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Research Article

Prevalence of begomoviruses associated with tomato leaf curl disease in the sub-Himalayan plains of West Bengal

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Abstract

Tomato is a solanaceous crop and one of the most economically important vegetables in the world. India ranks second in total production of tomato in the world. It has been referred to as a "functional food," a food that goes beyond providing just basic nutrition. ToLCD is one of the major constraints to tomato production in India. To study the disease incidence of tomato, a survey was made in the tomato crop growing fields of Darjeeling, Jalpaiguri, Coochbehar and Uttar Dinajpur districts of sub-Himalayan West Bengal during December 2015 to February 2016 and several infected and healthy leaf samples were collected based on the morphological symptoms like-vein clearing, leaf curling, leaf deformation and stunted growth of plants. Disease incidence ranged from 70% to 86.66% of the collected samples from different districts. All the samples collected from the present study area were tested by PCR with DengA and DengB primer and an expected amplicon of ~530bp was found. Two randomly selected PCR positive samples were sequenced and analyzed (Acc. Nos. KX108859 and KX108860). The SLG-1 isolate (Acc. No. KX108859) showed 95% nt identity with ToLCKV (Acc. No. KP178730) and the ISL-1 isolate (Acc. No. KX108860) showed 96% nt identity with ToLCNDV (Acc. No. KC513822). The threat of begomoviral spread to the north-eastern part of India has been taken into consideration.

Keywords: Solanaceous crop, Tomato leaf curl disease, *Begomovirus*, Coat protein.

Introduction

Tomato (*Lycopersicon esculentum* L.) is a solanaceous crop and one of the most economically important vegetables in the world (Hanssen *et al.*, 2010). India ranks second in total production of tomato in the world. In 2014, the production of tomato in India was 1,94,02,000 metric tons (1 t = 1000 kg) produced in a total area of 12,04,000 ha (1 ha = 10000 m^2), with an mean yield of 16.1 mt/ha(Indian Horticulture Database-2014). West Bengal stands eighth in the production of tomato in India, contributing about 17% of total production in India. Jalpaiguri and Coochbehar districts are the major tomato producing areas of West Bengal (Indian Horticulture Database-2014). It has been referred to as a "functional food," a food that goes beyond providing just basic nutrition. This is due to lycopene, a beneficial phytochemical. Tomatoes also play a role in preventing chronic diseases and deliver other health benefits (Batta, 2016).

Tomato leaf curl disease (ToLCD) is one of the major constraints to tomato production in India. ToLCD-associated begomovirus is a member of the family *Geminiviridae* and transmitted through whitefly (*Bemisia tabaci*) in a persistent circulative non-propagative manner (Czosnek *et al.*, 1988; Hong and Harrison, 1995; Rana *et al.*, 2016). *Tomato leaf curl virus* (ToLCV) is characterized by twinned particles consisting of a circular, single-stranded (ss) DNA-A genome (~2.7 kb) (Stanley, 1985). It is often associated with a DNA-B or alpha- and/or beta-satellite molecules for successful symptom development (Dean *et al.*, 2001; Sohrab *et al.*, 2016). In India, occurrence of ToLCD was first reported by Vasudeva and Samraj in 1948. Altogether forty two strains of ToLCV have been reported to cause serious damage in tomato production worldwide (Vasudeva and Samraj, 1948; Sastry and Singh, 1973; Saikia and Muniyappa, 1989;

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Reddy *et al.*, 2005; Kirthi *et al.*, 2002; Paximadis *et al.*, 2001; Ramappa *et al.*, 1998; Brown *et al.*, 2015). Occurrence of ToLCV was reported from several places of West Bengal (Reddy *et al.*, 2005; Saha *et al.*, 2013; Saha *et al.*, 2014). In this communication, PCR amplification, sequencing and diversity analysis of partial coat protein (CP) gene of ToLCV have been reported infecting tomato in sub-Himalayan West Bengal.

Materials and methods

Survey, disease incidence and collection of diseased Samples

To study the disease incidence of tomato, a survey was made in the tomato crop growing fields of Darjeeling, Jalpaiguri, Coochbehar and Uttar Dinajpur districts of sub-Himalayan West Bengal during December 2015 to February 2016 (Fig. 1A, 1B) and several infected and healthy leaf samples were collected based on the morphological symptoms. Disease incidence was estimated using the method of James and Teng (1979).

$$\text{Disease Incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Number of total plants}} \times 100$$

DNA isolation and PCR

Total DNA were extracted from the infected and healthy leaves following the method of Haible *et al.* (2006). Polymerase chain reaction (PCR) was done using Deng universal primer pair (DengA/DengB) and amplicons were visualized in 1.2% (w/v) agarose gels under UV-transilluminator (Fig. 1C).

Cloning, sequencing and sequence analysis

The purified PCR products were cloned into pGEM-T vector following the method of Sambrook and Russel (2001) and the clones were sent to Chromous Biotech Pvt. Ltd. for sequencing. The nucleotide sequences were aligned using ClustalW (Thompson *et al.*, 1994). The nucleotide were compared with the corresponding sequences of other isolates of ToLCV deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) using the BLAST analysis (Altschul *et al.*, 1997). Sequence identity matrix was generated using SDT 1.2 (Muhire *et al.*, 2014) and a phylogenetic tree was generated by neighbor-joining method and Kimura-2 parameter using MEGA 6 (Tamura *et al.*, 2013).

Results and discussion

Symptomatology

Disease infected plants showed typical begomoviral symptoms like- vein clearing, leaf curling, leaf deformation and stunted growth of plants. Disease incidence varies from location to location like 78.57% in Darjeeling, 86.66% in Uttar Dinajpur, 80% in Jalpaiguri and 70% in Coochbehar district. Similar types of symptoms were reported earlier by several workers (Padidam *et al.*, 1995; Saha *et al.*, 2014; Saha *et al.*, 2013).

Molecular characterization and phylogenetic analysis

All the samples collected from the present study area were tested by PCR using universal Deng primer pair. On PCR with DengA and DengB, all the infected samples gave an expected amplicon of ~530bp (Fig. 1D) but none of the healthy samples showed positive result. Two randomly selected PCR positive samples were sequenced and analyzed. Similar types of results were also described by Reddy *et al.* (2005), Briddon *et al.* (2008) and several other workers (Brown *et al.*, 2001; John *et al.*, 2006; Santoso *et al.*, 2008; Samad *et al.*, 2009; Haider *et al.*, 2007).

The partial CP (AV1), Pre-CP (AV2) region of the viruses were sequenced and submitted in the GenBank database (Acc. Nos. KX108859 and KX108860). When the nucleotide (nt) sequences of the isolates were compared with those of other *Begomovirus* available in the GenBank, the SLG-1 isolate (Acc. No. KX108859) showed 95% nt identity with *Tomato leaf curl Karnataka virus* (ToLCKV) (Acc. No. KP178730) and the ISL-1 isolate (Acc. No. KX108860) showed 96% nt identity with *Tomato leaf curl New Delhi virus* (ToLCNDV) (Acc. No. KC513822) as shown in the fig. 2A. In the phylogenetic tree, ToLCKV and ToLCNDV form different clusters with the other subsequent members of the respective viruses (Fig. 2B).

As the number of strains of ToLCV has increased a lot, the whole genome sequencing

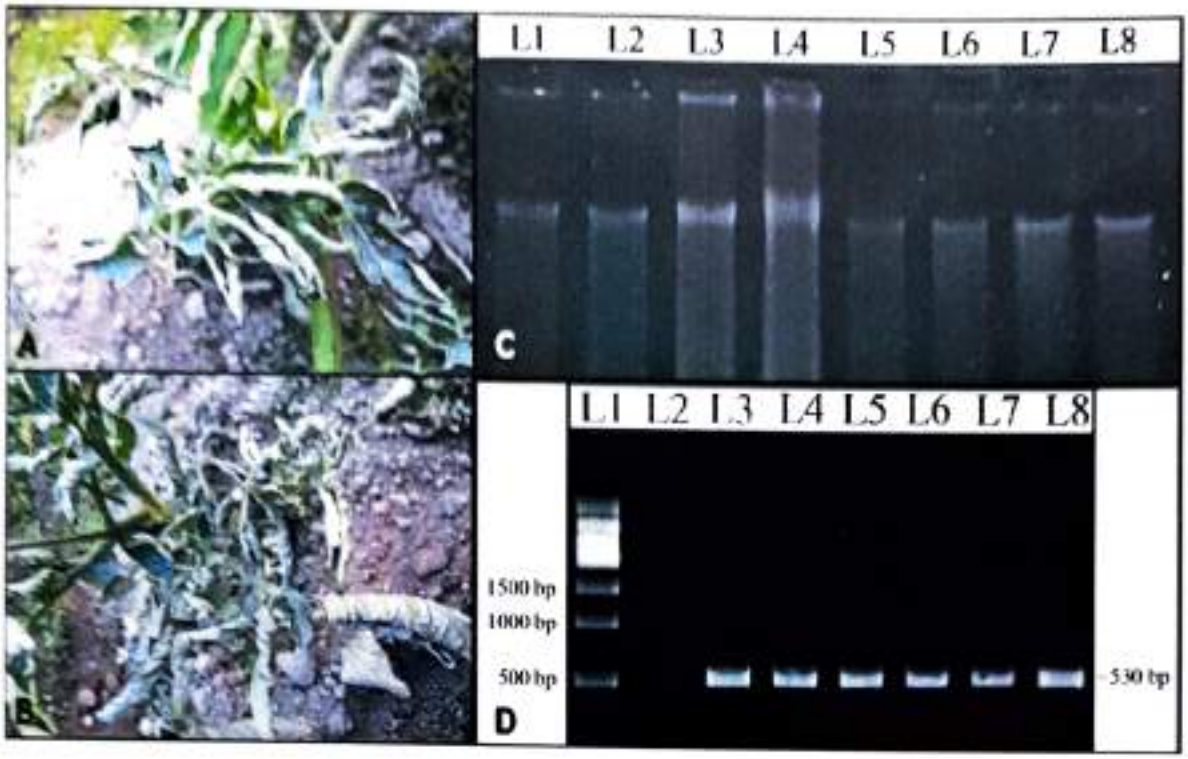

Fig. 1. (A & B) Naturally infected tomato plants; (C) Total DNA on agarose gel isolated from healthy (L1-L3) and infected (L4-L8) plants; (D) Amplified PCR product on agarose gel isolated from healthy (L2) and infected (L3-L8) plants; L1- 500 bp DNA ladder.

Table 1. ToLCKV and ToLCNDV isolates used in the study along with their GenBank Acc. No., host and place of occurrence

SL No.	Acc. No.	Organism	Place	*Host*	Country
1	KX108859**	ToLCKV	Siliguri	*Lycopersicon esculentum*	India
2	KP178730	ToLCKV	Maharashtra	*Lycopersicon esculentum*	India
3	KP178731	ToLCKV	Maharashtra	*Lycopersicon esculentum*	India
4	EU604297	ToLCKV	Lucknow	*Petunia* sp.	India
5	JX987088	ToLCKV	Lucknow	*Zinnia elegans*	India
6	AY375241	ToLCKV	Lucknow	*Lycopersicon esculentum*	India
7	KX219744	ToLCKV	Andhra Pradesh	*Helianthus annuus*	India
8	KF663699	ToLCKV	Punjab	*Lycopersicon esculentum*	India
9	KF551581	ToLCKV	Punjab	*Lycopersicon esculentum*	India
10	KP195261	ToLCKV	Punjab	*Lycopersicon esculentum*	India
11	AY754812	ToLCKV	Janti	*Lycopersicon esculentum*	India
12	AY753203	ToLCKV	Karnataka	*Lycopersicon esculentum*	India
13	FJ436982	ToLCKV	Bahraich	*Capsicum annum*	India
14	HM851186	ToLCKV	New Delhi	*Lycopersicon esculentum*	India
15	KX108860**	ToLCNDV	Islampur	*Lycopersicon esculentum*	India
16	KC545812	ToLCNDV	Delhi	*Cucumis sativus*	India
17	KM383743	ToLCNDV	Jamalpur	*Lycopersicon esculentum*	Bangladesh
18	KM383742	ToLCNDV	Jamalpur	*Lycopersicon esculentum*	Bangladesh
19	KM383741	ToLCNDV	jessore	*Lycopersicon esculentum*	Bangladesh
20	KM383739	ToLCNDV	Joydibpur	*Lycopersicon esculentum*	Bangladesh
21	KM383738	ToLCNDV	Joydibpur	*Lycopersicon esculentum*	Bangladesh
22	KM383737	ToLCNDV	Sylhet	*Lycopersicon esculentum*	Bangladesh
23	AF448058	ToLCNDV	Dargai	*Lycopersicon esculentum*	Pakistan
24	KC513822	ToLCNDV	Lucknow	*Lycopersicon esculentum*	India
25	AF448059	ToLCNDV	Islamabad	*Lycopersicon esculentum*	Pakistan

** Isolate under study

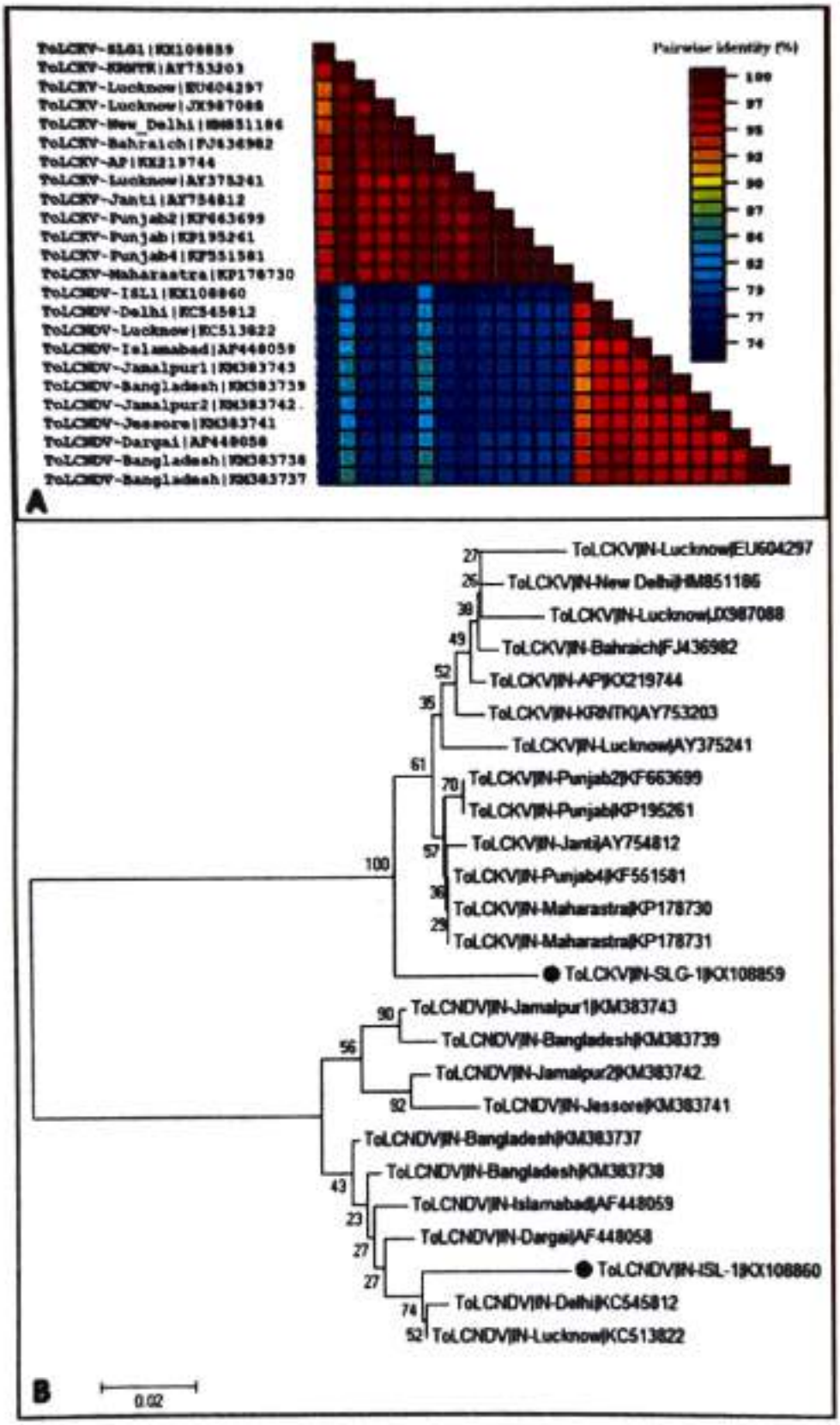

Fig. 2. (A) Sequence identity matrix of the 14 ToLCKV and 11 ToLCNDV isolates. Identity percent corresponds to the color matrix is indicated on the right side of the figure; (B) Phylogenetic tree generated by neighbour joining of ToLCKV- and ToLCNDV- CP alignments. Values at the nodes indicate percentage of bootstrap support (out of 1000 bootstrap replicates). GenBank accession numbers along with the collection spot of the viruses have been indicated at the end of each branch.

of the viruses are now-a-days essential for identifying different species as proposed by Kings *et al.* (2011). However, the ToLCV-CP gene analysis may provide valuable information to the recent occurrence of ToLCD in sub-Himalayan region of West Bengal. It can be said that, the high disease incidence may be attributed to prevalence of whitefly vector, warm tropical climate supporting year round survival of the whitefly, intensive cultivation of crops and polyphagous nature of the whitefly serving path of sustenance of begomovirus in alternative hosts (Saha *et al.*, 2014). The bipartite ToLCNDV has been reported to infect tomato and other solanaceous crops in the Indian subcontinent (Padidam *et al.*, 1995; John *et al.*, 2006; Santoso *et al.*, 2008; Saha et al, 2013). Although, ToLCKV was thought to be a recombinant strain and restricted to the southern part of India (Chatchawankanphanich and Maxwell, 2002). The threat of begomoviral spread to the north-eastern part of India has been taken into consideration and this may be correlated to different factors like- weather condition, tomato live stock import-export, and agricultural practices, that is operational in the study area.

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