Non-Transmission of *Tobacco streak virus* Isolate Occurring in India Through the Seeds of Some Crop and Weed Hosts

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Abstract

Tobacco streak virus *(TSV; genus* Ilarvirus, *family Bromoviridae) responsible for necrosis disease in sunflower, groundnut and several forage legumes has emerged as a major threat to several crops during last one decade in India. The virus has a wide host range comprising of variety of crops, wild species and weeds, and the virus is transmitted through pollen assisted by thrips (Thysanoptera: Thripidae). Certain strains of TSV are known to be transmitted in seed of a range of host species. The present study investigated possible transmission of TSV isolate occurring in India in seed of naturally and experimentally infected groundnut* (Arachis hypogaea)*, sunflower* (Helianthus annuus)*, mungbean* (Vigna radiata)*, marigold* (Tagetes erecta)*, French bean* (Phaseolous vulgaris)*, urdbean* (Vigna mungo)*, soybean* (Glycine max)*,* Chenopodium quinoa, Gomphrena globosa *and* Parthenium hysterophorus. Studies revealed that *TSV seed transmission was not observed in any of these host species. Implications of this finding on disease epidemiology are also discussed in this paper.*

Keywords: *Tobacco streak virus*, seed transmission, weeds, crop hosts

Introduction

Tobacco streak virus (TSV; genus *Ilarvirus*, family *Bromoviridae*), first confirmed in the year 2000 in necrosis disease-affected sunflower in India (Prasadarao *et al.*, 2000), emerged as a major threat to several annual crops during last one decade (Kumar *et al.*, 2008). The virus has a wide host range comprising of several crop, wild and weed species (Prasadarao *et al.*, 2003; Kumar *et al.*, 2006). The virus spreads through infected pollen, disbursed through wind currents and also by insects on their body parts. However, TSV infection is specifically associated with the thrips (*Thysanoptera: Thripidae*) feeding damage on leaves and presence of TSV-infected pollen at the feeding sites (Prasadarao *et al.*, 2003; Shukla *et al.*, 2005). Both adults and nymphs of several thrips species assist TSV transmission. Weeds such as *Parthenium hysterophorus* (congress weed) widely occurring on field bunds and fallow lands producing copious pollen is an asymptomatic host and a major source of TSV infected pollen to crop plants in India (Prasadarao *et al.*, 2003).

Studies elsewhere indicated that certain strains of TSV are transmitted through the seed of infected plants. TSV seed transmission has been reported in french beans (*Phaseolus vulgaris*) (Thomas and Graham, 1951); *Melilotus alba*, *Glycine max*, *Gomphrena globosa*, *Nicotiana clevelandi*, *Vigna unguiculata* (Kaiser *et al.*,1982); black raspberry (*Rubus occidentalis*) (Converse and Lister, 1969), *Nicandra physolodes* (Salazar *et al.*, 1982); strawberry (*Fragaria vesca* var. *semperflorens*) (Johnson *et al*, 1984); tomato (*Lycopersicon esculentum*) (Sdoode and Teakle, 1988); *Cicer arietinum* and adjuki bean (*Vigna angularis*) (Kaiser *et al.*, 1991); *P. hysterophorus* (Sharman *et al.*, 2009). Interestingly our earlier studies indicated lack of TSV seed transmission in seed of the naturally infected or experimentally inoculated groundnut (*Arachis hypogaea*), sunflower (*Helianthus annuus)* (Prasadarao *et al.*, 2003; Reddy *et al.*, 2007). However, seed transmission to an extent of 2.7 to 65.7% was reported in cucumbers (*Cucumis sativus*) and gherkins (*Cucumis anguaria*) from Southern Karnataka in India (Jain *et al.*, 2008). In the light of this report and also very recent report of TSV seed transmission in parthenium in Australia (Sharman *et al.*, 2009), we investigated the TSV seed transmission in forage legumes and five other plant species, including parthenium, groundnut and sunflower, susceptible to TSV in India.

Materials and methods

Plant species and source of seeds

TSV seed transmission was assessed in groundnut, sunflower,

mungbean (*Vigna radiata*), marigold (*Tagetes erecta*), french bean, urdbean (*Vigna mungo*), soybean (*Glycine max*), *Chenopodium quinoa, G. globosa* and parthenium.

Surveys for naturally infected TSV plants were conducted in 2002 to 2005 in farmers' fields in Anantapur and Ranga Reddy districts, in Andhra Pradesh, India. Leaf samples from symptomatic groundnut, sunflower, marigold and parthenium plants were collected prior to the flowering stage and tested for TSV by direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA) as detailed below. Virus positive plants were tagged and seeds were harvested at the end of the season and utilized in seed-transmission experiments. Seeds were also obtained from groundnut, sunflower, french bean, soybean, mungbean, urdbean, *G. globosa*, *C. quinoa* and parthenium plants experimentally inoculated with TSV under glass house conditions. The seeds harvested from the above crops were used in grow out tests within two months from the date of harvest and until then the seeds were stored at 4°C in a refrigerator.

Mechanical sap inoculation

Healthy seeds of test plant species were sown in sterilized potting mixture @ 2 seeds per 20 cm diameter plastic pots in a glasshouse and sap inoculation with TSV infected leaf sap extract was done as described in Reddy *et al.*, (2002). Groundnut cultivars (cvs.) JL-24 and TMV-2; sunflower cv. PAC-36 were inoculated at 20 days after sowing (DAS); french bean cvs. Top Crop, Early Ramshorn, Dark Red Kidney; soybean cvs. Bragg and JS -335 were inoculated at 14 DAS; and *G. globosa* and *C. quinoa* were inoculated at 25 DAS. All plants were tested for TSV infection by DAC-ELISA and seed from TSV positive plants were collected at maturity and used in grow-out tests to assess the seed transmission.

Grow-out test

Grow-out tests were conducted by raising seedlings in trays filled with sterilized potting mixture in a glasshouse at National Bureau of Plant Genetic Resources, Hyderabad. Seedlings were regularly monitored for symptoms and after four weeks of germination, leaves from all the seedlings were collected and tested in groups of 10 samples (bulk analysis) for TSV by DAC-ELISA. Seed from uninfected plants were used as controls.

DAC-ELISA

TSV detection by DAC-ELISA was performed as described by Reddy *et al.* (2002). Leaf tissues were extracted in 0.1 M carbonate buffer $_{p}$H 9.6 (1: 20 w/v) and 0.2 ml was loaded into wells of ELISA plates. TSV polyclonal antiserum (ICRISAT, Patancheru, India) was used at 1:10,000 dilution and Alkaline phosphatase (ALP) labeled anti-rabbit IgG (Sigma chemicals, USA) at 1:10,000 dilution and 0.5 mg/ml paranitrophenyl phosphate in 10% (v/v) diethanolamine buffer, pH 9.8 were used to detect antigen antibody complexes. Absorbance was recorded at 405nm after 1 h of incubation at room temperature.

Results and discussion

Evaluation of seed from naturally infected plants

TSV was detected in 79.6%, 56.1% and 76.1% of field collected groundnut plants tested in 2002, 2003 and 2004, respectively (Table 1). Significant proportion of the infected

Table 1. Incidence of *Tobacco streak virus* (TSV) in groundnut, sunflower, marigold and parthenium in Andhra Pradesh, India

Plant species	Year	District	No. of plants tested *	No. of TSV positive plants	Per cent TSV infection	Per cent plant mortality
Groundnut	2002	Anantapur	137	109	80	32
	2003	Anantapur	82	46	56	16
	2004	Anantapur	201	153	76	24
Sunflower	2003	Ranga Reddy	37	19	51	5
	2004	Ranga Reddy	18	11	61	0
	2005	Ranga Reddy	123	92	75	0
Marigold	2004	Anantapur	29	18	62	0.5
Parthenium	2002	Anantapur	427	133	31	0
	2003	Anantapur	172	39	23	0
	2004	Anantapur	89	46	52	0
	2005	NBPGR, Hyderabad	273	8	3	0
	2006	NBPGR, Hyderabad	116	5	4	0

*Detected by ELISA

plants had died prematurely (Table 1). Pods were harvested from surviving plants. Necrotic spots were observed on shells of many pods from infected plants. However, kernels from infected and uninfected plants were similar in appearance. In grow-out tests, 87.1% of the seeds from TSV infected plants germinated compared to 98.0% in seeds from uninfected plants (Table 2). All the seedlings germinated from virus infected groundnut tested negative to TSV in DAC-ELISA and they had normal growth pattern similar to that of seedlings from uninfected groundnut.

Sunflower infected at early stage (within 40 days after sowing) were stunted and majority of them did not produce any flowers. A few infected plants produced distorted flowers, which had very few seeds. Plants infected between 40 to 60 days produced flower heads containing normal but fewer seeds. In grow out tests, 69.5% of the seeds harvested from infected plants germinated compared to 89.0% in seeds from uninfected plants (Table 1). All the seedlings from virus infected plants regardless of time of infection tested negative to TSV in DAC-ELISA (Table 2).

Eighteen of 29 marigold plants tested positive to TSV. Only 62.4% seeds harvested from TSV infected plants germinated compared to 85.0% germination of seeds from uninfected plants (Table 1). All the seedlings germinated from TSV infected plants tested negative to TSV in ELISA tests (Table 2).

TSV was detected in 31.1%, 22.7% and 51.7% parthenium grown around groundnut fields in Anantapur during 2001, 2003 and 2004, respectively (Table 1). In plants obtained from NBPGR, TSV was detected in 2.9% and 4.3% plants during 2005 and 2006, respectively (Table 1). Seeds harvested from the TSV infected parthenium had 68.2% germination compared to 79.0% in seeds from healthy plants. TSV was not detected in any of the 1031 seedlings germinated from seeds obtained from infected parthenium plants in ELISA tests (Table 2).

Evaluation of seed from experimentally inoculated plants

Seedlings generated from the seed of nine susceptible plant species experimentally inoculated with TSV were also negative to TSV in ELISA (Table 3). Germination for the seeds collected from TSV infected plants ranged between 60% for *C. quinoa* and 93% for french bean cv. Top Crop (Table 3). All the germinated plants had normal growth pattern, similar to that of seed from healthy controls.

TSV infection was first observed in sunflower during 1997 at Bagepally village near Bangalore (Singh *et al.*, 1997), but actual identification of the virus was made in 2000 (Prasadarao *et al.*, 2000). The virus was shown to be mechanically transmissible under experimental conditions and its natural transmission is through infective pollen assisted by thrips (Prasadarao *et al.*, 2003). Some TSV isolates occurring in USA were reported to be seed transmitted in *C. quinoa* (Brunt *et al.*, 1996); soybean and *G. globosa* (Kaiser *et al*, 1982); french beans (Thomas and Graham, 1951) and other species (Kumar *et al.*, 2008), but the TSV isolates occurring in Andhra Pradesh in India were not transmitted through seed of 10 susceptible species. TSV seed transmission as high as 90.6 % was reported in soybean cv. Bragg (Ghanekar and Schwenk, 1974), whereas Indian isolate of TSV is not seed transmitted in soybean cvs. Bragg and JS-335. Evidence from seed material obtained from naturally and experimentally infected plants over different seasons and years clearly ruled out this mode of transmission (Tables 1, 2, 3). A previous study on seed transmission of TSV in groundnut and sunflower in India also concluded that Indian TSV isolate was not seed transmissible (Prasada Rao *et al.*, 2003; Reddy *et al.*, 2007).

The genetic basis for seed transmission was investigated with TSV pathotype I isolate Mel 40 and pathotype II isolate Mel F (Walter *et al.*, 1995). Electrophoresis of RNA from the infrequently seed transmitted TSV isolate Mel F revealed many minor RNA species not detected in the seed transmitted isolate Mel 40. Non-seed transmitted Mel F encapsulated one minor RNA designated RNA F5. Reddy *et al* (2002)

Table 2. Effect of *Tobacco streak virus* (TSV) on seed germination and seed transmission in plant species naturally infected with TSV

Plant species	No. of seeds germinated/ No. of seeds sown (% germination)		*No. positive plants/ No. tested
	Seed from TSV infected plants	Seed from healthy controls	
Groundnut	969/1130 (87)	295/300 (98)	0/969
Sunflower	1673/2408 (69)	269/300 (89)	0/1673
Marigold	486/779 (62)	85/100 (85)	0/486
Parthenium	1031/1511 (68)	397/500 (79)	0/1031

*Plants tested for TSV by ELISA

Table 3. Effect of *Tobacco streak virus* (TSV) infection on seed germination and seed transmission in different plant species mechanically inoculated with TSV in greenhouse trials

Plant species	No. of seeds germinated / No. of seeds sown (per cent germination)	No. TSV positive* / No. of plants tested
Groundnut cv. JL-24	649/737 (88)	0/649
Groundnut cv. TMV-2	292/335 (87)	0/292
Sunflower cv. PAC-36	387/521 (74)	0/387
French bean cv. Early Ramshorn	191/215 (89)	0/191
French bean cv. Dark Red Kidney	113/124 (91)	0/113
French bean cv. Top Crop	209/224 (93)	0/209
Soybean cv. Bragg	228/264 (86)	0/228
Soybean cv. JS-335	159/176 (90)	0/159
Mungbean cv. K-851	389/481 (81)	0/389
Urdbean cv. LBG 20	232/301 (77)	0/232
Gomphrena globosa	311/387 (80)	0/311
Chenopodium quinoa	368/639 (60)	0/368
Parthenium	631/852 (74)	0/631

*Plants tested for TSV by ELISA

observed that nucleic acid extracted from purified particle preparation of Indian TSV isolate contained, in addition to four RNA species, RNA components of 0.6 and 0.42 kb when analyzed by electrophoresis in denaturing gels. It has to be ascertained whether these minor RNA species observed in TSV of groundnut in India (Reddy *et al.*, 2002) are similar to the TSV isolate Mel F many minor RNA's, responsible for the non seed transmission of TSV (Walter *et al.*, 1995).

The sequence identity of 97-100% of all isolates of TSV occurring in India suggests that all the TSV isolates in India represent one species and have a common origin (PL Kumar, unpublished). The occurrence of minor RNA species in the purified virus preparations of one of the isolates (groundnut) (Reddy *et al.*, 2002) and non seed transmission of TSV in India in several plant species tested in the present study as well as by other workers (Reddy *et al.*, 2007; Prasadarao *et al.*, 2003) strongly support the view that the TSV isolate in India is non seed transmitted and continues to be non seed transmitted until a new seed transmitted strain is introduced and/ or mutation occurs. In spite of its occurrence, TSV is still considered to be of plant quarantine importance to India, because it exists in a variety of strains (Fulton, 1985) and included in the schedule V of Plant Quarantine Order 2003 (Regulation of Import into India). Many countries also consider TSV as a potential quarantine pest in view of its seed transmission in several plant species and also existence of strains. Legume seeds of above crops from India can therefore be imported safely without any apprehensions of disease movement in view of the present study and its outcome of occurrence of non-seed transmitted TSV strain.

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