Sunflower and groundnut are the two major oil seed crops cultivated in India. During the recent years, sunflower necrosis disease (SND) and groundnut stem necrosis disease (GSND) of viral etiology were reported has a major limitation in their productivity. The causal agent of SND and GSND was attributed as *Tobacco streak virus* (TSV). The virus is widely distributed in Southern states of India and has a wide host range. Therefore the present study was undertaken to determine the natural occurrence, distribution and the variability of the virus isolates infecting various hosts in India.

Surveys carried out during 2002 to 2004 showed a wide distribution of TSV in South Central India infecting several oil seed, vegetable, fibre, ornamentals and weed species. SND was endemic in major sunflower growing regions of Andhra Pradesh, Karnataka and Maharashtra with the incidence ranging upto 95%, 75% and 80% during the 2002, 2003 and 2004, respectively. GSND was highly confined to the groundnut growing regions of Andhra Pradesh (Anantapur and Kurnool) were the disease incidence ranged upto 15%, 3% and 80% during 2002, 2003 and 2004, respectively. GSND was also recorded in Karnataka (Raichur, Bellary and Bijapur) and Combatore (Tamil Nadu). High disease incidence and frequent epidemics in sunflower and groundnut resulted as a serious constraint to oil seed production in the recent years.

Apart from sunflower and groundnut, the diseases caused by TSV were was observed on cotton, okra and soybean with the incidence ranging upto 20%, 25% and 40%, respectively. In addition, TSV infection was also observed in several other crop and weed species present in and around the infected plots.
Detection of TSV in various crop and weed samples collected during the present survey was carried out by DAC-ELISA using homologous antiserum. TSV was detected from crop species viz., sunflower, groundnut, cotton, okra, soybean, cowpea, mungbean, safflower, sesame and niger; ornamentals viz., marigold, crossandra and ixora; and weed species viz., parthenium, xanthium, croton, achyranthus, acanthosperum, commelina and tridax.

TSV produced chlorotic or necrotic symptoms on the vegetative and reproductive parts of various hosts. TSV infected sunflower showed either necrosis or mosaic mottling depending on the genotype it infects. Systemic infection on sunflower showed necrosis of leaves, petiole, stem and head. Infected plants were stunted and produced heads either with poor seed set or filled with chaffy seeds. In legumes such as groundnut, soybean, mungbean and cowpea, TSV infection produced necrosis on leaves, petiole and stem. TSV infection in okra showed chlorotic ringspots, mosaic mottling and deformity of leaves with characteristics chlorotic streak on the distorted fruits. TSV infection in cotton produced either necrotic or mosaic mottling type of symptoms.

In virus transmission assay, TSV produced local necrotic lesion followed by systemic necrosis on cowpea; necrotic rings with the characteristic oak leaf like pattern in tobacco and chlorotic lesions, mosaic mottling and leaf deformation in sunflower upon mechanical sap inoculations. TSV was transmitted by pollen but not through seeds under green house condition. Transmission of TSV by pollen under green house condition suggest the crucial role played by pollen in transmitting the virus under field conditions which can be by means like insect carriers (Thrips) or wind.
In a host range study, the representative TSV-SF Jln-02 isolate infected 29 out of 33 plant species belonging to Asteraceae, Chenopodiaceae, Cucurbitaceae, Leguminosea, Malvaceae and Solanaceae. In a comparative host range study of 17 isolates, 16 TSV isolates showed a similar host range with respect to TSV-SF Jln-02 isolate indicating a low biological variability among the isolates. However, TSV-OK Rai-04 isolate was observed to produce milder symptoms on cowpea, sunflower and C. quinoa when compared to other isolates.

The studies on virus stability showed that TSV-SF Jln-02 isolate had a DEP between 1:1000 to 1:10000, TIP in between 55°C to 60°C and the LIV of 12 h at room temperature.

TSV was purified from the virus inoculated cowpea leaves using differential centrifugation, PEG precipitation and linear sucrose gradient ultra centrifugation method. In SDS-PAGE, the purified virus resolved into a major protein of ~28 kDa and a minor protein of ~57 kDa protein. In western analysis, both major and minor proteins reacted with TSV homologous antiserum.

RNA extracted from the purified virus resolved into 6 distinct bands comprising three major RNA of size ~3.8 kb, 3.1 kb, 2.2 kb and three minor RNA of ~0.9 kb, 0.65 kb and 0.45 kb in sizes. The concentration of 2.2 kb fragment (RNA3) was more compared to others.

Complete RNA3 component of TSV-SF Jln-02 was sequenced. The RNA3 component of TSV-SF Jln-02 isolate is 2213 nts long comprising two ORF’s in sense orientation. The RNA3 of Indian isolate shared 88.2% sequence homology with reference to the type strain, TSV-WC isolate (United States). The two ORF’s coding for movement (MP) and coat protein (CP) shared 87.6% and 88.9% sequence homology respectively with the type strain. Based on CP gene sequence homology the
TSV-SF Jln-02 which showed less than 90% identity with reference to the type strain (TSV-WC) could be considered as a distinct strain of TSV.

In order to look into the sequence diversity of TSV isolates from India, the CP gene from 52 isolates were cloned and sequenced. The TSV isolates showed high degree of sequence conservation (98 to 100%) in the coding region of CP gene. Phylogenetic tree constructed based on the CP gene sequences showed that the isolates collected from various hosts and geographical locations during 2002 to 2004 were closely clustered with each other and were diverging from the United States (TSV-WC) and Brazilian (TSV-BR) isolates.

Sequence analysis showed that all the TSV isolates characterised from India were belonging to single strain (but distinct from type strain) and were tightly clustered irrespective of the crop, location, season from which the isolate was collected.

TSV-CP gene was sub-cloned into pET20b (+) vector and expressed in E. coli BL21 DE3 pLysS strain by IPTG induction. The recombinant 6x His-tagged TSV CP fusion protein was purified by Ni-NTA agarose column and used for producing polyclonal antibodies. The antiserum titre of 1:5000 was optimized and further used for the detection of TSV by various sero-diagnostic tools. Western blot analysis detected specifically a ~28 kDa protein corresponding to the coat protein of TSV from sunflower leaves. Virus was detected from leaves, petiole and stem by tissue blot immuno assay (TBIA) and leaf imprint assay from sunflower, cotton, okra, cowpea and tobacco. TBIA can be used as a method for the epidemiological studies by blotting the antigen in the field during the survey and diagnosis in the laboratory condition.
A binary vector was constructed using the conserved TSV CP gene sequence driven by CsVMV promoter (Cassava vein mosaic virus) and NOS polyadenylation sequence and transformed into Nicotiana tabacum cv. Xanthi. PCR analysis of the putative transgenic tobacco plants confirmed the integration of TSV CP gene in 39 lines. Among the 39 lines, only 13 lines showed detectable levels of CP accumulation in the transgenic plants by ELISA and western analysis.

Upon virus challenge by sap inoculations, the progenies from CP expressing transgenic tobacco lines showed protection varying from 29 to 84% under greenhouse conditions. The progenies derived from non-CP expressing showed symptom at the same time as control tobacco plants. At the T_2 generation, the progenies derived from virus resistant transgenic plants of the three promising lines (TNX35, TNX53 and TNX66) showed varied levels of virus protection ranging from 36 to 100%. Southern analysis showed the integration of CP gene as single copy inserts in TNX66 and double copy inserts in TNX35 and TNX53.

Among the lines analyzed at T_3 generations, the transgenic tobacco lines TNX66/3/1, TNX66/3/2, TNX66/15/1 and TNX66/15/2 confirmed 95 to 100% complete virus resistance. And the lines (TNX53/1/1 and TNX53/1/2) showed 70 to 80% complete virus protection. A correlation of CP accumulation and virus protection was observed in the transgenic tobacco plants. Transgenic plants with CP accumulation conferred either complete resistance or delayed symptom expression. Whereas the transgenic plants with no or low levels of CP expression were found to be susceptible upon virus inoculations and showed symptoms similar to control tobacco plants. The present study showed that the CP-MR technology could be used for the development transgenic plants (sunflower, groundnut and other crops) resistant to the Indian TSV strain.
7. REFERENCES


replication. The European Journal of Molecular Biology. 18: 4856-4864.


gene confers cross protection in transgenic tobacco and tomato plants.


Valleau, W.D. (1940). Sweetclover, a probable host of *Tobacco streak virus.*


Van Dun, C. M., Overduin B, van Vloten-Doting, L., and Bol, J. F. (1988). Transgenic tobacco expressing *Tobacco streak virus* or mutated *Alfalfa mosaic virus* coat protein does not cross-protect against alfalfa mosaic virus infection.


Plant Molecular Biology, 37: 1055-1067.


