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Molecular Detection of *Tomato leaf curl New Delhi virus* Infecting Pumpkin Plants in Sub-Himalayan Plains of West Bengal, India

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ABSTRACT

Tomato leaf curl New Delhi virus is a silverleaf whitefly-transmitted bipartite *Begomovirus* that causes damage to various cultivated plant species mainly belonging to the family Solanaceae and Cucurbitaceae. A survey was carried in pumpkin fields of sub-Himalayan plains of West Bengal, India during 2018. During the survey, presence of silverleaf whitefly along with symptoms like leaf curling, leaf yellowing, yellow mosaic, vein clearing and leaf distortion were observed on pumpkin plants. Disease incidences in the range of 30% to 50% were also recorded. Collected infected leaf samples were analyzed for the presence of begomovirus using universal begomovirus primers, DengA/DengB. Amplified PCR products from severely infected plants were cloned and sequenced. All the sequences were completely identical, thus only one representative sequence was submitted to GenBank (Acc. No.MG721010). After BLASTn analysis the causal organism was identified as *Tomato leaf curl New Delhi virus.* Nucleotide sequence identity of CP gene showed close relationship with *Tomato leaf curl New Delhi virus.* This study confirmed the emergence of *Tomato leaf curl New Delhi virus* in the infected fields of pumpkin in sub-Himalayan plains of West Bengal, India.

Keywords Sub-Himalayan West Bengal, *Tomato leaf curl New Delhi virus*, Pumpkin, PCR.

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INTRODUCTION

Tomato leaf curl New Delhi virus (ToLCNDV) is one of the economically important viral pathogens of tomato in India (Varma and Malathi 2003, Varma *et al.* 2011). *Tomato leaf curl New Delhi virus* is a bipartite begomovirus belonging to the family Geminiviridae containing DNA-A and DNA-B as its genome and are known to infect various host plants throughout the world (Padidam *et al.* 1995). *Begomovirus* is the largest genus of the family Geminiviridae and are transmitted by silverleaf whitefly (*Bemisia tabaci*). Pumpkin (*Cucurbita pepo*) is an economically

important cucurbit, most widely cultivated in India that mainly consumed as fresh vegetables because of high nutritional value of its fruits. Pumpkin seeds are consumed as roasted, salted snack in some regions of Canada, Mexico, USA, Europe and China and produced oils that are highly valuable in central Europe (Paris *et al.* 2012). It is also used as hypoglycemic agent and has anti-cancerous, anti-diabetic and anti-oxidant properties (Perez Gutierrez 2016). So far, only very few *Begomovirus* species such as *Squash leaf curl China virus* (SLCCNV) associated with yellow vein mosaic disease of pumpkin in both northern and Southern India (Muniyappa *et al.* 2003, Maruthi *et al.* 2007, Singh *et al*. 2009) and ToLCNDV associated with leaf curl of pumpkin (*Cucurbita moschata*) in northern India (Phaneendra *et al.* 2012) has been reported in pumpkin.

However, *Begomovirus* infecting pumpkin in the present study area are less reported. The pumpkin fields of this region were surveyed, where leaf curl, yellow vein mosaic symptoms were prominent. The objective was to detect and identify the prevalent virus associated with leaf curl disease of pumpkin in this region.

MATERIALS AND METHODS

Survey, disease incidence and sample collection

During February 2018 a survey was carried in pumpkins fields of sub-Himalayan plains of West Bengal, India and disease incidences were estimated following the method of Sohrab *et al*. (2010). Infected (Ten) and healthy (five) leaf samples were collected with leaf curling, leaf yellowing, yellow vein mosaic, vein clearing and leaf distortion symptoms (Fig. 1).

DNA isolation and PCR amplification

Total DNA was isolated from both the infected and healthy leaf samples following the method of Haible *et al*. (2006). Isolated DNA were run on 1% agarose gel, observed under UV-transillumnator and stored at -20°C for further use.

For *Begomovirus* detection, PCR amplification was done using *Begomovirus* specific primers Deng A (5′-TAATATTACCKGWKGVCCSC-3′) and Deng B (5′-TGGACYTTRCAWGGBCCTTCACA-3′).The primers were used to amplify the movement protein (AV2) and partial coat protein (CP) genes of DNA-A genome of *Begomovirus* (Reddy *et al.* 2005).

Sequencing and phylogenetic analysis

The PCR products were then purified and cloned into pGEM-T vector following the method of Sambrook and Russel (2001). After cloning, the clones were sent to Chromous Biotech Pvt Ltd for sequencing. The obtained nucleotide sequences were aligned using Clustal W (Thompson *et al.* 1994). After BLASTn analysis the annotated genome sequences were sub-

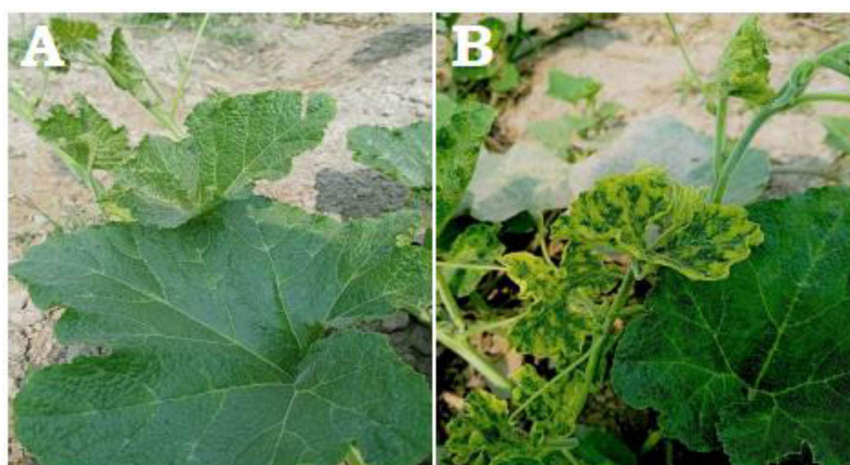

Fig. 1. a) Healthy pumpkin plant, b) Natural begomovirus infection in pumpkin plants showing yellow mosaic and leaf curl symptoms in leaf.

mitted to GenBank. Sequence identity matrix was generated using SDTv1.2 (Muhire *et al.* 2014) and a phylogenetic tree was generated by neighbour-joining method and Kimura-2 parameter in MEGA 6.0 (Tamura *et al.* 2013).

RESULTS

Survey and diagnosis of viral disease

During initial survey about 30-50% of the crops were found to be symptomatic in various pumpkin growing fields of sub-Himalayan plains of West Bengal, India. Total DNA was isolated from all the collected healthy and infected leaf samples and was tested for the detection of *Begomovirus* through PCR using *Begomovirus* specific primers 'Deng A' and 'Deng B' (Fig. 2). Five out of ten infected leaf samples were amplified by PCR and produced a prospective amplicon of ~530 bp (Fig. 2). None of the healthy leaf samples were amplified using PCR. Amplified products were purified, cloned and sequenced. All the sequences were completely identical, thus only one representative sequence was submitted to GenBank (Acc. No. MG721010). BLASTn analysis revealed that the obtained sequence (isolate ISL-02) showed 98% nucleotide identity with ToLCNDV.

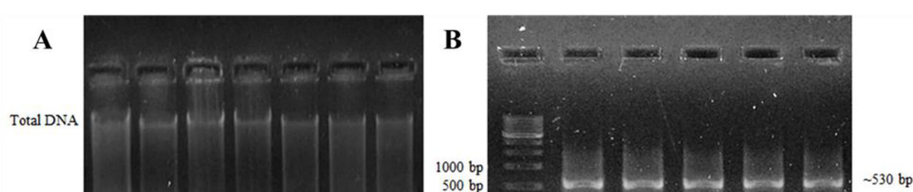

Fig. 2. A) Total DNA isolated from plant samples on 1% agarose gel under UV-trans-illuminator, B) Amplified PCR products from infected plant samples on 1% agarose gel under UV-trans-illuminator.

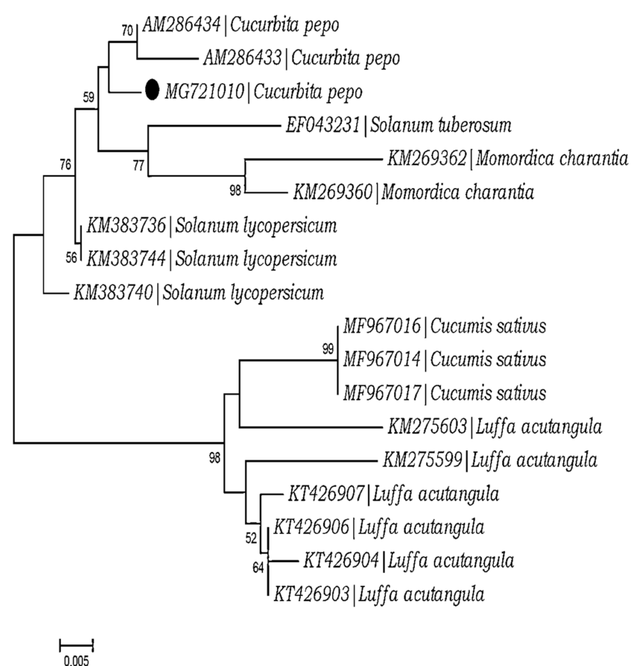

Fig. 3. Phylogenetic tree generated by neighbour-Joining method consisting of coat protein (CP) gene of ToLCNDV isolate of the present study and other ToLCNDV isolates infecting different hosts. Values at each node indicate percentage of bootstrap support (out of 1000 bootstrap replicates) and are indicated if greater than 50. GenSBank accession numbers along with the hosts of the viruses have been indicated at the end of each branch.

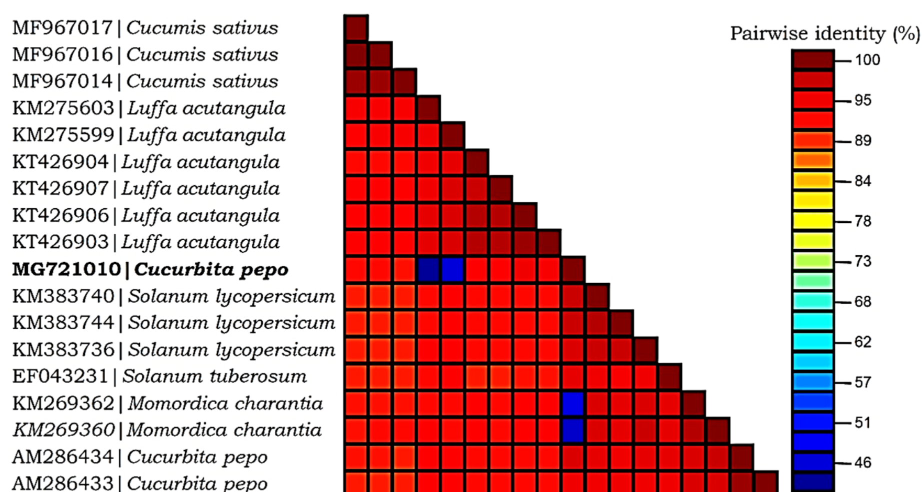

Fig. 4. Nucleotide sequence identity matrix of Coat Protein gene of ToLCNDV isolate of the current study and other ToLCNDV isolates infecting different hosts. Identity percentages are indicated on the right side corner of the matrix.

Phylogenetic analysis

Isolates of ToLCNDV infecting different hosts were taken from GenBank for phylogenetic analysis of present isolate (Fig. 3). In phylogenetic analysis, isolate of the present study showed very close relationship with other pumpkin-infecting ToLCNDV isolates and clustered together. The phylogenetic analysis was also supported by the two dimensional color-coded identity matrix developed using SDTv1.2 (Muhire *et al.* 2014) where present isolate (Acc. No. MG721010) shown similar color pattern with that of ToLCNDV isolates infecting same host reported from other agro-climatic area elsewhere (Fig. 4).

DISCUSSION

The present study stated the occurrence of disease caused by *Begomovirus* in pumpkin plants in sub-Himalayan plains of West Bengal, India. During initial survey, presence of silverleaf white fly along with symptoms like leaf curling, leaf yellowing, yellow mosaic, leaf distortion and vein clearing symptoms were found in this region. These symptoms are generally associated with *Begomovirus* infection as reported by different workers throughout the world (Leke *et al.* 2015, Sohrab *et al.* 2017, Subiastuti *et al.* 2019, Lavanya and Arun 2021, Wahyono *et al.* 2023). *Begomovirus* infection in pumpkin plants has been reported by different researchers from different region of the world (Bandaranayake *et al.* 2014, Diaz-Najera *et al.* 2020, Thuy *et al.* 2022, Selangga and Listihani 2022, Kushvaha *et al.* 2023). Since, the disease symptoms along with literature review reports indicated *Begomovirus* infection. Further confirmation and identification of *Begomovirus,* present in the infected pumpkin plants was done by PCR. PCR with *Begomovirus* specific primers, amplify the partial coat protein gene of *Begomovirus* and produced the expected amplicons which indicating the presence of virus in the symptomatic leaf samples. Coat protein genes are the most conserved gene present in the genome DNA-A of *Begomovirusis* and the sequencing of this gene is recognized as sufficient for the initial identification of begomoviruses (Fauquet and Stanley 2003). This coat protein gene has been used for the detection and identification of *Begomovirus* in different crops by different authors (Kumar *et al.* 2017, Prabhandakavi *et al*. 2018, Lavanya and Arun 2021, Kwak *et al.* 2022).

In this study, sequencing of the PCR products combined with BLASTn analysis identified the sequences as those of ToLCNDV.ToLCNDV has been reported to infect various crops worldwide, including tomato, cucurbits, potato, papaya, bitter gourd, and chili (Parrella *et al.* 2017, Zaidi *et al.* 2017, Venkataravanappa *et al.* 2019, Cai *et al.* 2023).

In phylogenetic analysis, present pumpkin-infecting isolate showed close relationship among them and they clustered together with other pumpkin-infecting ToLCNDV isolates. The phylogenetic analysis was also supported by the two dimensional color-coded identity matrix developed by using SDTv1.2 (Muhire *et al.* 2014) where present isolate (Acc. No. MG721010) showed similar color pattern with that of ToLCNDV isolates infecting same host. Similar type of clustering of ToLCNDV has also been reported by different workers worldwide (Bandaranayake *et al.* 2014, Venkataravanappa *et al.* 2019, Kwak *et al.* 2022). Unexpectedly, a high disease incidence was recorded in the surveyed areas. This may be due to the poor knowledge of farmers about the etiology of the disease, impractical control measures against the vector and the improper culture practices, such as crop overlapping, which provided continuous source of *Begomoviruses.*

CONCLUSION

In the present communication, based on PCR analysis, sequencing and phylogeny, confirmed the incidence of *Tomato leaf curl New Delhi virus* (ToLCNDV) infecting pumpkin plants in sub-Himalayan plains of West Bengal, India.

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