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Molecular characterization and genetic diversity of *Tobacco streak virus* infecting soybean (*Glycine max* L.)

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Soybean (*Glycine max* L.), the most popular oil seed crop, grown in the experimental fields of Tamil Nadu Agricultural University and adjoining areas exhibited symptoms of necrosis and stunting. The symptoms were similar to bud blight of soybean caused by the *Tobacco streak virus* (TSV) and the disease is most prevalent throughout the country. To study the nature of infection, sap inoculation of the soybean strain induced local as well systemic infection on cowpea plants cv. C 152 and resulted in the production of circular necrotic lesions and death of plants. The samples were also serologically positive in DAC-ELISA and it has also yielded a protein band of approximately 29 kDa corresponding to coat protein of TSV in Western blot assay. For the characterization of virus, RT-PCR was carried out with a newly designed coat protein gene specific primer, which resulted in amplification of the expected 929 bp size. Sequence analysis of the CP gene had nucleotide similarity of 80.6 to 99.3% with known isolates of TSV. The multiple sequence alignment revealed that CP gene showed single unique variation and some of deletion and addition mutation was found in nucleotide and amino acid sequences against the isolates of other soybean Brazil and USA isolates and produced single unique variation at position 344 where adenine was substituted with guanine. There was no deletion and addition between nucleotide sequences in the group of Indian isolates, further confirms the placement of the soybean isolate of TSV in a single subgroup.

Key words: Soybean, *Tobacco streak virus*, coat protein gene, diversity analysis.

INTRODUCTION

Soybean (*Glycine max* L.) is known as 'Golden bean' and is a native of North China, Asia belongs to family Fabaceae. It is a versatile and fascinating crop with innumerable possibilities of not only improving agriculture but also supporting industries. It is a rich source of lysine (6.4%) in addition to other essential amino acids, vitamins

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and minerals. Like other economically important crops soybean is susceptible to different diseases caused by viruses. *Yellow mosaic virus* (YMV), *Soybean mosaic virus* (SMV) and *Groundnut bud necrosis virus* (GBNV) are the major viral diseases of soybean in India, which causes considerable reduction in yield up to 80% under severe conditions (Thakur et al., 1998; Rebedeaux et al., 2004; Lal et al., 2005). In addition to above, a recent outbreak of soybean bud blight caused by *Tobacco streak virus* (TSV) being reported to be an emerging virus and considered as a major constraint in soybean (Arun Kumar et al., 2008). This may be an indication of the occurrence of a virus disease which also reduces yield and in some varieties 100% yield losses have been observed. The characteristic symptoms caused by the TSV in soybean under field conditions are stunting of plant and necrotic patches on the leaves of growing tip of plants. Johnson (1936) reported infection of TSV in tobacco and it is a member of the genus *Ilarvirus* under the family Bromoviridae. In India, the TSV infects several other crops in addition to soybean (Jain et al., 2005; Sivaprasad et al., 2010; Bhaskara Reddy et al., 2012). TSV can be transmitted mechanically, but the transmission of TSV commonly occurs through different species of thrips *viz*., *Megalurothrips usitatus, Frankliniella schultzei*, *Scirtothrips dorsalis*, *Thrips palmi* and *Thrips* tabaci under field conditions (Jagtap et al., 2012) Alternative host plants have been suspected to harbour TSV which have contributed in its transmission. The virus causes asymptomatic infections in several common weed species, including *Parthenium hysterophorus, Ageratum conyzoides* and *Corchorus trilocularis,* whose pollen is a major source of TSV and these plants, also harbour thrips (Prasada Rao et al., 2003; Shukla et al., 2005). Though the occurrence of TSV has been reported from many hosts in India, only limited reports are available on the biological and molecular characterization of these isolates and their exact identification remains unaddressed in soybean. In this study, we report the natural occurrence of TSV on soybean and its molecular properties and phylogenetic relationship with other TSV isolates.

MATERIALS AND METHODS

Virus isolates and maintenance of inoculum

Soybean (*Glycine max* L.) plants showing characteristic symptoms of TSV were collected from naturally infected field at Coimbatore (Tamil Nadu) and used as inoculum of virus. The infected plants were identified by the presence of veinal necrosis on the growing leaves, necrotic spots on the leaves and stunting of plants (Figure 1a). The TSV infected samples collected from field were subjected to direct antigen coating ELISA (DAC-ELISA) as per the procedure described by Hobbs et al. (1987) with the polyclonal antiserum specific to TSV (kindly provided by the ICRISAT, Hyderabad). The cowpea plants cv. C 152 was used for propagating the virus. The cowpea C 152 plants were raised in the glasshouse under insect proof conditions. The virus extract was prepared by macerating TSV infected leaf samples with 0.1 M sodium phosphate buffer pH 7.0 using ice tray and inoculated mechanically in cowpea cv. C125 cotyledonary leaves of six day old plants previously dusted with 600 mesh carborundum powder. The inoculated plants were kept under observation for 4 to 5 days for the expression of symptoms (Subramanian and Narayanasamy, 1973).

Purification of *Tobacco streak virus*

Virus infected leaves from cowpea C 152 were ground in 0.01 M potassium phosphate buffer, pH 8.0 (2:1w/v) containing 1% 2-mercaptoethanol. The slurry was filtered in a double cheesecloth, clarified with chloroform (1/2 vol) and stirred for 20 min at 4°C. The extract was centrifuged at 10,000 g for 10 min at 4°C and the supernatant was mixed with 6% PEG 8,000 at 4°C for 2 h. After low speed centrifugation (10,000 g for 10 min) the pellet was dissolved overnight at 4°C. After another low speed centrifugation the supernatant was centrifuged at 180,000 *g* for 2 h in a swinging bucket rotor using 25% sucrose frozen gradient (Baxter-Gabbard, 1972; Davis and Pearson, 1978). The virus was fractionated using an ISCO density gradient fractionator and UV analyzer. Fractions were diluted in 0.01 M potassium phosphate buffer, pH 8.0 and centrifuged at 100,000 g for 90 min. The pellet was dissolved and centrifuged at 10,000 g for 10 min and the supernatant rescued and stored. Virus yield was determined by assuming an extinction coefficient of 5.1 (Salazar et al., 1982).

Western blot analysis

A mixture of an equal amount of virus preparation and dissociation buffer (0.125 M Tris-HCl, pH 6.7, 3% SDS, 20% glycerol and 10% 2-mercaptoethanol) was boiled for 5 min. Electrophoresis was carried out in 12.5% (separating) polyacrylamide gels according to Laemmli's method (1970). The pre-stained molecular weight markers (Fermentas Life Sciences) along with the samples were loaded (20 µl) into the slot of the gel. The protein was electrophoretically transferred to nitrocellulose blotting membrane (NC, Sartorius, 0.2 µm pore size) in Tris-glycine buffer containing 20% (v/v) methanol overnight at 25 V at 4^{o}C. The membrane was washed and blocked for 1 h with 1% (w/v) hemoglobin in phosphate-buffered saline (PBS) and then separately incubated for 2 h with polyclonal antiserum specific to TSV (1:1,000) respectively in PBS-Tween (PBST). Nonspecific antibodies were removed by 10 min washing (4 times) with PBST. Goat-anti-rabbit IgG conjugated to alkaline phosphatase (Sigma) was used as the secondary antibody (1:3,000 in PBST). The NC membrane was incubated for 1.5 h at room temperature, excess antibody was removed and immuno-reactive proteins were visualized after subjecting the NC membrane to the reaction mixture containing 4-nitroblue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP).

RNA isolation and cDNA synthesis

The inoculum of TSV soybean isolate was maintained on cowpea cv. C152 and total RNA was extracted from 100 mg leaves of soybean using RNeasy plant extraction kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturers' protocol and resuspended in 50 µl nuclease free water. For cDNA synthesis of TSV, 1 µg total isolated RNA (200 ng/µl) was annealed with 0.3 µM downstream primers (GKTSV CPR-5'TGCTCGCATGGGTCATAGAC 3') at 70°C for 10 min. To the

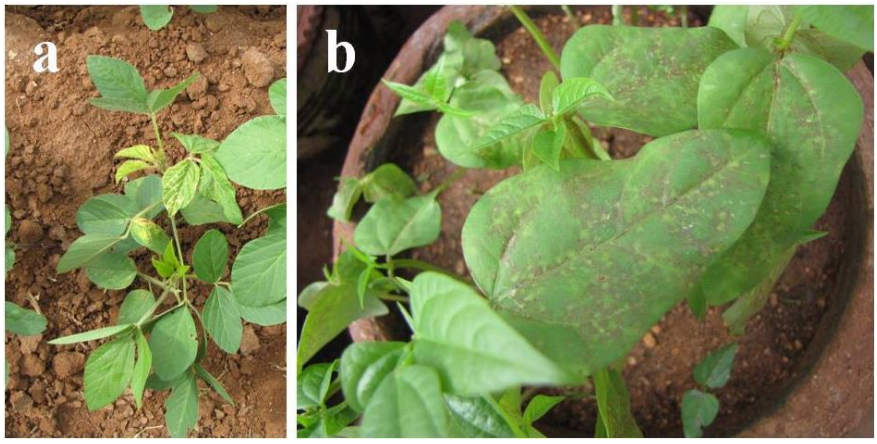

Figure 1. a. Symptoms of TSV on soybean leaves necrotic lesion under field condition; b. Development of circular necrotic lesions on the leaves of cowpea on artificial inoculation.

transcription mixture, various reaction components were added [(RNase inhibitor 1 µl (20 U); dNTPs 2 µl (10 mM); 4 µl 5× reverse transcriptase buffer containing Tris HCl 250 mM, pH 8.3 at 25°C, KCl 250 mM, $MgCl_2$ 20 mM, 1 µl DTT 50 mM)]. The reaction mixture was incubated at 37°C for 10 min, 40 U M-MuLV reverse transcriptase was added and the mixture re-incubated at 37°C for 60 min. The reaction was stopped by heating the mixture at 70°C for 10 min.

Reverse transcription polymerase chain reaction (RT-PCR)

TSV:cDNA product (5 µl) was added to 50 µl of PCR reaction mixture containing 0.20 mM each of dNTPs, 0.25 µM of each primer (GKTSV CPF - 5'AGATAAGTCGCTTCTCGGAC 3' and GKTSV CPR - 5' TGCTCGCATGGGTCATAGAC 3'), 5 µl 10X Taq polymerase buffer, 2.0 mM $MgCl_2$ and 2 U Taq DNA polymerase. The RT-PCR was performed in Eppendorf Mastercycler Gradient ES with the following thermal programme: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 30 s and extension at 72°C for 1 min and final extension of 72°C for 10 min.

Molecular cloning and sequencing

The amplicon of coat protein and replicase genes were purified using QIAGEN gel extraction kit (Qiagen Inc., Chatsworth, CA, USA) and cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) following the manufacturer's instructions and transformed into *Escherichia coli* DH5α by following standard molecular biology procedures (Sambrook et al., 1989). Plasmid DNA was isolated from the potential recombinant clones using Wizard plus DNA purification kit (Promega, Madison, WI) according to the manufacturer's protocol and the positive clones were identified by restriction digestion analysis using *EcoRI* enzyme. The three independent clones were sequenced at Chromos Biotech Pvt. Ltd., Bangalore from both orientations for each fragment separately. The sequences were then edited using the BIOEDIT Software (Hall, 1999). Sequence similarity search of the GenBank database was done using the Basic Local Alignment Search Tool (BLAST) program.

Sequence diversity and phylogenetic analysis

The amino acid sequences of the TSV coat protein gene was translated from the consensus nucleotide sequences using the EMBOSS Transeq program (Rice et al., 2000). Both the nucleotide and amino acid sequences were then aligned with selected sequences of TSV strains using the CLUSTAL W program (Larkin et al., 2007). Phylogenetic analysis was done on MEGA 5.1 (Tamura et al., 2011) and trees were created using the neighbour-joining method (Saitou and Nei, 1987). The robustness of the trees was determined by bootstrap using 1,000 replicates. *Prunus ringspot necrotic virus* (PRNV) was used as a reference out group member of the genus *Ilarvirus* for rooting the phylogenetic tree.

RESULTS

Isolation of virus and serodiagnosis

The soybean plant samples showing characteristic symptoms of TSV were collected and inoculated separately on cowpea cv. C152 plants through mechanical sap inoculation. The assay host cowpea cv. C152 expressed distinct local lesions on three to four days after inoculation. The inoculated cowpea cotyledonary leaves developed necrotic lesions and then the systemic veinal necrosis occurred. The veinal necrosis resulted in severe stem necrosis and lead to the collapse of the entire inoculated plants (Figure 1b). The results of DAC-ELISA revealed that, the samples exhibited characteristic symptoms of TSV showed strong positive reaction with approximately five fold increase in absorbance values than the apparently healthy samples.

Western blot analysis

The molecular weight of coat protein was determined by

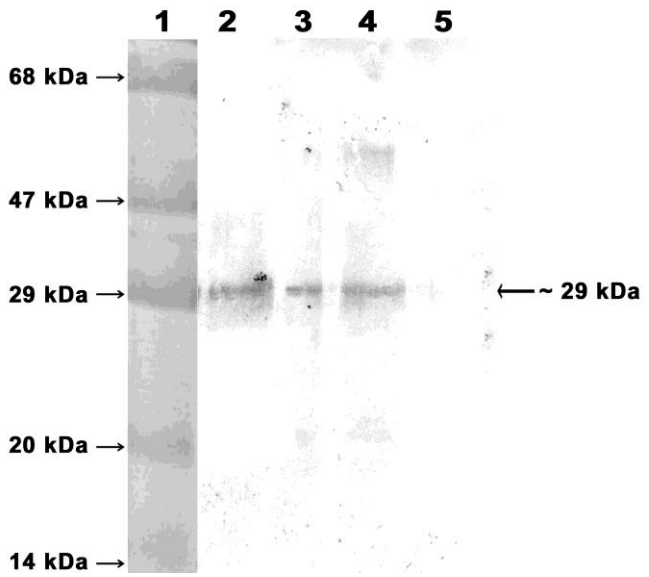

Figure 2. Western blot assay of TSV infected cowpea (primary inoculum from naturally infected soybean); Lane 2-4: TSV infected sample; Lane 5: Healthy sample (Control).

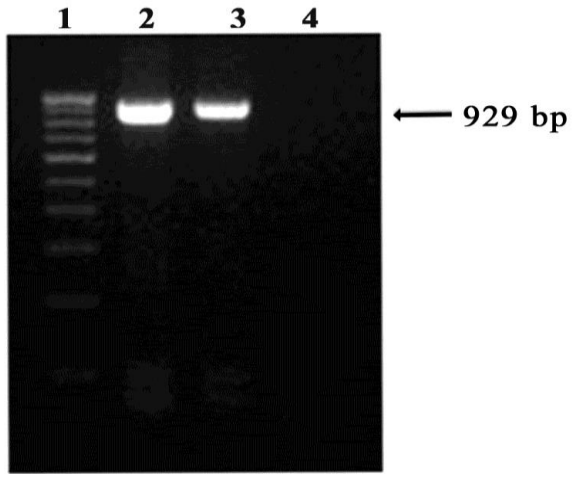

Figure 3. RT-PCR amplification of TSV coat protein gene from naturally infected leaves of soybean. Lane 1: 100 bp ladder; lanes 2-3: Amplified DNA fragment from infected samples; lane 4: Healthy samples (Control).

comparing its mobility with the marker proteins on a 12% PAGE under denaturing conditions. The isolated virus particles revealed the presence of a single band corresponding to ~29 kDa coat protein subunit. Western blot immuno assay was performed by using 1:1000 dilution of primary antibody specific to TSV (ICRISAT, Hyderabad). A 1:1500 dilution of IgG conjugated to alkaline phosphatase (secondary antibody) produced a positive reaction with the ~29 kDa band obtained with SDS PAGE. The resulted band was of viral coat protein and confirming the virus isolate under study was TSV (Figure 2).

Cloning and sequencing

Total RNA extracted from cowpea samples infected with TSV inoculum from soybean was analyzed by RT-PCR with specific primers corresponding to coat protein gene. The result revealed the infected samples resulted in the amplification of 929 bp corresponding to CP gene (Figure 3). The amplified DNA fragment of coat protein gene was excised, cloned into pGEM-T easy vector and sequence determined. The gene sequence was edited using BIOEDIT software and obtained full length nucleotide sequence. The nucleotide sequence analysis using NCBI BLAST confirmed the association of TSV.

Coat protein gene sequence analysis

The amplified fragment corresponding to CP gene contains single ORF consist of 717 bases encoded the protein with 239 amino acids. The coat protein gene sequence of soybean strain of TSV was submitted in NCBI Genbank database (Accession No. KJ825822). The sequence (KJ825822) was compared with corresponding genes from known TSV isolates at the nucleotide and amino acid sequence levels. The sequence had 98.8 and 98.4%, nucleotide homology with other soybean TSV strains of Indian isolates (DQ518916 and DQ864457), 80.6 and 87.9 % nucleotide homology with other TSV strains from soybean of Brazil and USA respectively (AY354406 and FJ403377) (Table 1). Multiple nucleotide sequence alignment and phylogenetic analysis revealed very high homologies between the TSV strains and confirmed the formation of single subgroup (Figure 4). Multiple sequence alignment further revealed a near perfect homology between the nucleotide sequence of the soybean strain and the nucleotide sequences of other strains except for a single unique variation at position 344 where adenine was substituted with guanine. Also strain produced variation at the position 15 where cytosine was substituted with adenine (DQ518916 and DQ864457). Similarly strain had variation at position 52 where cytosine was substituted with thiamine, position 521 where thiamine was substituted with cytosine and position 536 where cytosine was substituted with thiamine with other Indian isolates (DQ518916 and DQ864457) (Figure 5). The results revealed that nucleotide sequences in the group, further confirms the placement of the soybean strain of TSV as a single subgroup. Analysis of the 239 deduced amino acid sequence of the 3' end of the coat protein gene of RNA 3 revealed that the soybean strain of our TSV had 79.5 to 99.5% homology with other strains of the same virus (AY354406 and AY940154). The amino

Table 1. Nucleotide (nt) and amino acid (aa) identities of the coat protein gene of *Tobacco streak virus* (TSV) soybean strain (KJ825822) with corresponding sequences of selected isolates of TSV.

Accession No.	Strain/host	Country	% identity	
			nt	aa
DQ518916	Soybean	India	98.8	99.1
DQ864457	Soybean	India	98.4	97.4
EU368962	sunflower	India	98.8	98.7
DQ058079	Cowpea	India	98.8	99.1
FJ561304	Okra	India	99.1	98.7
JQ269831	Guar	India	98.8	99.1
FJ561301	Pumpkin	India	98.4	97.8
FJ655172	Gherkin	India	99.0	98.7
AY590139	Chilli	India	98.0	97.8
FJ608537	Watermelon	India	98.8	98.3
AF515823	Mungbean	India	98.7	98.7
FJ749259	Blackgram	India	98.4	97.4
HM131487	Greengram	India	98.8	98.7
AY940154	Cotton	India	99.3	99.5
FJ417083	Marigold	India	98.8	98.7
FJ355949	Groundnut	India	97.9	97.4
FJ655170	Squash	India	99.0	99.1
AY354406	Soybean	Brazil	80.6	79.5
FJ403377	Soybean	USA	87.9	90.0
AJ969095	Rose - PRNV	India	37.3	18.2

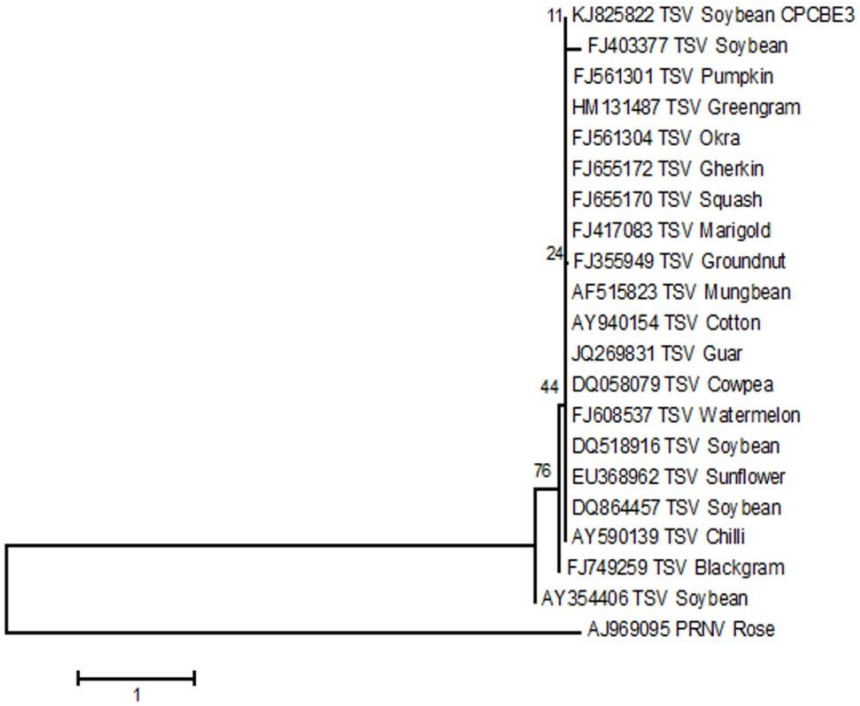

Figure 4. Neighbour-joining phylogenetic tree based on the nucleotide sequences of the coat protein gene of TSV (KJ825822) and *P. ringspot necrosis virus* is defined as an out-group.

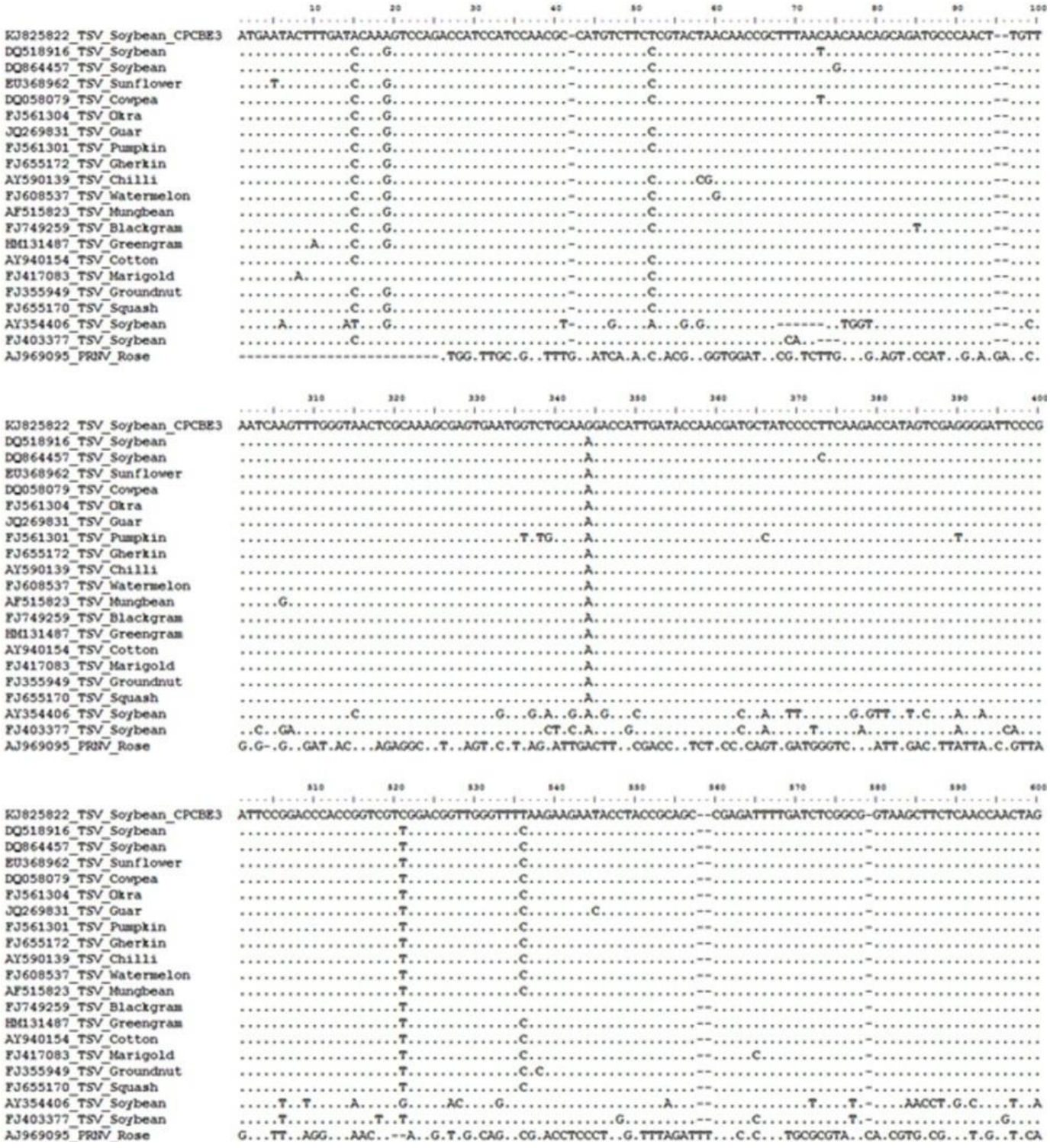

Figure 5. Multiple sequence alignment of the 717 nucleotide sequences of the coat protein gene of the soybean strain of TSV and corresponding sequences of 19 selected strains of TSV. *P. ringspot necrosis virus* is defined as an out-group.

acid sequence had 99.1 and 97.4% homology with other soybean TSV strains of Indian isolates (DQ518916 and DQ864457), 79.5 and 90.0% amino acid homology with other TSV strains from soybean of Brazil and USA

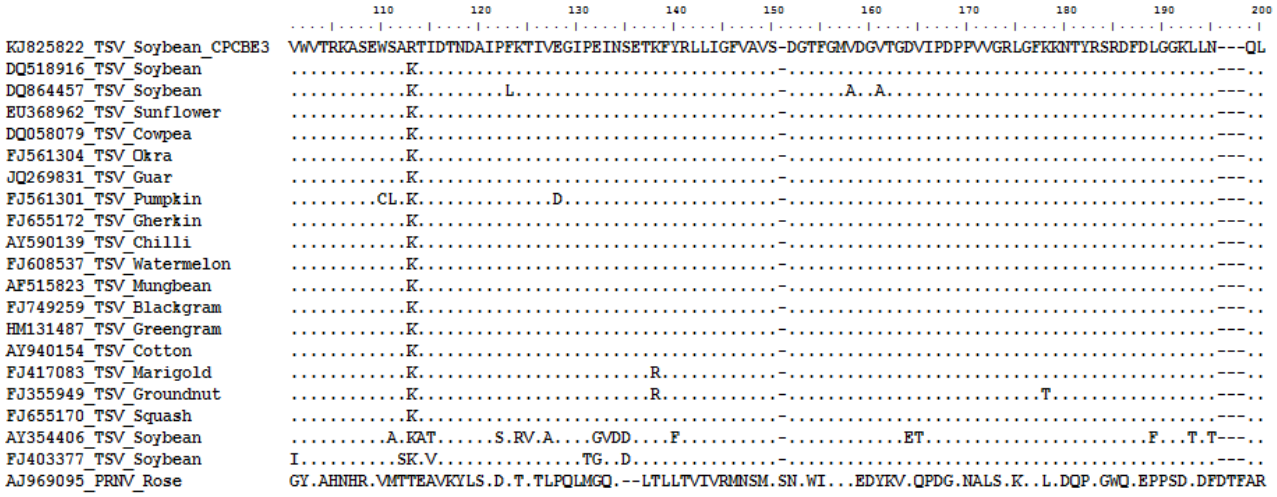

Figure 6. Multiple sequence alignment of the 239 amino acid sequences of the 3' end of the coat protein gene of the soybean strain of TSV and corresponding sequences of 19 selected strains of TSV. *P. ringspot necrosis virus* is defined as an out-group.

respectively (AY354406 and FJ403377). The soybean strain had single unique amino acid variation at position 113 where Lysine substituted with arginine (Figure 6). Also amino acid sequence has deletion mutation from the position 43 and 44 against the TSV soybean strains from Brazil and USA respectively (AY354406 and FJ403377).

DISCUSSION

Characterization of *Tobacco streak virus* infecting soybean (*Glycine max* L.) in Tamil Nadu provides knowledge of better understanding the genetic composition, variation caused by mutation and recombination and correct taxonomic position. TSV had wider host range and extending its host range day by day. Due to different environmental constraints on the evolution of new strains, it is important to study the phylogenetic relationship of the viruses locally. TSV on soybean showed symptoms *viz*., stunting and necrotic patches on leaves of growing tip of plants under field conditions. The soybean exhibiting typical symptoms of TSV were inoculated on cowpea cv. C 152 plants, resulted in production of typical necrotic lesions on inoculated primary leaves, systemic veinal necrosis and death of plants under glasshouse conditions. The circular necrotic lesions on cowpea are the characteristic symptoms of TSV by mechanical inoculation (Ramiah et al., 2001; Ladhalakshmi et al., 2006; Arun Kumar et al., 2008). Serological or immunological assays have been developed and successfully used for a number of years for the detection of plant viruses. TSV infected soybean collected from field were found to be positive for TSV specific polyclonal antibody. This type of results was supported by Bhaskara Reddy et al. (2012) who raised the polyclonal antibody against the TSV and showed positive reaction for sample collected from natural infection of *Hibiscus cannabinus* in DAC-ELISA. Prasad Rao et al. (2003) also proved the detection of a new strain SB-10 of TSV from potato through DAC-ELISA. In our study virus preparation has reacted with the antiserum specific to TSV, which was produced approximately 29 kDa protein in western blot assay. This results was supported by Almeida et al. (2005) reported coat protein gene of TSV from infected soybean in Brazil had a molecular mass of 29.880 kDa. This suggested that necrosis disease of soybean was caused by the TSV.

PCR has been shown effective in rapid and sensitive detection of many plant viruses (Candresse et al., 1998). To detect TSV infection in soybean plants, RT-PCR technique was used to amplify CP gene with self-designed primers. Approximately 929 bp including UTR corresponding to CP gene was amplified using specific primer, while no such band was observed when total RNA extracted from healthy tissue. The products were cloned and its nucleotide sequences were determined. The CP gene of TSV isolate was compared with corresponding gene from known TSV isolates at the nucleotide and amino acid sequence levels. Phylogenetic analysis revealed soybean strain has very high homologies between the TSV strains of other crops and confirmed the formation of single subgroup (Figure 4). Multiple sequence alignment revealed a near perfect homology between the nucleotide sequence of the soybean strain and the nucleotide sequences of other strains. The CP gene showed single unique variation and some of deletion and addition mutation was found in

nucleotide sequences against the strains from soybean Brazil (AY354406) and USA (FJ403377) isolates. Rajamanickam and Karthikeyan (2014) characterized the CP gene of TSV okra stain and reported sequence had two unique variations at the position 15 where cytosine was substituted with adenine and it produced unique variation at the position 526 where cytosine was substituted with thiamine. There was no deletion and addition between nucleotide sequences in the group. Cornelissen et al. (1984) cloned and sequenced TSV RNA3 genome reveals that, complete sequence of 2,205 nucleotides of TSV RNA 3, confirming 140 bp 3'-terminal residues. Two long open reading frames starting with a methionine codon are revealed by this sequence. Similarly, Bhat et al. (2002) conducted serology and characterization of coat protein studies for the sunflower *Ilarvirus* from India and they reported that it should be regarded as a strain of TSV belonging to subgroup I, designated as TSV-SF, which shared 90% amino acid sequence identity with TSV (strain WC). Almeida et al. (2005) amplified coat protein gene of TSV with a size of 717 nucleotides along with 287 nucleotides at 3' untranslated region using RT-PCR and the results revealed that nucleotides and amino acids showed 96 to 98% similarity to other TSV isolates. They also reported TSV isolate causing soybean bud blight disease in Brazil was reported to be a distinct strain of TSV (TSV-BR), which shared 81.3 and 80.7% nucleotide sequence homology with the CP gene of TSV-WC and TSV-MB (mungbean isolate from India). In conclusion, the CP gene of TSV soybean strain revealed, single unique variation and some of deletion and addition mutation was found in nucleotide and amino acid sequences of CP gene of TSV. But, there were no amino acid changes except, three positions compared to other Indian isolates used in the study. Such studies would help in the development of strategies for the control of viral diseases.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Almeida AMR, Sakai J, Hanada K, Oliveira TG, Belintani P, Kitajima EW, Souto ER, Novaes TG, Nora PS (2005). Biological and molecular characterization of an isolate of *Tobacco streak virus* obtained from soybeans in Brazil. Fitopatol. Bras. 30(4):366-373.

Arun Kumar N, Lakshminarasu M, Zehr UB, Ravi KS (2008). Molecular characterization of *Tobacco streak virus* causing soybean necrosis in India. Indian J. Biotechnol. 7:214-217

Baxter-Gabbard KL (1972). A simple method for the large scale preparation of sucrose gradients. FEBS Lett. 20:117-119

Bhaskara Reddy BV, Sivaprasad Y, Naresh Kumar CVM, Sujitha A, Raja Reddy K, Sai Gopal DVR (2012). First Report of *Tobacco streak virus* infecting kenaf (*Hibiscus cannabinus*) in India. Indian J. Virol. 23(1):80-82.

Bhat AI, Jain RK, Kumar A, Ramiah M, Varma A (2002). Serological and coat protein sequence studies suggest that necrosis disease on sunflower in India is caused by a strain of *Tobacco streak ilarvirus.* Arch. Virol. 147:651-658.

Candresse T, Hammond RW, Hadidi A (1998). Plant virus disease control. APS Press, USA. pp 399-416.

Cornelissen BJC, Janssen HK, Zuidema D, Bol JF (1984). Complete nucleotide sequence of *Tobacco streak virus* RNA 3. Nucleic Acids Res. 12:2427-2437.

Davis PB, Pearson CK (1978). Characterization of density gradients prepared by freezing and thawing a sucrose solution. Anal. Biochem. 91:343-349.

Hall TA (1999). Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symp. Series 41:95-98

Hobbs HA, Reddy DVR, Rajeshwari R, Reddy AS (1987). Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. Plant Dis. 71: 747-749.

Jagtap GP, Jadhav TH, Utpal D (2012). Occurrence, distribution and survey of *Tobacco streak virus* (TSV) of cotton. Sci. J. Crop Sci. 1(1):16-19.

Jain RK, Bag S, Awasthi LP (2005). First report of natural infection of *Capsicum annuum* by *Tobacco streak virus* in India. Plant Pathol. 54: 257.

Johnson J (1936). Tobacco streak, a virus disease. Phytopathol. 26: 285-292.

Ladhalakshmi D, Ramiah M, Ganapathy T, Reddy MK, Salaheddin K, Merin B, Kamalakannan A (2006). First report of the natural occurrence of *Tobacco streak virus* on blackgram (*Vigna mungo*). Plant Pathol. 55:569.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:1018-1020.

Lal SK, Rana VKS, Sapra RL, Singh KP (2005). Screening and utilization of soybean germplasm for breeding resistance against *Mungbean yellow mosaic virus*. Soybean Genet News Lett. 31: 45.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947-2948

Prasada Rao RDVJ, Reddy AS, Reddy SV, Thirumaladevi K, Chander Rao S, Manoj Kumar V, Subramanyam K, Yellamanda Reddy T, Nigam SN, Reddy DVR (2003). The host range of *Tobacco streak virus* in India and transmission by thrips. Ann. Appl. Biol. 142:365-368.

Rajamanickam S, Karthikeyan G (2014). Sequence diversity analysis of *Tobacco streak virus* infecting okra (*Abelmoschus esculentus* L.) in India. J. Pure Appl. Microbiol. 8(6): 4759-4767

Ramiah M, Bhat AI, Jain RK, Pant RP, Ahlawat YS, Prabhakar K, Verma A (2001). Partial characterization of an isometric virus causing sunflower necrosis disease. Indian Phytopathol. 54:246-250

Rebedeaux PF, Gaska JM, Kurtzweil NC, Grau CR (2004). Seasonal progression and agronomic impact of *Tobacco streak virus* on soybean in Wisconsin. Plant Dis. 89: 391-396.

Rice P, Longden I, Bleasby A (2000). EMBOSS: The European molecular biology open software suite. Trends Gen. 16(6): 276-277.

Saitou N, Nei M (1987). The neighbour-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Salazar LF, Abad JA, Hooker WJ (1982). Host range and properties of a strain of *tobacco streak virus* from potatoes. Phytopathology 72:1550-1554.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Shukla S, Kalyani G, Kulkarni N, Waliyar F, Nigam SN (2005). Mechanism of transmission of *Tobacco streak virus* by *Scirtothrips dorsalis, Frankliniella schultzei* and *Megalurothrips usitatus* in groundnut, *Arachis hypogaea* L. Oilseeds Res. 22:215-217.
Sivaprasad Y, Bhaskara Reddy BV, Rekha Rani K, Raja Reddy K, Sai Gopal DVR (2010). First report of *Tobacco streak ilarvirus* infecting onion (*Allium cepa*). New Dis. Rep. 22:17.
Subramanian KS, Narayanasamy P (1973). Mechanical transmission of *Whitefly borne yellow mosaic virus* of *Lablab niger* Medikus. Curr. Sci. 47:92-99.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731-2739.
Thakur MP, Verma KP, Agrawal KC (1998). Characterization and management of bud blight disease of soybean in India. Int. J. Pest Manage. 44:87-92.