Molecular characterization of *Tobacco streak virus* causing soybean necrosis in India

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A virus isolate infecting soybean (*Glycine max* L.) with characteristic symptoms of necrosis was collected from various locations of Maharashtra, India. The virus was detected as *Tobacco streak virus* (TSV) by direct antigen-coating-enzyme-linked immunosorbent assay (DAC-ELISA) using TSV specific antisera. ELISA positive TSV soybean isolates upon mechanical transmission onto indicator hosts, viz. *Vigna unguiculata* cv. C-152, *Glycine max* and *Nicotiana tabacum* cv. Xanthi, produced characteristic local necrotic lesions on primary inoculated leaves, followed by systemic infection. Coat protein (CP) gene from a representative soybean isolate (TSV-SB) was amplified using TSV CP specific primers. The amplicon of ~750 bp was cloned and sequenced. The CP gene consists of 717 nucleotides, potential of coding a polypeptide of 238 amino acid residues. The CP gene of TSV-SB isolate shared 98.3 to 99.3% nucleotide and 96.6 to 98.3% amino acid sequence homology with the TSV isolates characterized from India. With TSV-WC (White clover isolate from America, type strain) and TSV-BR (Soybean isolate from Brazil), TSV-SB isolate shared 88.7% and 79.2% amino acid sequence homology, respectively. Phylogenetic tree constructed based on amino acid sequence analysis showed a close clustering of TSV-SB isolate with other TSV isolates from India than with the American and Brazilian strains. Based on CP gene sequence analysis, it is concluded that the TSV-SB isolate is a strain of TSV that is prevalent in India. This is the first report of molecular characterization of TSV infecting soybean from India.

**Keywords**: coat protein gene, *Tobacco streak virus*, soybean

**Introduction**

A disease characterized as bud blight with symptoms of chlorosis, necrosis of leaves, stem, and buds, and stunting was reported to cause serious damage to soybean (*Glycine max* L.) in India\(^1\). Based on the serological and molecular characterization, the causal agent of soybean bud blight was identified as a strain of *Groundnut bud necrosis virus* (GBNV)\(^2,3\). In recent years, *Tobacco streak virus* (TSV)—an economically important virus in India—was reported to produce similar symptoms of necrosis\(^3,5\) as described in the bud blight disease of soybean\(^3\). Such similarity in the necrotic symptoms due to TSV or GBNV infections was earlier reported on groundnut\(^6\). Serological reaction\(^5\) and reverse transcription polymerase chain reaction (RT-PCR)\(^4\) confirmed the TSV infection in soybean. The necrosis of soybean was reported to be prevalent up to 40% in Maharashtra resulting in severe yield losses\(^5\). The association of TSV with bud blight of soybean was earlier reported from USA\(^7,8\) and Brazil\(^9,10\), where it contributed to yield losses of 25 and 100%, respectively.

In recent years, TSV has emerged as an important virus infecting several economically important crops in India\(^3,5,11-19\). In India, based on coat protein (CP) gene sequence analysis, the virus isolate infecting sunflower crop was identified as a strain of TSV distinct from the type strain, TSV-WC (White clover isolate from America)\(^19,20\). Further, the CP gene sequence analysis of the TSV isolates characterized from various crops and locations of India was reported to share 99 to 100% nucleotide sequence homology among each other and shared 88 to 89% sequence homology with the type strain\(^19,21\). Recently, a TSV isolate causing soybean bud blight disease in Brazil (TSV-BR) was reported to be a distinct strain of TSV, which shared 81.3 and 80.7% nucleotide sequence homology with the CP gene of TSV-WC and TSV-MB (mungbean isolate from India), respectively\(^10\). However, the complete molecular identity of the soybean isolate causing necrosis disease in India was not addressed. Thus, the present study was aimed to determine the molecular identity of the TSV isolate infecting soybean from India.
characterization and phylogenetic relationship of the TSV soybean isolate from India.

Materials and Methods

Virus Isolates

Soybean plants showing symptoms of chlorosis, mosaic mottling, necrosis of leaves, petiole, stem and pods were collected from Jalna, Beed and Osmanabad regions of Maharashtra. Symptomatic samples collected from various locations were diagnosed by direct antigen-coating-enzyme-linked immunosorbent assay (DAC-ELISA) using TSV specific polyclonal antiserum (Mahyco Research Center). The ELISA positive samples were further inoculated onto Vigna unguiculata cv. C-152 (cowpea), G. max and Nicotiana tabacum cv. Xanthi (tobacco) by mechanical sap inoculations, using 0.05% HCN (Applied Biosystems). Nucleotide sequence data was compiled using ContigExpress and AlignX program in Vector NTI version 6.0 software. Translations of the primary nucleotide sequences, multiple sequence alignment and the sequence homologies with the corresponding CP gene sequence of other TSV isolates were determined using Bio Edit Sequence Alignment Editor. Based on the sequence homologies, a phylogenetic tree was generated using NTSYS-pc version 2.11 software. The CP gene sequences of TSV isolates used in the present sequence analysis were downloaded from NCBI GenBank (Table 1).

Results and Discussion

Infected soybean plants showing the symptoms of chlorosis and necrotic lesions on leaves, veins, midrib, petioles, stem and pods reacted positive with the TSV polyclonal antiserum in DAC-ELISA, suggesting association of TSV with the necrosis disease. Of 30 soybean samples collected from Jalna, Osmanabad and Beed regions of Maharashtra, 25

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Cloning, Sequencing and Sequence Analysis

The amplicon was gel eluted using QIAEXII gel extraction kit (Qiagen Inc., Chatsworth, CA, USA) and ligated into pGEM-T Easy vector (Promega, Madison, WI, USA) following the manufacturer’s instructions. The ligated product was transformed into Escherichia coli DH5α competent cells by following standard molecular biology procedures. Recombinant clones were identified by restriction digestion analysis using Ncol and Xhol enzymes. The recombinant TSV CP plasmid was sequenced with universal sequencing primers (T7 and M13 reverse) using BigDye termination cycle sequencing kit (Applied Biosystems, USA) and analyzed in an automated DNA sequencer (ABI Prism377, Applied Biosystems). Nucleotide sequence data was compiled using ContigExpress and AlignX program in Vector NTI version 6.0 software. Translations of the primary nucleotide sequences, multiple sequence alignment and the sequence homologies with the corresponding CP gene sequence of other TSV isolates were determined using Bio Edit Sequence Alignment Editor. Based on the sequence homologies, a phylogenetic tree was generated using NTSYS-pc version 2.11 software. The CP gene sequences of TSV isolates used in the present sequence analysis were downloaded from NCBI GenBank (Table 1).
reacted to TSV antiserum with absorbance values at A_{405 \text{ nm}} ranged from 0.48 to 1.80. The symptomatic leaf (ELISA positive) samples upon mechanical sap inoculation produced necrotic lesions on the inoculated primary leaves of cowpea cv. C-152, soybean and tobacco followed by systemic necrosis confirming the natural infection of TSV on soybean plants.

In RT-PCR analysis, total RNA isolated from the symptomatic soybean leaf tissue amplified ~750 bp fragment corresponding to the CP gene of TSV. No such amplification was observed when total RNA extracted from a healthy soybean leaf tissue (data not shown). Sequence analysis showed that the amplified product was 717 nucleotides long coding for a protein of 238 amino acids, which was similar to the size of CP gene of the TSV isolates characterized from India\(^{7,18,20,21}\) and Brazil (TSV-BR)\(^{10}\). Whereas, the CP sequence of TSV soybean (TSV-SB, present study) and other Indian isolates possessed an additional amino acid residue at 25\(^{th}\) position compared to the 237 amino acid encoding CP of TSV-WC, type strain\(^{23}\). The primary nucleotide sequence of TSV-SB isolate has been deposited in NCBI database (Acc.No. AY940151). Comparative sequence analysis revealed that the CP gene of TSV-SB shared 98.3 to 99.3% and 96.6 to 98.3% homologies at nucleotide and amino acid sequence levels, respectively with the TSV isolates characterized from other crop species and locations of India (Table 2). TSV-SB isolate showed 5 amino acid substitutions in the CP at 7\(^{th}\), 25\(^{th}\), 120\(^{th}\), 154\(^{th}\) and 157\(^{th}\) positions from glycine to serine, arginine to serine, phenylalanine to leucine, valine to alanine and valine to alanine, respectively with reference to the conserved CP amino acid sequence of the Indian TSV isolate. With TSV-WC (American) and TSV-BR (Brazilian), CP of TSV-SB isolate shared 88.7 and 79.2% amino acid sequence homology, respectively. The phylogenetic analysis based on the deduced amino acid sequences showed a close clustering of TSV-SB with other Indian TSV isolates, irrespective of the hosts and the locations from which/where the isolates were characterized; while it diverged from the type strain as well as the Brazilian soybean isolate (Fig. 1).

In the present study, the association of TSV with necrosis disease of soybean in India was established based on serology, transmission and CP gene sequence analysis. CP gene of TSV-SB isolate shared a high degree of sequence homology with other TSV

![Phylogenetic tree showing the relationship of Tobacco streak virus soybean isolate (TSV-SB) with other isolates from India, America (TSV-WC, type strain) and Brazil (TSV-BR) based on the coat protein sequence homologies.](Image)

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isolates from India. Based on the CP gene sequence analysis it is concluded that TSV-SB should be regarded as a strain of TSV-SF prevalent in India. Further, the CP gene sequence homology of the Indian isolates with the TSV isolates from other parts of the world was below 90% (which was proposed as a threshold level for demarcating virus isolates at species and strain level), strengthen the argument of a new strain of TSV from India. This is first report of molecular characterization of TSV on soybean from India. The high degree of CP gene sequence homology among various TSV isolates suggest that the virus isolates so far characterized from various crops and locations in India are of common origin.

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