Assessment for *Tobaccos streak virus* (TSV) Transmission through Seed in Groundnut and Sunflower

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Stem necrosis disease caused by *Tobacco streak virus* (TSV, genus *Ilarvirus;* family *Bromoviridae*) was first recognized in 2000 in Anantapur District, Andhra Pradesh, India (Reddy et al 2002). It has emerged as a potential threat to groundnut (*Arachis hypogaea* L.), sunflower (*Helianthus annuus* L.) and several cucurbits and other annual crops in southern and western parts of India (Kumar et al 2006). All the currently grown peanut cultivars in India are highly susceptible to the virus. TSV infection in groundnut results in severe necrosis of shoots leading to death of the plant. A few plants that survived TSV infection showed malformed growth and severe reduction in pod yield. TSV is transmitted through pollen, assisted by thrips and also through seed (<1 to 90%) of several susceptible crops like *Cicer arietinum, Datura stramonium, Chenopodium quinoa, Phaseolus vulgaris, Glycine max, Gomphrena globosa, Nicotiana clevelandii, Nicandra physalodes, Vigna unguiculata,* black raspberry and tomato (Kaiser et al 1982; 1991). However, the TSV strain occurring in India was shown to be transmitted through pollen that was assisted by the thrips, but not through seed (Prasadarao et al 2003; Shukla et al 2005). Very recently, TSV transmission through seed (3 – 68%) was reported in cucumber (*Cucumis sativus* L.) and gherkins (*Cucumis anguria* L.) in Karnataka, India (Jain et al 2006; Krishna Reddy, personal communication). Usually, seed transmission depends on the virus, its strains, host species and cultivar that is affected, and is also influenced by environment. In the light of the new observation, the potential of TSV transmission through groundnut seed was reassessed by testing seed from naturally infected groundnut plants in Anantapur district, Andhra Pradesh, India.

Materials and Methods

In the 2004 rainy season (Jul – Nov), groundnut fields in Anantapur district were surveyed for TSV infection and severely affected fields were selected. Leaf samples from suspected plants were tested for TSV by direct antigen coating-enzyme-linked immunosorbent assay (DAC-ELISA) as described by Reddy et al (2000). Briefly, leaves were extracted in 0.1 M carbonate buffer, pH 9.5, (1:20 w/v), and 100 µl were loaded into wells of ELISA plates. TSV-polyclonal antiserum (ICRISAT, Patancheru, India) was used at a 1:15000 dilution after cross-adsorption with healthy groundnut leaf extract (1:20 w/v). Alkaline phosphatase (ALP)-labelled anti-rabbit IgGs (Sigma, USA) at 1:5000 dilution and 0.5 mg/ml paranitrophenyl phosphate in 10% (v/v) diethanolamine buffer, pH 9.8, were used to detect antigen-antibody complexes. Optical density at 405 nm was measured in a Titertek Multiskan ELISA reader after 60 min. Readings were considered virus positive if the absorbance values of samples were three-fold higher than those of the healthy control samples. Similarly seeds were tested for TSV by DAC-ELISA as described in Bharatan et al (1984). Seeds from TSV infected groundnut and sunflower plants along with healthy controls (cv JL 24) were evaluated for virus transmission in grow-out tests by raising seedlings in 20 cm plastic pots (8 - 9 seeds/pot) filled with sterilized potting mixture in a greenhouse at ICRISAT, Patancheru, India, during Jun 05 - Feb 06 (day 24-36C and night 16-22C). Seedlings were regularly monitored for symptoms and after six weeks all seedlings were tested for virus by DAC-ELISA as described earlier (Table 2).

Results and Discussion

Seed transmission can be a serious problem with some plant viruses, as it forms a major mechanism for spread of the virus (eg. *Barley stripe mosaic virus*). However, in some cases seed transmission rate can be low, but they are significant as a route for the introduction of viruses into new areas where they may spread further and become established if suitable vectors and hosts are available. This mode of virus spread is of great concern

in groundnut, where it is a common practice to move a large amount of seed stock between regions. Seven viruses are known to be seed transmitted in groundnut: *Cowpea aphid-borne mosaic virus* (<1%), *Cucumber mosaic virus* (2 – 4%), *Indian peanut clump virus* (IPCV; up to 6%), *Peanut clump virus* (up to 6%), *Peanut mottle virus* (PMV, up to 8.5%), *Peanut stripe virus* (PStV, up to 35%) and *Peanut stunt virus* (up to 5%) (Sreenivasulu et al 2006). Of these, IPCV and PMV occur in India. TSV, a newly recognized virus in India, was recently reported to spread through seed in cucurbits. This study was made to ascertain if TSV is transmitted through seed in groundnut and sunflower.

Table 1. Plants tested for TSV infection

Crop	Plants tested	TSV positive plants
Groundnut	900	434
Sunflower	5	4

Nearly 48% of the filed samples tested positive for TSV (Table 1). Infected plants in the field were tagged and pods from these plants were harvested separately. The number of pods per infected plant was low (up to 60% reduction compared to uninfected plants), but they were normal in appearance. Of the 2030 seeds, 97% germinated and showed normal growth pattern similar to that of healthy controls (Table 2). None of the seedlings showed any symptoms or tested positive to TSV by ELISA suggesting that the virus was not transmitted through seed. None of the 100 cotyledons tested contained the viral antigen. In addition to groundnut, seeds from TSV infected sunflower from adjacent fields were also evaluated by grow-out tests (Tables 1 and 2). Even in sunflower, virus transmission through seed was not found (Table 2). However, only 71% of seeds sown germinated, which indicated poor viability presumably due to virus infection.

Table 2. Grow-out test for seed transmission assay

Seed	Seedlings germinated/sown	TSV ELISA Positive seedlings /tested
Groundnut	1969/2030	0/1969
Sunflower	588/828	0/588

Previous studies in 2000-01 using seed obtained from the naturally infected and experimentally inoculated groundnut (2435 seeds) and sunflower (826 seeds) reported lack of seed transmission in these crops (Prasadarao et al 2003). It is likely that seed transmission observed in two cucurbit species could be a host-virus specific phenomenon. However, it may be worthy to characterize TSV isolated from cucurbits to rule out the emergence of a seed-transmissible TSV strain.

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