomato leaf curl New Delhi virus - [India:Bahraich:Chilli:2006]	EU309045	-	ToLCNDV-[IN:Bah:Chi:06]
30	Tomato leaf curl New Delhi virus - [India:New Delhi:Chilli:2009]	HM007113	-	ToLCNDV-[IN:ND:Chi:09]
31	Tomato leaf curl New Delhi virus - [India:Tumkur:Chilli:2008]	HM007120	-	ToLCNDV-[IN:Tum:Chi:08]
32	Tomato leaf curl New Delhi virus - [Pakistan:Khalawal:Chili:2004]	DQ116880	DQ116882	ToLCNDV-[PK:Kha:Chi:04]
33	Tomato leaf curl New Delhi virus - [Pakistan:Multan:Momordica:2007]	AM747291	-	ToLCNDV-[PK:BG:07]
34	Tomato leaf curl Palampur virus - [India:Palampur:Pumpkin:2008]	FJ931537	-	ToLCPalV[IN:Var:Pum:08]
35	Tomato leaf curl Palampur virus - [India:Palampur:2007]	AM884015	AM992534	ToLCPalV[IN:HP:Tom:07]
36	Tomato leaf curl Palampur virus - [Iran:Jiroft 1:T55X:Cucumber:2008]	FJ660444	FJ660443	ToLCPalV[IR:Jir:T55X:Cuc:08]
37	Tomato leaf curl Palampur virus - [Iran:Kahnooj:T9X:Cucumber:2007]	FJ660434	FJ660424	ToLCPalV[IR:Kah:T9X:Cuc:07]
38	Tomato leaf curl Palampur virus - [Iran:Kerman:T8X:Cucumber:2007]	FJ660433	FJ668379	ToLCPalV[IR:Ker:T8X:Cuc:07]
39	Tomato leaf curl Palampur virus - [Iran:Roodan:T7X:2007]	EU547682	FJ660442	ToLCPalV-[IR:Roo:07]
40	Tomato leaf curl Palampur virus - [Iran:Khash:W9P:Citrullus lanatus:2010]	JF501728	-	ToLCPalV[IR:Kha:W9P:Wat:10]
41	Tomato leaf curl Palampur virus - [Iran:Jiroft:T5X:Cucumis sativus:07]	JF501724	-	ToLCPalV[IR:Jir:T5X:Cuc:07]
42	Tomato leaf curl Palampur virus - [Iran:Jiroft:T13X:Cucumis melo:2006]	JF501719	-	ToLCPalV[IR:Jir:T13X:Me:06]
43	Tomato leaf curl Palampur virus - [Iran:Jiroft:T56X:Cucumis sativus:2008]	JF501721	-	ToLCPalV[IR:Jir:T56X:Cuc:08]
44	Tomato leaf curl Palampur virus - [Iran:Jiroft 1:T1X:Cucumber:2007]	FJ660440	-	ToLCPalV[IR:Jir:T1X:Cuc:07]
45	Tomato leaf curl Palampur virus - [Iran:Jiroft 9:T7X:Cucumber:2007]	FJ660437	FJ660435	ToLCPalV[IR:Jir9:T7X:Cuc:07]
46	Tomato leaf curl Palampur virus - [Iran:Jiroft:Melon:2007]	EU547683	EU547681	ToLCPalV[Jiroft:mel:07]
47	Tomato leaf curl Palampur virus - [Iran:Jiroft 4:T6X:Cucumber:2007]	FJ660436	FJ660429	ToLCPalV[IR:Jir5:T6X:Cuc:07]
48	Tomato leaf curl Palampur virus - [Iran:Jiroft 8:T58P:Cucumber:2007]	FJ660431	FJ660425	ToLCPalV[IR:Jir8:T58P:Cuc:07]
49	Tomato leaf curl Palampur virus - [Iran:Jiroft 3:T4X:Cucumber:2007]	FJ660439	FJ660430	ToLCPalV[IR:Jir3:T4X:Cuc:07]
50	Tomato leaf curl Palampur virus - [Iran:Jiroft 5:T51X:Cucumber:2007]	FJ660432	FJ660428	ToLCPalV[IR:Jir5:T51X:Cuc:08]
51	Tomato leaf curl Palampur virus - [Iran:Iranshahr:M4P:Cucumis melo:2009]	JF501725	-	ToLCPalV[IR:Ira:M4P:Mel:09]
52	Tomato leaf curl Palampur virus - [Iran:Jiroft:T65X:Cucumis sativus:2008]	JF501720	-	ToLCPalV[IR:Jir:T65X:Cuc:08]
53	Tomato leaf curl Palampur virus - [Iran:Jiroft 6:T3X:Cucumber:2007]	FJ660441	FJ660427	ToLCPalV[IR:Jir6:T3X:Cuc:07]
54	Tomato leaf curl Palampur virus - [Iran:Jiroft 7:T11X:Cucumber:2007]	FJ660438	FJ660426	ToLCPalV[IR:Jir7:T11X:Cuc:07]
55	Tomato leaf curl Palampur virus - [Iran:Jiroft:T61X:Cucumis sativus:2008]	JF501723	-	ToLCPalV[IR:Jir:T61X:Cuc:08]
56	Tomato leaf curl Palampur virus - [Iran:Jiroft:T69P:Cucumis sativus:2008]	JQ825226	-	ToLCPalV[IR:Jir:T69P:Cuc:08]
57	Tomato leaf curl Palampur virus - [Iran:Jiroft:T63X:Cucumis sativus:2008]	JF501722	-	ToLCPalV-[IR:Jir:T63X:Cuc:08]
58	Tomato leaf curl Palampur virus - [Iran:Jiroft:K1P:Cucurbita pepo:2009]	JF501727	-	ToLCPalV-[IR:Jir:K1P:Squ:09]
59	Tomato leaf curl Palampur virus - [Iran:Barantin:B908P:Phaseolus vulgaris:2010]	JF501726	-	ToLCPalV-[IR:Bar:B908P:Pv:10]
60	Mungbean yellow mosaic India virus - [India:New Delhi:Cowpea 7:1998]	AF481865	AF503580	MYMIV-[IN:ND:Cp7:98]
61	Mungbean yellow mosaic India virus - [India:Akola]	AY271893	AY271894	MYMIV-[IN:Ako]
62	Squash leaf curl China virus - [India:pumpkin:IARI:2010]	JN587811	-	SLCCNV-[IN:ND:Pum:10]
63	Squash leaf curl China virus - India [India:Varanasi:Pumpkin:2008]	EU573715	FJ859881	SLCCNV-[IN:Var:Pum:08]
64	Squash leaf curl China virus - India [India:Coimbatore:Pumpkin:03]	AY184487	AY184488	SLCCNV-[IN:Coi:Pum:03]
65	Squash leaf curl China virus - India [India:Lucknow:Pumpkin:03]	DQ026296	-	SLCCNV-[IN:Luc:Pum:03]
66	Squash leaf curl China virus - India [India:Varanasi:Pumpkin2:2008]	GU967381	GU967382	SLCCNV[IN:Var:Pum:08]
67	Tomato leaf curl New Delhi virus - [India: Ash gourd:2011]	-	JN208137	ToLCNDV[IN:AG:11]
68	Tomato leaf curl New Delhi virus - [India: New Delhi:Cucucmber:2012]	-	KC545813	ToLCNDV-[IN:ND:Cuc:12]
69	Tomato leaf curl New Delhi virus - [India:Bangalore:Chilli::2011]	-	JN663848	ToLCNDV-[IN:BLR:Chi:11]
70	Tomato leaf curl New Delhi virus - [India:Bangalore:Chilli::2011]	-	JN663867	ToLCNDV-[IN:BLR:Chi:11]
71	Tomato leaf curl New Delhi virus - [India:New Delhi:Severe:1992]	-	U15017	ToLCNDVIN[IN:ND:Svr:92]
72	Tomato leaf curl New Delhi virus - [India:Pal:Chilli::2011]	-	JN663871	ToLCNDV-[IN:Pal:Chi:11]
73	Tomato leaf curl New Delhi virus - [Pakistan:Solanum nigrum:2009]	-	FN435312	ToLCNDV-[PK:Sol:09]
74	Tomato leaf curl New Delhi virus - [India:2009]	-	HM159455	ToLCNDV-[IN:09]
75	Tomato leaf curl New Delhi virus - [India:Tamil Ndau:Okra::2006]	-	HQ586007	ToLNDV-[IN:TN:OK:06]
76	Tomato leaf curl New Delhi virus - [India:West Bengal:Tomato::2013]	-	KF577604	ToLCNDV-[IN:WB:Tom:13]
77	Tomato leaf curl New Delhi virus - [India:Gujarat:Potato:2013]	-	KC874498	ToLCNDV-[IN:Guj:Pot:13]
78	Tomato leaf curl New Delhi virus - [India:Haryana:Potato:2010]	-	KC874495	ToLCNDV-[IN:HR:pot:10]
79	Tomato leaf curl New Delhi virus - [India:Punjab:Potato:2013]	-	KC874501	ToLCNDV-[IN:PJ:Pot:13]
80	Tomato leaf curl New Delhi virus - [India: Maharashtra :Tomato:2010]	-	HM803117	ToLCNDV-[IN:MH:Tom:10]
81	Tomato leaf curl New Delhi virus - [India: Uttar Pradesh:Potato:2013]	-	KC874497	ToLCNDV-[IN:UP:Pot:13]
82	Tomato leaf curl New Delhi virus - [India: New Delhi: Bitter Gourd:2005]	-	DQ020490	ToLCNDV-[IN:ND: BG:05]
83	Tomato leaf curl New Delhi virus - [Pakistan:Lahore:2004]	-	AM778833	ToLCNDV-[PK:Lah:04]
84	Tomato leaf curl New Delhi virus - [Pakistan:Lahore:2004]	-	AM392426	ToLCNDV-[PK:Lah:04]
85	Tomato leaf curl New Delhi virus - [Pakistan:Solanum nigrum:1997]	-	AJ620188	ToLCNDV-[PK:Sn:97]
86	Tomato leaf curl New Delhi virus - [India:Punjab:Tomato:2013]	-	KF571462	ToLCNDV-[IN:PJ:tom:13]
87	Tomato leaf curl Palampur virus - [India:Punjab:Tomato:2013]	-	KC456162	ToLCPalV-[IN:PJ:Tom:13]
88	Tomato leaf curl Palampur virus - [Iran:Jiroft:T63X:Cucumis sativus:2008]	-	FJ660423	ToLCPalV-[IR:Jir1:T55P:Cuc:08]
89	Squash leaf curl China virus - India [India: New Delhi:Pumpkin:11]	-	JN624306	SLCCNV-[IN:ND:Pum:11]

Supplementary Table 2.

Details of recombination between ToLCNDV and other begomoviruses detected using RDP4.

DNA-A	Break point begin-end [a]	Parent-like sequences		P-Values					
		Major Parent	Minor parent	RDP	GENECOV	Max Chi	Chimera	Si Scan	3Seq
SnG	139-1108 (IR, CP, AC3)	ToLCPaV[IN:HP:Tom:06].AM884015	SLCCNV-[IN:Luc:Pum:03].DQ026296	2.239X10-9	2.656X10-11	1.044X10-6	1.180X10-7	2.228X10-18	5.787X10-3
	543-626(CP)	ToLCNDV-[IN:ND:AG:11].JN208136 ToLCNDV-	ToLCNDV-[BG:cuc:07].EF450316	7.081X10-4	1.822X10-2	NS	NS	NS	2.285X10-5
	2511-2705 (AC4, IR)	[IN:ND:Chi:09].HM007113	ToLCNDV[IN:Pune:tom:08].FJ468356	1.24X10-3	NS	3.618X10-3	4.548X10-4	7.459X10-4	2.71X10-6
DNA-B									
SnG	2576-2692 (BC1)	ToLCNDV-[IN:UP:Pot:13].KC874497	ToLCPaV-[IR:Jir3:T4P:Cuc:07].FJ660430	NS	NS	NS	9.265X10-1	1.452X10-2	NS

NS- Recombination Non-significance
Definition for acronyms in the supplementary Table 1
[a] The text in the parenthesis of this column indicates ORF's in which break points are identified

Fig. 1a Infected SKG

Fig. 1b Healthy SKG

Fig. 1: Snake gourd sample collected from farmers field are showing (a) severe mosaic symptoms, (b) healthy samples

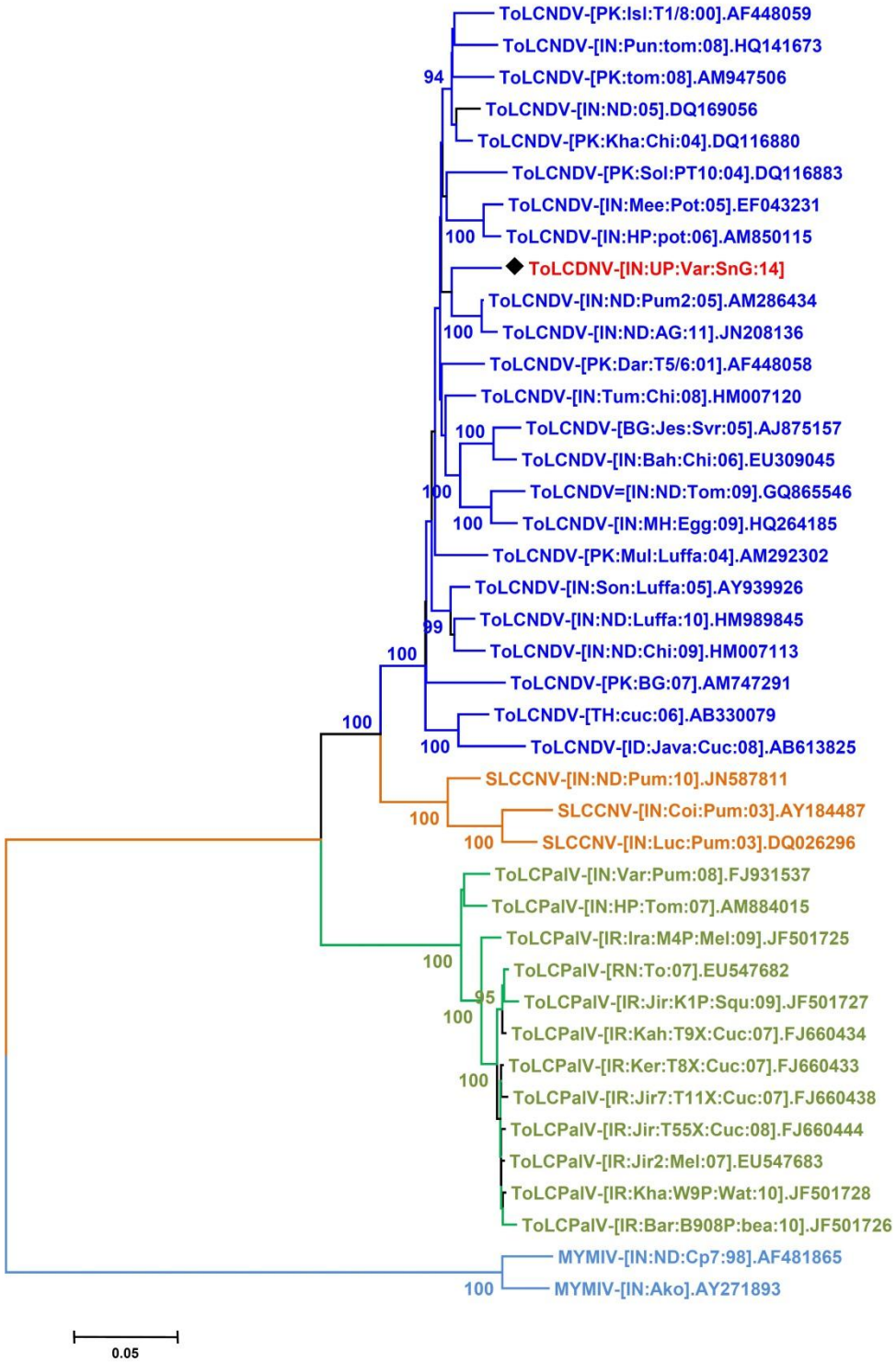
ToLCNDV-[PK:Isl:T1/8:00].AF448059
ToLCNDV-[IN:Pun:tom:08].HQ141673
94
ToLCNDV-[PK:tom:08].AM947506
ToLCNDV-[IN:ND:05].DQ169056
ToLCNDV-[PK:Kha:Chi:04].DQ116880
ToLCNDV-[PK:Sol:PT10:04].DQ116883
ToLCNDV-[IN:Mee:Pot:05].EF043231
100
ToLCNDV-[IN:HP:pot:06].AM850115
ToLCDNV-[IN:UP:Var:SnG:14]
ToLCNDV-[IN:ND:Pum2:05].AM286434
100
ToLCNDV-[IN:ND:AG:11].JN208136
ToLCNDV-[PK:Dar:T5/6:01].AF448058
ToLCNDV-[IN:Tum:Chi:08].HM007120
100
ToLCNDV-[BG:Jes:Svr:05].AJ875157
ToLCNDV-[IN:Bah:Chi:06].EU309045
100
ToLCNDV=[IN:ND:Tom:09].GQ865546
100
ToLCNDV-[IN:MH:Egg:09].HQ264185
ToLCNDV-[PK:Mul:Luffa:04].AM292302
ToLCNDV-[IN:Son:Luffa:05].AY939926
99
ToLCNDV-[IN:ND:Luffa:10].HM989845
100
ToLCNDV-[IN:ND:Chi:09].HM007113
ToLCNDV-[PK:BG:07].AM747291
100
ToLCNDV-[TH:cuc:06].AB330079
100
ToLCNDV-[ID:Java:Cuc:08].AB613825
SLCCNV-[IN:ND:Pum:10].JN587811
100
SLCCNV-[IN:Coi:Pum:03].AY184487
100
SLCCNV-[IN:Luc:Pum:03].DQ026296
ToLCPalV-[IN:Var:Pum:08].FJ931537
ToLCPalV-[IN:HP:Tom:07].AM884015
ToLCPalV-[IR:Ira:M4P:Mel:09].JF501725
100
ToLCPalV-[RN:To:07].EU547682
95
ToLCPalV-[IR:Jir:K1P:Squ:09].JF501727
100
ToLCPalV-[IR:Kah:T9X:Cuc:07].FJ660434
ToLCPalV-[IR:Ker:T8X:Cuc:07].FJ660433
100
ToLCPalV-[IR:Jir7:T11X:Cuc:07].FJ660438
ToLCPalV-[IR:Jir:T55X:Cuc:08].FJ660444
ToLCPalV-[IR:Jir2:Mel:07].EU547683
ToLCPalV-[IR:Kha:W9P:Wat:10].JF501728
ToLCPalV-[IR:Bar:B908P:bea:10].JF501726
MYMIV-[IN:ND:Cp7:98].AF481865
100
MYMIV-[IN:Ako].AY271893
0.05

Fig. 2: DNA-A

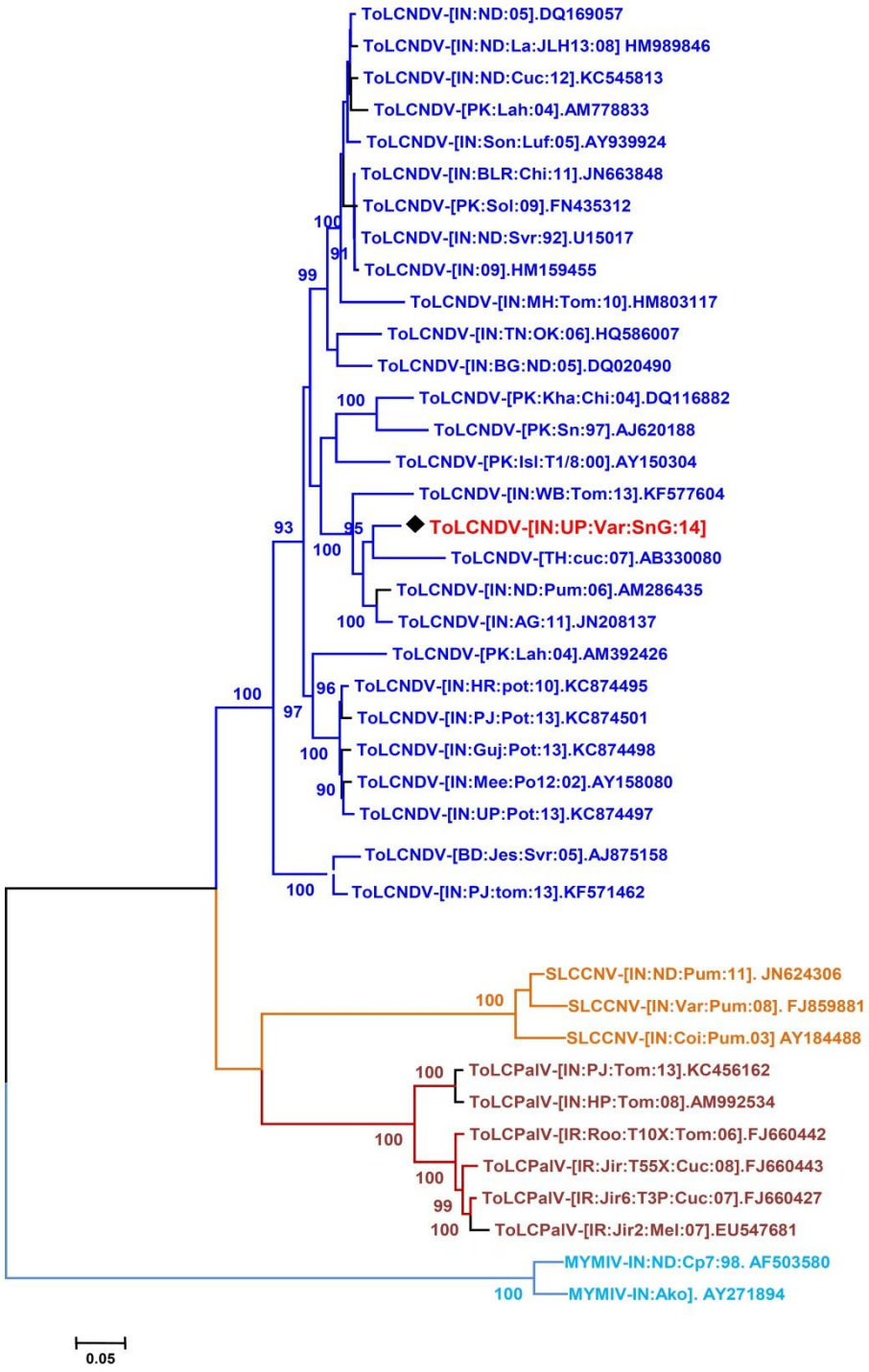

Fig. 3: DNA-B

Fig 2.

The phylogeny was constructed using the Maximum parsimony method. (2a) DNA-A sequence of begomovirus (SnG-1) and (2b) DNA-B sequence of begomovirus (SnG-1) associated with severe mosaic disease of Snake gourd in India. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown below the branches. The begomoviruses acronyms given are: Tomato leaf curl New Delhi virus (ToLCNDV), Tomato leaf curl Palampur virus (ToLCPaV), Squash leaf curl China virus (SLCCNV), Mungbean yellow Mosaic Indian virus (MYMIV) and Bhendi yellow vein mosaic virus (BYVMV). The database accession number in each case is given. Isolate and strain descriptors are as given in Brown et al. (2015).

CONCLUSION

Snake gourd is one of the most popular perennial climber grown in different parts of India. The data presented here provides useful information on occurrence of a recombinant ToLCNDV on snake gourd. Therefore further studies is required to know the role of climate change to favour persistence of vector and spread of virus and development of infectious clones to screen the snake gourd genotypes could be an interesting aspect of future investigations where the present findings can serve as a focal case study.

Acknowledgements

The research was supported by the project **"Consortium platform on Vaccines and diagnostics"** funded by Indian Council of Agricultural Research, Government of India, New Delhi, India.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. Altschul, S.F., W. Gish, W. Miller, E.W., Myers and D.J. Lipman, Basic local alignment search tool. *J Mol Biol*. **215**: 403-410 (1990).
2. Arguello-Astorga, G.R., Guevara-Gonzalez L.R., Herrera-Estrella L.R. and Rivera-Bustamante R.F., Geminivirus replication origins have a group-specific organization of iterative elements: a model for replication. *Virology*. **203**: 90-100 (1994).
3. Ariyaratne, I., Weeraratne , W.A.P.G. and Ranatunge, R.K.R. Identification of a new mosaic virus disease of snake gourd in Sri Lanka. Annals of the Sri Lanka Department of Agriculture. **7**: 13-21 (2005).
4. Bandaranayake, W. M. E. K., Wickramarachchi W. A. R. T., Wickramasinghe H. A. M., Rajapakshe R. G. A. S. and Dissanayake D. M. K. K., Molecular detection and characterization of begomoviruses associated with cucurbitaceous vegetables in SriLanka. *J natn Sci Foundation Sri Lanka,* **42(3)**: 265-271 (2014).
5. Briddon, R.W., Bull S.E., Mansoor S., Amin I. and Markham P.G., Universal primers for the PCR-mediated amplification of DNA beta A molecule associated with some monopartite begomoviruses. *Mol Biotechnol*. **20**: 315-318 (2002).
6. Brown, J.K., Zerbini F.M., Navas-Castillo J., Moriones E., Ramos-Sobrinho R., Silva J.C.F., Fiallo-Olive E., Briddon R.W., Hernandez-Zepeda C., Idris A., Malathi V.G., Martin D.P., Rivera-Bustamante R,, Ueda S. and Varsani A., Revision of Begomovirus taxonomy based on pairwise sequence comparisons. *Arch Virol*. **160(6)**: 1593-619 (2015).
7. Bull, S.E., Briddon R.W. and Markham P.G., Universal primers for the PCR-mediated amplification of DNA 1: a satellite-like molecule associated with begomovirus-DNAb complexes. *Mol Biotechnol*. **23**: 83-86 (2003).
8. Chakravarthy, H.L. Fascicles of Flora of India. (Fascicle 11) Botanical Survey of India, Howra, India (1982).
9. Choudhury, B. Vegetables National Book Trust, New Delhi, India. pp. 152-154 (1967).
10. Doyle, J.J., Doyle J.L., Isolation of plant DNA from fresh tissue. *Focus*. **12**: 13-15 (1990).
11. Garg, I.D., Paul-Khurana S.M., Kumar S. and Lakra B.S., Association of a geminivirus with potato apical leaf curl in India and its immunoelectron microscopic detection. *J Indain Potato Assoc*. **28**: 227-32 (2001).
12. Hanley-Bowdoin, L., Settleage S.B., Orozco B.M., Nagar S. and Robertson D., Geminivirus: models for plant DNA replication, transcription, and cell cycle regulation. *Crit Rev Plant Sci*. **18**:71-106 (1999).
13. Heyraud, F., Matzeit V., Kammann M., Schafer S., Schell J. and Gronenborn B., Identification of the initiation sequence for viral-strand DNA synthesis of wheat dwarf virus. *EMBO J*. **12**: 4445-4452 (1993).
14. Jadao, A.S., Buriola J.E. and Rezende J.A.M., First Report of Papaya ringspot virus-Type W and Zucchini yellow mosaic virus Infecting Trichosanthes cucumerina in Brazil. *Plant dis*. **94(6)**: 789 (2010).

15. Jain, R.K., Pappu H.R., Krishna Reddy M. and Vani A., Watermelon bud necrosis tospovirus is a distinct virus species belonging to serogroup IV. *Arch Virol* **143**: 1637-1644 (1998).
16. Jyothsna, P., Haq Q.M.I., Priyanka S., Sumiya K.V., Praveen S., Rawat R., Briddon R.W. and Malathi V.G., Infection of tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites. *Appl Microbiol Biotechnol*. **97(12)**: 5457-71 (2013).
17. Khan, M.S, Raj S.K. and Singh R., First report of tomato leaf curl New Delhi virus infecteing chilli in India. *Plant Pathol*. **55**: 289 (2006).
18. Kirtikar and Basu., Indian Medicinal Plants. Sri Satguru Publications, New Delhi p. 5: 1545-1547 (2000).
19. Kumar, S., Sankarlingam, A., Rabindran, R. Characterization and Confirmation of *Papaya ringspot virus*-W Strain Infecting *Trichosanthese cucumerina* at Tamil Nadu, India. *J Plant Pathol Microboil*. **5**: 225 (2014).
20. Mandal, B., Mandal S., Sohrab S.S., Pun K.B. and Varma A., A new yellow mosaic disease of chayote in India. *Plant Pathol*. **53**: 797 (2004).
21. Mandal, B., Chaudhary V.and Jain R. K., First report of Natural infection of Luffa acutangula by Watermelon bud necrosis virus in India. *Plant Dis*. **87**: 598 (2003).
22. Martin, D.P., Murrell B., Golden M. Khoosal A. and Muhire B., RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evolution* 1: vev003 doi: 10.1093/ve/vev003 (2015).
23. Maruthi, M.N., Rekha A.R. and Muniyappa V., Pumpkin yellow vein mosaic disease is caused by two distinct begomoviruses: complete viral sequences and comparative transmission by an indigenous Bemisia tabaci and the introduced B-biotype. *EPPO Bulletin*. **37**: 412-419 (2007).
24. Muhire, B.M., Varsani A. and Martin D.P., SDT: A Virus Classification Tool Based on Pairwise Sequence Alignment and Identity Calculation. *PLoS ONE*. **9(9)**: e108277. doi:10.1371/journal.pone.0108277 (2014).
25. Muniyappa, V., Maruthi M.N., Babiha C.R., Colvin J., Briddon R.W. and Rangaswamy K.T. Characterisation of pumpkin yellow vein mosaic virus from India. *Ann Appl Bio*. **142**: 323-331 (2003).
26. Namrata, J., Saritha R.K., Datta D., Singh M., Dubey R.S., Rai A.B.and Rai M., Molecular Characterization of Tomato leaf curl Palampur virus and Pepper leaf curl betasatellite naturally infecting pumpkin (*Cucurbita moschata*) in India. *Indian J Virol*. **21(2)**: 128-132 (2010).
27. Namrata, J., Saritha R.K., Datta D., Singh M., Dubey R.S., Rai A.B. and Rai M., Mixed infections of begomoviruses in pumpkins with yellow vein mosaic disease in north India. *Arch Phytopathol Plant Prot*. **45**: 8 (2012).
28. Padidam, M., Beachy, R.N. and Fauquet C.M., Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *J Gen Virol*. 76: 25-35 (1995).
29. Padidam, M., Beachy R.N. and Fauquet C.M., Classification and identification of geminiviruses using sequence comparisons. *J Gen Virol*. **76**: 249-263 (1995).
30. Phaneendra, C., Rao K.R. S.S., Jain R.K. and Mandal B., Tomato leaf curl New Delhi virus is Associated With Pumpkin Leaf Curl: A New Disease in Northern India. *Indian J Virol*. **23(1)**: 42-45 (2012).
31. Prasanna, H.C., Sinha D.P., Verma A., Singh M., Singh B., Rai M. and Martin D.P., The population genomics of begomoviruses: global population structure and gene flow. *Virology J*. **7**: 220 (2010).
32. Raj, S.K., Snehi S.K., Khan M.S., Tiwari A.K. and Rao G.P., First report of Pepper leaf curl Bangladesh virus strain associated with bitter gourd (*Momordica charantia* L.) yellow mosaic disease in India. *Australas Plant Dis*. **5**: 14-16 (2010).
33. Raj, S.K., Snehi S.K., Khan M.S., Tiwari A.K. and Rao G.P., Molecular

identification of Ageratum enation virus associated with mosaic disease of pointed gourd (*Trichosanthes dioica* Roxb.) in India. *Phytoparasitica*. **39**: 497-502 (2011).

34. Rajinimala, N. and Rabindran R., First report of Indian cassava mosaic virus on bittergourd (*Momordica charantia*) inTamil Nadu, India. Australas. *Plant Dis Notes*. 2: 81-82 (2007).
35. Reddy, C.R.V., Colvin J., Muniyappa, V. and Seal S., Diversity and distribution of begomoviruses infecting tomato in India. *Arch Virol*. **150**: 845-867 (2005).
36. Roy, A., Spoorthi P., Panwar G., Bag M. K., Prasad T.V., Kumar G., Gangopadhyay K. K. and Dutta M., Molecular Evidence for Occurrence of Tomato leaf curl New Delhi virus in Ash Gourd (*Benincasa hispida*) Germplasm Showing a Severe Yellow Stunt Disease in India. *Indian J Virol*. **24(1):**74-77 (2013).
37. Sohrab, S.S., Mandal B., Pant R.P. and Varma A., First reports of association of Tomato leaf curl New Delhi virus with yellow mosaic disease of *Luffa cylindrica* in India. *Plant Dis*. **87**: 1148 (2003).
38. Sohrab, S.S., Mandal B., Ali A.and Varma A., Molecular diagnosis of emerging begomovirus diseases in cucurbits occurring in northern India. *Indian J Virol*. **17(2)**: 88-95 (2006).
39. Sohrab, S.S., Mandal B., Ali A., and Varma A., Chlorotic curly stunt: a severe begomovirus disease of bottle gourd in Northern India. *Indian J Virol*. **21**: 56-63 (2010).
40. Tamura, K., Stecher, G., Peterson D., Filipski A. and Kumar S., 2013.Molecular Evolutionary genetics analysis verison 6.0. *Mol Biol Evol*., **30**: 2725-2729.
41. Tiwari, A.K., Sharma, P.K, Khan M.S., Snehi S.K., Raj S.K. and Rao G.P., Molecular detection and identification of Tomato leaf curl New Delhi virus isolate causing yellow mosaic disease in Bitter gourd (*Momordica charantia*), a medicinally important plant in India. *Medicinal Plants*. **2(2)**: 117-123 (2010).
42. Tiwari, A.K., Snehi S.K., Singh R., Raj S.K., Rao G.P. and Sharma P.K., Molecular identification and genetic diversity among six Begomovirus isolates affecting cultivation of cucurbitaceous crops in Uttar Pradesh, India. *Arch Phytopathol Plant Prot.* **45(1)**: 62-72 (2011)
43. Varma, A. and Malathi V.G., Emerging geminivirus problems: a serious threat to crop production. *Ann Appl Biol* **142**: 145-164 (2003).
44. Venkataravanappa, V., Swarnalatha, P., Lakshminarayana Reddy, C.N., Neha Chauhan, Krishna Reddy, M., Association of recombinant Chilli leaf curl virus with enation leaf curl disease of tomato: a new host for chilli begomovirus in India. *Phytoparasitica* **44**: 213-223 (2016).
45. Venkataravanappa, V., Reddy C.N.L., Jalali S.and Krishna Reddy M., Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. *Virus Genes*. **44 (3)**: 522-535 (2012a).
46. Venkataravanappa, V., Prasannaa H.C., Lakshminarayana Reddy C.N. and Krishna Reddy M., Evidence for two predominant viral lineages, recombination and subpopulation structure in begomoviruses associated with yellow vein mosaic disease of okra in India. *Plant Pathol*. **64(3)**: 508-518 (2014).
47. Venkataravanappa, V., Swarnalatha P., Reddy C.N.L., Mahesh B., Rai A.B. and Krishna Reddy M., Molecular Evidence for Association of Tobacco Curly Shoot Virus and a Betasatellite with Curly Shoot Disease of Common Bean (Phaseolus vulgaris L.) from India. *J. Plant Pathol Microbiol*. **3**: 148 (2012b).
48. Venkatasubbaiah, K., Gopal D.V.R.S. and Sai Gopal D.V.R., Characterization and identification of a tobamovirus infecting snakegourd (*Trichosanthes anguina* Linn) in Andhra Pradesh. *Indian J Virol*. **13(2)**: 153-157 (1997).