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## Serodiagnosis of *Tobacco streak ilarvirus* infecting peanut (*Arachis hypogea*) in villages of Chittoor district, Andhra Pradesh

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### Abstract

*Tobacco streak virus* (TSV) is most devastating plant pathogen; causing severe impact on the crop production and productivity. TSV is distributed throughout the globe; infecting species under families of the dicots and monocots. Epidemics of the TSV on the peanut and sunflower occur in India. In the present study, we have collected leaf and stem samples from the peanut plant showing chlorotic and necrotic lesions from the fields of Gajulamandam, Tirupathi and Chandragiri villages of Chittoor district. Collected samples were serodiagnosed by direct antigen coating (DAC)-ELISA using TSV antisera. Twenty six field samples were found positive towards the TSV antisera out of 165. The ELISA positive samples were further confirmed through Dot immunobinding assay (DIBA). The remaining 139 field samples were found negative for against TSV antisera; may be infected other plant pathogens or physiological conditions.

**Keywords:** *Tobacco streak virus*, DAC-ELISA, peanut, DIBA

### Introduction

TSV is a fast emerging and devastating plant virus transmitted by the Arthropod vector thrips, belongs to the family Bromoviridae and genus *Iilarvirus*. TSV was first discovered on tobacco (*Nicotiana tabacum*) in 1936 by Johnson (Johnson, 1936) [1]. Later, it has been reported from more than 26 countries worldwide. TSV has a wide host range infecting more than 200 plant species belonging to 30 dicotyledonous and monocotyledonous plant species (Fulton, 1985) [2]. It is pollen borne and transmitted by thrips (Sdoodee & Teakle, 1987, Sdoodee & Teakle, 1993) [3, 4]. However, some strains of TSV is transmitted by the seed is also reported (Kaiser *et al.*, 1991; Sharman *et al.*, 2008) [5, 6].

TSV is a newly emerging virus predominantly occurring in Indian subcontinent and infecting diverse groups of crop plants causing severe economical losses is a major constraint in sunflower and peanut crops since 1997 in Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. According to a recent report presented in the abstract of 16<sup>th</sup> Annual Convention and International Symposium of Indian Virological Society on "Management of Vector-Borne Viruses" at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-2006) by Dr. K.S. Ravi's research group at Mahyco Seeds Company, Jalna, Maharashtra, the disease incidence is recorded up to 95%, 80%, 20%, 25% and 40% in sunflower, peanut, cotton, okra and soybean crops respectively with characteristic symptoms of necrosis on the affected plant parts except in okra with mosaic mottling and veinal chlorosis. Since this virus is transmitted by the vector thrips that also transmit Peanut bud necrosis tospovirus, a disease epidemic resulted in the death of young groundnut plants in kharif 2000 in Anantapur district of Andhra Pradesh state over an area of 2.25 lakh hectares out of 7 lakh hectares grown shows the potential loss of the crop. Initially, the disease was suspected to be caused by Peanut bud necrosis virus (PBNV), because of the characteristic necrosis of terminal buds. In subsequent studies TSV was found associated with the disease and was named as peanut stem necrosis.

In the present study, we have conducted field survey in the peanut farmer's field in the villages of Chittoor district, Andhra Pradesh. We identified the natural incidence of the TSV on the Peanut plant.

### Materials and methods

#### Virus Culture

Virus infected peanuts plants were collected from three village of chittoor district of Andhra Pradesh during field survey. Selected village has more infestation of the virus and all areas come within the circumference of about 20 km from Tirupathi.

The number of the samples collected from each village is mentioned in table no 1. The virus presence was confirmed by direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA) and then maintained on cowpea (C-152) following three successful single lesion transfer by sap inoculations.

#### DAC-ELISA

DAC-ELISA was used to screen the virus infected sample by directly coating the antigen (plant sap) in the microtitre plate wells (Hobbs *et al.*, 1987 & Mowat and Dawson, 1987) [7, 8]. Approximately, 200 mg of infected plant leaf was macerated with 500 µl of 0.05 M carbonate buffer (pH 9.6) using pestle and acid washed sand and 200 µl was added to each well of ELISA plate. Plate was incubated for 2 h at room temperature (RT) and this was washed with PBS-T (0.15 M NaCl in 0.1 M phosphate buffer, 0.05% Tween 20) thrice at 5-minute interval. The traces of solution were removed by tapping ELISA plate on four-fold of tissue paper. The antisera were diluted to required volume in PBS-TPO buffer (0.15 M NaCl, 0.1 M phosphate buffer, 0.05% Tween 20, 2% polyvinyl pyrrolidone, 0.2% ovalbumin). Then 200 µl of respective antisera (1: 20,000 dilution) were added into the well and incubated for 2h at room temperature. After incubation, decant the solution and washing steps were repeated as above to remove the unbound antibody. After that 200 µl of secondary antibody (1:10,000 dilution)-alkaline phosphatase conjugate was added to the wells, incubated and washing steps repeated with PBS-T thrice at 5 minutes interval. The reaction was developed by adding 200 µl of the substrate solution (9.7% diethanolamine, 50 mg p-nitrophenyl phosphate pH 9.8) and kept for incubation at room temperature for 10 min in the dark. The reaction was terminated by adding 50 µl of 3 M NaOH solution to each well. The positive samples were screened either visually (yellow colour intensity) or by measuring the absorbance at 405 nm wavelength.

#### Dot Immunobinding Assay (DIBA)

DIBA was performed using the total soluble proteins of healthy and infected leaf samples. Approximately 500 mg of leaf samples were macerated with 2 ml of TB Buffer (50 mM Tris Acetate pH 7.4, 10 mM MgCl<sub>2</sub>, 250 mM KCl and 20% glycerol) at 4 °C, then 1 mM PMSF and 1 mM DTT were added and centrifuged at 15,000 rpm for 10 min. The supernatant was collected and used for further study. The PVDF (polyvinylidene difluoride) membrane of required size

was cut and soaked in methanol. 5µl total soluble proteins from healthy and ELISA confirmed infected peanut leaves were placed as dot on the PVDF membrane. After the dots were dried, the membrane was kept in blocking buffer (5% milk Nestle every day, 0.02 M Tris, 0.5 M NaCl, 0.05% tween-20, pH 7.5) for 40 min at the room temperature on the rocker. After incubation respective primary antibody (1:10,000 dilutions) was added and kept for two hours at room temperature on the rocker. Decant the solution and membrane was washed with TBS-T 3 times at 5 min interval. It was incubated again in the antibody buffer (0.02 M Tris, 0.5 M NaCl, 0.05% tween-20, pH 7.5 and 5% milk Nestle every day) containing secondary antibody-enzyme conjugate for a period of 2 h at RT on the rocker. It was washed with TBS-T thrice followed by TBS. Membrane was incubated with 300 µl of BCIP/NBT substrate till colour developed. The reaction was stopped by adding distilled water and membrane was dried on tissue paper.

#### Inoculation and sap transmission

As plant viruses cannot invade plant cells through natural opening like leaf stomata or stem lenticel unlike bacteria or fungi. They can only enter into the cell through wound caused by mechanical damage. This mechanical damage was created by celite and carborundum 500 mesh. The ELISA and DIBA positive samples were taken for further study. The 200mg of positive sample was ground in inoculation buffer (20 mM phosphate buffer pH 8, 0.01% β-Mercapto ethanol) using mortar and pestle. Healthy plant was dusted with carborundum 500 mesh as an abrasive then muslin cloth was wetted in solution and gently applied on the leaf. Leaf was washed with the tap water then kept in growth chamber.

#### Results and Discussion

##### Serodiagnosis of field collected samples by ELISA

Serodiagnosis was performed by Direct Antigen Coating-ELISA (DAC-ELISA) using *Tobacco streak virus* (TSV) antisera provided by Dr. Varsha Wesley, ICRIAT, Hyderabad. Results were identified by visual scoring on ELISA plates (data not shown). ELISA results of peanut infected samples from three villages indicate that maximum infection occurred in the Gajulamandyam which account for 18% and the lowest infestation occurs in the Tirupathi i.e. 14.3%. The disease incidence due to TSV showed 16 % (mentioned in table no. 2. To authenticate, further ELISA positive samples were confirmed through DIBA assay.

**Table 1:** ELISA results of peanut infected samples from three villages of Chittoor district of Andhra Pradesh. All villages coming 20 km radius of Tirupathi

S. No.	Field visited in	No. of samples collected	TSV	Percentage of Incidence
1	Tirupathi	70	10	14.30
2.	Chandragiri	55	9	16.00
3.	Gajulamandyam	40	7	18.00
	Total	165	26	
	Percentage		16.00%	

#### Dot Immuno Binding Assay (DIBA)

To confirm the ELISA results, total seventeen symptomatic field samples were selected randomly. Total soluble proteins were extracted and spotted onto PVDF membrane as mentioned in material and methods. Randomly, 15 samples were tested with TSV antisera; All samples were gave positive result and concurrent with the ELISA result.

#### Inoculation and sap transmission

The identified peanut strain of TSV was found to be mechanically transmissible by sap inoculation to the

experimental host plant. It is similar with various investigations for TSV (Costa & Carvalho, 1961; Salazar *et al.*, 1982; Reddy *et al.*, 2002; Kaiser *et al.*, 1982) [9, 10, 11]. However we have also noticed decline in infectivity with different seasons (data not shown).

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