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RESEARCH ARTICLE

Molecular characterization of Begomovirus and Beta Satellite Virus associated with leaf curl disease of Chilli (*Capsicum annum* L.) in southern parts of Tamil Nadu

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Abstract

Chilli is one of the widely cultivated commercial vegetable crops in India. Chilli leaf curl is the most devasting disease and causes a yield loss of 100 per cent in Chilli. Fourteen begomovirus species cause chilli leaf curl disease (ChiLCD). An extensive survey was carried out in 2023 to determine the prevalence of the leaf curl disease of Chilli caused by begomovirus in major chilli-growing areas of the Southern Districts of Tamil Nadu. During the survey, 21 isolates of begomovirus infecting Chilli were collected. Among the 21 isolates, the isolate collected from Kalakudi village (BV-KI) exhibited severe symptoms. DNA extracted from 21 isolates of begomovirus were subjected to PCR by using Rojas universal primers, Chilli leaf curl virus (ChiLCV) Coat protein primers and beta satellite virus primers and obtained 1380 bp, 1100 bp and 1400 bp amplicons of top and coat protein region of DNA-A of begomovirus, Chilli leaf curl virus coat protein region and beta satellite virus associated with ChiLCV respectively. The 1380 bp, 1100 bp and 1400 bp amplicons obtained from the BV-KI isolate were sequenced and submitted to the NCBI. The sequences were blast analyzed, and it showed that the nucleotide sequence of the top and coat protein region of DNA-A of begomovirus and CP gene of ChiLCV BV-KI isolate was found to have 90.61 and 99.78% similarity with Bhavanisagar (NC 055130.1) isolate respectively. The nucleotide sequence of the beta satellite virus of BV-KI isolate was found to have 99.93% similarity with the Chilli leaf curl beta satellite virus Meerut (MH355642.1) isolate.

Keywords

Coat protein; Gemini virus; Chilli leaf curl virus; DNA-A

Introduction

Chilli (*Capsicum annum* L.) is one of the critical spice cum vegetable crops, which is used for flavouring foods for its pungency. Chilli is a member of the Solanaceae family, and the genus Capsicum is originated from South America. Chilli was introduced to India by the Portuguese during the year 1584 (1). Chilli requires a lengthy warm season with the temperature of 20 °C to 30 °C for its growth and development and it is primarily grown in tropical parts of the world.

India is being the world's largest producer, consumer and exporter of

Chilli and has the largest area of 0.85 Mha with a production of 1.95 MT. It is grown across all the states and union territories of India. Among them, Andhra Pradesh, Orissa, Maharashtra, West Bengal, Karnataka, Rajasthan, and Tamil Nadu are the central states that produce Chilli in India. Andhra Pradesh is the largest chilli producer in India with the production of 0.62 MT. Tamil Nadu is one of the important states in India for the output of Chilli. In Tamil Nadu, chilli crop is grown over an area of 0.04 Mha with a production of 0.02 MT (2).

Chilli is affected by fungal, bacterial and viral diseases. Among them, viral diseases are causing severe damage and yield loss. Among the viral diseases, Chilli leaf curl disease (ChiLCD) caused by Chilli leaf curl virus (ChiLCV), belongs to the genus begomovirus, has become a significant issue in reducing the cultivation of Chilli in the tropical and subtropical parts of the Indian subcontinent (3).

Begomovirus has two genomic components, *DNA-A and DNA-B,* of approximately 2.7 kb each. DNA-A encodes a replication-associated protein essential for viral replication, a replication enhancer protein, a transactivator protein that controls late gene expression, and the coat protein (4, 5). DNA-B encodes a nuclear shuttle protein (NSP) and a movement protein (MP), both of which are essential in efficient systemic spread and symptom expression (6). However, many begomoviruses have a single genomic component resembling DNA-A, capable of autonomous replication and movement within the plant (7). Leaf curl disease of Chilli (ChiLCD) is now a severe problem in India. The disease is characterized by leaf curling, puckering, vein clearing and swelling symptoms. The size of leaves and branches is reduced considerably in severely affected plants, resulting in a bushy appearance. Such plants bear very few flowers and very few fruits (8). Senanayake *et al.* (9) first reported the Chilli Leaf Curl Disease in India. ChiLCD is transmitted by the Whitefly, *Bemisia tabaci* (Gennadius), in a persistent, non-propagative manner. In India, due to adverse climatic conditions, whitefly prevails throughout the year (10). ChiLCD causes yield loss up to 100 percent (11). Chilli leaf curl disease is caused by 14 begomovirus species (12). To develop a management practice for ChiLCD, identification and molecular characterization of species of begomovirus that causes the disease is most important.

Materials and Methods

Survey and collection of ChiLCD-infected plant samples of Chilli

An extensive roving survey was carried out to determine the prevalence of the Leaf Curl disease of Chilli caused by *Begomovirus* in the Southern Districts of Tamil Nadu as per the procedure given by Wahyono *et al.* (13). The study was carried out during the year 2023 in major Chilli growing areas of Thoothukudi, Tirunelveli, Tenkasi and Ramanathapuram districts *viz.,* Kasilingapuram, Koonarkulam, Peikulam, Pudhur pandiyapuram, Solapuram, Ettayapuram, Vallioor, Panangudi, Thalvaipuram, Kavalkinaru, Radhapuram, Kalakudi, Athiyuthu, Pavoor Chathiram, Keezhapavur, Peraiyur, Kamuthi, Keelasirupothu, Sadaiyaneri, Mudhukulathur and Idhampadal. ChiLCD-infected plant samples were collected from all the surveyed areas and maintained in the Department of Plant Pathology glass house, VOC Agricultural College and Research Institute, Killikulam (8.7063° N, 77.8550° E). Totally twenty-one isolates of begomovirus were collected, and an isolated number was given for each sample (Table 1). Based on the disease severity, chilli plants expressing severe leaf curl symptoms were collected from Kalakudi village (BV-KI) of Tirunelveli district and used as a virus source for further studies (Fig 1).

Extraction of DNA

One hundred milligrams of begomovirus-infected chilli leaves of twenty- -isolates were separately ground with 800 µl of CTAB buffer (Cetyltrimethylammonium bromide buffer) using a pestle and mortar. CTAB buffer was commonly used in DNA extraction to help the breakdown of cell membranes and to separate DNA from other cellular components. The grounded mixture of leaves was transferred into a centrifuge tube and added with 52.8 µl of 20% SDS (Sodium Dodecyl Sulfate). The mix in the centrifuge tubes was then incubated in a water bath at 65 ° C for 15 min. After the incubation, 800 µl of phenol: chloroform (9:1) was added to the centrifuge tube. The tube was then centrifuged in an Eppendorf centrifuge (Model Number: 5427R) at 12000 rpm for 10 min. The supernatant was collected in a separate centrifuge tube and added with an equal volume of phenol: chloroform (1:1). The tube was centrifuged again at 12000 rpm for 5 min. The supernatant was collected again and transferred to a new centrifuge tube. The supernatant was added with 700 µl of chloroform and centrifuged at 12000 rpm for 5 min. The supernatant was collected, and 600 µl of isopropanol was added into that and incubated overnight at 4 °C for precipitation. After incubation, the tube was centrifuged at 12000 rpm for 15 min. The supernatant was discarded, and the DNA pellet was washed with 300 µl of 70 percent ice-cold ethanol to remove the remaining impurities. The tube was centrifuged again at 12000 rpm for 5 min, and the supernatant was discarded. The DNA pellet was air-dried for 30 min to remove the remaining ethanol. Finally, 30 µl of nucleus-free water was added to the pellet to resuspend the DNA, and the DNA sample was stored at four °C (14).

Detection of begomovirus causing ChiLCD by using Universal primers

DNA extracted from 21 begomovirus isolates was subjected to Polymerase Chain Reaction (PCR) by using Rojas primer to amplify the begomovirus's top and coat protein region. The following reaction mixture prepared a total volume of 10 µl of reaction.

Table 1. Survey for the incidence of ChiLCD in southern districts of Tamil Nadu

S.No	District	Block	Village	GPS	Name of the Isolate *
1.	Thoothukudi	Karungulam	Kasilingapuram	8.76°N 77.87°E	BV-KP
			Koonarkulam	8.75°N 77.86°E	BV-KM
		Srivaikundam	Peikulam	8.53°N 77.88°E	BV-PK
		Ottapidaram	Pudhur pandiyapuram	8.90°N 78.10°E	BV-PP
		Kayathar	Solapuram	9.10°N 78.00°E	BV-SP
		Kovilpatti	Ettayapuram	8.33°N 77.60°E	BV-EP
2.	Tirunelvlei	Vallioor	Vallioor	8.33°N 77.60°E	BV-VR
			Panangudi	8.34°N 77.60°E	BV-P
			Thalvaipuram	8.34°N 77.57°E	BV-TP
			Kavalkinaru	8.27°N 77.57°E	BV-KAK
		Radhapuram	Radhapuram	8.34°N 77.57°E	BV-RP
		Manoor	Kalakudi	8.88°N 77.63°E	BV-KI
3.	Tenkasi	Aalangulam	Athiyuthu	8.88°N 77.46°E	BV-AU
		Keezhapavur	Pavoor chathiram	8.88°N 77.42°E	BV-PC
			Keezhapavur	8.89°N 77.38°E	BV-KP
4.	Ramanathapuram	Kamuthi	Peraiyur	9.37°N 78.43°E	BV-PR
			Kamuthi	9.40°N 78.38°E	BV-KA
		Mudhukulathur	Keelasirupothu	9.28°N 78.59°E	BV-KS
			Sadaiyaneri	9.30°N 78.56°E	BV-SN
			Mudhukulathur	9.31°N 78.55°E	BV-MK
		Kadaladi	Idhampadal	9.24°N 78.68°E	BV-IP

* BV -Begomovirus

Fig. 1. Chilli Leaf curl disease infected chilli plant

Reaction mixtures

2X Master mix	:	4 µl
Nuclease free water	:	4 µl
Roja's Forward primer	:	0.5 µl
Roja's Reverse Primer	:	0.5 µl
Template DNA	:	1 µl
		10 µl

Initial Denaturation	: 94 °C for 5 min
Denaturation	: 94 °C for 30 sec
Annealing	: 52 °C for 30 sec
Extension	: 72 °C for 60 sec
Final Extension	: 72 °C for 10 min

35 Cycles

The top and coat protein region of DNA - A of begomovirus was amplified from the DNA of 21 isolates by following the below mentioned PCR conditions

The PCR reaction was carried out in BIORAD T100 Thermal cycler PCR machine. PCR products were checked by one per cent agarose gel electrophoresis. The primers used for the amplification of top and coat protein region begomovirus are listed below (15)

Rojas 1 -

2X Master mix	:	3 µl
Nuclease free water	:	4 µl
ChiLCV Forward primer	:	1 µl
ChiLCV Reverse Primer	:	1 µl
Template DNA	:	1 µl
		10 µl

5'ACNGGNAARACNATGTGGGC3'

Rojas 2 - 5'GGNAARATHTGGATGGA3'

Initial Denaturation	: 94 °C for 4 min
Denaturation	: 94 °C for 60 sec
Annealing	: 58 °C for 60 sec
Extension	: 72 °C for 60 sec
Final Extension	: 72 °C for 10 min

35 Cycles

Detection of ChiLCV using CP primer

DNA extracted from 21 begomovirus isolates was subjected to PCR using CP primer to amplify the coat protein region of ChiLCV. A total volume of 10 µl of reaction was prepared by following the reaction mixture.

Reaction mixtures

The CP region of ChiLCV was amplified from the DNA of 21 isolates by following the below mentioned PCR conditions

2X Master mix	:	8 µl
Nuclease free water	:	8 µl
Beta Forward primer	:	1 µl
Beta Reverse Primer	:	1 µl
Template DNA	:	2 µl
		20 µl

PCR reaction was carried out in BIORAD T100 Thermal cycler PCR machine. PCR products were checked by one per cent agarose gel electrophoresis.

Initial Denaturation	:94 °C for 5 min
Denaturation	:94 °C for 30 sec
Annealing	:50 °C for 60 sec
Extension	:72 °C for 90 sec
Final Extension	:72 °C for 10 min

35 Cycles

The primers used for the amplification of CP gene of Chilli leaf curl virus

CLA 5F – 5'AAGAACCGTTCACGGTTTTAGGTT3'

CLA 8R - 5'TTCATTTCTTAAGGGTATTTAGGACAA3'

Detection of Beta satellite virus associated with ChiLCV

DNA extracted from 21 isolates of begomovirus were subjected to PCR by using beta satellite virus specific primer to amplify the beta satellite virus. The preparation of total volume of 20 µl of reaction was carried out by the following reaction mixture.

Reaction mixtures

The beta satellite virus associated with ChiLCV was amplified from the DNA of 21 isolates with the following the below mentioned PCR condition

The PCR reaction was carried out in BIORAD T100 Thermal cycler PCR machine. PCR products were checked by one per cent agarose gel electrophoresis.

The primer was used for the amplification of beta satellite virus associated with ChiLCV.

Beta01– 5'GGTACCACTACGCTACGCAGCAGCC3'

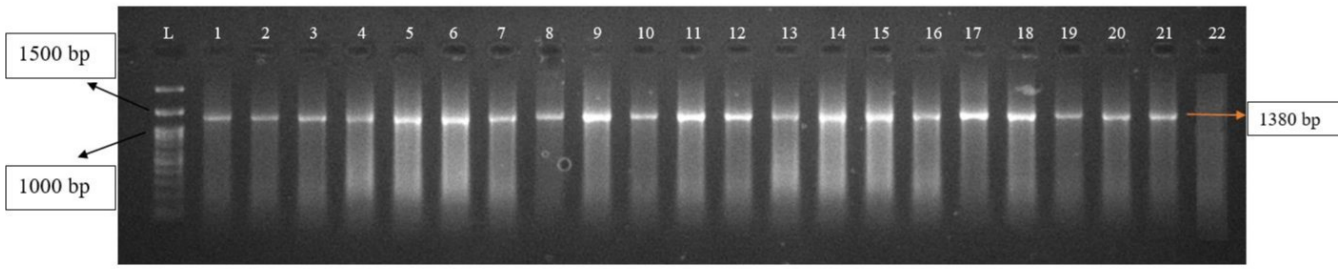
Beta02- 5'GGTACCTACCTCCCAGGGGTACA3'

Sequencing and Phylogenetic Analysis

The amplified product of the top and coat protein region of DNA -A of begamovirus, CP gene of ChiLCV, beta satellite virus associated with ChiLCV of BV-KI isolate was sent to Eurofins Genomics, Bangalore, for sequencing. After obtaining the sequence, it was submitted to the NCBI Gene Bank. CLUSTAL-W was used to compare and analyze the sequence of top and coat protein region of DNA -A of begomovirus, CP gene of ChiLCV, beta satellite virus associated with ChiLCV of BV-KI isolate with the sequence of other known begomovirus isolates from Gene bank. MEGA version 11.0 was used to create the phylogenetic tree at the molecular level. Sequences for comparison were retrieved from Gene Bank through BLAST. The analysis was done on top and coat protein regions of DNA -A of begamovirus, CP gene of ChiLCV and beta satellite virus associated with ChiLCV sequences with a bootstrap percentage (1000 replication).

Results and Discussion

Detection of begomovirus causing ChiLCD by using Rojas Universal primers



L- 100 bp Ladder
1- BV-KP
2- BV-KM
3- BV-PK
4- BV-PP
5- BV-SP
6- BV-EP
7- BV-VR
8- BV-P
9- BV-TP
10- BV-KAK
11- BV-RP
12- BV-KI
13- BV-AU
14- BV-PC
15- BV-KP
16- BV-PR
17- BV-KA
18- BV-KS
19- BV-SN
20- BV-MK
21- BV-IP
22- Healthy chilli sample

Fig. 2. Detection of begomovirus associated with ChiLCD-infected chilli samples by PCR using Rojas universal primer

Sequences producing significant alignments Download Select columns Show 100

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
Chilli leaf curl virus-[Bhavanisagar:India:2010] segment DNA A, complete sequence	Chilli leaf curl vir...	479	479	38%	5e-130	90.61%	2785	NC_055130.1
Chilli leaf curl virus isolate CL-15 from Sri Lanka, complete genome	Chilli leaf curl virus	473	473	38%	2e-128	90.33%	2754	JN555600.1
Corchorus yellow vein mosaic virus clone pDatB2 segment DNA-A, complete sequence	Corchorus yellow...	429	429	38%	5e-115	88.12%	2742	KX513862.1
Chilli leaf curl Ahmedabad virus isolate CHL31, complete genome	Chilli leaf curl Ah...	429	429	38%	5e-115	87.77%	2748	MW795673.1
Eggplant leaf curl Chhattisgarh virus isolate BLC4-CH segment DNA-A, complete sequence	Eggplant leaf cur...	425	425	36%	7e-114	88.99%	2771	OM315015.1
Chilli Leaf curl Salem virus-India [India/Salem/2008] clone pChSalH36 segment DNA-A, complete sequence	Chilli leaf curl Sal...	425	425	39%	7e-114	87.20%	2783	HM007119.1
Corchorus yellow vein mosaic virus isolate CEA8, complete genome	Corchorus yellow...	424	424	38%	3e-113	87.85%	2743	NC_020473.1
Corchorus yellow vein mosaic virus isolate CEA8, complete genome	Corchorus yellow...	424	424	38%	3e-113	87.85%	2743	KC223600.1
Corchorus yellow vein mosaic virus clone CEA9, complete sequence	Corchorus yellow...	424	424	38%	3e-113	87.85%	2742	KC196077.1
Chilli leaf curl Ahmedabad virus isolate CHL3, complete genome	Chilli leaf curl Ah...	422	422	35%	9e-113	89.32%	2752	MW795670.1
French bean severe leaf curl virus isolate FbLCV-Cas-Jal, complete genome	French bean sev...	412	412	37%	5e-110	87.82%	2735	KC699544.1
Chilli leaf curl Ahmedabad virus-India isolate CHL44, complete genome	Chilli leaf curl Ah...	407	407	36%	3e-108	87.90%	2744	MN417110.1
Chilli leaf curl Ahmedabad virus isolate CHL62, complete genome	Chilli leaf curl Ah...	407	407	38%	3e-108	86.68%	2746	MW861357.1
Chilli leaf curl virus isolate AKS1-VNS segment DNA-A, complete sequence	Chilli leaf curl virus	403	403	36%	3e-107	87.83%	2760	MH346125.1
Chilli leaf curl virus isolate TC-Vns segment DNA A, complete sequence	Chilli leaf curl virus	403	403	36%	3e-107	87.83%	2762	KP868762.1
Chilli leaf curl virus isolate KLD_RVA segment DNA-A, complete sequence	Chilli leaf curl virus	401	401	36%	1e-106	87.61%	2764	OQ148478.1

Fig. 3. NCBI blast search using top and CP region sequence of DNA-A of BV-KI isolate

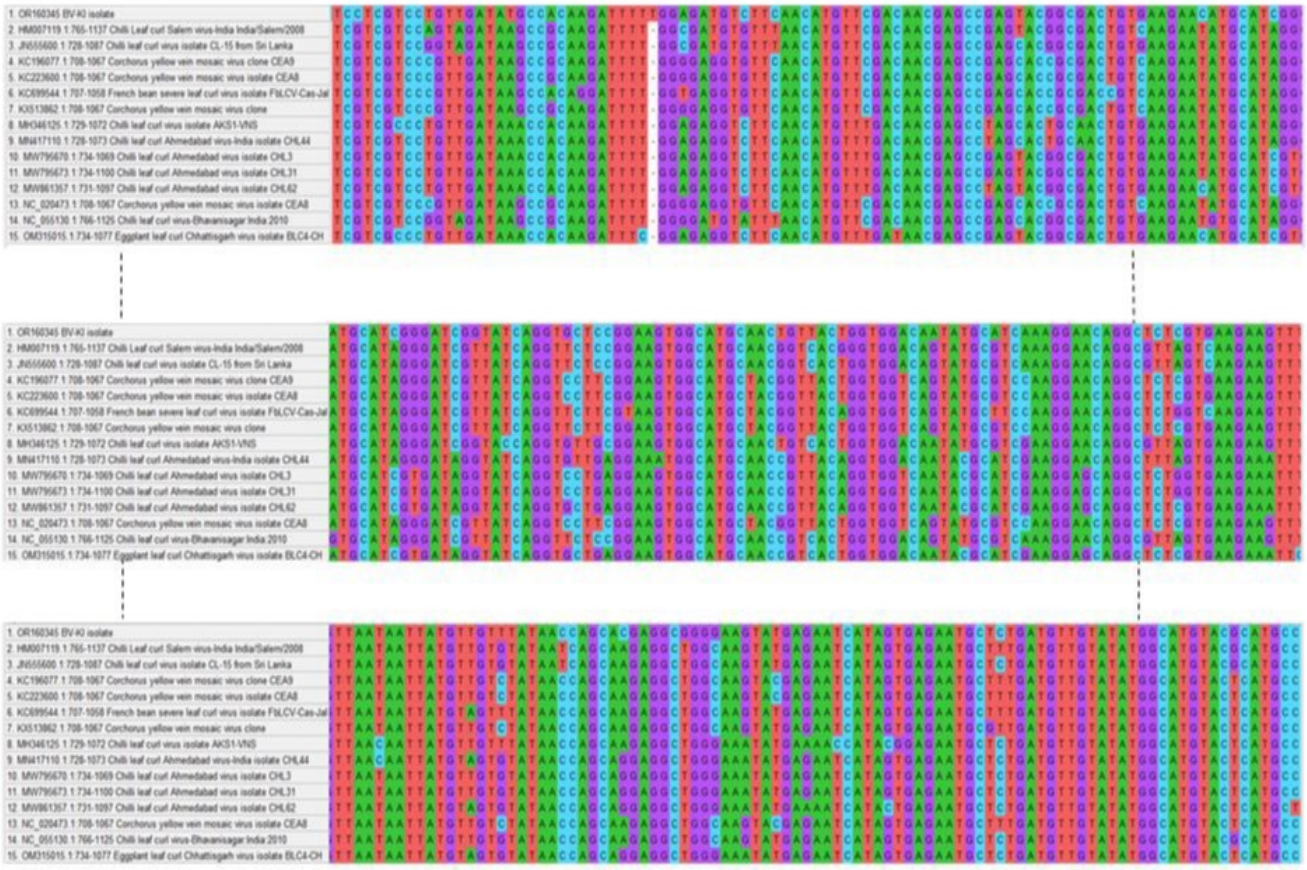


Fig. 4. Multiple alignments of the nucleotide sequence of the top and CP region of BV-KI isolate with other isolates of begomovirus sequence from Gene Bank

Total DNA was extracted from the ChiLCD-infected chilli leaves collected from 21 villages (21 isolates) of Southern districts of Tamil Nadu, and healthy chilli plant tissue was collected from the plants maintained in the insect-proof cage under a glass house. The total DNA was subjected to PCR using Rojas Universal primer to amplify the top and coat protein region of DNA - A of begomovirus. In the PCR analysis, 1380 bp amplicon sizes were obtained from all the (21 isolates) infected samples, whereas no amplicon was obtained in healthy samples (Fig 1). The amplicon obtained at 1380 bp from the isolate BV-KI was sequenced and submitted to the NCBI Gene Bank database (Accession Number: OR160345). The sequence was analyzed through Blast and CLUSTAL-W. The results revealed that the nucleotide sequence of the top and coat protein region of DNA-A of BV-KI isolate was found to be 90.61 per cent and 90.33 percent, similar to the ChiLCV isolate of Bhavanisagar (NC 055130.1) and Srilankan isolates respectively (Fig. 2 and 3). A cluster dendrogram was constructed based on MEGA 11.0 analysis of nucleotide sequence of top and coat protein gene of DNA-A of BV-KI isolate (Accession Number: OR160345) with other begomovirus sequences already available in the NCBI database. The results revealed that the BV-KI isolate was closely related to multiple ChiLCV isolates from different hosts, forming one cluster (Fig. 4). This result is relevant to the reports of Senanayake *et al*. (10), who sequenced and constructed a phylogenetic tree for ChiLCV infecting Chilli from Rajasthan. This isolate showed 96.10 % similarity with ChiLCV Varanasi isolate (EF190217).

Detection of ChiLCV using CP primer

Total DNA extracted from all the 21 isolates of ChiLCD infected samples and the healthy sample were subjected to PCR using ChiLCV CP gene-specific primers. In the PCR analysis, 1100 bp amplicon was obtained from all the infected samples. In contrast, no amplicon was obtained in the healthy sample (Fig. 5). This is in accordance with the findings of Kumar *et al.* (16). They obtained 1100 bp size of the CP gene of ChiLCV from the total DNA extracted from the ChiLCV infected chilli tissues using CP gene-specific primers in PCR.

In the present study, the amplicon obtained at 1100 bp from the isolate BV-KI was sequenced and submitted to the NCBI Gene Bank database (OR 612058). The sequence was analyzed through Blast and CLUSTAL-W. The results revealed that the nucleotide sequence of the CP gene of BV-KI isolate was found to be 99.78 and 92.17%, similar to the ChiLCV isolate of Bhavanisagar (NC 055130.1) and Srilankan isolates (JN555600.1) respectively (Fig. 6 and 7). A clustal dendrogram was constructed based on MEGA 11.0 analysis of the nucleotide sequence of the ChiLCV CP gene of BV-KI isolate (OR 612058) with other ChiLCV CP gene sequences already available in the NCBI database. The results revealed that the BV-KI isolate was closely related to multiple ChiLCV isolates from different hosts, forming one cluster (Fig. 8). This result is relevant to the reports of Kumar *et al*. (16). They reported that the CP gene sequence of Chilli leaf curl virus isolate has 99.4 %

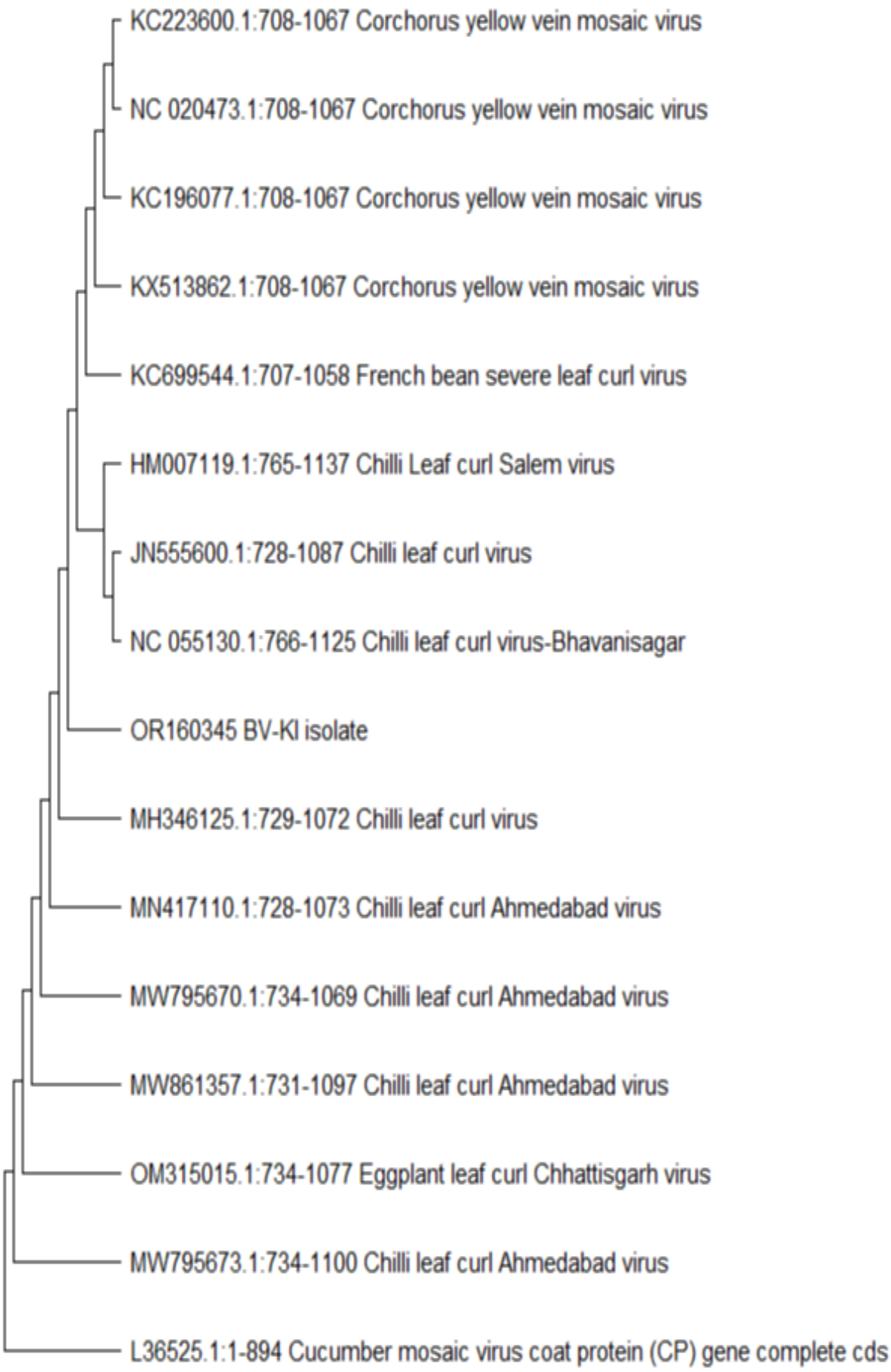
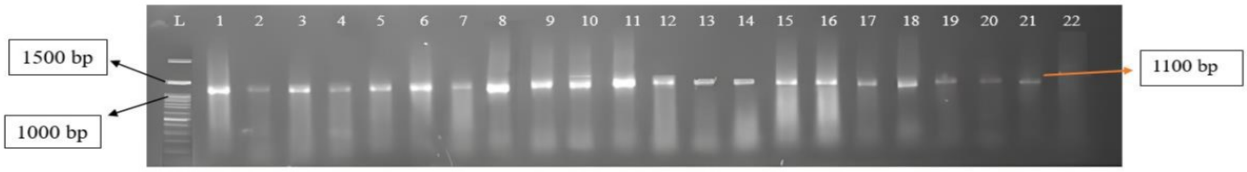
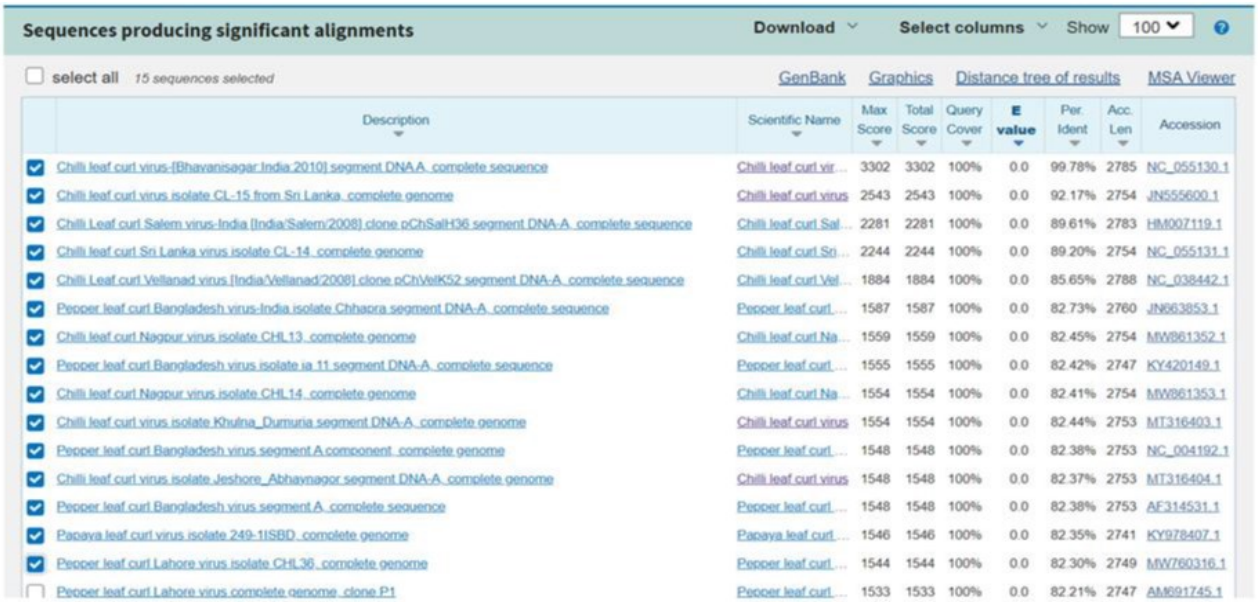


Fig. 5. Phylogenetic tree of top and coat protein region of DNA-A sequence of BV-KI Isolate



L- 100 bp Ladder
1- BV-KP
2- BV-KM
3- BV-PK
4- BV-PP
5- BV-SP
6- BV-EP
7- BV-VR
8- BV-P
9- BV-TP
10- BV-KAK
11- BV-RP
12- BV-KI
13- BV-AU
14- BV-PC
15- BV-KP
16- BV-PR
17- BV-KA
18- BV-KS
19- BV-SN
20- BV-MK
21- BV-IP
22- Healthy chilli sample

Fig 6. Detection of ChiLCV in ChiLCD-infected samples by PCR using coat protein-specific primer



Sequences producing significant alignments Download Select columns Show 100

select all *15 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chilli leaf curl virus-[Bhavanisagar India 2010] segment DNA A, complete sequence	Chilli leaf curl vir...	3302	3302	100%	0.0	99.78%	2785	NC_055130.1
Chilli leaf curl virus isolate CL-15 from Sri Lanka, complete genome	Chilli leaf curl virus	2543	2543	100%	0.0	92.17%	2754	JN555600.1
Chilli Leaf curl Salem virus-India [India:Salem:2008] clone pChSalH36 segment DNA-A, complete sequence	Chilli leaf curl Sal...	2281	2281	100%	0.0	89.61%	2783	HM007119.1
Chilli leaf curl Sri Lanka virus isolate CL-14, complete genome	Chilli leaf curl Sri...	2244	2244	100%	0.0	89.20%	2754	NC_055131.1
Chilli Leaf curl Vellanad virus [India:Vellanad:2008] clone pChVelK52 segment DNA-A, complete sequence	Chilli leaf curl Vel...	1884	1884	100%	0.0	85.65%	2788	NC_038442.1
Pepper leaf curl Bangladesh virus-India isolate Chhapra segment DNA-A, complete sequence	Pepper leaf curl...	1587	1587	100%	0.0	82.73%	2760	JN663853.1
Chilli leaf curl Nagpur virus isolate CHL13, complete genome	Chilli leaf curl Na...	1559	1559	100%	0.0	82.45%	2754	MW861352.1
Pepper leaf curl Bangladesh virus isolate ia 11 segment DNA-A, complete sequence	Pepper leaf curl...	1555	1555	100%	0.0	82.42%	2747	KY420149.1
Chilli leaf curl Nagpur virus isolate CHL14, complete genome	Chilli leaf curl Na...	1554	1554	100%	0.0	82.41%	2754	MW861353.1
Chilli leaf curl virus isolate Khulna_Dumuria segment DNA-A, complete genome	Chilli leaf curl virus	1554	1554	100%	0.0	82.44%	2753	MT316403.1
Pepper leaf curl Bangladesh virus segment A component, complete genome	Pepper leaf curl...	1548	1548	100%	0.0	82.38%	2753	NC_004192.1
Chilli leaf curl virus isolate Jeshore_Abhaynagor segment DNA-A, complete genome	Chilli leaf curl virus	1548	1548	100%	0.0	82.37%	2753	MT316404.1
Pepper leaf curl Bangladesh virus segment A, complete sequence	Pepper leaf curl...	1548	1548	100%	0.0	82.38%	2753	AF314531.1
Papaya leaf curl virus isolate 249-1ISBD, complete genome	Papaya leaf curl...	1546	1546	100%	0.0	82.35%	2741	KY978407.1
Pepper leaf curl Lahore virus isolate CHL36, complete genome	Pepper leaf curl...	1544	1544	100%	0.0	82.30%	2749	MW760316.1
Pepper leaf curl Lahore virus complete genome, clone P1	Pepper leaf curl...	1533	1533	100%	0.0	82.21%	2747	AM691745.1

Fig. 7. NCBI blast search using ChiLCV CP sequence of BV-KI isolate

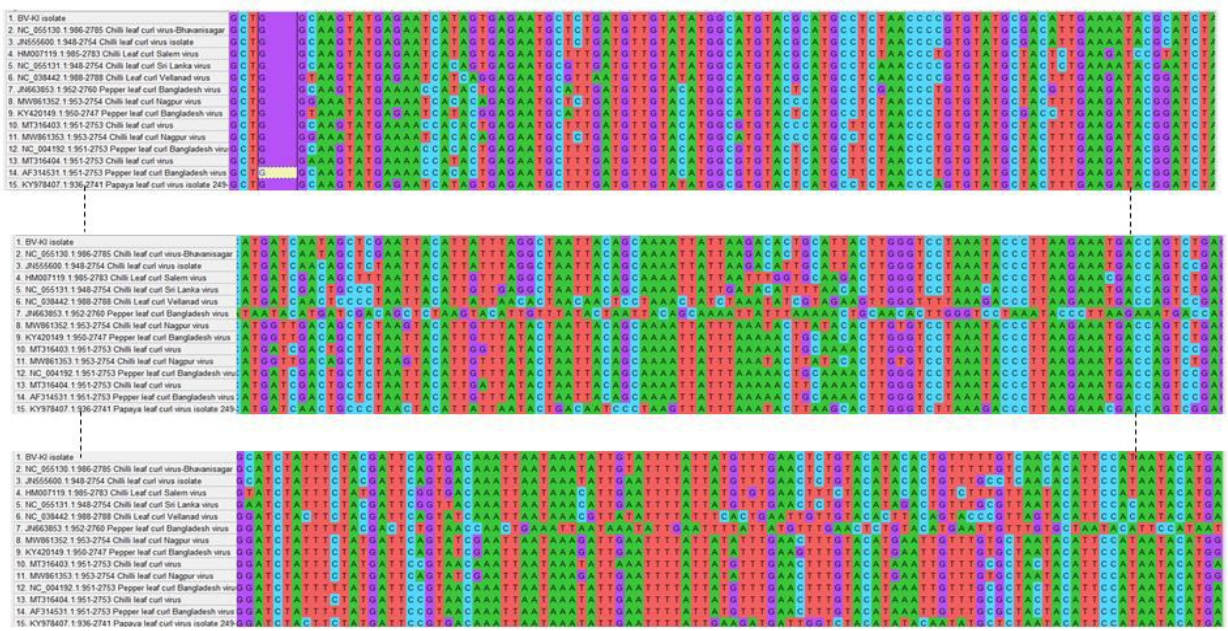


Fig. 8. Multiple alignments of ChiLCV CP nucleotide sequence of BV- KI isolate

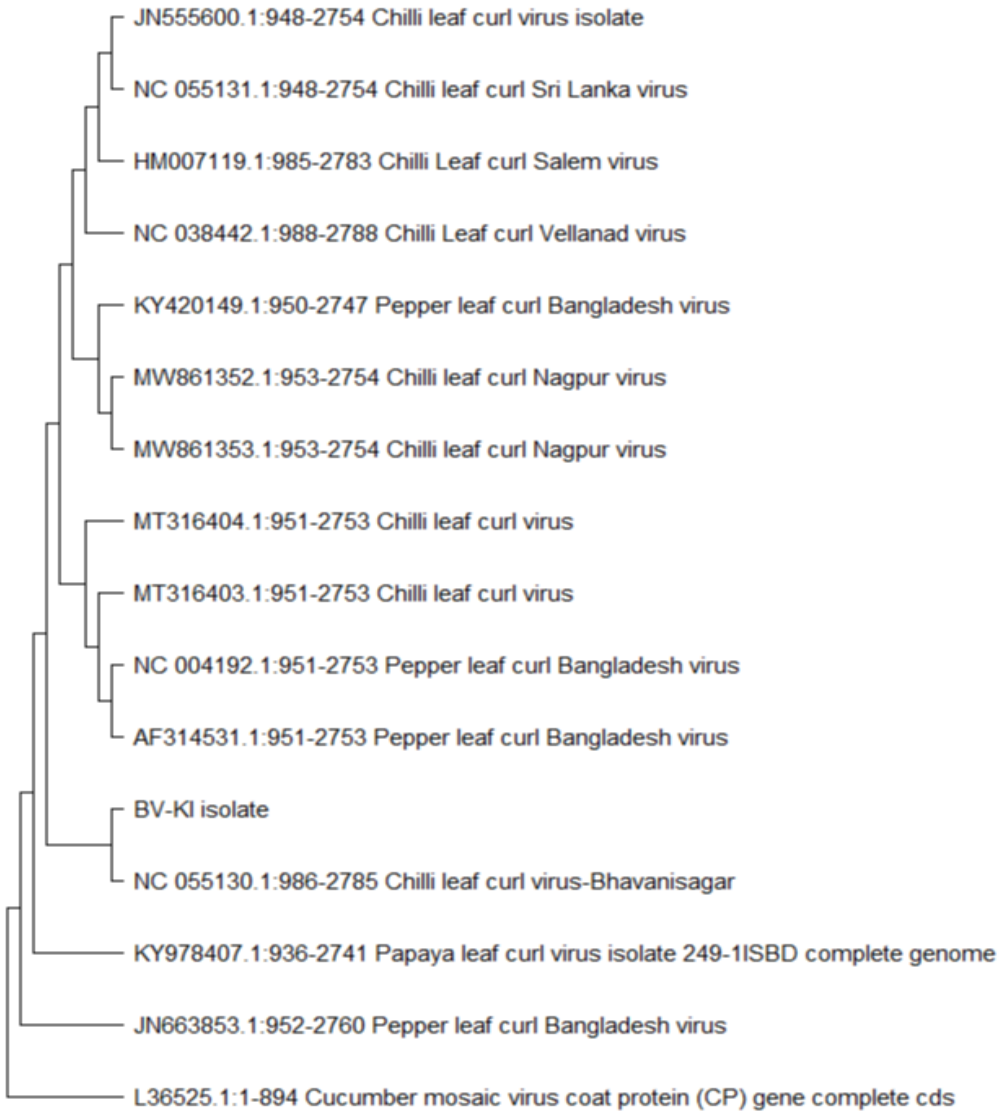
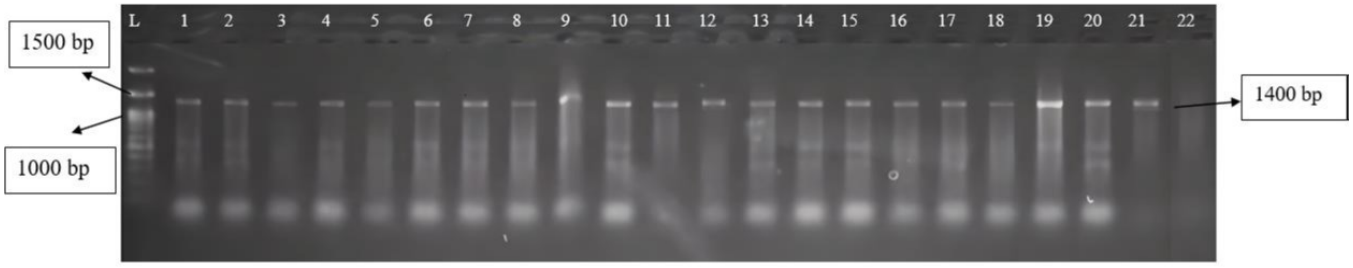
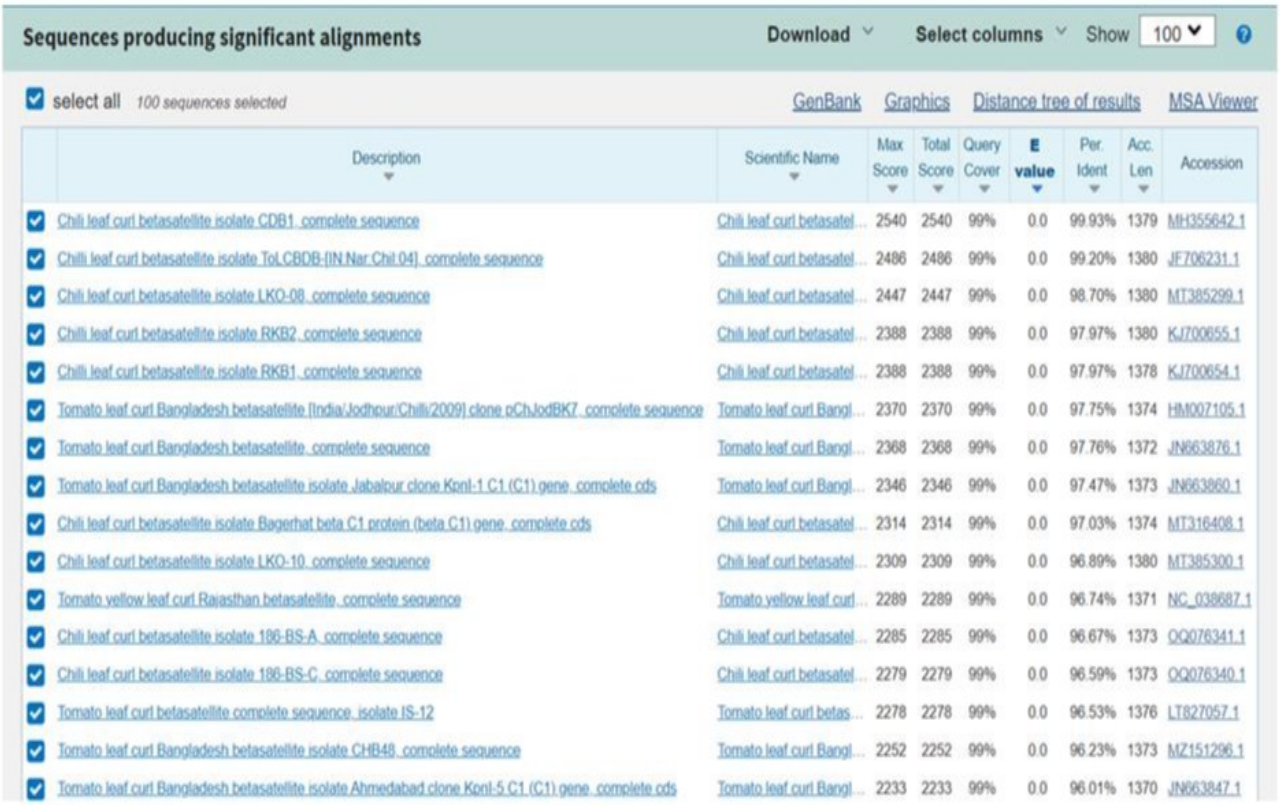


Fig. 9. Phylogenetic tree of ChiLCV based on Coat Protein nucleotide sequences of BV-KI isolate



L- 100 bp Ladder
1- BV-KP
2- BV-KM
3- BV-PK
4- BV-PP
5- BV-SP
6- BV-EP
7- BV-VR
8- BV-P
9- BV-TP
10- BV-KAK
11- BV-RP
12- BV-KI
13- BV-AU
14- BV-PC
15- BV-KP
16- BV-PR
17- BV-KA
18- BV-KS
19- BV-SN
20- BV-MK
21- BV-IP
22- Healthy chilli sample

Fig. 10. Detection of beta satellite virus associated with ChiLCV in the infected chilli samples by using specific primers



Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chili leaf curl betasatellite isolate CDB1, complete sequence	Chili leaf curl betasatel...	2540	2540	99%	0.0	99.93%	1379	MH355642.1
Chilli leaf curl betasatellite isolate ToLCBDB-[IN:Nar:Chil:04], complete sequence	Chili leaf curl betasatel...	2486	2486	99%	0.0	99.20%	1380	JF706231.1
Chili leaf curl betasatellite isolate LKO-08, complete sequence	Chili leaf curl betasatel...	2447	2447	99%	0.0	98.70%	1380	MT385299.1
Chilli leaf curl betasatellite isolate RKB2, complete sequence	Chili leaf curl betasatel...	2388	2388	99%	0.0	97.97%	1380	KJ700655.1
Chilli leaf curl betasatellite isolate RKB1, complete sequence	Chili leaf curl betasatel...	2388	2388	99%	0.0	97.97%	1378	KJ700654.1
Tomato leaf curl Bangladesh betasatellite [India/Jodhpur/Chilli/2009] clone pChJodBK7, complete sequence	Tomato leaf curl Bangl...	2370	2370	99%	0.0	97.75%	1374	HM007105.1
Tomato leaf curl Bangladesh betasatellite, complete sequence	Tomato leaf curl Bangl...	2368	2368	99%	0.0	97.76%	1372	JN663876.1
Tomato leaf curl Bangladesh betasatellite isolate Jabalpur clone KpnI-1 C1 (C1) gene, complete cds	Tomato leaf curl Bangl...	2346	2346	99%	0.0	97.47%	1373	JN663860.1
Chili leaf curl betasatellite isolate Bagerhat beta C1 protein (beta C1) gene, complete cds	Chili leaf curl betasatel...	2314	2314	99%	0.0	97.03%	1374	MT316408.1
Chili leaf curl betasatellite isolate LKO-10, complete sequence	Chili leaf curl betasatel...	2309	2309	99%	0.0	96.89%	1380	MT385300.1
Tomato yellow leaf curl Rajasthan betasatellite, complete sequence	Tomato yellow leaf curl...	2289	2289	99%	0.0	96.74%	1371	NC_038687.1
Chili leaf curl betasatellite isolate 186-BS-A, complete sequence	Chili leaf curl betasatel...	2285	2285	99%	0.0	96.67%	1373	OQ076341.1
Chili leaf curl betasatellite isolate 186-BS-C, complete sequence	Chili leaf curl betasatel...	2279	2279	99%	0.0	96.59%	1373	OQ076340.1
Tomato leaf curl betasatellite complete sequence, isolate IS-12	Tomato leaf curl betas...	2278	2278	99%	0.0	96.53%	1376	LT827057.1
Tomato leaf curl Bangladesh betasatellite isolate CHB48, complete sequence	Tomato leaf curl Bangl...	2252	2252	99%	0.0	96.23%	1373	MZ151296.1
Tomato leaf curl Bangladesh betasatellite isolate Ahmedabad clone KpnI-5 C1 (C1) gene, complete cds	Tomato leaf curl Bangl...	2233	2233	99%	0.0	96.01%	1370	JN663847.1

Fig. 11. NCBI blast search using sequence of beta satellite virus associated with ChiLCV of BV-KI isolate

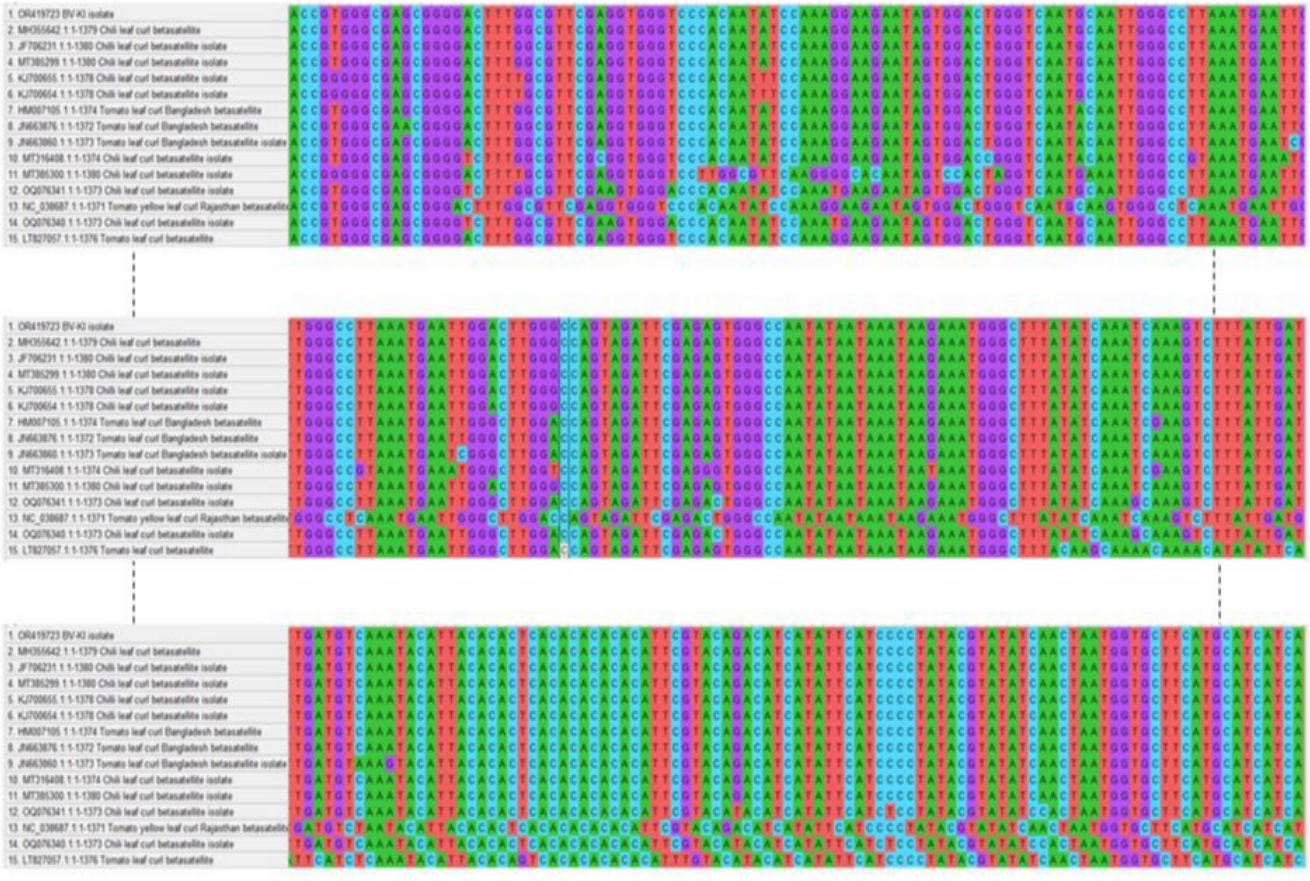


Fig. 12. Multiple alignments of the nucleotide sequence of beta satellite virus associated with ChiLCV BV-KI isolate with other isolates of ChiLCV sequence

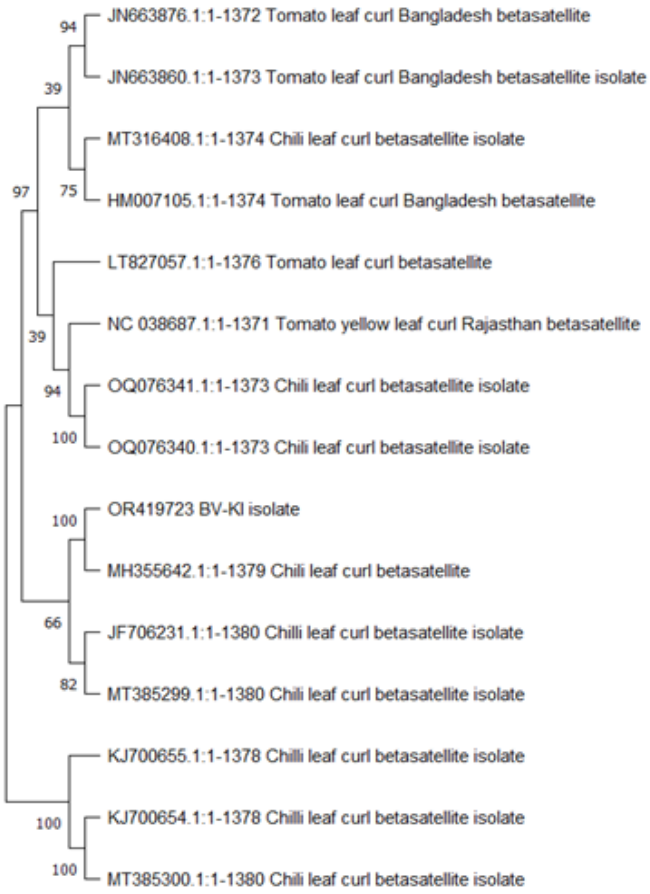


Fig. 13. Phylogenetic tree of beta satellite virus associated with ChiLCV of BV-KI isolate

similarity with the Chilli leaf curl virus Ahmadabad isolate.

Detection of Beta satellite virus associated with ChiLCV

Total DNA extracted from all 21 isolates of ChiLCD-infected samples and healthy samples were subjected to PCR by using Beta satellite primer. The PCR analysis obtained 1400 bp amplicon sizes from all the infected samples. In contrast, no amplicon was obtained from healthy samples (Fig. 9). The amplicon obtained at 1400 bp from the isolate BV-KI was sequenced and submitted to the NCBI Gene Bank database (Accession Number: OR419723). The sequence was analyzed using blast and CLUSTAL-W. The results revealed that the nucleotide sequence of the beta satellite virus of BV-KI isolate was found to be 99.93, 99.20 and 98.70% similarity with the beta satellite virus isolate of Chili leaf curl beta satellite isolate from Meerut (MH355642.1), Narwan (JF706231.1) and Lucknow (MT385299.1) respectively (Fig. 10 and 11). A clustal dendrogram was constructed based on MEGA 11.0 analysis of the nucleotide sequence of the beta satellite virus of BV-KI isolate (Accession Number: OR419723) with other beta satellite virus sequences already available in the NCBI database. The results revealed that the BV-KI isolate was closely related to multiple beta satellite virus isolates associated with ChiLCV from different hosts and forming one cluster (Fig. 12 and 13). Senanayake *et al*. (10) constructed a phylogenetic tree for the sequence of beta satellite virus associated with ChiLCV from Narwan (AY438558). The results revealed that the beta satellite virus associated with ChiLCV has a 97.3% identity with the Tomato leaf curl beta satellite virus Rajasthan isolate.

Conclusion

Fourteen begomovirus species from different countries of India and its subcontinents were reported as causative agents for ChiLCD. The present study concludes that, in Tamil Nadu, ChiLCD is caused by ChiLCV, which belongs to the begomovirus species. ChiLCV is also associated with the beta satellite virus, which has main role in symptom expression. Identification of the type of begomovirus species causing ChiLCD will pave the way for developing a management strategy for this disease.

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Authors' contributions

Author NR carried out the molecular work and designed the study, JM carried out sequence alignment and part of the molecular work, KE drafted the manuscript, ES carried out the survey, and MT, MIM and JS assisted in the

molecular work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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