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RESEARCH ARTICLE

Prevalence of chilli leaf curl virus and tomato leaf curl New Delhi virus with chilli leaf curl disease in India

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Abstract

The members of the family, *Geminiviridae*, cause severe diseases in a wide range of economically important crops across continents. In the Indian sub-continent, chilli leaf curl disease (ChiLCD) caused by begomoviruses has emerged as a major constraint for chilli cultivation. Here, we report the identification of 16 begomoviruses and 6 betasatellites with ChiLCD from 16 locations covering 7 states in India. In the regions surveyed, chilli leaf curl virus and tomato leaf curl New Delhi virus were identified as the pre-dominantly distributed begomovirus species. Similarly, tomato leaf curl Bangladesh betasatellites and tomato leaf curl Joybebpur betsatellites were detected in the samples collected from 6 out of 16 locations. However, we have failed to detect any DNA-B component in these samples. Further, inter-species recombination has possibly contributed to the emergence of these cloned viral components. This study further emphasized the current status on the distribution of begomoviruses and betasatellites with ChiLCD in the major chilli-growing regions of India. This epidemiological data might help in devising efficient antiviral strategies to curb this disease.

Keywords *Capsicum* spp. · Detection · Diversity · Geminivirus infection · Recombination · Satellite · Solanaceous crop

Introduction

Members of the family *Geminiviridae* are circular, single-stranded and insect vector-transmitted plant-infecting DNA viruses (Fiallo-Olive et al. 2021; Roumagnac et al. 2022). The whitefly (*Bemisia tabaci*)-transmitted virus species of the *Begomovirus* genus causes diseases in a wide range of monocotyledons and dicotyledons crops and weeds in the 'Old World' and 'New World' regions. Further, they are subdivided into bipartite (containing equal-sized DNA-A and DNA-B components) or monopartite (with a homologous DNA-A like component) begomoviruses (Brown et al. 2015). In general, DNA-A encoded proteins help in virus replication, transcription, encapsidation of virus particles, and vector transmission, whereas the DNA-B encoded proteins assist in virus movement within and between cells (Kumar 2019; Kumar and Shivaprasad 2020). Importantly, the majority of the 'Old World' monopartite begomoviruses often co-exist with sub-viral agents (~ 1.4 kb sized), namely betasatellites (Family: *Tolecusatellitidae*) and alphasatellites (Family: *Alphasatellitidae*) (Briddon et al. 2016; Briddon and Stanley 2006; Kumar et al. 2017a). The proteins encoded by these sub-viral molecules function as pathogenicity determinants and also

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help in virus movement (Gnanasekaran et al. 2019; Luo et al. 2019; Gong et al. 2021; Zhao et al. 2022). The rapid evolution of these viral molecules is largely driven by genetic reassortment, mutation/substitution, and/or recombination (Kumar et al. 2015, 2017a; Lefeuvre et al. 2007; Duffy and Holmes 2008; George et al. 2015; Lefeuvre and Moriones 2015; Lima et al. 2017).

Chilli or pepper (*Capsicum* spp.) of the *Solanaceae* family is cultivated as an essential spice cum vegetable crop worldwide. Globally, India is one of the leading producers of chillies amounting to 5.74 million tons in 1.06 million ha. The Indian states such as Andhra Pradesh, Madhya Pradesh, Rajasthan, Tamil Nadu and Uttar Pradesh contribute nearly 75% of chilli production in the country (DACFW 2018). However, chilli leaf curl disease (ChiLCD) caused by begomoviruses has emerged as a major constraint for its production in this part of the world. The disease symptoms associated with ChiLCD include leaf curling, stunting of plants, puckering and reduced size of leaves, puckering and thickening of leaves, yellowing of veins and veinlets. In severe cases, the infected plants failed to produce any fruits resulting in up to 80% crop loss. The association of a begomovirus with ChiLCD in India was first documented in the early 1960s (Mishra et al. 1963). Subsequently, various isolates/strains belonging to several begomovirus species such as chilli leaf curl virus (ChiLCV), chilli leaf curl India virus (ChiLCINV), chilli leaf curl Kanpur virus (ChiLCKV), chilli leaf curl Vellanad virus (ChiLCVV), cotton leaf curl Multan virus (CLCuMuV), papaya leaf curl virus (PaLCuV), pepper leaf curl Bangladesh virus (PepLCBV), pepper leaf curl Lahore virus (PepLCLaV), tomato leaf curl virus (ToLCV), tomato leaf curl Gujarat virus (ToLCGV), tomato leaf curl Joydebpur virus (ToLCJV), and tomato leaf curl New Delhi virus (ToLCNDV) have been reported to be associated with ChiLCD in the past decades (Kumar et al. 2011, 2015; Khan et al. 2006; Shih et al. 2007; Chattopadhyay et al. 2008; Singh et al. 2016; Nagendran et al. 2019a; Mishra et al. 2020). Furthermore, the indispensable role of betasatellites in modulating the pathogenicity of ChiLCD-associated begomoviruses have been demonstrated (Kumar et al. 2015; Singh et al. 2016; Mishra et al 2020). This present study has been carried out to understand the current status of ChiLCD-associated begomovirus and betasatellite in the major chilli growing regions in India. Furthermore, this study also highlighted the evolutionary significance of this disease complexes.

Materials and methods

Survey and collection of ChiLCD-affected chilli samples

During 2016–2017, a survey conducted across 46 locations in major chilli growing regions of 7 Indian states (Andaman, Andhra Pradesh, Bihar, Madhya Pradesh, Rajasthan, Tamil Nadu and Uttar Pradesh). A total of 101 plant samples showing symptoms of ChiLCD such as leaf curling, leaf rolling, leaf puckering, mosaic, thickening of leaves, vein enation and/or stunting were collected (Fig. 1; Table 1). The sampling was based solely on observed disease symptoms in field plants, and not on the specific chilli cultivars. For sample collection, 1–5 agricultural fields were surveyed at each location. In each field, five random one-meter rows were selected for observation. To calculate the percent disease incidence (PDI), both healthy and diseased plants in these rows were counted. The PDI was calculated using the formula:

$$\mathrm{PDI} = \left[\text{Number of affected plants in 5 rows} \,/\, \text{Total number of plants in 5 rows}\right] \times 100$$

Most samples were collected in person, while a few of them were received from the volunteers through post. The collected chilli plants were surface cleaned, and packed with the perforated papers for transportation to the Virology Laboratory at Indian Institute of Vegetable Research (ICAR-IIVR), Varanasi, Uttar Pradesh, India.

Cloning of begomoviruses and betasatellites associated with ChiLCD

The total DNA was extracted from the leaf tissues of symptomatic and asymptomatic chilli plants as described by Doyle and Doyle, (1990). The extracted DNA was then subjected to rolling circle amplification (RCA) using Φ29 DNA polymerase as per manufacturer's (GE Healthcare, Waukesha, WI, USA) instructions to enrich viral concatemers. The resultant RCA products were used as a template for PCR analysis with degenerate primers (PAL1c1960 and PAR1v722) specific to DNA-A component of the begomovirus genome (Chatchawankanphanich and Maxwell 2002). In addition, to detect the presence of begomovirus species associated with the diseased plant samples, PCR was performed using primers specific to diverse begomovirus species such as ToLCNDV, ToLCJoV (currently considered as a distinct strain of ChiLCV), ChiLCV, ToLCBV, ToLCKV, ToLCPalV and ToLCGV (Chakraborty et al. 2008; Kumar 2015; Reddy et al. 2005; Table S1). Cloned viral fragments of the respective viruses obtained in our previous studies were used as positive controls (Nagendran et al. 2019a). PCR assays were conducted in a 20 µL reaction mixture containing 1 µL (50 ng) of DNA template, 10 µL of 2 × Red Dye PCR master mix (Genei, Bangalore), 1 µL (25 pmol each) of forward and reverse primers, and final volume made up

Fig. 1 **Types of symptoms observed on ChiLCD-affected chilli plants in the agricultural fields.** The location of the samples collected are provided with each panel

with nuclease-free water. The conditions used for PCR include initial denaturation for 3 min at 95 °C followed by 40 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 56 °C, and elongation for 1 min at 72 °C, with a final elongation step for 5 min at 72 °C. The total DNA extracted from the healthy chilli plants served as a negative control for this PCR assay. The PCR amplicons were visualized on 1% agarose gel electrophoresis, and the presence of begomovirus species in symptomatic chilli plants was documented.

To isolate the associated begomovirus, the RCA-derived viral concatemers were further digested individually with unique restriction endonucleases such as *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I, *Xba*I and *Sac*I. Subsequently, the resulting ~ 2.7 kb DNA fragments (corresponding to the size of a begomovirus genome) were cloned into pBluescript SK + vector which was previously linearized with the respective restriction endonucleases. To investigate the presence of DNA-B and betasatellites associated with the samples, PCR was performed with an RCA-derived product as a template using DNA-B and betasatellite-specific primers as described previously (Briddon et al. 2002; George et al. 2014). The resulting PCR amplicons (~ 1.3 kb in size) were gel eluted and subsequently cloned into pTZ57R/T vector. Further, the recombinant clones were subjected to restriction fragment length polymorphic analysis for analyzing the sequence variability, and the unique clones (two of each restriction patterns) were sequenced to obtain the full-length genome of the associated begomoviruses and betasatellites.

Sequence identity, phylogeny and recombination analyses

The color-coded pairwise sequence identity matrices of full-length begomoviral and betasatellite genomes were generated using the MUSCLE method available in SDT v1.2 (Muhire et al. 2014). The maximum-likelihood-based phylogenetic trees of the selected begomoviruses and betasatellites were constructed using MEGA X with 1000 bootstrap repetitions (Kumar et al. 2018). Recombination analyses of the isolated begomoviral and betasatellite sequences were performed using RDP v.4.97 (Martin et al. 2015) with default settings using a 0.05 *P* value cutoff as described by George et al. (2015).

Results

Incidence of ChiLCD and the detection of begomoviruses

The ChiLCD incidence was found to be ranged between 20 and 85% in the 46 agricultural fields surveyed during

Table 1 Survey and detection of begomovirus species with ChiLCD in India

State	PDI^ (%)		Number of fields surveyed	Symptoms in the field grown plants[#]	*PCR positive samples with the primers specific for				
					Universal	ChiLCV	ToLCJV	ToLCNDV	ToLCBV, ToLCGV, ToLCKV, ToLCPalV
Andaman & Nicobar Is	46.8	Manglutan	4	ULC, LR	7/8	1/8	1/8	5/8	0/8
		Wandoor	2	ULC, LR, St, VT, YL	6/7	0/7	0/7	6/7	0/7
Andhra Pradesh	40.0	Kurnool	2	ULC, LR, St, YL	2/2	0/2	0/2	2/2	0/2
		Tirupati	2	ULC, LCr, LR, SL, VT	2/2	2/2	2/2	2/2	0/2
Bihar	28.3	Muzzaffarpur	1	ULC, LR, SL, YL	1/3	1/3	1/3	0/3	0/3
		Pusa, Samastipur	1	ULC, LR, St, YL	1/3	0/3	0/3	1/3	0/3
Madhya Pradesh	56.6	Sirauli	5	ULC, LCr, LR, SL, St, VT	14/16	10/16	8/16	2/16	0/16
		Burhanpur	5	ULC, LCr, LR, St, VT, YL	14/15	0/15	0/15	14/15	0/15
Rajasthan	27.5	Daulatpura	2	ULC, LCr, LR, St, VT	1/2	1/2	1/2	0/2	0/2
		Nimeda	2	ULC, LR, St, YL	1/2	0/2	0/2	1/2	0/2
Tamil Nadu	50.0	Anthiyur	2	DLC, LCr, VT, YL	3/5	0/5	0/5	3/5	0/5
		Mettur	2	DLC, LCr, SL, VT, St	3/3	0/3	0/3	0/3	0/3
Uttar Pradesh	21.9	Deoria	4	ULC, LR	8/8	7/8	7/8	1/8	0/8
		Varanasi	4	ULC, LCr, LR, SL, VT	10/10	9/10	9/10	1/10	0/10
		Pindra	4	ULC, LR, SL, St, VT, YL	9/9	0/9	0/9	0/9	0/9
		Faizabad	4	ULC, LR, St, YL	6/6	0/6	0/6	6/6	0/6

^The percent disease incidence (PDI) for every field was calculated using the number of plants exhibiting symptoms and the total number of plants observed in the field

[#] *DLC*: downward leaf curling, *LCr*: leaf crinkling, *LR*: leaf rolling, *SL*: small leaves, *St*: stunting, *ULC*: upward leaf curling, *VB*: vein thickening, *YL*: yellowing of leaves

[*] The number of PCR positive samples with the primers mentioned / The total number of samples used for PCR analysis. The abbreviated begomovirus species are ChiLCV—chilli leaf curl virus; ToLCBV—tomato leaf curl Bangalore virus; ToLCJV—tomato leaf curl Joydevpur virus; ToLCGV- tomato leaf curl Gujarat virus; ToLCKV—tomato leaf curl Karnataka virus; ToLCNDV—tomato leaf curl New Delhi virus and ToLCPalV—tomato leaf curl Palampur virus. + and—indicate the presence and absence of the begomovirus species as mentioned, respectively

2016–2017. Higher average PDI was noticed in the chilli-growing regions of Madhya Pradesh (56%) followed by Tamil Nadu (50%), whereas least average PDI (21.94%) was observed in the Uttar Pradesh regions (Table 1). In the PCR assay performed using universal begomovirus primers, begomovirus infection was detected in 88 out of 101 samples tested (Table 1). In all the regions surveyed, the presence of ToLCNDV was detected, whereas ChiLCV was found in all the regions except from the Tamil Nadu (Table 1). Similarly, ToLCJoV was noticed only in the Andaman and Uttar Pradesh regions. However, no viral amplicons specific to ToLCBV, ToLCGV, ToLCKV or ToLCPalV were detected in any of these 101 samples collected (Table 1).

Characterization of ChiLCD-associated begomoviruses

The begomovirus sequence identified from Mettur in Tamil Nadu (OQ921396) shared a maximum nucleotide identity of 95.6% with an isolate of dolichos yellow mosaic virus (DoYMV; AY271891). Similarly, a sequence each from the Faizabad (OQ921386), Burhanpur (OQ921387), Wandoor (OQ921388), Anthiyur (OQ921389), Kurnool (OQ921390) and Pusa (OQ921391) samples possess highest sequence identities between 93 and 99.3% with the isolates of tomato leaf curl New Delhi virus 1 (ToLCNDV-1; AJ875157). Hence, they are considered as the isolates of DoYMV and ToLCNDV-1, respectively, and so they are placed separately as distinct clades in the phylogenetic tree (Table 2; Fig. 2A and B). A begomovirus sequence isolated from Nimeda (OQ921392) showed highest nucleotide identities of 90% to 98.5% with the isolates of yet to be named ‘strain’ of ToLCNDV (Figs. 2A and S1). As per ICTV-devised guidelines for begomovirus species and strain demarcation (Brown et al. 2015), it can be placed in a newly proposed ‘strain’ of ToLCNDV for which we propose the name, Tomato leaf curl New Delhi virus—Chilli (ToLCNDV—Chilli). In consistent with its sequence identity, this sequence was found to be placed in a distinct clade in the phylogenetic tree (Fig. 2B).

Distribution of betasatellites with ChiLCD in India

The betasatellite sequences identified from the Pindra (OR180506) and Varanasi (OR180504) shared sequence identities between 98.2 and 92.2% with the isolates of

Table 2 The characterization of ChiLCD-associated begomoviruses and betasatellites in India

Sample collection			Enzyme used for cloning	Begomovirus identified*		Betasatellite identified*	
State	Location	GPS co-ordinates		Isolate name	GenBank accession number	Isolate name	GenBank accession number
Andaman and Nicobar Is	Manglutan	11°35’12.2”N 92°39’26.4”E	*Bam*HI	ChiLCV	MK330665	ToLCJoB	MN066162
	Wandoor	11°35’46.4”N 92°37’29.6”E	*Xba*I	ToLCNDV	OQ921388	ToLCJoB	OR180507
Andhra Pradesh	Kurnool	15°42’32.2”N 78°18’01.5”E	*Hind*III	ToLCNDV	OQ921390	–	–
	Tirupati	13°39’32.5”N 79°22’41.1”E	*Xba*I	ChiLCV	OQ921393	–	–
Bihar	Muzzaffarpur	26°06’17.1”N 85°24’59.5”E	*Xba*I	ChiLCV	OQ921394	ToLCJoB	OR180508
	Pusa, Samastipur	26°00’26.1”N 85°37’47.3”E	*Sac*I	ToLCNDV	OQ921391	–	–
Madhya Pradesh	Sirauli	24°07’09.1”N 81°41’49.8”E	*Xba*I	ChiLCV	MT636373	–	–
	Burhanpur	21°09’13.3”N 76°09’26.9”E	*Xba*I	ToLCNDV	OQ921387	–	–
Rajasthan	Daulatpura	27°05’04.9”N 75°49’39.9”E	*Xba*I	ChiLCV	OQ921395	–	–
	Nimeda	26°30’34.6”N 75°30’50.6”E	*Sac*I	ToLCNDV	OQ921392	–	–
Tamil Nadu	Anthiyur	11°35’26.9”N 77°35’25.7”E	*Xba*I	ToLCNDV	OQ921389	ToLCJoB	OR180505
	Mettur	11°51’07.0”N 77°44’56.0”E	*Eco*RI	DoYMV	OQ921396	–	–
Uttar Pradesh	Deoria	26°18’55.2”N 83°59’48.6”E	*Xba*I	ChiLCV	MT636371	–	–
	Varanasi	25°10’54.8”N 82°52’33.9”E	*Kpn*I	ChiLCV	MT636374	ToLCBDB	OR180504
	Pindra	25°29’19.5”N 82°49’41.9”E	*Bam*HI	PepLCBV	MT636372	ToLCBDB	OR180506
	Faizabad	26°32’38.1”N 81°51’57.4”E	*Xba*I	ToLCNDV	OQ921386	–	–

*The abbreviated names of begomovirus/betasatellite are ChiLCV-chilli leaf curl virus; DoYMV- dolichos yellow mosaic virus, ToLCNDV-tomato leaf curl New Delhi virus; ToLCBDB-tomato leaf curl Bangladesh betsatellite and ToLCJoV- tomato leaf curl Joydevpur betsatellite

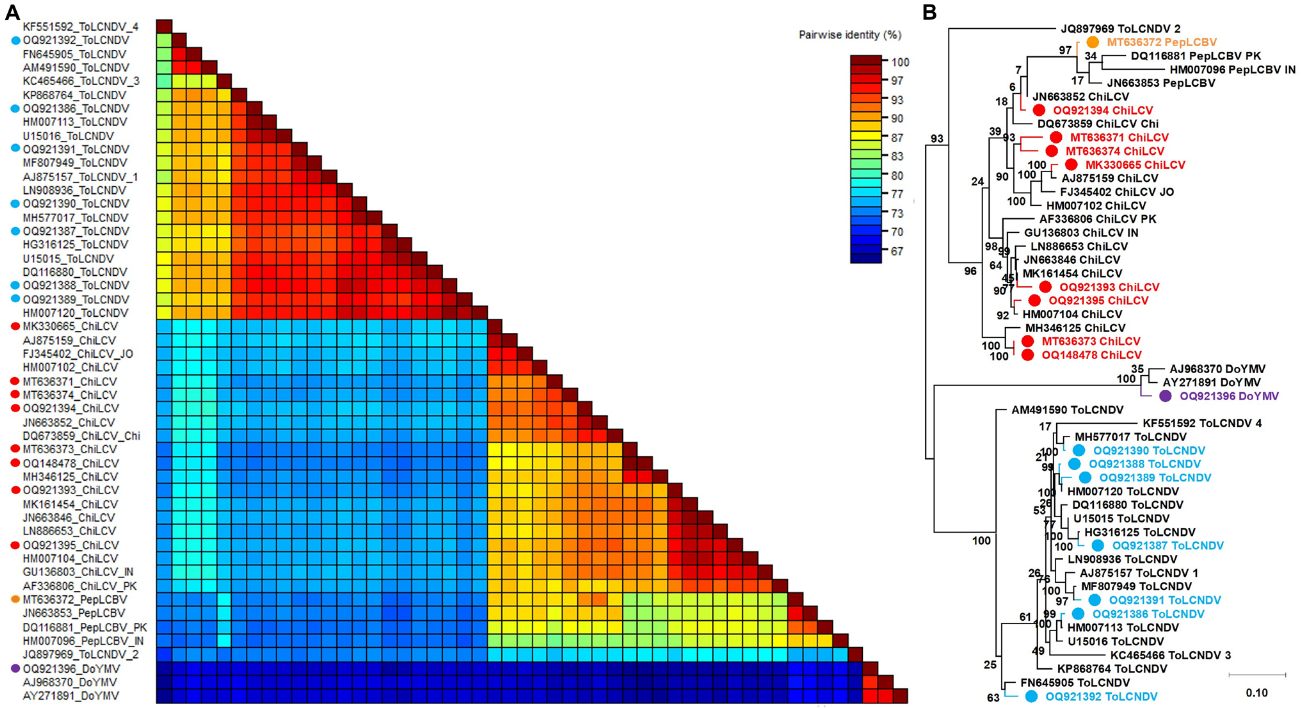

Fig. 2 Characterization of begomoviruses associated with ChiLCD in India. Pairwise nucleotide sequence identity matrix (**A**) and maximum-likelihood phylogenetic tree (**B**) of the selected DNA-A sequences. The scale bar represents the genetic distance and the percent bootstrap values of 1000 replicates are indicated at the nodes. The color-coded filled circles indicate the genomes identified in this study. Each color represents a distinct begomovirus species

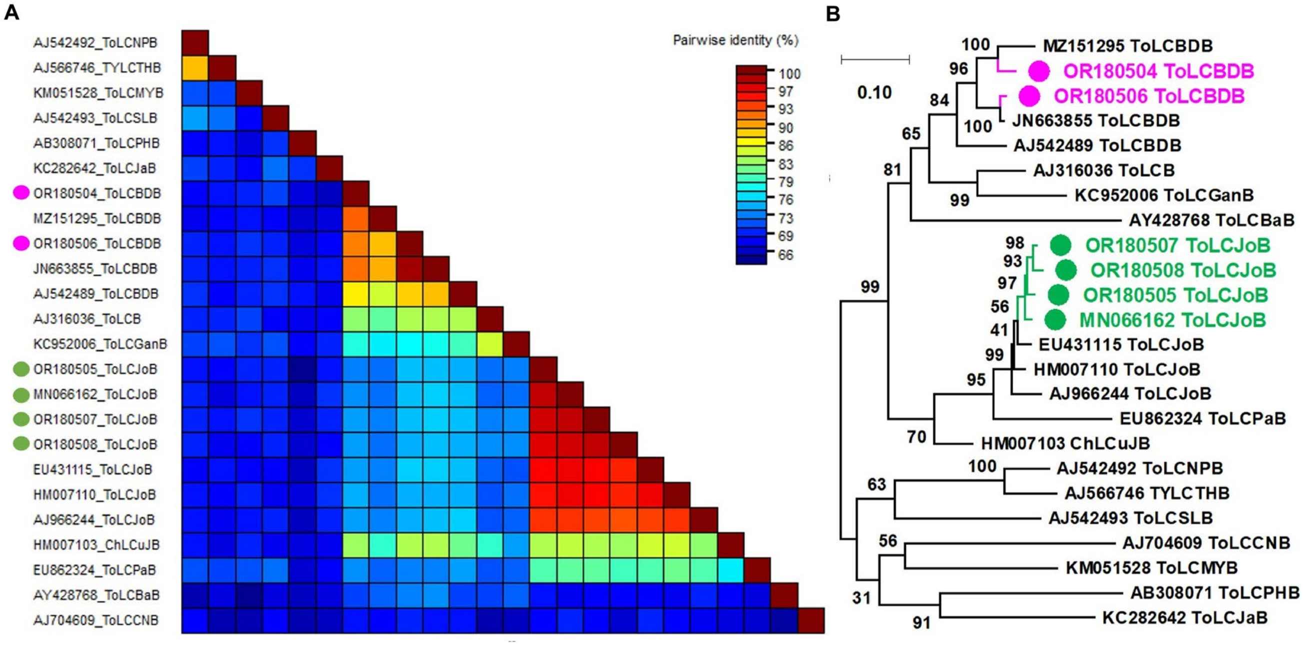

Fig. 3 Characterization of ChiLCD-associated betasatellites in India. Pairwise nucleotide sequence identity matrix (**A**) and maximum-likelihood phylogenetic tree (**B**) of the selected betasatellite sequences. The scale bar represents the genetic distance and the percent bootstrap values of 1000 replicates are indicated at the nodes. The color-coded filled circles indicate the genomes identified in this study. Each color represents an individual betasatellite group

tomato leaf curl Bangladesh betasatellite (ToLCBDB) (Fig. 3A). So, according to betasatellite demarcation threshold value of 91% (Briddon et al. 2016), these betasatellites can be considered as the isolates of ToLCBDB. Similarly, a sequence each from the Manglutan (MN066162), Anthiyur (OR180505), Wandoor (OR180507) and Muzzaffarpur (OR180508) samples possess the highest sequence identity of 92.7–96% with tomato leaf curl Joydebpur betasatellite (ToLCJoB) isolates. Hence, they are clustered along with the other ToLCJoB isolates in the phylogenetic tree (Fig. 3B).

Recombination analysis

Recombination analysis was performed to identify the recombined regions in the viral genome and their putative

Table 3 Recombination analysis of the cloned viral genomes associated with ChiLCD in India

Isolate	Recombinant sequences	Recombination breakpoint position (in nts)			Putative parental sequences		RDP methods detected*	Average *p*-value range detected
		Start	End	Location in the genome#	Major	Minor		
ChiLCV	MT636371	39	1141	V1	MK161454; ChiLCV-PK	AJ875159; ChiLCV	RGBMCST	2.452×10^{-10}–7.060×10^{-19}
		1153	2036	C2, C3	AJ875159; ChiLCV	DQ116881; PepLCBV_PK	RMCST	8.437×10^{-04}–1.549×10^{-08}
	MT636372	329	971	V1	HM007104; ChiLCV	HM991146; ToLCJoV	RGBMCST	2.058×10^{-03}–2.286×10^{-12}
		2132	2741	CR, C1/C4	JN663854; ChiLCV	Undetermined	RGBMCST	7.318×10^{-18}–7.634×10^{-40}
	MT636373	2750	508	CR, V2	JN663853; PepLCBV	HM007104; ChiLCV	RGBCST	3.069×10^{-04}–7.583×10^{-14}
	MT636374	2028	274	CR	KF515619; ToLCGuV	MT636371; ChiLCV	RGMCST	2.534×10^{-04} – 1.821×10^{-27}
	MK330665	398	1075	V1	HM007104; ChiLCV	HM991146; ToLCJoV	RGBMCST	2.058×10^{-03}–2.286×10^{-12}
		2367	14	CR, C1/C4	JN663853; ChiLCV_Chi	Undetermined	RGMCST	1.314×10^{-10}–2.205×10^{-24}
	OQ921393	600	1079	V1	DQ116881; PepLCBV_PK	HM007096; PepLCBV_IN	RGBMCST	4.383×10^{-09}–1.050×10^{-15}
	OQ921394	329	977	V1	HM007104; ChiLCV	HM991146; ToLCJoV	RGBMCST	2.058×10^{-03}–2.286×10^{-12}
	OQ921395	513	918	V1	DQ116881; PepLCBV_PK	HM007096; PepLCBV_IN	RGBMCST	4.383×10^{-09} – 1.050×10^{-15}
ToLCNDV	OQ921386	2455	2646	CR	KC465466; ToLCNDV	AF188481; ToLCBV	RGBMCST	1.681×10^{-11} – 7.334×10^{-44}
	OQ921387	2597	137	CR	AJ875157; ToLCNDV	EF450316; ToLCBV	RGBMCST	2.081×10^{-05} – 2.128×10^{-09}
	OQ921388	2469	2683	CR	KC465466; ToLCNDV	AF188481; ToLCBV	RGBMCST	1.681×10^{-11} – 7.334×10^{-44}
	OQ921389	2469	2683	CR	KC465466; ToLCNDV	AF188481; ToLCBV	RGBMCST	1.681×10^{-11} – 7.334×10^{-44}
	OQ921390	2455	2683	CR	KC465466; ToLCNDV	AF188481; ToLCBV	RGBMCST	1.681×10^{-11} – 7.334×10^{-44}
	OQ921391	2455	2714	CR	KC465466; ToLCNDV	AF188481; ToLCBV	RGBMCST	1.681×10^{-11} – 7.334×10^{-44}
	OQ921392	2056	2715	CR, C1/C4	KC465466; ToLCNDV	AF188481; ToLCBV	RGBMCST	7.228×10^{-14} – 4.605×10^{-43}
ToLCBDB	OR180504; OR180506	407	680	βC1	AJ542489; ToLCBDB	AJ316036; ToLCB	RGBMCST	1.394×10^{-03} – 1.253×10^{-07}
	MN066162	1058	93	SCR	EU862324; ToLCPaB	AJ542489; ToLCBDB	RBMST	1.074×10^{-05} – 4.012×10^{-19}
ToLCJoB	OR180505, 07, 08	1075	67	SCR	EU862324; ToLCPaB	JN663855;		
ToLCBDB	RGBMCST	1.338×10^{-04}–1.178×10^{-07}						

#*CR*; common region and *SCR*; satellite conserved region

*The abbreviated names are R–RDP; G–GENECONV; B–BOOTSCAN; M–MAXCHI; C—CHIMERA; S—SISCAN; T–3SEQ parental sequences (Table 3). The RDP analysis of ToLCNDV isolates detected recombination events around the common region (CR) which could be possibly contributed by ToLCBV and ToLCNDV. In the cloned ChiLCV isolates, recombination events were found around either the coat protein (CP)-CR and C1-CR, with the exception of a single recombination event around C2/C3 region. The ChiLCD-associated begomoviruses (such as ChiLCV, ChiLCV_IN, ChiLCV_PK, PepLCBV_IN and PepLCBV_PK) were identified as possible parents of these recombinant ChiLCV isolates (Fig. 4). Similarly, the recombination breakpoints were detected around satellite conserved region (SCR) or βC1 coding region. Further, tomato-associated betasatellites (such as ToLCB and ToLCPaB) have contributed to the emergence of these cloned betasatellites.

Discussion

Begomoviruses have emerged as prominent plant-infecting viruses that cause significant crop losses in several economically important agricultural and horticultural plants in India (Reddy et al. 2024; Kumari et al. 2022). Among the diseases caused by begomoviruses, ChiLCD is widespread in major chilli growing regions across the country resulting in severe yield losses. The various monopartite and bipartite

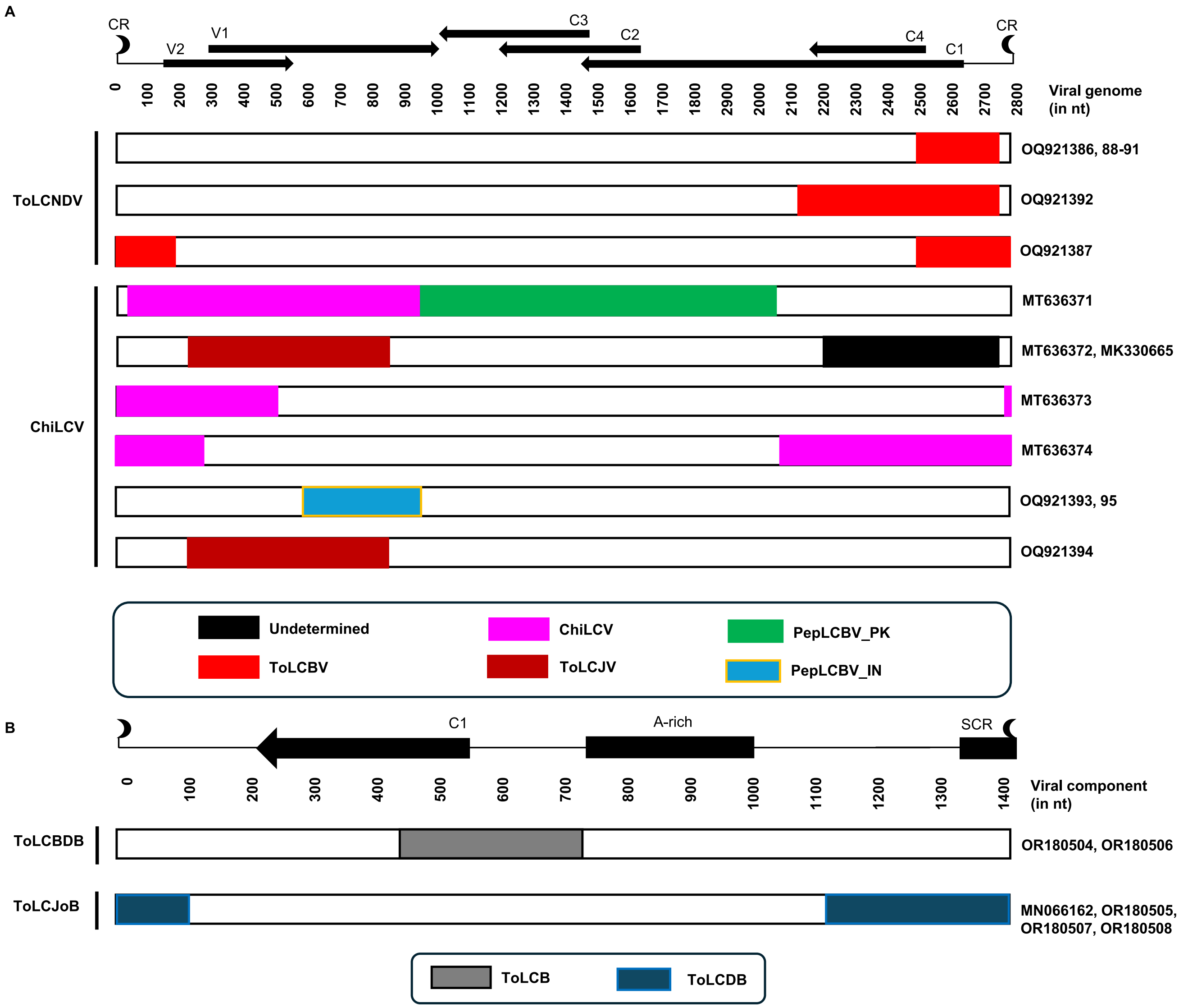

Fig. 4 The recombination analyses of the cloned begomoviruses and betasatellites. Each bar represents an individual sequence. The schematic representations of begomovirus and betasatellite genomes are provided. The name of the isolate and its corresponding accession number are indicated accordingly

begomoviruses along with satellite molecules have been reported to be associated with ChiLCD in India (Kumar et al. 2015; 2016a; 2016b; 2017a; Chattopadhyay et al. 2008; Nagendran et al. 2019a). The present study reports the current status of the distribution of ChiLCD-associated begomoviruses and betasatellites in the major chilli-growing regions in the country. Our analysis also highlighted the recombinant nature of the cloned viral genomes.

In order to study the begomovirus diversity among the surveyed ChiLCD samples, RCA-based PCR assay using the virus species-specific primers is considered the most helpful approach. Based on this PCR assay, ChiLCV, ToLCJoV and ToLCNDV were found to be associated with ChiLCD, whereas begomovirus species such as ToLCBV, ToLCKV, ToLCPalV and ToLCGV were not detected (Table 1). The full-length genome characterization has identified the association of ChiLCV, DoYMV, PepLCBV and ToLCNDV with these samples (Table 2). Strikingly, this is the first study that reports the association of DoYMV with ChiLCD. Similarly, ToLCBDBs were identified only from the Uttar Pradesh regions, whereas ToLCJoBs were reported from Andaman, Bihar, and Tamil Nadu regions (Fig. 5). Previously, Kumar et al., (2015) have documented the association of 6 begomovirus species and 5 betasatellite groups. However, this study reports the predominant distribution of ChiLCV and ToLCNDV with ChiLCD; thereby highlighting the spread of ToLCNDV to major chilli growing regions in India. It is worth mentioning that the overlapping cropping pattern and/or season of chillies and tomatoes might have facilitated the distribution of ToLCNDV to chilli to a great extent as previously mentioned by Nagendran et al., (2019b). Our data also highlight that such frequent occurrences of ToLCNDV with ChiLCD could potentially emerge as a serious threat (along with ChiLCV) to chilli cultivation in India. This finding is consistent with previous studies that reported the frequent association of ToLCNDV causing serious threat to cotton and cucurbits productivity globally (Zaidi et al. 2016; Juarez et al. 2019; Cai et al. 2023; Vignesh et al. 2023). The necessity of ChiLCD-associated betasatellites or DNA-B for the development of leaf curl disease in chili have been demonstrated previously (Kumar et al. 2015; Singh et al. 2016). Interestingly, the majority of the begomoviruses (10 out of 16) identified in this study lack either DNA-B and/or betasatellites (Fig. 5). Interestingly, ToLCNDV isolates lacking DNA B component have been identified with several plant species (Brown et al. 2015; Zaidi et al. 2017; Sangeeta et al. 2023). Furthermore, this necessitates to understand the ability of these ChiLCV and/or ToLCNDV isolates (that lack DNA-B and/or betasatellite) in developing typical disease symptoms in chilli plants.

Recombination and reassortment are widely considered as major driving forces in the emergence and evolution of begomovirus-betasatellite disease complexes worldwide (George et al 2015; Lefeuvre and Moriones 2015; Kumar et al 2017b). Recombination analyses of the cloned begomoviruses have identified breakpoints encompassing either CR/C1/C4 or CP regions (Fig. 4). The exchange of C4 region through interspecies recombination has been demonstrated to elevate the pathogenicity of a mild virus species into a severe one (Xie et al 2013). This necessitates to assess the functional significance of such exchanged region on the disease development of the cloned recombinant isolates. Similarly, the recombination breakpoints in the cloned betasatellites were detected around SCR or βC1 regions. These findings have corroborated the earlier literatures suggesting that such regions are considered as recombination hotspots (Lefeuvre et al. 2007; George et al 2015; Kumar et al. 2015; Prasanna et al. 2010; Hou and Gilbertson 1996; Prasanna and Rai 2007). The chilli- and tomato-associated begomoviruses and betasatellites might have contributed to the emergence of these cloned viral components via interspecies recombination. It is worth mentioning that such interspecies recombination events (contributed by monopartite begomoviruses) might have been attributed to the existence of ToLCNDV isolates without DNA B component. However, such assumption requires experimental verification of studying the biology and interaction of such 'monopartite nature of ToLCNDV' in disease development and its host range expansion. Such interspecies recombination might have helped such 'recombinant' begomoviruses (infecting chilli and tomato) to acquire improved ecological fitness (than 'parental' virus species) and persists with various solanaceous crops interchangeably. Further, the host range expansion of ToLCNDV to chillies and the tendency of its rapid evolution makes it difficult to devise any genetic based-resistance (GBR) strategy to limit its infection. So, for its effective disease management, GBR approach can be implemented along with any integrated disease management approaches such as avoiding continuous cropping or intercropping of various solanaceous crops in the field.

The present study reports the current status of ChiLCD-associated begomoviral disease complexes from the agricultural fields in India. Both ChiLCV and ToLCNDV were predominantly distributed in these regions. However, the majority of these isolates were not identified along with either DNA B or betasatellites. Moreover, these cloned genomes might have emerged via interspecies recombination between chilli- and tomato-associated viral genomic components. Further, the frequent occurrence of ToLCNDV with these samples necessitates to include this begomovirus species in devising any potential antiviral strategies to curb ChiLCD.

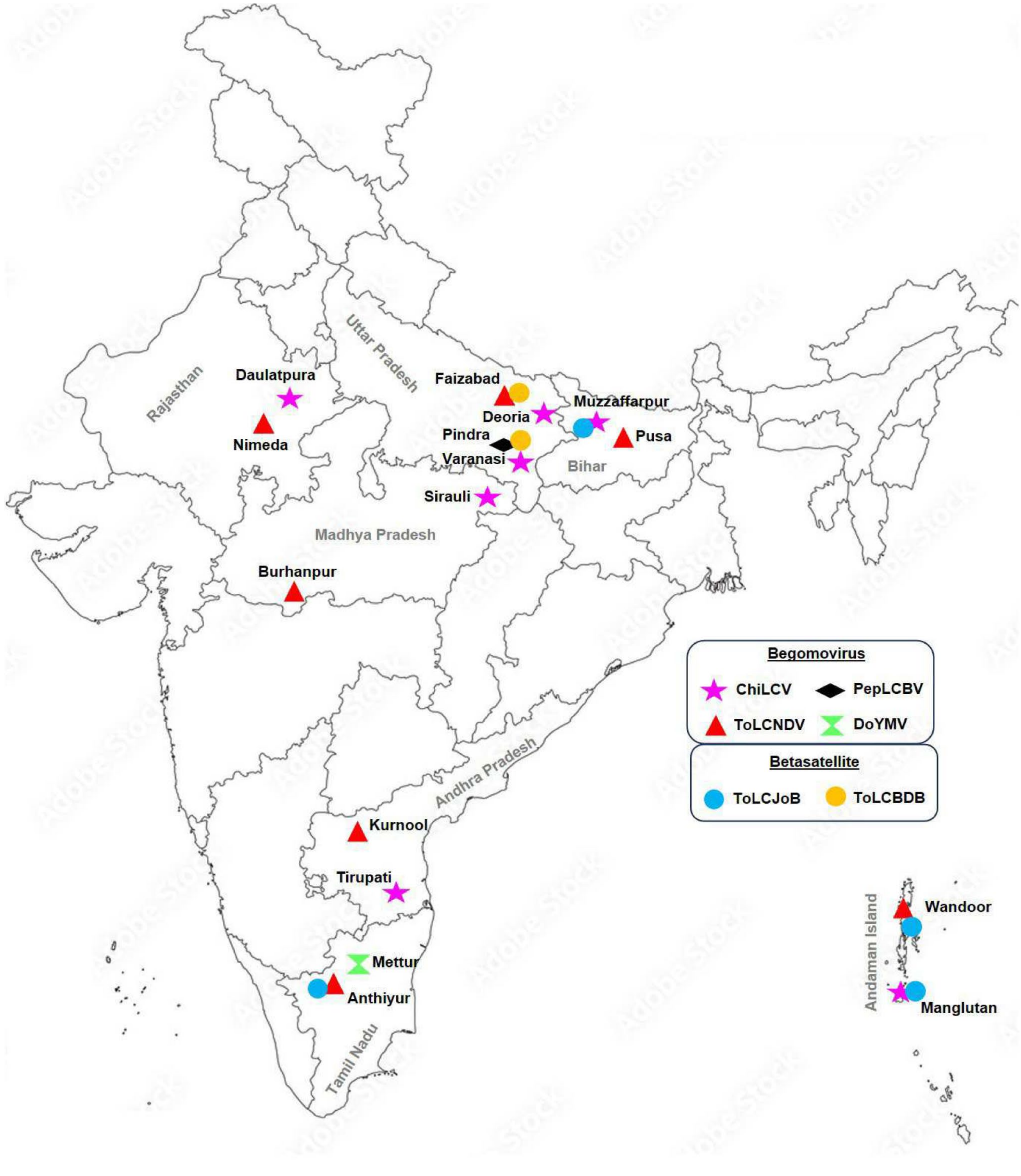

Fig. 5 Distribution of the identified ChiLCD-associated begomovirus disease complexes in India. Distinct components of DNA A and betasatellites are provided in different shapes and colors. The abbreviated names of the cloned begomoviruses and satellites are ChiLCV—chilli leaf curl virus, DoYMV—dolichos yellow mosaic virus, PepLCBV—pepper leaf curl Bangladesh virus, ToLCNDV—tomato leaf curl New Delhi virus, ToLCBDB—tomato leaf curl Bangladesh betasatellite and ToLCJoB—tomato leaf curl Joydebpur betasatellite

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Declarations

Conflict of interest Authors declare that they have no conflict of interest.

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