Chlorotic Curly Stunt: A Severe Begomovirus Disease of Bottle Gourd in Northern India

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Abstract Bottle gourd (*Lagenaria siceraria*) an important vegetable crop in India was observed to be affected by a chlorotic curly stunt disease (CCSD) during 2003–2006 in the vegetable growing areas of Delhi and adjoining state of Haryana. The affected plants are severely stunted and bear very small chlorotic and mildly curled leaves. Incidence of the disease varied from 4.7 to 36%. The disease could be easily transmitted by whitefly, *Bemisia tabaci* but not by sap. The causal virus was found to be a *Begomovirus* on the basis of whitefly transmission and sequence identity of putative coat protein (CP) and replication initiator protein (Rep) genes. The virus was transmitted to *Cucumis sativus*, *Luffa acutangula*, *L. cylindrica*, *Lycopersicon esculentum*, *Nicotiana tabacum* and *Praecitrullus fistulosus* but not to *Citrullus lunatus*, *Cucumis melo*, *Cucurbita moschata* and *Vigna unguiculata*. The N-terminal 60 amino acids of CP of the virus had 100% sequence identity with all the isolates of *Tomato leaf curl New Delhi virus* (ToLCNDV) and two isolates of *Squash leaf curl China virus* (SLCCV). The full length amino acid sequence of the CP and Rep genes had 100% similarity with ToLCNDV-Svr and -Luffa isolates. The phylogenetic analysis showed that the virus associated with CCSD of bottle gourd belongs to ToLCNDV cluster of the begomoviruses. This is the first record of emergence of a *Begomovirus* associated severe disease in bottle gourd in India.

Keywords Bottle gourd · Chlorotic curly stunt · *Begomovirus* · *Tomato leaf curl New Delhi virus*

Introduction

Bottle gourd (*Lagenaria siceraria*), a popular cucurbitaceous vegetable is grown throughout India. In northern India, bottle gourd is cultivated during summer-rainy seasons under hot and humid conditions. Several viruses, like *Cucumber mosaic virus* [36] *Cucumber green mottle mosaic virus* [23] *Papaya ring spot virus-W* [19], *Watermelon mosaic virus* [1] and *Zucchini yellow mosaic virus* [38] are known to affect bottle gourd cultivation in India. During the last two decades, begomoviruses have emerged as a severe constraint in cultivation of cucurbits in India [35]. In the early sixties, yellow vein mosaic (YVM) disease of pumpkin (*Cucurbita moschata*) caused by a whitefly transmitted virus (apparently a begomovirus) was reported from central-western India. Since then, the diseases caused by begomoviruses have been reported in bitter gourd, chayote, cucumber, muskmelon, pumpkin and sponge gourd [17, 20, 22, 30, 34]. Many different begomoviruses infecting cucurbits have been reported from other countries such as *Squash leaf curl Yunnan virus* from China [39], *Melon chlorotic leaf curl virus* from Guatemala [3] *Chayote yellow mosaic virus* from Nigeria [33], ToLCNDV from Thailand [26, 27], *Watermelon chlorotic curly stunt virus* from Sudan and Iran [13], *Squash leaf curl Philippines virus* from the Philippines [14], *Cucurbit leaf curl virus* [2], *Cucurbit leaf crumple virus* [9], *Squash leaf curl virus* [8, 16] and *Squash mild leaf curl virus* [4] from USA, and *Luffa yellow mosaic virus* from Vietnam [24].

The genus *Begomovirus*, family *Geminiviridae*, consists of a group of viruses infecting dicot plants, which are exclusively transmitted by whitefly, *Bemisia tabaci* [15].

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The begomoviruses have either monopartite genome having one circular ssDNA component of about 2.7 kb designated as DNA A, or bipartite genomes having two similar size components designated as DNA A and DNA B. The DNA A genome contains four major genes encoding coat protein (CP), replication initiator protein (Rep), transcriptional transactivator protein and replication enhancer protein [11, 31, 32]. The DNA B contains two genes encoding proteins required for movement of virus, host range and pathogenicity [10, 28]. The CP gene of begomoviruses is highly conserved and the N-terminal sequence identity of CP has been found useful in identifying begomovirus isolates [21, 26].

During 2003–2006, while conducting surveys of begomovirus diseases in cucurbits in the major cucurbit growing areas of Delhi and Haryana, bottle gourd was observed to be affected by begomovirus like disease. The affected plants exhibited symptoms like chlorotic mottling, mild curling and serious stunting and the disease was designated as chlorotic curly stunt of bottle gourd. In the present study, we describe a new disease of bottle gourd from northern India. Based on the biological and molecular studies, a *Begomovirus* under ToLCNDV evolutionary cluster was identified to be associated with the disease.

Materials and Methods

Disease Survey and the Virus Isolate

Surveys were conducted during the cropping seasons of 2003–2005 in the major cucurbit growing areas of Delhi and Haryana states. The disease incidence was estimated by recording symptomatic and non-symptomatic plants in one square meter area, at ten different randomly selected spots of each field.

The virus in this study was isolated from a young leaf of a severely infected bottle gourd plant collected from a commercial field in Gannore (Panipat District, Haryana State) and maintained in bottle gourd through whitefly inoculation at regular intervals. The isolate was designated as bottle gourd-Gannore-1 (BotGn-1).

Electron Microscopy and Dot Blot Hybridization

Leaf samples of bottle gourd plants collected from various fields were examined in an electron microscope (Jeol 100×) by leaf dip method to detect virus particles [6]. For dot blot, DNA isolated from 100 mg of leaf tissue of field samples was dotted on nylon membrane and the blots were hybridized with the radio-labeled probe to CP gene of ToLCNDV-[Luffa] [30].

Inoculations by Whitefly and Sap

The test cv. Pusa Naveen plants were raised in four-inch plastic pots containing soil mixture. Single plant per pot was grown from seeds under insect free conditions in a greenhouse. The test plants were inoculated at one to two true leaf stage.

Aviruliferous stock of whitefly was cultured and maintained on egg plant (*Solanum melongena*). Adult whiteflies were allowed acquisition access period (AAP) of 24 h on diseased leaf. After the AAP, 5–10 whiteflies were transferred to each test plant covered with small plastic cages (10.0 cm × 6.5 cm), and allowed for inoculation access period (IAP) of 24 h. After inoculation, whiteflies were killed by spraying plants with systemic insecticide, Karate (0.01%). The inoculated plants were grown under insect-proof conditions in the greenhouse. The observations were taken up to 30 days post inoculation (dpi).

For sap inoculation, the inoculum was prepared by extracting symptomatic leaves in 0.1 M sodium phosphate buffer, pH 7.0 having 0.01% beta mercapto-ethanol and 0.2% sodium sulphite. The extract was gently applied with cotton swab on fully expanded leaves and cotyledons of the healthy test plants predusted with Carborundum powder (600 mesh). The inoculated plants were observed for symptom development as described above.

Polymerase Chain Reaction (PCR)

PCR was conducted using the CP primers AVF30: 5'TT GGATCCATGGCGA AGCCGACCA3', AVR31: 5'A AGAGCTCTTAATTTGTGACCGA3' and Rep primer AVF66:5'AAGAGCTCATGGCTTCGCCACGTCGTTT T3' and AVR67: 5'AAGGA TCCTCAACTCGCCTCCT GCGAAT3' designed from the putative CP (AY309957) and Rep (AY557219) genes of ToLCNDV-Luffa. The PCR reaction mixture consisted of 2.5 unit of Taq DNA polymerase (MBI Fermentas, USA), 5 µl of 10× PCR buffer and 1 µl of 10 mM dNTPs, 1 µl (100 ng) each of forward and reverse primers and template DNA of about 200 ng. The total reaction volume was made up of 50 µl using autoclaved distilled water. PCR amplification conditions were: hot start at 94°C for 2 min then 35 cycles of denaturation at 90°C for 1 min, annealing at 40°C for 30 s and extension at 72°C for 1 min. At the end of 35 cycles, the final extension was at 72°C for 10 min. The PCR products were analyzed in 1% agarose gel stained with ethidium bromide (0.5 µg/ml) and results were recorded by a gel documentation system.

Cloning of Putative CP and Rep Genes, Sequencing and Sequence Analysis

The amplified fragments of CP and Rep genes of BotGn-1 were purified from gel using QIAquick Gel Extraction Kit

(Qiagen, USA) and separately ligated in pDrive vector using PCR cloning kit (Qiagen, USA). The *E. coli* strain, DH5 α was transformed [25] and the positive clones were identified by colony PCR using specific primers.

Two recombinant clones, one each for CP and Rep genes were sequenced from both the directions in an automated DNA sequencer (ABI Prism, Perkin Elmer, at University of Delhi, South Campus, New Delhi). The sequence data were assembled and analyzed using the software programme, BioEdit (version 5.0.9). The following sequences were obtained from GenBank database and used for comparisons: *Chayote yellow mosaic virus* (ChaYMV: AJ223191), *Cucurbit leaf crumple virus* [California] (CuLCrV-[Cal]: AF224760), *Cucurbit leaf crumple virus* [Arizona] (CuLCrV-[AZ]: AF256200), *Cotton leaf curl Rajasthan virus* (CLCuRV: AF363011), *Luffa yellow mosaic virus* (LYMV: AF509739), *Melon chlorotic leaf curl virus* [Costa Rica] (MCLCuV-[CR]: AY064391), *Melon chlorotic leaf curl virus* [Guatemala] (MCLCuV-[GT]: AF325497), *Mungbean yellow mosaic India virus* [Mungbean] (MYMIV-[Mb]: AF416742), *Papaya leaf curl virus* (PaLCuV: NC_004147), *Squash leaf curl virus* (SLCV: NC_001936), *Squash leaf curl virus* [Cairo] (SLCV-[C]: DQ285019), *Squash leaf curl virus* [Israel] (SLCV-[I]: AY206998), *Squash leaf curl China virus* (SLCCNV: AB027465), *Squash leaf curl China virus* [B] (SLCCNV-[B]: AF509743), *Squash leaf curl China virus* [K] (SLCCNV-[K]: AF509741), *Squash leaf curl China virus* [Pumpkin: Coimbatore] (SLCCNV-[P:C]: AY184487), *Squash leaf curl China virus* [Pumpkin: Delhi] (SLCCNV-[P:D]: AY686500), *Squash leaf curl China virus* [Pumpkin: Lucknow] (SLCCNV-[P:L]: DQ026296), *Squash leaf curl China virus* [Philippine] (SLCCNV-Ph: AB085793), *Squash leaf curl Yunnan virus* [Yunnan 23] (SLCYNV-[Y23]: AJ420319), *Squash mild leaf curl virus* [Imperial valley] (SMLCV-[IV]: NC_004645), *Squash mild leaf curl virus* [R] (SMLCV-[R]: AF421552), *Tomato leaf curl Karnataka virus* (ToLCKV: AY738100), *Tomato leaf curl Bangalore virus* (ToLCBV: NC_003891), *Tomato leaf curl Bangalore virus* [Ban 4] (ToLCBV-[Ban 4]: AF165098), *Tomato leaf curl New Delhi virus* [Mild] (ToLCNDV-Mld: U15016), *Tomato leaf curl New Delhi virus* [Severe] (ToLCNDV-Svr: U15015), *Tomato leaf curl New Delhi virus* [Lucknow] (ToLC-V-[Luc]: Y16421), *Tomato leaf curl New Delhi virus* [AVT1] (ToLCNDV-[AVT1]: AY428769) and *Tomato leaf curl New Delhi virus* [Luffa] (ToLCNDV-[Luffa]: AJ557219), *Watermelon chlorotic curly stunt virus* (WmCSV: AJ012081), *Watermelon chlorotic curly stunt virus* [IR] (WmCSV-[IR]: AJ245652). The phylogenetic relationships among begomoviruses were analyzed comparing amino acids sequences using ClustalW algorithm of the DNASTAR programme, Version

5.01 (DNASTAR Inc., Madison, WI, USA) with the gap weight of 10 and gap length of 0.2.

Results

Field symptoms, Begomovirus Detection and Disease Incidence

In 2003, a severe disease of bottle gourd was observed for the first time in a farmer's field in Gannore, Haryana. Later, the disease was also observed in other areas of vegetable growing tracts of Delhi and Haryana. The affected plants develop a variety of symptoms characterized by chlorotic mottle, leaf smalling, leaf curling and acute stunting (Fig. 1a, b). The symptoms are most severe in plants infected at an early stage of growth. These plants develop small chlorotic leaves with upward curling and severe stunting symptoms. Such plants do not produce flower and fruit. The plants infected at a later stage develop chlorotic mottling and downward curling of the older leaves. The incidence of the disease in Haryana (Gannore and Baktawarpur) was 23.6–36% in 2003–2004, and in Delhi (Asmanpur and IARI) as 4.7–20% in 2005.

Field samples from five places in Delhi and Haryana that exhibited typical symptoms were found to contain geminate particles of 18×30 nm (Table 1; Fig. 1d). The plants, which showed yellow mosaic with or without blistering also, contained flexuous particles of about 750 nm (Table 1). No virus like particle was detected in plants showing only yellow patches or general yellowing. The begomovirus association with the disease was confirmed by dot blot hybridization with the radio-labeled probe to CP gene of ToLCNDV-Luffa and by PCR with the CP specific primers AVF30 and AVR31 (Fig. 2). In 2006, 16 out of 20 bottle diseased gourd plants tested from the experimental field at IARI showed presence of begomovirus in dot blot (Fig. 2a).

Biological Characterization of the Virus

Transmission

The virus isolated from the severely affected bottle gourd plants infected 50–60% bottle gourd test plants by whitefly inoculation. The infected plants developed characteristic symptoms within 7–10 days of inoculation. The first symptoms appeared in second or third true leaf, which showed chlorotic mottling and downward curling. The plants were severely stunted and produced very small new leaves with their margins curled upward (Fig. 2b). The symptoms on the test plants were remarkably similar to those observed in the field. The virus could not be

Fig. 1 Disease symptoms of chlorotic curly stunt of bottle gourd (*Lagenaria siceraria*). **a**, **b** Field symptoms. **c** Greenhouse symptoms following inoculation through whitefly, *Bemisia tabaci*. **d** Geminate particles in the diseased leaf of bottle gourd

Table 1 Detection of begomovirus in the field samples of bottle gourd (*Lagenaria siceraria*) plants collected during 2003–2005 from Haryana and Delhi

+: Positive result; θ : negative result; –: not tested

^a Determined by electron microscopy

^b Transmission by whitefly to bottle gourd

^c Dot blot hybridization with the probe to CP gene ToLCNDV

^d PCR amplification using AVF30 and AVR31 primers

State Place	Symptoms	Particle ^a observed	Transmission ^b	Dot blot ^c	PCR ^d
Haryana					
Gannore	Chlorotic mottle, smalling	Geminate	+	+	+
	Yellowing	No particle	–	θ	–
	Yellow mosaic	Geminate, filamentous	–	+	–
	Pale yellowing with curling	Geminate	+	+	–
Bakhtawarpur	Yellow mosaic, green blisters	Geminate, filamentous	–	+	+
Delhi					
Asmanpur	Yellow mottle, mild curling	Geminate	+	+	–
Gandhi Nagar	Mottling, margin rolling	Geminate	–	+	–
IARI	Yellowing, curling	Geminate	–	–	+
	Pale yellowing, smalling	Geminate	+	–	+
	Yellow mottle and curling	Geminate	–	+	+
	Yellow spots, no curling, smalling	No particle	–	–	θ
	Yellow mottle, marginal bending	Geminate	–	–	+

transmitted by sap inoculation through the extracts prepared from field infected plants as well as from the experimentally infected bottle gourd plants to bottle gourd and ridge gourd seedlings. The sap inoculated plants neither produced any symptoms nor tested positive in dot blot hybridization (data not shown).

Host Range

For determining the host range, the virus isolate was inoculated by whitefly to eleven plant species (Table 2). Of these, five plant species belonging to Cucurbitaceae [bottle gourd, cucumber (*Cucumis sativus*), ridge gourd (*Luffa*

Fig. 2 Molecular detection of the begomovirus in bottle gourd (*Lagenaria siceraria*) affected by chlorotic curly stunt disease. **a** Dot blots of DNA from field samples of bottle gourd hybridized with the radio-labeled probe to CP gene of ToLCNDV-[Luffa]. **b, c** Polymerase chain reaction showing amplification of putative coat protein (CP) gene (750 bp) in field samples (lanes: 1–4) of bottle gourd (**a**) and both CP and replication initiator protein gene (1000 bp) in greenhouse sample of BotGn-1 isolate (lanes 1 and 2: with CP primer and lane 3 with Rep primer) (**c**). M: marker, 1 kb ladder (MBI Fermentas); +ve: positive control; -ve: negative control

acutangula), sponge gourd (*L. cylindrica*) and round melon (*Praecitrullus fistulosus*), and two plant species belonging to Solanaceae [tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*)] developed symptoms at 7–11 dpi. The infection in these plant species was further confirmed by dot blot hybridization with probe to the putative CP gene of BotGn-1. Cucumber, ridge gourd, sponge gourd produced yellow spots followed by mosaic. Round melon

produced chlorotic spots and mottling symptoms on true leaves, which gradually wilted and withered. The symptom on round melon was most severe, as the infected plants died within 4 weeks of infection. Tobacco and tomato plants developed leaf curl symptoms.

Molecular Characterization of the Virus

PCR using CP and Rep genes primers of ToLCNDV resulted in amplification of about 750 and 1000 bp fragments, respectively (Fig. 2). These amplicons were cloned and sequence. The CP consisted of 771 nucleotides (nt) (GenBank accession DQ272540), and the Rep 1081 nt (GenBank accession DQ272539). The BLAST search in GenBank showed closest identity of these sequences with that of ToLCNDV and SLCCNV isolates reported from India (Table 3). The amino acid sequence of the complete CP shared 98.4–100% identity with the various isolates of ToLCNDV and SLCCNV. The N-terminal sequence of CP (first 60 amino acids) was identical with SLCCNV-[P:D] and SLCCNV-[P:L] and with all the isolates of ToLCNDV (Table 3). The Rep was identical with the ToLCND-[Luf] and shared 91.3–97.2% identity with the remaining four other isolates of ToLCNDV (Table 3). The SLCCNV-[P:C], -[P:D] and -[P:L] although were near identical in CP had only 87.5–88.3% identity in Rep. The cucurbit infecting begomoviruses, ChaYMV, CuLCrV, MLCuV, SLCV, SMLCV and WmCSV, which are not known to occur in India shared only 72.4–77.4% and 51.0–72.8% identity with the BotGn-1 isolate in complete CP and Rep sequences, respectively. The analysis of phylogenetic relationship based on N-terminal 60 aa (Fig. 3a) and complete Rep (Fig. 3b) showed that the BotGn-1 belonged to the ToLCNDV cluster.

Table 2 Host range of the begomovirus (BotGn-1 isolate) associated with chlorotic curly stunt disease of bottle gourd (*Lagenaria siceraria*)

Plant species inoculated ^a	No. of infected plants out of five inoculated	Symptoms at dpi	Symptoms	Dot blot
<i>Citrullus lunatus</i>	0	—	No symptoms	θ
<i>Cucumis melo</i>	0	—	No symptoms	θ
<i>Cucumis sativus</i>	1	10	Yellow dots	+
<i>Cucurbita moschata</i>	0	—	No symptoms	θ
<i>Lagenaria siceraria</i>	3	7–9	Chlorotic mottling, leaf curling and stunting	+
<i>Luffa cylindrica</i>	3	7	Yellow dots	+
<i>Lycopersicon esculentum</i>	4	7–10	Leaf curling	+
	2	11	Leaf curling	+
<i>Nicotiana tabacum</i>				
<i>Praecitrullus fistulosus</i>	2	9	Yellow spots, mottling and withering	+
<i>Vigna unguiculata</i>	0	—	No symptoms	θ

+: Positive, θ: negative hybridization, dpi: days post inoculation

^a *Bemisia tabaci* (5–10 number per plant) were used with 24 h each of acquisition and inoculation access periods for inoculation of the test plant species

Table 3 Per cent identity of amino acids sequences of putative coat protein (CP) and replication initiator protein (Rep) of the begomovirus associated with the chlorotic curly stunt disease of bottle gourd (BotGn-1 isolate) with that of the selected begomoviruses

Virus	CP		Rep
	FAA	NAA	FAA
ChaYMV	74.3	70.0	72.8
CLCuRV	91.8	81.7	75.6
CuLCrV-[AZ]	74.7	41.7	52.1
CuLCrV-[CF]	74.7	40.0	52.1
LYMV	96.5	93.3	72.8
MCLCuV-[CR]	72.5	41.7	49.0
MCLCuV-[GT]	75.5	41.7	51.0
MYMIV-[Mg]	74.8	25.0	70.6
PaLCuV	91.4	86.7	66.9
SLCCNV	94.9	91.7	79.4
SLCCNV-[B]	95.3	91.7	88.2
SLCCNV-[K]	96.1	91.7	87.5
SLCCNV-[P:C]	98.8	98.3	87.8
SLCCNV-[P:D]	98.4	100	88.3
SLCCNV-[P:L]	99.2	100	88.3
SLCCNV-Ph	94.9	91.7	81.3
SLCV	74.5	43.3	51.7
SLCV-[C]	73.7	41.7	52.6
SLCV-[I]	74.5	45.0	—
SLCYNV-[Y32]	77.0	73.3	67.8
SMLCV-[IV]	74.9	41.7	51.6
SMLCV-R	74.9	41.7	51.6
ToLCBV	86.3	65.0	76.1
ToLCBV-[Ban4]	85.2	65.0	76.9
ToLCKV	94.1	90.0	78.1
ToLCNDV-[Luc]	98.0	100	93.1
ToLCNDV-[Luf]	99.2	100	100
ToLCNDV-Mld	99.2	100	94.4
ToLCNDV-Svr	100	100	97.2
ToLCNDV-[AVT1]	99.2	100	91.3
WmCSV	75.5	70.0	68.1
WmCSV-[IR]	77.4	68.3	67.5

FAA full length amino acids sequence, NAA N-terminal 60 amino acids sequence, —: sequence not available

Discussion

This paper reports a new disease of bottle gourd from northern India. The disease is designated as bottle gourd chlorotic curly stunt due to the characteristic symptoms, chlorotic mottling, mild curling and excessive reduction in leaf size, and stunting of plants. The disease is highly destructive when plants are infected at early stage of growth, as the affected plants become unproductive and die prematurely.

Fig. 3 Phylogenetic relationship of the begomovirus associated with the bottle gourd chlorotic curly stunt disease (BotGn-1 isolate) with selected begomoviruses based on **a** N-terminal 60 amino acids sequence of putative coat protein and **b** complete amino acids sequence of putative replication initiator protein. Trees were constructed using the ClustalW algorithm of the DNASTAR with the gap weight of 10 and gap length of 0.2

Symptoms of the disease are similar to watermelon leaf curl disease described from Pakistan [18] and watermelon chlorotic curly stunt disease described from Iran and Sudan [5, 13] which also result in mottling, curling of leaves and stunting of plants. The virus associated with the CCS disease of bottle gourd did not infect watermelon by whitefly inoculation, indicating differences between the viruses or

their strains causing these diseases. Whether the viruses associated with the watermelon diseases in Pakistan, Iran and Sudan infect bottle gourd or not is not known. The YVM disease of pumpkin reported from southern India was shown to induce only mild mosaic symptoms on bottle gourd and no symptoms on tomato following whitefly inoculation [20]. The CCS of bottle gourd differs from the YVM disease of pumpkin as it does not induce any symptoms in pumpkin and induces leaf curl symptoms in tomato.

The analysis of putative CP and Rep sequences of the virus showed very close identities to ToLCNDV strains/isolates reported from northern India. The CP and Rep were 100% identical with ToLCND-Svr and ToLCNDV-[Luf], respectively. The phylogenetic analysis based on CP and Rep showed the closest relationship with ToLCNDV cluster. The current convention of *Begomovirus* species identification is based on less than 89% nucleotide sequence identity of DNA A, transmission and host range properties [7]. However, the N-terminal region of 60–70 aa of CP of begomoviruses is a close representation of the genome and the sequence of this region is shown to be sufficient to identify a virus isolate [21]. In the present study, the N-terminal region of CP of BotGn-1 was 100% identical with all the isolates of ToLCNDV and two isolates of SLCCNV occurring in northern India. However, biological properties indicated that BotGn-1 is closer to ToLCNDV as it induces leaf curl in tomato and does not infect pumpkin. On the basis of biological and molecular characterization, we conclude that the virus associated with CCS of bottle gourd is a strain of ToLCNDV. ToLCNDV is emerging as a serious constraint in the production of cucurbits in India as it has been shown to cause severe diseases of bitter gourd [12], chayote [17], cucumber [22], winter squash [29], sponge gourd [30] and bottle gourd found in the present study.

Begomoviruses are major threat in numerous crops in India due to favorable environmental conditions and abundance of vector, *B. tabaci* [35]. Leaf curl of tomato was known to occur in India for nearly 60 years [37], but whitefly transmitted diseases of cucurbits were not serious concern until 1980s [34]. In recent years, however cucurbit-infecting begomoviruses [12, 17, 20, 29, 30] have emerged as a serious threat to cucurbit cultivation, particularly in northern India. As ToLCNDV, which seems to be the major cause of these diseases in northern India has been known for a long time to cause diseases in tomato and luffa but it did not move to the other cucurbits, which are traditionally grown in the summer months.

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