Biological and molecular characterization of a Tospovirus isolate from tomato and its relationship with other Tospoviruses

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ABSTRACT: A Tospovirus was isolated from tomato (Lycopersicon esculentum Mill) plants showing severe necrosis of leaves and growing buds. Host range studies indicated that the tomato Tospovirus isolate was closely related to Groundnut bud necrosis virus (GBNV). Sequence analyses of nucleocapsid protein (N) gene showed that the amino acid sequence identity with GBNV was 96% while the identities with other Tospoviruses were in the range of 16-85%. On this basis, it is concluded that the tomato Tospovirus should be considered as a strain of GBNV and designated as GBNV-To.

Key words: Tospovirus, tomato, nucleocapsid protein gene, Groundnut bud necrosis virus

Tospoviruses belonging to the family Bunyaviridae (Van Regenmortel et al., 2000) are causing substantial losses worldwide to crops such as groundnut, potato, tobacco, vegetables and ornamental plants (Moyer, 1999). They are exclusively vectored by several thrips species in a circulative and propagative manner (Moyer, 1999). Tospoviruses are quasispherical (80-110 nm diameter) enveloped particles consisting of three linear single stranded RNA species designated small (S) RNA, medium (M) RNA and large (L) RNA. A set of descriptors such as host range, vector specificity and nucleocapsid protein (N) gene sequence identities have been identified, based on which thirteen distinct Tospovirus species were described (Moyer, 1999).

In India, so far three distinct Tospovirus species namely Groundnut bud necrosis (GBNV) (Reddy et al., 1992); Groundnut yellow spot (GYSV) (Satyanarayana et al., 1998) on groundnut and Watermelon bud necrosis (WBNV) (Jain et al., 1998) on watermelon have been identified. Recently, association of GBNV was also established with soybean bud blight (Bhat et al., 2002). Occurrence of Tospovirus infections have also been recorded on other crops including tomato (Prasada Rao et al., 1980, 1987; Bhat et al., 2001), but their exact identification remained unaddressed. We report here the biological and molecular characterization of tomato Tospovirus isolate and discuss its taxonomic position within the genus Tospovirus.

MATERIALS AND METHODS

Virus Isolate

Tomato plants showing bronzing and severe necrosis of leaves and growing buds were collected from experimental farm of College of Agriculture, Kerala Agricultural University, Vellayani. Association of the Tospovirus with symptomatic tomato plants was confirmed by bio- and immuno-assays.

The virus was rub inoculated on to cowpea (Vigna unguiculata cv. Pusa Komal) in a glasshouse at primary leaf stage using 0.01 M potassium
phosphate buffer (pH 7.2) containing 0.1% β-mercaptoethanol. For immuno-assay, direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) was conducted using polyclonal antisera to Watermelon silver mottle (WSMV) and Groundnut bud necrosis (GBNV) viruses (Clark and Bar-Joseph, 1984).

Host range

Thirty-five plant species belonging to five families Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae and Solanaceae were tested (Table 1). The test plants were raised in pots in an insect-free glasshouse and rub inoculated. The inoculated seedlings (10 each) were observed for symptoms over a period of four weeks after inoculation. ELISA tests were used in some cases in addition to symptomatology to check the virus presence.

RNA isolation

Total nucleic acid from infected tissues (ca. 10 mg) was extracted using RNeasy Kit (Qiagen Inc., GmbH, Hilden, Germany) according to the manufacturer's instructions and was used as a template for amplification in the reverse transcription and polymerase chain reaction (RT-PCR).

PCR amplification

RT-PCR (Pappu et al., 1993) was performed using the primer pair (5'ATGTCTAACGT(C/T)AAGCA(A/G)CTC3' and 5'TTACAATTCAGCGAAGGACC3') derived from the nucleocapsid protein (N) gene sequence of GBNV (Satyanarayana et al., 1996) and WSMV (Yeh and Chang, 1995). Amplification was performed in an automated thermal cycler (Power Block II, Ericomp. Inc. San Diego, CA, USA) programmed for one cycle of 42°C for 45 min for cDNA synthesis and 40 cycles of amplification with 30s of denaturation at 94°C, 1 min of annealing at 56°C and 1 min of extension at 72°C followed by one cycle of final extension for 60 min at 72°C. The PCR products were analysed by 1.0% agarose gel electrophoresis.

Cloning and sequencing

The ca. 800 bp band corresponding to the N gene was excised from the gel and purified using QiaX II gel extraction kit (Qiagen Inc., GmbH, Hilden, Germany). The purified PCR product was ligated into pGEM-T Easy vector (Promega, Madison, WI, USA) and competent Escherichia coli cells (Strain DH5α) were transformed by following standard molecular biology procedures (Sambrook and Russell, 2001). Recombinant clones were identified by EcoR1 restriction endonuclease digestion and selected clones were sequenced at the automated DNA sequencing facility at the Department of Biochemistry, South Campus, University of Delhi, Delhi. Sequence data were initially analysed and compared using Seqaid Version 3.6 (Rhoads and Roufa, 1985). Multiple sequence alignments were made using CLUSTAL W (Thompson et al., 1994). Sequence phylograms were constructed using PHYLIP package (Bootstrap analysis with 1000 replicates) and unrooted trees generated using TREEVIEW software (Page, 1996). The N gene nucleotide and amino acid sequences of other known Tospoviruses from India used for comparison were obtained from GenBank (Benson et al., 1999).

RESULTS AND DISCUSSION

Necrosis affected tomato plants reacted with polyclonal antisera directed against nucleocapsid protein of GBNV (A 405 = 1.348) and WSMV (A 405 = 2.105), suggesting the association of a Tospovirus antigenically related to WSMV serogroup (Van Regenmortel et al., 2000). Bio-assay revealed that the virus was easily transmitted to cowpea (cv. Pusa Komal), a diagnostic assay for Tospovirus (Bhat et al., 2001). Both localized as well as systemic infections characterized by chlorotic/necrotic lesions, veinal and systemic necrosis were observed.

The experimental host range of the tomato Tospovirus was ascertained. The virus could infect 15 of 26 different hosts tested from four families (Table 1). None of the Cucurbitaceae species tested were infected. Symptoms induced by the virus in most cases were similar to those caused by GBNV (Ghanekar et al., 1979). The virus caused only chlorotic and necrotic lesions on plant species belonging to Amaranthaceae and Chenopodiaceae families. Both localized as well as systemic
Table 1. Experimental host range studies of tomato Tospovirus isolate

<table>
<thead>
<tr>
<th>Host</th>
<th>Symptoms*</th>
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<td></td>
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<td>Amaranthaceae</td>
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<td>Chenopodiaceae</td>
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<td>CS, NLL</td>
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<td>CS, NLL</td>
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<td>C. quinoa</td>
<td>CS, NLL</td>
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<td>Cucurbitaceae</td>
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<td>Benincasa hispida</td>
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<td>Cucumis melo</td>
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<td>Cucurbita pepo</td>
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<td>Luffa acutangula</td>
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<td>Cajanus cajan</td>
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<td>Glycine max</td>
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<td>VN, SN</td>
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<td>V. unguiculata cv. Pusa Komal</td>
<td>CS, NLL</td>
<td>VN, SN</td>
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<td>NLL</td>
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<td>Datura stramonium</td>
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<td>Lycopersicon esculentum</td>
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<td>Nicotiana benthamiana</td>
<td>CS, NLL</td>
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<td>N. glutinosa</td>
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<td>N. tabacum</td>
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<td>Physalis floridana</td>
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<td>Solanum melongena</td>
<td>CS, NLL</td>
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*BN – Bud Necrosis; CS – Chlorotic spot; NLL – Necrotic local lesion; SN – Stem necrosis; VN – Veinal necrosis; – = No symptoms

Infections were observed on Fabaceae and Solanaceae species. The virus induced chlorotic and/or necrotic lesions followed by veinal necrosis, stem necrosis, leaf deformation and bud necrosis in Arachis hypogaea, Vigna mungo, V. radiata, V. unguiculata, Macrotyloma uniflorum and Physalis floridana. Symptomatic plants were ELISA positive and A. hypogaea plants supported maximum virus concentration (A 405 = 1.074) followed by N. benthamiana (0.757), V. unguiculata (0.543), P. floridana (0.407), V. radiata (0.327) and Phaseolus vulgaris (0.278).

The nucleotide and translated amino acid sequences of the nucleocapsid protein (N) gene of the tomato Tospovirus are presented in Fig. 1. Sequence has been submitted to GenBank as accession number AF515817. The cloned region contained a single open reading frame of 831 nucleotides long that could potentially encode a protein of 276 amino acids, which was 1 and 30 amino acids longer than that of corresponding gene of WBNV (Jain et al., 1998) and GYSV (Satyanarayana et al., 1998) respectively.

The N gene was compared with corresponding gene from other known Tospoviruses recorded from India. Cluster dendrogram revealed that the tomato Tospovirus was most closely related to GBNV (Fig. 2), forming one cluster. Comparative sequence analyses showed that the tomato Tospovirus shared maximum sequence identity with GBNV at nucleotide (94%) as well as amino acid (96%) levels (Fig. 3). In contrast, 45-81% and 16-85% nucleotide and amino acid sequence identities were observed with N genes of other Tospoviruses. Close sequence relationship between the N genes of the tomato Tospovirus and GBNV was in agreement with the serological data.

Isolates in the Tospovirus genus with greater than 90% nucleocapsid protein (N) gene sequence identity are delineated as strains of the same virus (Moyer, 1999). In view of this and considering biological characteristics, the tomato Tospovirus should be regarded as a strain of GBNV and designated as GBNV-To. Since GBNV has been reported to possess broad experimental host range (Ghanekar et al., 1979), it is anticipated that GBNV could become wide-spread and has moved from...
Fig.1. Nucleotide and deduced amino acid sequences of nucleocapsid protein (N) gene of tomato Tospovirus (GBNV-To)
ACKNOWLEDGEMENTS

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REFERENCES


Fig.2. Cluster dendrogram showing the relationship among the deduced amino acid sequences of the nucleocapsid protein (N) gene of tomato Tospovirus (GBNV-To) with those of known Tospoviruses recorded from India

Fig.3. CLUSTAL W generated multiple alignment of nucleocapsid protein (N) gene sequences of Groundnut bud necrosis virus (GBNV) and tomato Tospovirus (GBNV-To). Asterisks indicate identical amino acid residue at a given position. Differences have been shown with bold letters.


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