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Full Length Research Paper

Identification of *Zinnia leaf curl virus* infecting *Zinnia elegans* in India

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In a survey during 2007 to 2009 at Gorakhpur and nearby locations of North Eastern Uttar Pradesh, India leaf curling, foliar deformation and distortion symptoms were observed on *Zinnia elegans* plants. The associated White fly population indicated the possible presence of begomovirus in the field. Therefore, Polymerase chain reaction (PCR) was performed with the begomovirus specific primers (TLCV-CP). Total genomic DNA was isolated from infected as well as healthy leaf samples. In gel electrophoresis expected ~500 bp amplicons was obtained in symptomatic leaf sample while, no amplicon was found in healthy leaf samples. Amplicon obtained were directly sequenced and submitted in the GenBank (GQ412352) and phylogeny were constructed with the available identical sequences in the Genbank. Based on the highest similarity 97% at nucleotide and 99% at amino acid level and closest relationship with isolates of *Zinnia leaf curl virus*, the present study isolate was considered an isolate of *Zinnia leaf curl virus*.

Key words: *Zinnia elegans*, *Zinnia leaf curl virus*, polymerase chain reaction (PCR), phylogenetic analysis.

INTRODUCTION

Zinnia is a common Mexican wildflower and members of the family Asteraceae. The early Spanish colonists in Mexico found the *Zinnia* sp lowers ugly and called them *mal de ojos* (evil eyes). The Plants are known to have good medicinal properties and used for various purposes. Roots of *Medicago sativa* (Lucern) and whole plants of *Zinnia elegans* were subjected to phytochemical and antifungal screening. *Z. elegans* inhibited the growth of *Fusarium moniliforme* (Hafiza et al., 2002).

Begomoviruses are an important group of plant viruses, and a major problem for economic important plants, causing huge economic losses to food and fiber crops worldwide, mainly in tropical and sub tropical parts of the world (Varma and Malathi, 2003; Tiwari et al., 2008). They belong to the family *Geminiviridae* and are transmitted by the white fly vector *Bemisia tabaci*. In early's *Tobacco leaf curl virus* from Tanzania was the first reported virus on *Zinnia* (Storey, 1931). Later, *Zinnia mosaic virus* (Huertos, 1953), *Zinnia mild mottle virus* from India (Padma et al., 1974), *Bidens mottle virus* (Logan et al., 1984), *Zinnia leaf curl virus* (Haider, 2005), *Ageratum enation virus* (Kumar et al., 2010), *Zinnia leaf curl virus*, *Zinnia potyvirus* (Maritan et al., 2004) and *Zinnia leaf curl Pakistan virus* (AM040438) were reported by different workers around the world. Recently, Ha et al. (2008), found *Alternanthera yellow vein virus* affecting *Zinnia* with leaf curling symptoms in Vietnam.

During a routine visit of the Gorakhpur locations, leaf curl along with mosaic symptoms were observed and symptomatic leaves were analyzed through PCR and on the basis of sequence analysis the isolate was considered as an isolates of *Zinnia leaf curl virus* in the present study.

MATERIALS AND METHODS

Survey and sample collection

During survey in 2007 to 2009 at Gorakhpur and it's nearby

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locations yellow mosaic and leaf curling of leaves on *Z. elegans* were recorded. The symptomatic leaves were collected along with the healthy ones and stored at -80°C for further study.

DNA extraction, PCR and gel electrophoresis

Total DNA was extracted from infected as well as healthy leaf samples using the method described earlier by Dellaporta et al. (1983). PCR was performed using a pair of primers designed from the coat protein gene region of a well characterized begomovirus (*Tomato leaf curl New Delhi virus*). The forward and reverse primers were: 5′-ATGGCGAAGCGACCAG-3′ and 5′-TTAATTTGTGACCGAATCAT-3′, respectively (Tiwari et al., 2010 a). The PCR was set up in a 50 µl reaction mixture containing DNA extract (~100 ng, 5 µl), dNTPs (10 mm each, 1 µl), 2 primers (25 pm each, 1 µl), *Taq* DNA polymerase (3 U/µl, 1 µl), *Taq* buffer (10×, 5 µl), $MgCl_2$ (25 mM, 2 µl) and 34 µl double distilled sterile water to make up the reaction volume.

The amplification was performed in an automated thermal cycler with the following parameters: initial denaturation at 94°C for 5 min; 30 cycles consisting of 30 s of denaturation at 94°C, 30 s of primers annealing at 47°C and 40 s of extension at 72°C and a final extension of 5 min at 72°C at the end of the cycle. The PCR product obtained was checked by electrophoresis on 1% agarose gel prepared in 1 × TAE gel buffer.

Sequencing

Fragments with sizes corresponding to the expected amplicon (~500bp) were excised from the gel and eluted using the QIAquick Gel Extraction Kit (Qiagen) and were sequenced by ABI's AmpliTaq FS dye terminator cycle sequencing chemistry, based on Sanger's Sequencing method, in an automated ABI 3100 Genetic Analyzer.

Sequence comparison and phylogenetic analysis

The nucleotide and amino acid sequence data of the virus isolate were analyzed by basic local alignment search tool (BLAST) with the sequence data of various begomoviruses submitted in NCBI Entrez databases (Bethesda, MD, USA).

Multiple sequence alignment and pairwise similarities at nucleotides and amino acids of the isolate with other begomoviruses were obtained using the DiAlign2 program (Morgenstern, 1999).

Phylogenetic analyses were perused using the molecular evolutionary genetics analysis tool with 100 replicates bootstrapping and a dendrogram was generated with the neighbour joining (NJ) method and viewed by the NJ plot programme (Tamura et al., 2007).

RESULTS

Symptoms

During the course of survey in different parts of Eastern Uttar Pradesh in 2007 to 2009, infected *Z. elegans* plants were found with symptoms of leaf curling, and yellow mosaic as compared to healthy plants (Figure 1a and b). The affected plants became deformed with smaller and few flowers, the infestation with whiteflies was also noticed on symptomatic *Z. elegans*.

Detection by PCR amplification

The results of the PCR investigation with specific primer (ToLCV-CP) revealed that the *Z. elegans* samples were positive for *Begomovirus* infection. A ~500 bp amplicon was consistently amplified in 1% agrose gel electrophoresis of PCR assay products in all the symptomatic sample. No amplificon was obtained from any of the healthy non-symptomatic samples (Figure 2).

Sequence similarity of *Z. elegans* isolate

Sequence similarity of *Zinnia* isolate from Gorakhpur at amino acid and nucleotide level comparison showed that the isolates in the present investigation are highly similar to *Zinnia leaf curl virus*.

Comparison at nucleotide level showed highest 97% similarity with *Zinnia leaf curl virus* (AM040438). 89 to 85% similarity was found with *Tomato leaf curl virus* (FJ002571), *Tomato leaf curl Pakistan virus* (FM164938), *Begomovirus* (DQ339124), *Tomato leaf curl Karnataka virus* (FJ514798), *Cotton leaf curl virus* (DQ343283), *Papaya leaf curl virus* (EU126824, DQ376038), *Pedelanthus leaf curl virus* (AM292303). Lowest similarity of 85% was found with *Tomato leaf curl virus* (EU263016). Comparison at amino acid level indicated highest similarity of 99% with *Zinnia leaf curl virus* (AM040438), *Tomato leaf curl Pakistan virus* (FM164938), *Tomato leaf curl virus* (FJ02571), while, 91% similarity was found with *Whitefly transmitted begomovirus* (DQ339124). *Cotton leaf curl virus* (DQ343283), *Papaya leaf curl virus* (EU126824, DQ376038) and *Tomato leaf curl virus* (EU263016) showed 90% similarity, however 88% similarity of *begomovirus* was observed with rest of the analyzed isolates namely; *Tomato leaf curl Karnataka virus* (FJ514798) and *Pedelanthus leaf curl virus* (AM292303) (Table 1). Sequence similarity at nucleotide and amino acid level confirmed that *Zinnia* isolate from Gorakhpur is highly similar to the isolate of *Zinnia leaf curl virus.*

Phylogenetic analysis

Phylogenetic analysis at nucleotide level showed close relationship with *Zinnia leaf curl virus* (AM040438) followed by *Tomato leaf curl virus* (FJ002571, EU263016). *Begomovirus* (DQ339124), *Tomato leaf curl Karnataka virus* (FJ514798), *Pedilanthus leaf curl virus* (AM292303), *Cotton leaf curl virus* (DQ343283), *Papaya leaf curl virus* (EU126824, DQ376038) and *Tomato leaf curl Pakistan virus (*FM164938) showed distance relationship with the *Zinnia* Gorakhpur isolates. The analysis indicated that the Gorakhpur isolate is close to the *Zinnia leaf curl virus* (Figure 3).

Figure 1. Naturally infected *Zinnia* plants showing yellow mosaic and leaf curling symptoms (1 b) as compare to healthy plant (1 a).

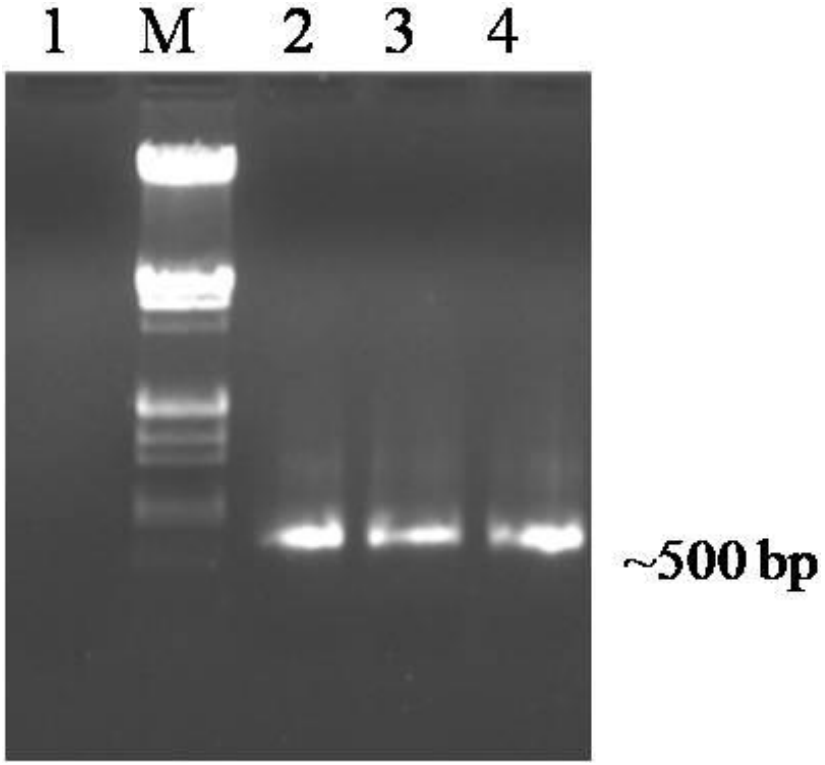

Figure 2. Agrose gel electrophoresis of PCR products obtained from *Zinnia elegans* samples showing positive amplicon of ~800 bp (lanes 2, 3,4) but not in healthy (lanes 1) samples collected from the same vicinity. M = Lambda DNA digested with *Hind* III and *Eco*R I (Genei, Bangalore, India).

DISCUSSION

During the survey of medicinal plants of Asteraceae family for the incidence of viral infections in Gorakhpur, Maharajganj, Kushinagar, Khalilabad, Basti and Deoria districts of North- Eastern Uttar Pradesh, symptoms of leaf curling and yellow mosaic were observed on *Z. elegans* at the flower garden of Gorakhpur. The symptomatic *Zinnia* leaves were analyzed through PCR with specific primers and positive ~500bp amplicons were

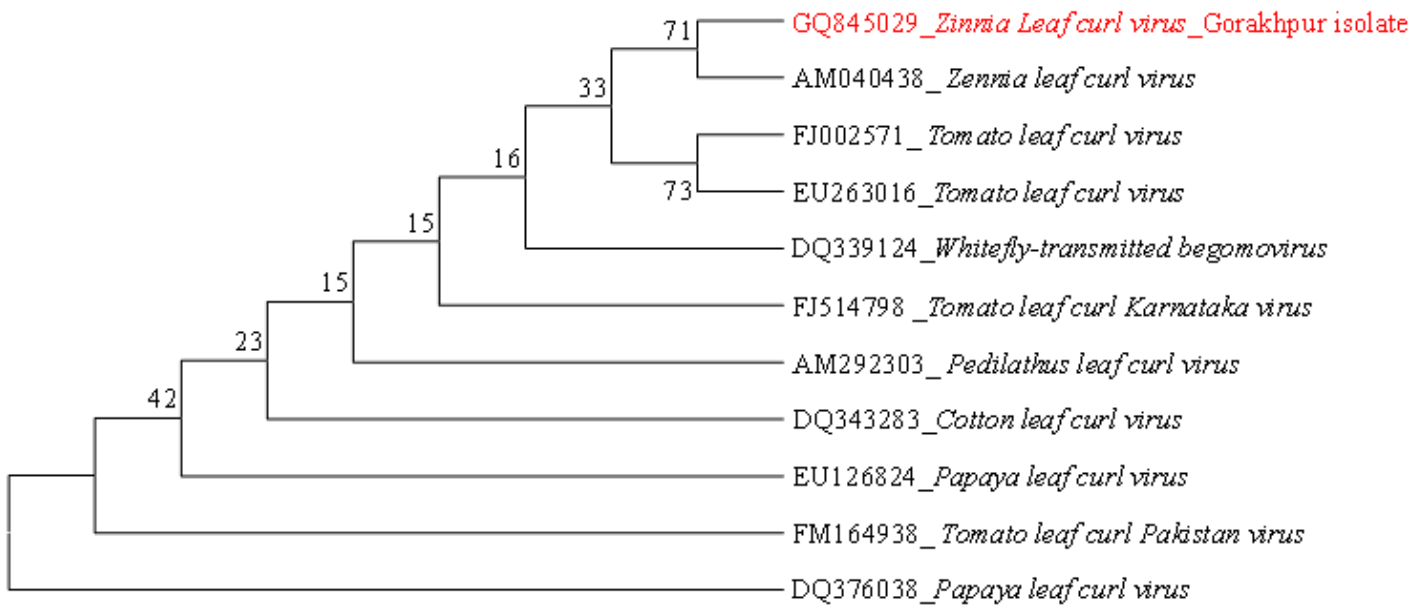

Figure 3. A neighbor-joining phylogenetic tree obtained from the coat protein sequence at nucleotide level of *Zinnia elegans* virus isolate (GQ845029) and other begomoviruses AV1 components. Tree was created by MEGA 4.0 tool.

obtained. The PCR amplified product was directly sequenced and sequence were submitted in GenBank with accession no. (GQ845029). Sequence similarity and phylogenetic analysis confirmed the presence of *Zinnia leaf curl virus* on *Zinnia elegans* in the present study.

Leaf curling symptoms on *Zinnia* were previously reported from Tanzania and study showed the presence of *Tobacco leaf curl virus* (Storey, 1931). Padma et al. (1974) did serological assay and confirmed the existence of *Zinnia mild mosaic mottle virus* on *Zinnia* plants from India. However leaf curling and mosaic were found as main symptoms on *Zinnia* associated with *Zinnia leaf curl virus* (NCBI) from Pakistan.

Literature survey confirmed limited host of Zinnia leaf curl virus from other part of the world. *Zinnia leaf curl virus* was mainly reported in Asian countries (NCBI), while, little information is available on the occurrence of *Zinnia leaf curl virus* from other parts of the world. The number of begomoviruses has increased in the last two decades from different regions of the world. In India this problem has been given more attention on the cultivated and non cultivated crop species namely; *Momordica charantia* (Tiwari et al., 2010a; Raj et al., 2010a), *Cucurbita pepo* (Tiwari et al., 2010b), *Cleome gynandra* (Raj et al., 2010b), *T. dioica* (Raj et al., 2011), *Ageratum conyzoides* (Pandey et al., 2011; Kumar et al., 2010), *C. maxima* (Singh et al., 2007), *Dimorphotheca sinuate* (Raj et al., 2007), *Amaranthus cruentus* (Raj et al., 2008), *Luffa cylindrica* (Tiwari et al., 2012a), *Luffa actuanga* (Tiwari et al., 2012b).

Various weed species namely; *Croton bonplandianum*, *Acalypha indica, Malvesrtum coromandalianum, Eclipta alba, Ageratum conyzoides*, *Coccinia grandis*, *Corchorus olitorius, Nicotiana plumbaginifolia*, *Parthenium hysterophorus*, *Solanum nigrum, Sonchus oleraceus* and *Trigonella corniculata* hich grow naturally in or nearby the fields of cultivated crops in many places of India are proven to be the alternate host or reservoir hosts of one or many begomoviruses (Raj et al., 2011).

The virus diseases cannot be controlled by any chemical treatment in the field (Valkonen, 1998). However, they can be managed based on strategies that prevent infection. Both conventional and non-conventional methods should be applied. Therefore, an immediate attention is required to check the further spread of this *begomovirus* in nature.

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