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EMERGENCE OF NEW VARIANT OF CHILLI LEAF CURL VIRUS IN NORTH INDIA

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Summary

The DNA from four leaf samples of Chilli Leaf Curl Virus (ChiLCV) symptomatic chilli plants collected from Varanasi, Mirzapur, Gorakhpur and Mahrajganj districts of Uttar Pradesh, India were isolated. Using coat protein gene (*CP*) specific primers, highly conserved coat protein regions of four isolates were amplified, sequenced and compared with 25 similar begomovirus sequences obtained from National Centre for Biotechnology Information (NCBI). The CP sequence identity of Varanasi isolate was 86% with Gorakhpur and Mirzapur isolates and 87% with Mahrajganj isolate. Phylogenetic relationships based on CP sequence similarity index grouped 29 sequences into three major clusters. The Gorakhpur, Mahrajganj and Mirzapur isolates were clustered together with tomato leaf curl Joydebpur virus-[Kalyani] isolated from chilli, whereas Varanasi isolate clustered with chilli leaf curl virus-[Amritsar:Papaya] in a separate cluster. Thus, based on very low CP sequence identity between Varanasi isolate (ChiLCV-VNS) and other three isolates from Gorakhpur (ChiLCV-GKP), Mahrajganj (ChiLCV-MAH) and Mirzapur (ChiLCV-MZP), it is concluded that there is existence of a new variants of begomovirus, ChiLCV, infecting chilli crop in North India.

सारांश

मिर्च के पत्तीमोंड़ विषाणु रोग के लिए हाइली कन्जर्ब्ड कोट प्रोटीन जीन (CP) सेक्वेंस के अध्ययन के लिए रोग ग्रसित पत्तियां उत्तर प्रदेश (इण्डिया) के चार जनपदों वाराणसी, मीरजापुर, गोरखपुर तथा महराजगंज से एकत्रित किया गया। डी.एन.ए. निकालने के बाद CP स्पेशाफिक प्राइमर को सभी चारों नमूनों पर एम्प्लीफाई कराया गया। एम्प्लीफाइड प्रोडक्ट को सेक्वेंस किया गया तथा उसको नेशनल सेन्टर फार वायोटेक्नोलाजी इन्फार्मेसन (NCBI) से प्राप्त 25 समान वेगमोंवायरस से मिलान कराया गया। कन्जर्ब्ड प्रोटीन (CP) वाराणसी 86% गोरखपुर तथा मीरजापुर से और 87% महराजगंज आइसोलेट मिलता पाया गया। CP सेक्वेंस की समानता के आधार पर फाइलोजेनेटिक रिश्तो में 29 स्ट्रेन्स प्रयोग किए गये जो तीन मेजर ग्रुप में विभाजित हुए। आइसोलेट गोरखपुर, महराजगंज तथा मीरजापुर के साथ टोमैटो लीफ कर्ल (जयदेवपुर जो कि मिर्च में पाये गये थे) एक ग्रुप में पाये गये जबकि वाराणसी आइसोलेट चिली लीफ कर्ल वायरस (अमृतसर : पपाया) दूसरे ग्रुप में एक साथ पाये गये। वाराणसी आइसो लेट का मीरजापुर, गोरखपुर तथा महराजगंज के कन्जर्व रीजन से अलगाव पूर्वी उत्तर प्रदेश में एक नये स्ट्रेन की उपस्थिति को प्रदर्शित कर रहा है।

Introduction

The genus *Begomovirus* encompasses geminiviruses, transmitted by the whitefly *Bemisia tabaci* that infect dicotyledonous plants (Stanley *et al*., 2005). Begomoviruses have emerged as a major threat to the production of crops such as chilli, tomato, cucurbits, cassava, beans and cotton (Varma and Malathi, 2003). An increasingly large number of distinct begomoviruses have been isolated from different plant species and characterized as strains and variants based on certain criteria (Fauquet and Stanley, 2005). Chilli Leaf Curl Virus (ChiLCV) causing leaf curl disease along with thrips and mites infestation are threatening chilli production in India (Kumar *et al*., 2006). Until the last decade, the major control measure employed against ChiLCV was intensive use of insecticides targeting whitefly. However, now efforts are underway to identify and develop ChiLCV resistant/tolerant germplasm (Kumar *et al*., 2006; Rai, 2010). The ChiLCV has a chimeric genome perhaps derived through recombination between ChiLCV and ToLCV (Khan *et al*., 2006). Understanding on diversity and distribution of ChiLCV variants/strains is crucial for effective screening of *Capsicum* germplasm against virus and to conduct investigations on genotype-virus interactions. The emergence and existence of different variants of ChiLCV, infecting chilli in North India, has been demonstrated in this communication.

Materials and Methods

DNA isolation and ChiLCV detection: Leaf samples of ChiLCV symptomatic chilli plants (Kumar *et al.*, 2006) were collected from the chilli fields in Varanasi, Mirzapur, Gorakhpur and Mahrajganj districts of Uttar Pradesh, India. The viruses were preserved as leaf samples in deep freezer (-80ºC) until total DNA was isolated using cetyl-trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). The DNA samples were used as template for polymerase chain reaction (PCR) using degenerate primers (Wyatt and Brown, 1996) to detect begomoviral genome.

Diversity analysis: The coat protein gene sequences of reported ChiLCV accessions were obtained from GenBank (http://www.ncbi.nlm.nih.gov) and primers specific to ChiLCV coat protein gene were designed using primer-BLAST (NCBI).

ChiLCV-CP (F) 5'- AGGGCTAAGGTCTAGATGTCCACACA -3'

ChiLCV-CP (R) 5'-TGTTCAATCACAACCTGAGGAAAGCG-3′

Coat protein (CP) sequence of each sample was amplified, products were separated on a 1% agarose gel and visualized by staining with ethidium bromide on UV transilluminator (Bangalore Genei, Bangalore, India). The DNA bands were extracted and purified from the gel by Quiquick Gel Extraction Kit (Quiagen Inc., CA, USA) and sequencing were performed (Applied Biosystems, CA, USA). Sequence similarities were performed using BLASTN 2.2.24 (Basic Local Alignment Search Tool) (Zhang *et al.*, 2000) at NCBI database to obtain information on sequence similarity of the collected samples. Based on sequence similarity index, 25 most similar CP or whole sequences were selected and used for diversity analysis (Table 1). The nucleotide sequences were aligned using ClustalW 2.0.9 (Larkin *et al.*, 2007) with default parameters and phylogenetic relationships of 29 sequence data were determined by using the neighbour-joining method (Mega4.1b program). Bootstrap analyses with 1000 replicates were performed to assess the robustness of the branches.

Results and Discussion

Sequence comparison: In all the four DNA samples from Varanasi, Mirzapur, Gorakhpur and Maharajganj, coat protein gene specific ~650 bp fragments were successfully amplified (Fig. 1) and sequenced. These sequences have been submitted to NCBI database. The sequence comparisons among these four isolates revealed sequence identity ranging from 87% to 99%. The alignment of sequence homology and variation is

Table 1. Reference begomovirus sequences and their GenBank accession numbers

S.No.	Virus type (bi and monopartite DNA-A)	Code used	GenBank no.
1	Pepper leaf curl virus isolate Varanasi	PepLCV-[VNS]	EF190217.1
2	Pepper leaf curl Bangladesh virus segment A, complete sequence	PepLCBV	DQ116881.1
3	Pepper leaf curl virus from soybean coat protein gene, complete cds	PepLCV-[SOY]	DQ343285.2
4	Chilli leaf curl India virus segment A	ChiLCV-[IND]	FM877858.1
5	Chilli leaf curl virus isolate India:Amritsar:Papaya	ChiLCV-[AMT]	GU136803.1
6	Chilli leaf curl Multan virus segment A	ChiLCV-[MUL]	FM179613.1
7	Pepper leaf curl Lahore Virus	PepLCLV	AM404179.1
8	Tomato leaf curl New Delhi virus - chili pepper	ToLCNDV	DQ119573.2
9	Tomato leaf curl New Delhi virus - chili pepper isolate Kanpur	ToLCNDV-[KAN]	DQ431844.1
10	Tomato leaf curl New Delhi virus - chili pepper isolate Bahraich	ToLCNDV-[BAH]	DQ431845.2
11	Tomato leaf curl New Delhi virus-Chilli pepper isolate Lucknow	ToLCNDV-[LUC]	AY883570.2
12	Tomato leaf curl Karnataka virus isolate Lucknow	ToLCKV-[LUC]	EU604297.2
13	Tomato leaf curl Bangalore virus - [India:Kerala]	ToLCBV-[KER]	DQ852623.2
14	Tomato leaf curl Pune virus - [India:Pune]	ToLCV-[PUN]	AY754814.1
15	Tomato leaf curl Bangalore virus-Cotton [Fatehabad]	ToLCBV-[FAT]	AY456684.1
16	Tomato leaf curl Pakistan virus isolate Rahim Yaar Khan	ToLCPV-[RYK]	DQ116884.1
17	Tomato leaf curl Karnataka virus - Bangalore [India:Punjab:Mentha]	ToLCKV-[PJB]	FJ514798.1
18	Tomato leaf curl Joydebpur virus	ToLCJV	DQ673859.1
19	Tomato leaf curl Joydebpur virus-[Kalyani] from chili	ToLCJV-[KLY]	EF194765.1
20	Cabbage leaf curl virus	CbLCuV	U65529.2
21	Croton yellow vein mosaic virus	CrYVMV	AJ507777.1
22	Papaya leaf curl virus from Carica papaya cv. Coimbatore-2	PaLCuV	EU126824.1
23	Mungbean yellow mosaic virus	MYMV	AJ132575.1
24	Groundnut mosaic virus	GMV	HM770022
25	Cucumber mosaic virus	CuMV	AY600989

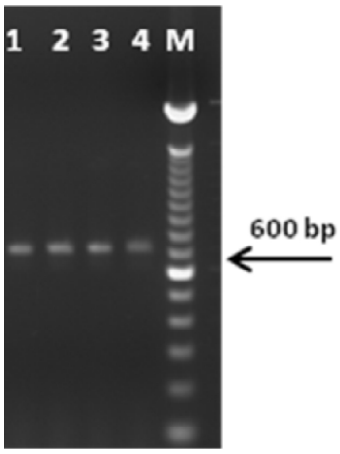

Figure 1. Presence of viral genome in the identified lines. Lane number corresponds to isolates from: (1) Mahrajganj (2) Gorakhpur (3) Varanasi (4) Mirzapur and M:100 bp marker

given in Fig. 2. The maximum coat protein sequence identity was observed between isolates of Gorakhpur and Mahrajganj (99%) and both these isolates had 92% nucleotide sequence identity with Mirzapur isolate. The Varanasi isolate had very less sequence identity with Gorakhpur and Mirzapur isolates (86%) and with Mahrajganj isolates (87%). Thus, based on pair wise comparison of sequence identity (Fauquet and Stanley, 2005), Varanasi isolate differs from the other three isolates. Hence, Varanasi isolate can be considered as different variant of begomovirus infecting chilli. Recombination between DNA-A molecules is considered to be a major reason for frequent occurrence of new begomovirus strains (Kirthi *et al.*, 2002).

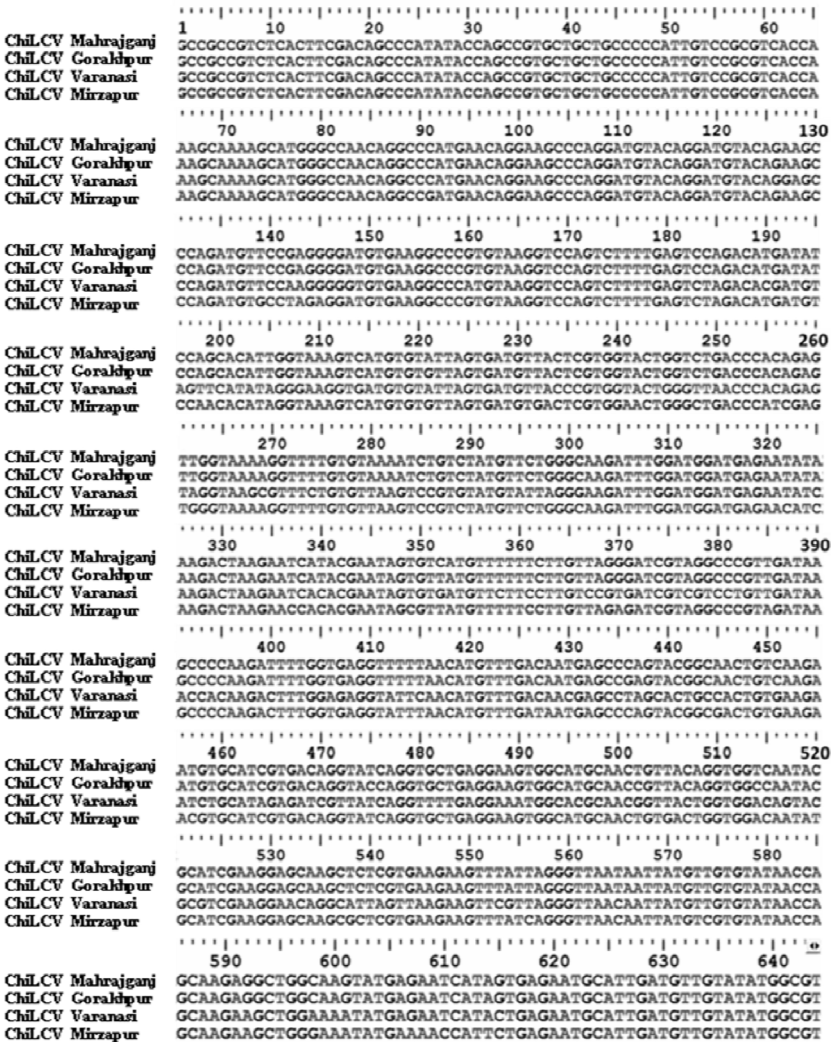

Figure 2. Comparision in coat protein nucleotide sequences of four collected ChiLCV isolates by CLUSTAL-W alignment.

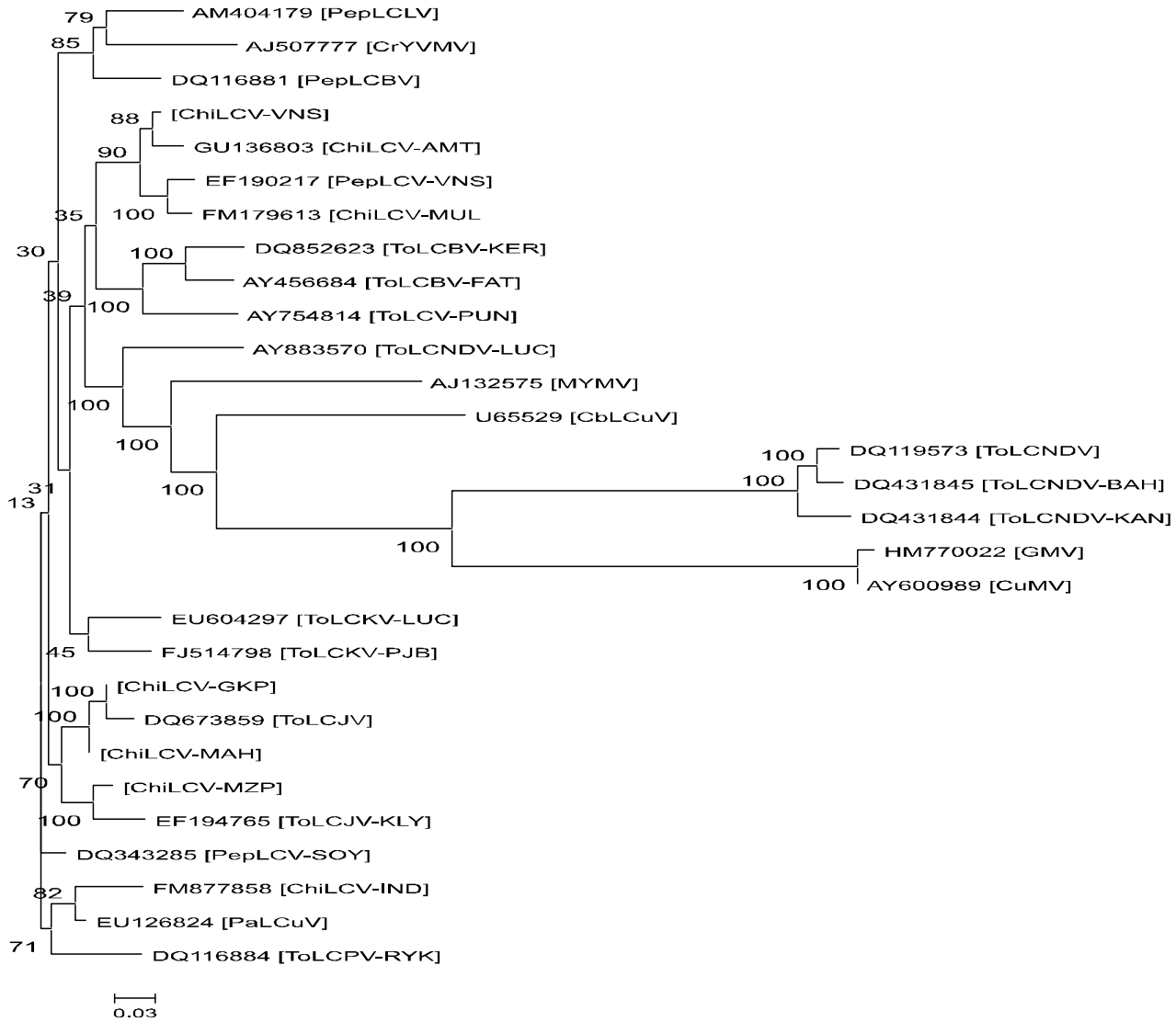

Figure 3. Neighbour-joining tree showing the relationship of CP sequences of ChiLCV from various places with other GenBank-published related sequences. Numbers at nodes indicate the bootstrap percentage scores out of 1000 replicates.

Sequence identity searches among the available sequence database resulted in listing of closely related 25 begomoviruses (Table 1). The CP sequence of all the four isolates showed very high sequence identity (98%) with ToLCV-Joydebpur (DQ673859.1). This support the report of Khan *et al*. (2006) indicating ChiLCV has a chimeric genome derived through recombination between ChiLCV and ToLCV (Khan *et al.*, 2006).

Phylogenetic analysis : The CP DNA sequences of four isolates and 25 representative sequences from GenBank were used to generate the phylogenetic tree (Fig. 3). The phylogenetic tree for the aligned sequences formed three major clusters. The first cluster consisted of 20 isolates, which were further separated into four sub-clusters. The second and third clusters consisted of 5 and 4 isolates, respectively. The Varanasi isolate (ChiLCV-VNS) grouped with ChLCV[AMT] (GU136803.1) in first cluster. Isolates from Gorakhpur (ChiLCV-GKP), Mahrajganj (ChiLCV-MAH) and Mirzapur (ChiLCV-MZP) showed 99% nucleotide identity and clustered with ToLCJV (DQ673859.1 and EF194765.1) in second cluster. The *CP* gene is most conserved gene family, so it is used as virus identification and to infer geographic and vector relationships (Reddy *et al.*, 2005). Percentage sequence identity within each cluster was in agreement with the criterion of $<90\%$ CP sequence identity representative for different geminivirus species (Fauquet and Stanley, 2005).

Occurrence of variants in eastern Uttar Pradesh has caused a complex of chilli leaf curl disease. In begomovirus, recombination between the variants of the same virus, between species and even between genera, has resulted in rapid diversification. Most virulent variants have developed through recombination of viral genomes such as those associated with cassava mosaic, cotton leaf curl, and tomato leaf curl diseases (Ito *et al*., 2008). Pathogenicity with cloned DNA components of ChiLCV of a Varanasi variant has indeed demonstrated that chilli leaf curl disease is caused by a complex consisting of the monopartite chilli leaf curl virus and a DNA- â satellite component (Chattopadhyay *et al*., 2008). The groundnut mosaic virus (GMV) and cucumber mosaic virus (CuMV) grouped in the first cluster with ToLCNDV which have high nucleotide identity with begomoviruses. This indicates that these species probably shares common regions which cluster them together. We have been able to locate resistant germplasm against ChiLCV-VNS strain (Kumar *et al*., 2006; Rai, 2010) and found that resistance is governed by major recessive genes with some minor genes and found markers significantly linked to ChiLCV-VNS resistance (Rai, 2010). Although ChiLCV genome from Varanasi alone has been found to be infectious that can produce mild-delayed symptoms, its association with a DNA-â satellite increases symptom severity and considerably shortens the incubation period (Chattopadhyay *et al*., 2008). The ChiLCV-VNS variant and the remaining three isolates (ChiLCV-GKP, ChiLCV-MAH, ChiLCV-MZP) may apparently have different infection capabilities that could be further investigated. Hence studies on ChiLCV-VNS taxonomic level using complete viral genome sequencing and infectivity of this variant alone and in combination with other three newly collected isolates will give more illustration on symptom expression pattern, like reported in case of pumpkin at Varanasi (Jaiswal *et al*., 2010). Such information coupled with diversity distribution study made herein using four ChiLCV isolates in the region are prerequisites for developing sustainable disease resistance strategy against this devastating virus of chilli crop.

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