VirusDisease (July–September 2023) 34(3):421–430
https://doi.org/10.1007/s13337-023-00837-8

ORIGINAL ARTICLE

Geographical distribution of tomato-infecting begomoviruses in major cucurbits in India: a diagnostic analysis using begomovirus species specific PCR

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Received: 20 July 2023 / Accepted: 21 August 2023 / Published online: 8 September 2023

Abstract
Cucurbits are an essential summer-season vegetable crops, but they are highly vulnerable from a range of abiotic and biotic factors. One of the significant biotic factors posing a growing menace to the production of major cucurbits in India is the emergence of tomato-infecting begomoviruses. In this study, we utilized PCR-based species-specific primers, developed earlier in our laboratory for the detection of begomoviruses infecting tomato and chilli plants, to identify begomoviruses in cucurbits across various regions of India. Leaf samples from major cucurbits were collected from different regions of Haryana, Delhi, Uttar Pradesh, Chhattisgarh, Maharashtra, Telangana and Karnataka, during the year 2020–2021. Total nucleic acid (TNA) was extracted from the samples and subjected to PCR using a generic primer specific to begomoviruses. The samples that exhibited positive amplification were further tested using six different species-specific primers targeting specific begomovirus species, namely *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Palampur virus* (ToLCPalV), *Tomato leaf curl Bangalore virus* (ToLCBV), *Tomato leaf curl Joydebpur virus* (ToLCJoV), *Tomato leaf curl Gujarat virus* (ToLCGuV), and *Chilli leaf curl virus* (ChiLCV). The PCR analysis revealed that among the 551 plant samples tested, a total of 124 samples exhibited positive amplification using the universal begomovirus PCR. Specifically, 47 samples tested positive for ToLCNDV, 73 samples were positive for ToLCPalV and only one sample showed positive amplification for ChiLCV. However, none of the samples tested positive for ToLCJoV, ToLCGuV and ToLCBV. These findings from our study indicate the prevalence of ToLCNDV and ToLCPalV in major cucurbits across India. Furthermore, the study highlights the varied distribution of begomoviruses in major cucurbits between northern and southern regions of India.

Keywords Cucurbits · Begomovirus · PCR · ToLCNDV · ToLCPalV · ToLCJoV · ToLCGuV · ToLCBV · ChiLCV

Introduction

Cucurbits, a significant group of vegetable crops belonging to the *Cucurbitaceae* family, consist of 118 genera and 1000 species [10]. They contribute to approximately 50% of global vegetable production, including India, and are extensively cultivated throughout the country. Cucurbits are well known for being rich sources of vitamins, minerals, and medicinal properties. They form the largest category of summer-season vegetables grown in India. Important cucurbits cultivated in India include cucumber (*Cucumis sativus*), bottle gourd (*Lagenaria siceraria*), muskmelon (*Cucumis melo*), watermelon (*Citrullus lanatus*), bitter gourd (*Momordica charantia*), ridge gourd (*Luffa acutangula*), sponge gourd (*Luffa cylindrica*), pumpkin (*Cucurbita moschata*), Chayote (*Sechium edule*), ivy gourd (*Coccinia cordifolia*), pointed gourd (*Trichosanthes dioica*), ash gourd (*Benincasa hispida*), round melon (*Praecitrullus fistulosuos*), long melon (*Cucumis melo var. utilissimus*), snapmelon (*Cucumis melo var. momordica*), summer squash (*Cucurbita pepo*), sweet gourd (*Momordica cochinchinensis*), and winter squash (*Cucurbita maxima*). Among these cultivated cucurbits, cucumber, bottle gourd, sponge gourd, ridge gourd, bitter gourd, pumpkin, chayote, muskmelon, and watermelon are the most economically important in terms of production [3].

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India documented virus diseases in cucurbits in the late 1930s. The first cucurbit-infecting virus to be reported in India was *Cucumber green mottle mosaic virus,* which belongs to the genus, *Tobamovirus*. This virus caused a mild green mottle disease in bottle gourd and was first reported in India by Capoor and Varma [8]. In the 1950s, a disease called yellow vein mosaic was noticed in pumpkins in Pune, Maharashtra. The transmission of this disease was attributed to the whitefly species *Bemisia tabaci*, and it was named pumpkin yellow vein mosaic virus (PYVMV) [55]. It is important to note that during this time, the existence of geminiviruses or begomoviruses was unknown. However, subsequent research led to the identification of PYVMV as the first confirmed begomovirus to infect cucurbits in India [29]. Between 1960 and 1977, three additional RNA viruses, *Cucumber mosaic virus* (CMV), *Papaya ring spot virus* (PRSV), and *Watermelon mosaic virus* (WMV), causing mosaic diseases in various cucurbits including ash gourd, bottle gourd, chayote, cucumber, long melon, muskmelon, pointed gourd, pumpkin, snake gourd, sponge gourd, summer squash, and watermelon were identified [1, 4, 5, 12, 27, 28, 38–40, 43]. In the 1990s, a new and highly destructive viral disease called bud necrosis emerged in watermelon. This disease was identified as *Watermelon bud necrosis virus* (WBNV) and was determined to be a *Tosspovirus* [15]. Additionally, in 2004, a potyvirus known as *Zucchini yellow mosaic virus* (ZYMV) was identified in bottle gourds [56]. Numerous viruses from 14 genera are known to affect cucurbit production worldwide. The members of the viruses belonging to *Begomovirus, Crinivirus, Cucumovirus, Potyvirus,* and *Tospovirus* genera have a significant negative impact on cucurbits production in different regions globally. However, in India, begomoviruses pose a major threat to a wide range of cucurbit crops [51].

Begomoviruses, which belong to the family *Geminiviridae,* are characterized by their twin-quasiisometric virions measuring approximately 20 × 30 nm. These viruses are specifically spread by the whitefly species *Bemisia tabaci* through a persistent mode of transmission. From 1980 onwards, diseases caused by begomoviruses gained notable prominence across diverse cucurbit crops. Particularly within the Indian context, begomovirus species have exerted a considerable influence on cucurbit cultivation. In India, so far, a total of eight species of begomovirus have been recognized to affect cucurbits namely, *Mesta yellow vein mosaic virus* (MeYVMV) [16], *Pumpkin yellow vein mosaic virus* (PYVMV) [29], *Tomato leaf curl New Delhi virus* (ToLCNDV) [25, 26, 47], *Indian cassava mosaic virus* (ICMV) [37], *Squash leaf curl China virus* (SLCCNV) [25, 26, 45], *Pepper leaf curl Bangladesh virus* (PepLCBV) [35], *Ageratum enation virus* (AEV) [36] and *Tomato leaf curl Palampur virus* (ToLCPaV) [31]. In recent times, begomovirus infection has emerged as a significant challenge for the commercial production of cucurbits in India. Although there are reports that suggested the occurrence of begomoviruses in cucurbits in India, a comprehensive study examining the diversity and distribution of begomoviruses, infecting cucurbits in India is lacking. Therefore, conducting a thorough investigation into the diversity of begomovirus occurrence in cucurbits across various regions of India is crucial for the effective management of the virus and to prevent it from negatively impacting production. Our laboratory had previously developed half a dozen of species-specific primers for the detection of begomoviruses infecting tomato. In this study, we conducted a comprehensive PCR-based detection using these specific primers on diverse samples of cucurbits collected from various regions across India. Subsequently, the distribution of begomoviruses in cucurbits across different parts of the country was mapped.

Materials and methods

Survey and sample collection

In order to obtain a representative cucurbit samples across different regions of India, a total of 551 leaf samples were collected from both symptomatic and asymptomatic plants belonging to eight different cucurbit species, namely cucumber, muskmelon, bottle gourd, bitter gourd, sponge gourd, ridge gourd, pumpkin and chayote. To avoid biases, these samples were randomly collected from farmers' fields across 12 locations in seven states of India, during 2020–2021 (Table 1). Specifically, 255 leaf samples were collected from five cucurbit species in three locations in northern India. In central India, 121 leaf samples were collected from five cucurbit species at two different locations. Additionally, in southern India, 175 leaf samples were collected from six cucurbit species across seven locations. The disease incidence in the fields was determined by examining the plants visually, and based on the presence of symptoms, the percent disease incidence was calculated.

Isolation of total nucleic acid from the cucurbit leaf samples

The DNA extraction was carried out using the cetyltrimethylammonium bromide (CTAB) method, following the protocol described by Doyle and Doyle [11]. From each of the collected plant samples, approximately 100 mg of leaf tissue was homogenized and incubated in a CTAB-based extraction buffer to release the DNA. Subsequently, phenol–chloroform-isoamyl alcohol extraction and ethanol precipitation steps were performed to purify the DNA. The concentration and quality of the purified DNAs were assessed using a NanoDrop spectrophotometer (Nabi-Microdigital, Genetix

Table 1 List of states and locations from where cucurbits samples were collected

Sl/No	State	Location	Crop	No. of samples	PDI (%)*	Date of collection
1	Haryana	Karnal	Cucumber	15	30–60	24/04/2021
			Bottle gourd	15	35–70	
			Bitter gourd	15	20–50	
2	Delhi	IARI, Pusa	Cucumber	45	40–80	15/10/2020
			Bottle gourd	30	35–80	18/11/2020
			Sponge gourd	20	40–70	26/10/2020
			Bitter gourd	15	30–70	06/03/2021
			Muskmelon	65	30–80	08/09/2020
3	Uttar Pradesh	Varanasi	Bitter gourd	20	20–60	12/05/2021
			Cucumber	15	30–60	
4	Chhattisgarh	Raipur	Cucumber	25	30–70	17/11/2020
			Bottle gourd	20	40–80	
			Ridge gourd	25	30–60	
			Pumpkin	21	20–50	
5	Maharashtra	Pune	Bitter gourd	15	30–50	12/06/2021
			Bottle gourd	15	20–40	
6	Telangana	Hyderabad	Sponge gourd	15	40–60	
			Bottle gourd	30	30–50	07/02/2021
		Dindigoul	Bitter gourd	15	20–40	
7	Karnataka	Arasikere	Cucumber	20	30–60	20/05/2020
		Bengaluru	Ridge gourd	10	30–60	08/10/2022
		Channarayapatna	Bitter gourd	15	30–70	11/05/2020
			Chayote	15	30–80	
		Kolar	Bitter gourd	10	30–60	15/06/2021
		Mandya	Cucumber	15	30–50	5/07/2020
			Bitter gourd	15	20–60	
			Chayote	15	30–70	
			Total	551		

*PDI: percent disease incidence

Biotech, India). These ensured the availability of sufficient and quality DNA for further PCR analyses.

Detection of begomovirus through PCR

The isolated DNA obtained from the cucurbit leaf samples was used as templates for the detection of begomovirus infection through PCR amplification using a generic begomovirus primer pair, BM783F/BM784R [21]. The PCR was carried in a 25 µl reaction volume containing 2.5 µl of 10X buffer, 1 µl of template DNA, 0.5 µl each of forward and reverse primer, 0.5 µl of dNTPs, 0.125 µl of *Taq* polymerase, and 19.875 µl of nuclease-free water. After mixing, the reaction was performed using a thermal cycler (Applied Biosystems, Singapore). The amplification process involved a hot start step, where the reaction mixture was initially subjected to a temperature of 95 °C for 3 min to activate the DNA polymerase enzyme. Subsequently, 35 cycles were performed, each consisting of denaturation at 95 °C for 30 s to separate the DNA strands, followed by annealing at 58 °C for 45 s to allow the primers to bind to their complementary target sequences. The extension step was carried out at 72 °C for 45 s, during which the DNA polymerase enzyme synthesized new DNA strands. Finally, a final extension step at 72 °C for 10 min was performed to ensure the complete extension of any remaining partial DNA strands.

Species-specific PCR for specific detection of begomovirus species

The cucurbit samples that exhibited positive amplification in the generic PCR were subjected to further analysis for the specific detection of begomovirus species. This specific detection was accomplished using species-specific PCR, employing six different tomato-infecting begomoviruses specific primers. Details of the primers used in the study were given in Table 2 [20]. The PCR reactions for the specific detection of begomovirus species were performed using a thermocycler (Applied Biosystems, Singapore). The PCR amplification was carried out with the following program:

Table 2 Detailed list of primers used in PCR detection of begomoviruses

Sl.No	Primer specific for-	Primer name	Primer sequence* (5'-3')	Primer location	Annealing temperature (℃)	Amplicon size (bp)
1	*Begomovirus*	BM783F	CCCCTGTGCGTGAATCCGT	AC1	58	538
		BM784R	SDVTBCMGCTGCGCGGCC			
2	*Tomato leaf curl New Delhi virus*	BM794F	CCTTGTAAGGTGCAGTCC	AV1	56	702
	(ToLCNDV)	BM795R	AACCCAGGTCCTTAAGTACC			
3	*Tomato leaf curl Banglore virus*	BM796F	CACGGATTCAGGTGTATGCTT	AV2	62	215
	(ToLCBV)	BM797R	ACAGCAGCACGCTTGCTG			
4	*Tomato leaf curl Palampur virus*	BM798F	AGACTTGCTCACCAAGC	AV2/AV1	56	647
	(ToLCPalV)	BM799R	GAAATCTTGTGGCGCAC			
5	*Tomato leaf curl Gujrat virus*	BM800F	GAAGCGACCAGCAGATATGC	AV2/AV1	60	396
	(ToLCGuV)	BM801R	GTGTTGGTGTGGTTCTTCAC			
6	*Tomato leaf curl Joydebpur virus*	BM802F	AGAATGTGCATCGTGACAGG	AV1	62	732
	(ToLCJoV)	BM803R	TCGTCGCGTTGACCTGGAC			
7	*Chilli leaf curl virus (ChiLCV)*	BM861F	GAGTCTAGACACGATGTA	AV1	56	500
		BM862R	CATCAGAGCATTCTCACT			

*S = G/C; D = A/T/G; V = A/C/G; B = C/G/T; M = A/C

hot start at 95 °C for 3 min, followed by subsequent 35 cycles of denaturation at 95 °C for 30 s, annealing at different temperatures for different primers as mentioned in Table 2 for 45 s, extension at 72 °C for 45 s. The final extension at 72 °C was allowed for 10 min.

Results

Disease incidence in cucurbits and begomovirus detection

In the survey and sample collection during the year 2020–2021, a range of begomovirus-like disease symptoms i.e., yellow mosaic, leaf curling, puckering, and stunting were observed in eight different cucurbit species (Fig. 1) and the disease incidence was estimated in cucumber (30–80%), muskmelon (30–80%), bottle gourd (20–80%), bitter gourd (20–70%), sponge gourd (40–70%), ridge gourd (30–60%), pumpkin (20–50%) and chayote (30–80%) (Table 1). Through PCR test using the generic primer for begomovirus, it was observed that out of the 551 cucurbits samples collected, 124 (approximately 23%) samples were confirmed to be associated with begomovirus (presence of expected amplification at 538 bp), indicating valuable insights into the occurrence of the viral infection in the samples (Table 3). 32% of total, 255 cucurbit samples from northern India, 17% of total, 121 cucurbit samples from central India, and 5% of total, 175 cucurbit samples from southern India were detected positive for begomovirus. In seven out of 8 species of cucurbit the virus was detected and the total number of samples found positive for begomovirus in each species includes cucumber (42), bottle gourd (33), muskmelon (24), ridge gourd (10), sponge gourd (9), chayote (5) and bitter gourd (1) (Table 3). All the 21 pumpkin samples were found negative for the presence of begomovirus.

Species-specific PCR detection of begomoviruses

Through PCR analysis using species-specific primers, we detected the presence of different begomoviruses in 124 positive cucurbit samples identified in the generic PCR. Specifically, out of the seven cucurbit species tested (cucumber, bottle gourd, ridge gourd, chayote, sponge gourd, muskmelon, and bitter gourd), 38% of total, samples showed positive amplification for ToLCNDV (amplicon size 702 bp). These positive samples included cucumber (19), bottle gourd (9), ridge gourd (7), chayote (5), sponge gourd (4), muskmelon (2), and bitter gourd (1) (Table 3). Additionally, 59% of total, samples from cucumber (26), bottle gourd (22), muskmelon (17), and sponge gourd (8) exhibited positive amplification for ToLCPalV (amplicon size 647 bp). We found a single cucumber sample that tested positive for ChiLCV (amplicon size 500 bp). Moreover, 10 samples from cucumber (5), sponge gourd (3), and muskmelon (2) showed a mixed infection of ToLCNDV and ToLCPalV. However, none of the samples exhibited positive reactions for ToLCJoV, ToLCGuV, and ToLCBV. Furthermore, the association of β satellite could not be found in any of the begomovirus-positive samples (Table 3).

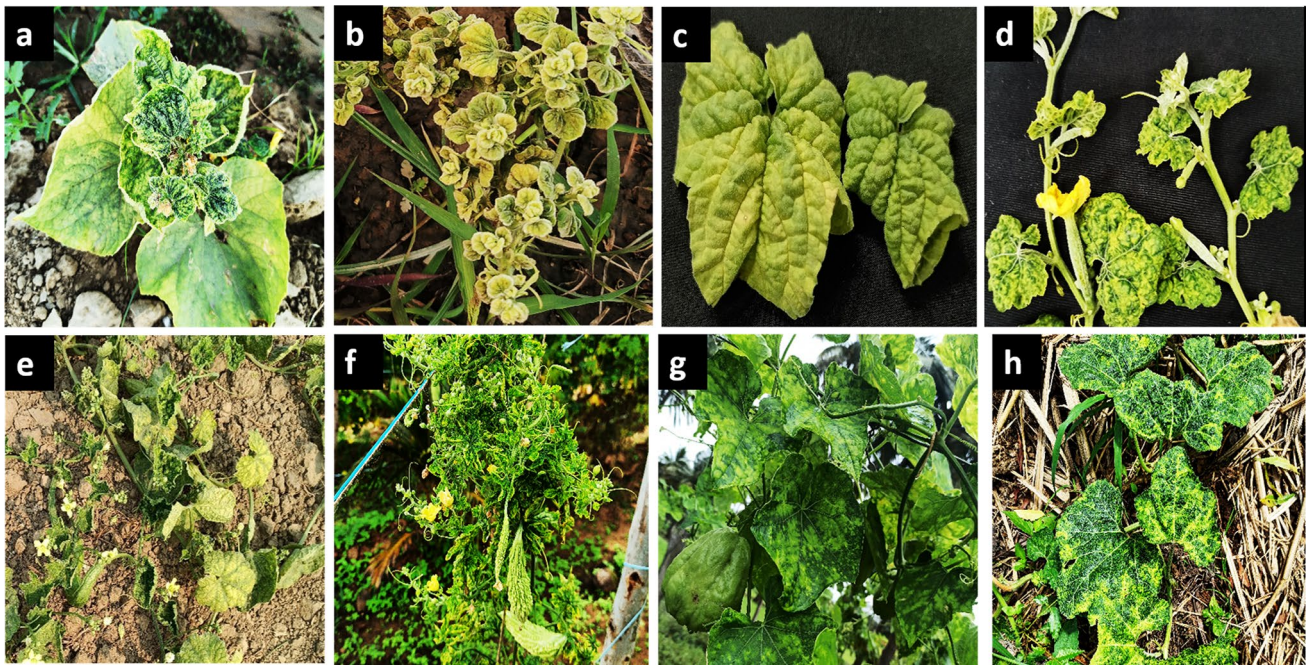

Fig. 1 Begomovirus disease symptoms observed in eight different cucurbits species across 12 locations from Haryana, Delhi, Uttar Pradesh, Chhattisgarh, Maharashtra, Telangana and Karnataka during sample collection; **a:** mosaic, leaf surl and stunting in cucumber; **b:** crinkle leaves, leaf curl and stunting in bottle gourd; **c:** yellow mosaic and leaf puckering in muskmelon; **d:** mosaic, leaf puckering and leaf curl in sponge gourd; **e:** leaf curl, puckering and fruit deformation in ridge gourd; **f:** leaf curl, mosaic and deformed fruit in bitter gourd; **g:** mosaic and fruit deformation in chayote and **h:** leaf puckering and yellow mosaic in pumpkin

Diversity and distribution of cucurbit-infecting begomoviruses

Through specific detection using begomovirus species-specific primers, we identified the presence of ToLCNDV in 38% cucurbit samples and ToLCPalV in 59% cucurbit samples. Among the six tomato-infecting begomoviruses, ToLCPalV and ToLCNDV were found to be the predominant species infecting major cucurbits in India. The diversified PCR detection of begomoviruses in eight major cucurbits revealed that ToLCNDV is present in northern, central and southern India, while ToLCPalV is predominantly limited to northern India (Fig. 2). In northern India, out of the 47 cucurbit samples that tested positive for ToLCNDV, 12% were cucumber, 8% were sponge gourd, and 4% were muskmelon. Central India exhibited 19% positive cases in cucumbers, 19% in bottle gourds, and 15% in ridge gourds out of the 47 samples tested. In southern India, ToLCNDV was detected in 10% of chayote samples, 8% of cucumber samples, and 2% of bitter gourd samples among the 47 tested cucurbits. From northern India, total of the 73 cucurbit samples tested positive for ToLCPalV, 36% were cucumbers, 30% were bottle gourds, 23% were muskmelon and 11% were sponge gourd samples (Table 3).

Discussion

Cucurbits, comprising a diverse group of vegetable crops, hold substantial economic importance due to their extensive cultivation throughout India. However, the increasing incidence of begomovirus-related diseases in cucurbit crops presents a growing concern, as highlighted in the previous studies [46, 51]. Begomoviruses have turned into a major obstacle to cucurbit production in India since the beginning of the 1980s [51]. Since that time, researchers have identified begomovirus-related diseases in a diverse range of cucurbit crops, including cucumber, muskmelon, bitter gourd, sponge gourd, ridge gourd, ivy gourd, chayote, pumpkin and bottle gourd. The detection of begomoviruses has been accomplished through nucleic acid hybridization tests or by PCR amplifications utilizing primers specific to the putative coat protein (CP) gene [16, 19, 24, 29–36, 37, 45–47, 49, 50, 53, 54]. This expanded understanding of begomovirus infections underscores their presence in various cucurbit species, highlighting the need for continued vigilance in disease management and prevention. Many begomoviruses infecting cucurbitaceous crops have been documented in various countries as well. For instance, *Melon chlorotic leaf curl virus* has been reported in Guatemala [6], while *Squash leaf curl Yunnan virus*

Table 3 PCR detection of begomoviruses in major cucurbits in India using species-specific primers

Sl/No	State	Location	Crop	No. of samples	Unibegomo	ToLCNDV	ToLCPalV	ToLCJoV	ToLCGuV	ToLCBV	ChiLCV	β satellite
1	Haryana	Karnal	Cucumber	15	5	–	5	–	–	–	1	–
			Bottle gourd	15	3	–	3	–	–	–	–	–
			Bitter gourd	15	–	–	–	–	–	–	–	–
2	Delhi	IARI, Pusa Campus	Cucumber	45	18	6	16	–	–	–	–	–
			Bottle gourd	30	19	–	19	–	–	–	–	–
			Sponge gourd	20	9	4	8	–	–	–	–	–
			Bitter gourd	15	–	–	–	–	–	–	–	–
			Muskmelon	65	24	2	17	–	–	–	–	–
3	Uttar Pradesh	Varanasi	Bitter gourd	20	–	–	–	–	–	–	–	–
			Cucumber	15	5	–	5	–	–	–	–	–
4	Chhattisgarh	Raipur	Cucumber	25	10	9	–	–	–	–	–	–
			Bottle gourd	20	11	9	–	–	–	–	–	–
			Ridge gourd	25	10	7	–	–	–	–	–	–
			Pumpkin	21	–	–	–	–	–	–	–	–
5	Maharashtra	Pune	Bitter gourd	15	–	–	–	–	–	–	–	–
			Bottle gourd	15	–	–	–	–	–	–	–	–
6	Telangana	Hyderabad	Sponge gourd	15	–	–	–	–	–	–	–	–
			Bottle gourd	30	–	–	–	–	–	–	–	–
		Dindigoul	Bitter gourd	15	1	1	–	–	–	–	–	–
7	Karnataka	Arasikere	Cucumber	20	1	1	–	–	–	–	–	–
		Bengaluru	Ridge gourd	10	–	–	–	–	–	–	–	–
		Channarayapatna	Bitter gourd	15	–	–	–	–	–	–	–	–
			Chayote	15	2	2	–	–	–	–	–	–
		Kolar	Bitter gourd	10	–	–	–	–	–	–	–	–
		Mandya	Cucumber	15	3	3	–	–	–	–	–	–
			Bitter gourd	15	–	–	–	–	–	–	–	–
			Chayote	15	3	3	–	–	–	–	–	–
			Total	551	124	47	73	–	–	–	1	–

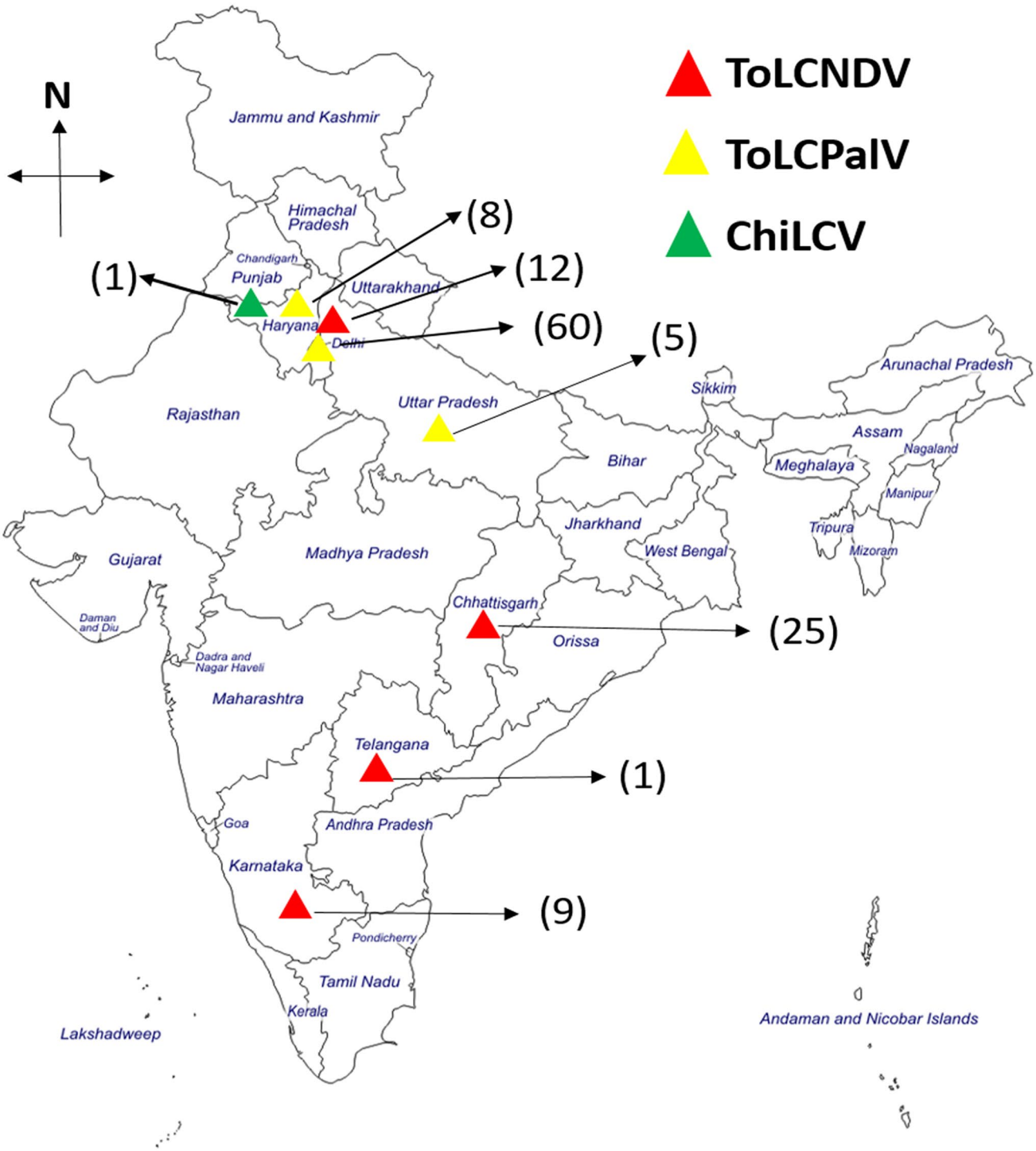

Fig. 2 Outline map of India showing cucurbits-infecting begomoviruses distribution in various parts of India and the respective number of PCR positive cucurbit samples for particular species of begomovirus in the represented state; tomato leaf curl New Delhi virus (ToLCNDV) is distributed in northern, central and southern India and tomato leaf curl Palampur virus & chilli leaf curl virus are distributed and restricted only in northern India

was identified in China [57]. *Watermelon chlorotic stunt virus* was found in Sudan and Iran [17], while *Tomato leaf curl New Delhi virus*-[Luf] reported in Thailand [42]. *Squash leaf curl Philippines virus* has been documented in the Philippines [18], while the USA has reported several begomoviruses, including *Cucurbit leaf curl virus*,

Cucurbit leaf crumple virus, *Squash leaf curl virus* [14, 22], and *Squash mild leaf curl virus* [7]. In Vietnam, *Luffa yellow mosaic virus* was identified [41] and *Squash yellow mottle virus* and *Pepper golden mosaic virus* were reported in Nicaragua [2]. These reports emphasize the global distribution and diversity of begomoviruses affecting cucurbit crops.

For the detection of begomoviruses, PCR has been regarded as the quickest and most precise technique [25, 26, 51]. PCR-based diagnosis using generic primers has been successfully utilized for determining the occurrence and distribution of begomoviruses in tomato, pepper, and cucurbits [9, 21] and by employing CP gene-based primers, begomoviruses were successfully detected by PCR in various cucurbits [23, 45, 46, 48, 49]. In the present study, we vastly demonstrated PCR-based diagnostics in the detection of begomoviruses in the field-collected cucurbit samples from various locations from 7 different states covering northern, central, and southern India using both generic and species-specific primers that were previously developed in our laboratory. Through PCR assay using the generic primer for begomovirus, we were able to observe that out of 551 cucurbit samples tested, 124 samples (approximately 23%) were found to be infected with the virus. This initial screening played a crucial role in narrowing down the number of samples to be further tested with species-specific PCR. Our PCR diagnostic systems successfully detected the presence of ToLCNDV (38%) and ToLCPalV (59%) in major cucurbit crops in India, while ToLCJoV, ToLCGuV, ToLCBV and beta satellite were not detected in any of the samples. The species-specific PCR approach allowed for the identification and differentiation of begomovirus species in the cucurbit samples, providing valuable insights into their diversity and prevalence. ToLCNDV and ToLCPalV were identified as the dominant begomovirus species affecting various cucurbit crops in India and negatively impacting production. The primary rationale behind focusing only on these six tomato-infecting begomoviruses from their prevalence and distribution across major cucurbit crops in India. This emphasis is driven by the recurrent presence and documented instances of these begomovirus species infecting both solanaceous and cucurbitaceous hosts within the Indian subcontinent. The continuous presence of susceptible host plants in vegetable fields across different regions of India likely contributes to the spread and prevalence of ToLCNDV and ToLCPalV. The restricted distribution of ToLCPalV to northern India can be attributed to a combination of factors, including climate suitability, host range, vector dynamics, human activity, natural barriers, research focus, and evolutionary processes. Additionally, the overlapping growing seasons of cucurbits and other vegetables may lead to cross-infections of different begomovirus species due to the migration of viruliferous whiteflies from the old to new crop fields in the adjoining areas [13, 52].

Our study unveiled a distinct distribution pattern of predominant begomoviruses affecting cucurbit crops in India. ToLCNDV was found to be prevalent in northern, central, and southern regions of the country, while ToLCPalV was restricted to northern India. These findings provide valuable insights into the diversity and prevalence of begomoviruses that infect major cucurbit crops in India. This information serves as a crucial resource for breeders, as it allows them to develop and deploy region-specific resistant varieties, leading to an effective management strategies and overcoming the limitations posed by begomoviruses in cucurbit production.

Acknowledgements The first author is thankful to the Ministry of Tribal Affairs, Govt. of India for providing the Ph.D. fellowship. The financial support from the research grant under the 16-11/PP/ICAR-CRP/20-22/04 Consortia Research Platform on Vaccines & Diagnostics, Indian Council of Agricultural Research, New Delhi is thankfully acknowledged.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We are declaring that to the best of our knowledge, there are no conflict of interest exists in the content of the manuscript.

References

1. Ahlawat YS, Kulshreshtha UK. A new graft transmissible disease of *Sechium edule*. Indian Phytopathol. 1977;30:268–9.
2. Ala-Poikela M, Svensson E, Rojas A, Horko T, Paulin L, Valkonen JPT, Kvarnheden A. Genetic diversity and mixed infections of begomoviruses infecting tomato, pepper and cucurbit crops in Nicaragua. Plant Pathol. 2005;54(4):448–59.
3. Anonymous. FAOSTAT, Food and Agriculture Organization of the United Nations. 2002
4. Bhargava B, Bhargava KS. Cucurbit mosaic viruses in Gorakhpur. Indian J Agric Sci. 1977;47(1):1–5.
5. Bhargava KS, Joshi RD. Detection of watermelon mosaic virus in U.P. Curr Sci. 1960;29:443–4.
6. Brown JK, Idris AM, Rogan D, Hussein MH, Palmieri M. Melon chlorotic leaf curl virus, a new begomovirus associated with *Bemisia tabaci* infestations in Guatemala. Plant Dis. 2001;85(9):1027–1027.
7. Brown JK, Idris AM, Alteri C, Stenger DC. Emergence of a new cucurbit-infecting begomovirus species capable of forming viable reassortants with related viruses in the Squash leaf curl virus cluster. Phytopathology. 2002;92(7):734–42.
8. Capoor SP, Varma PM. A mosaic disease of *Lagenaria vulgaris* Ser in the Bombay province. Curr Sci. 1948;17:274–5.
9. Charoenvilaisiri S, Seepiban C, Phironrit N, Phuangrat B, Yoohat K, Deeto R, Chatchawankanphanich O, Gajanandana O. Occurrence and distribution of begomoviruses infecting tomatoes, peppers and cucurbits in Thailand. Crop Prot. 2020;127:104948.

10. Chomicki G, Schaefer H, Renner SS. Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. New Phytol. 2020;226(5):1240–55.
11. Doyle JJ, Doyle JL A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem bull*. 1987.
12. Dubey GS, Nariani TK, Prakash N. Identification of Snake gourd mosaic virus. Indian Phytopathology. 1974;27:470–4.
13. Ellango R, Singh ST, Rana VS, Gayatri Priya N, Raina H, Chaubey R, Naveen NC, Mahmood R, Ramamurthy VV, Asokan R, Rajagopal R. Distribution of Bemisia Tabaci genetic groups in India. Environ Entomol. 2015;44(4):1258–64.
14. Flock RA, Mayhew DE. Squash leaf curl, a new disease of cucurbits in California. Plant Dis. 1981;65(1):75–6.
15. Jain RK, Pappu HR, Krishna RM, Vani A. Watermelon bud necrosis tospovirus is a distinct virus species belonging to serogroup IV. Arch Virol. 1998;143:1637–44.
16. Khan JA, Siddiqui MK, Singh BP. The association of begomovirus with bitter melon in India. Plant Dis. 2002;86(3):328–328.
17. Kheyr-Pour A, Bananej K, Dafalla GA, Caciagli P, Noris E, Ahoonmanesh A, Lecoq H, Gronenborn B. Watermelon chlorotic stunt virus from the Sudan and Iran: sequence comparisons and identification of a whitefly-transmission determinant. Phytopathology. 2000;90(6):629–35.
18. Kon T, Dolores LM, Bajet NB, Hase S, Takahashi H, Ikegami M. Molecular characterization of a strain of Squash leaf curl China virus from the Philippines. J Phytopathol. 2003;151(10):535–9.
19. Krishnan N, Kumari S, Chaubey T, Kumar RV, Chinnappa M, Dubey V, Pandey KK, Singh J, Singh AK. Study on mosaic disease of sponge gourd (*Luffa cylindrica* L.) caused by tomato leaf curl New Delhi virus. J Plant Pathol. 2023;105:573–80.
20. Kumar P. Development of species specific PCR based diagnostics for begomoviruses infecting tomato in India (M.Sc. thesis). New Delhi: Submitted to the Division of Plant Pathology, IARI. http://krishikosh.egranth.ac.in/handle/1/5810021708. 2015
21. Kumar P, Oraon PK, Yadav P, Roy A, Goel S, Reddy MK, Mukherjee SK, Mandal B. Random distribution of nucleotide polymorphism throughout the genome of tomato-infecting begomovirus species occurring in India: Implication in PCR based diagnosis. Virus Dis. 2022;33(3):270–83.
22. Lazarowitz SG, Lazdins IB. Infectivity and complete nucleotide sequence of the cloned genomic components of a bipartite squash leaf curl geminivirus with a broad host range phenotype. Virology. 1991;180(1):58–69.
23. Malathi VG, Renukadevi P, Chakraborty S, Biswas KK, Anirban Roy PN, Sivalingam VV, Mandal B. Begomoviruses and their satellites occurring in India: distribution, diversity and pathogenesis. In: Mandal B, Rao GP, Baranwal VK, Jain RK, editors. A Century of plant virology in India. Singapore: Springer; 2017. p. 75–177. https://doi.org/10.1007/978-981-10-5672-7_5.
24. Mandal B, Mandal S, Sohrab SS, Pun KB, Varma A. A new yellow mosaic disease of chayote in India. Plant Pathol. 2004;53(6):797.
25. Maruthi MN, Rekha AR, Mirza SH, Alam SN, Colvin J. PCR-based detection and partial genome sequencing indicate high genetic diversity in Bangladeshi begomoviruses and their whitefly vector, *Bemisia tabaci*. Virus Genes. 2007;34:373–85.
26. Maruthi MN, Rekha AR, 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 Bull. 2007;37(2):412–9.
27. Mayee CD, Nandpuri KS, Lal T. A Mosaic disease muskmelon in Punjab. Veg Sci. 1976;3:87–90.
28. Mitra DK, Nariani TK. A mosaic disease of Tori (*Luffa acutangula*). Indian Phytopathol. 1965;18:233–6.
29. Muniyappa V, Maruthi MN, Babitha CR, Colvin J, Briddon RW, Rangaswamy KT. Characterization of pumpkin yellow vein mosaic virus from India. Ann Appl Biol. 2003;142(3):323–31.
30. Nagendran K, Karthika C, Parthasarathy S, Kumar SM, Karthikeyan G. Molecular evidence for the occurrence of Tomato leaf curl New Delhi virus on bottle gourd in Tamil Nadu. India Veg Sci. 2016;43(1):73–7.
31. Namrata J, Saritha RK, Datta D, Singh M, Dubey RS, Rai AB, 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. 2010;21:128–32.
32. Patil CV, Ramdas SV, Premchand U, Shankarappa KS. Survey, symptomatology, transmission, host range and characterization of begomovirus associated with yellow mosaic disease of ridge gourd from southern India. VirusDisease. 2017;28(2):146–55.
33. Raj SK, Singh BP. Association of geminivirus infection with yellow green mosaic disease of *Cucumis sativus*: Diagnosis by nucleic acid probes. Indian J Exp Biol. 1996;34(6):603–5.
34. Raj SK, Kumar S, Srivastava A. Association of tomato leaf curl Palampur virus with yellow mosaic disease of Armenian cucumber (*Cucumis melo* var. Flexuoses) and wild melon (*C. callosus* var. Agrestis) in India. Archiv Phytopathol Plant Prot. 2015;48(9–12):751–9.
35. Raj SK, Snehi SK, Khan MS, Tiwari AK, Rao GP. First report of Pepper leaf curl Bangladesh virus strain associated with bitter gourd (*Momordica charantia* L.) yellow mosaic disease in India. Australas Plant Dis Notes. 2010;5(1):14–6.
36. Raj SK, Snehi SK, Khan MS, Tiwari AK, Rao GP. Molecular identification of an Ageratum enation virus isolate associated with mosaic disease of pointed gourd (*Trichosanthes dioica*) in India. Phytoparasitica. 2011;39:497–502.
37. Rajinimala N, Rabindran R. First report of Indian cassava mosaic virus on bitter gourd (*Momordica charantia*) in Tamil Nadu. India Australas Plant Dis Notes. 2007;2(1):81–2.
38. Ram S, Sharma GS, Varma HN. Epidemology of virus disease of cucurbits. Proc Indian Nat Sci Acad. 1971;37:345–51.
39. Raychaudhuri, M. (1973). *Studies on cucurbit viruses. Ph.D. Thesis.* P.G. School, IARI, New Delhi.
40. Reddy HR, Nariani TK. Studies on mosaic disease of vegetable marrow (*Cucurbit pepo)*. Indian Phytopathol. 1963;16:260–7.
41. Revill PA, Ha CV, Porchun SC, Vu MT, Dale JL. The complete nucleotide sequence of two distinct geminiviruses infecting cucurbits in Vietnam. Adv Virol. 2003;148:1523–41.
42. Samretwanich K, Chiemsombat P, Kittipakorn K, Ikegami M. Tomato leaf curl geminivirus associated with cucumber yellow leaf disease in Thailand. J Phytopathol. 2000;148(11/12):615–7.
43. Shankar G, Nariani TK. A mosaic disease of watermelon (*Citrullus lanattus*). Curr Sci. 1974;43:281–3.
44. Singh R, Raj SK, Chandra G. Association of a monopartite begomovirus with yellow mosaic disease of pumpkin (*Cucurbita maxima*) in India. Plant Dis. 2001;85(9):1029–1029.
45. Singh R, Raj SK, Prasad V. Molecular characterization of a strain of squash leaf curl china virus from north India. J Phytopathol. 2008;156(4):222–8.
46. Sohrab SS, Mandal B, Ali A, Varma A. Molecular diagnosis of emerging begomovirus diseases in cucurbits occurring in northern India. Indian J Virol. 2006;17(2):88–95.
47. Sohrab SS, Mandal B, Pant RP, Varma A. First report of association of Tomato leaf curl virus-New Delhi with yellow mosaic disease of *Luffa cylindrica* in India. Plant Dis. 2003;87(9):1148–1148.
48. Tiwari AK, Snehi SK, Raj SK, Rao GP, Sharma PK. Begomovirus: a major problem for cucurbitaceous crops in Eastern part of UP India. Indian J Virol. 2008;19:124.
49. Tiwari AK, Snehi SK, Singh R, Raj SK, Rao GP, Sharma PK. Molecular identification and genetic diversity among six

Begomovirus isolates affecting cultivation of cucurbitaceous crops in Uttar Pradesh, India. Archiv Phytopathol Plant Protect. 2012;45(1):62–72.
50. Varma A, Giri BK. Virus disease of cucurbits in India. *Cucurbits*. New Delhi: Oxford and IBH Publishing House Pvt Ltd; 1998.
51. Varma A, Malathi VG. Emerging geminivirus problems: a serious threat to crop production. Ann Appl Biol. 2003;142(2):145–64.
52. Venkataravanappa V, Swarnalatha P, Lakshminarayana Reddy CN, Mahesh B, Rai AB. 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 Microb. 2012;3(148):2.
53. Venkataravanappa V, Reddy CL, Chauhan N, Bhardwaj DR, Reddy MK. Association of tomato leaf curl New Delhi virus, a bipartite begomovirus with mosaic disease of snake gourd in India. Int J Pure Appl Biosci. 2017;5(5):558–70.
54. Venkataravanappa V, Prasanna HC, Reddy CL, Chauhan N, Shankarappa KS, Reddy MK. Molecular characterization of recombinant bipartite begomovirus associated with mosaic and leaf curl disease of Cucumber and Muskmelon. Indian Phytopathol. 2021;74:775–85.
55. Verma PM. Ability of whitefly to carry more than one virus simultaneously. Curr Sci. 1955;24:317–8.
56. Verma R, Ahlawat YS, Tomar SPS, Prakash S, Pant RP. First report of Zucchini yellow mosaic virus in bottle gourd (*Langeneria siceraria*) in India. Plant Dis. 2004;88:426.
57. Xie Y, Zhou XP. Molecular characterization of squash leaf curl Yunnan virus, a new begomovirus and evidence for recombination. Archiv Virol. 2003;148(10):2047–54.