New Experimental Hosts of *Tobacco streak virus* and Absence of True Seed Transmission in Leguminous Hosts

K. Vemana · R. K. Jain

Received: 6 October 2010 / Accepted: 21 December 2010 / Published online: 6 March 2011
© Indian Virological Society 2011

Abstract Of 70 plant species tested, 50 species were susceptible to *Tobacco streak virus* (TSV) on sap inoculation. Both localized (necrotic and chlorotic spots) and systemic (necrotic spots, axillary shoot proliferation, stunting, total necrosis and wilt) symptoms are observed by majority of plant species. Eleven new experimental hosts were identified viz., *Amaranthus blitum* var. *oleracea* (Chaulai sag), *Celosia cristata* (Cocks comb), *Beta vulgaris* var. *bengalensis* (Palak/Indian spinach), *Calendula officinalis* (Pot marigold), *Chrysanthemum indicum*, *Cosmos sulphurens* (Yellow cosmos), *Citrullus lunatus* (Watermelon), *Lagenaria siceraria* (Bottle gourd), *Coriandrum sativum* (Coriander), *Hibiscus subderiffa* var. *subderiffa* (Roselle) and *Portulaca oleraceae* (Little hogweed). Detected groundnut seed infection with TSV for the first time by Direct antigen coated immuno-sorbent assay (DAC-ELISA) using whole seed. The seed infection ranged from 18.9 to 28.9% among the seeds collected from naturally infected and sap inoculated groundnut varieties (JL 24, TMV 2, Prasuna, Kadiri 6, Kadiri 9, Anantha and Kadiri 7 Bold) belonging to spanish and virginia types. Further, TSV was detected both in pod shell and seed testa and none of the samples showed the presence of TSV either in cotyledon or embryo. Grow-out and bio-assay tests proved the absence of seed transmission in groundnut and other legume crops. Hence, TSV isolate was not a true seed transmission case under Indian conditions in legumes.

Keywords Groundnut · *Tobacco streak virus* · Host range · Seed transmission

Introduction

Tobacco streak virus (TSV) belongs to the genus *Ilarvirus* of family *Bromoviridae* and its virions are characterized by quasi-isometric particles measuring about 27–35 nm in diameter [10]. The virus is distributed worldwide including India. Its epidemic on groundnut (*Arachis hypogaea*) crop was observed during the year 2000–2001 in parts of India, inflicting losses to the tune of 42 million pounds [29]. The virus has also been recorded on sunflower (*Helianthus annuus*) since 1997 in India, amounting to annual loss of 1.0 lakh pounds [28].

So far, natural occurrence of TSV in India has been reported from sunflower (*H. annuus*) [3, 27, 28], groundnut (*A. hypogaea*) [29], cotton (*Gossypium hirsutum*), sunn-hemp (*Crotalaria juncea*), mungbean (*Vigna radiata*) [3], okra (*Abelmoschus esculentus*), cucumber (*Cucumis sativa*), gherkin (*C. anguria*) [21, 22], safflower (*Carthamus tinctorius*) [4], chilli (*Capsicum annuum*) [15], urdbean (*V. mungo*) [23], nizer (*Guizotia abyssinica*) [1], soybean (*Glycine max*) [2]. In the similar manner, natural infection of TSV has been reported from other countries: horticultural crops: Black raspberry (*Rubus occidentalis*) [18], crane berry (*Vaccinium macrocarpon*) [17], dahlia (*Dahlia variabilis*) [24]; agricultural crops: Solanaceous crops: pepper (*Capsicum spp.*) [14], tobacco (*Nicotiana tabacum*) [11], Potato (*Solanum tuberosum*) [30], tomato (*S. lycopersicum*) [8], Legume crops: soybean (*G. max*) [13], cowpea (*V. unguiculata*), white clover (*Melilotus alba*) [19], groundnut (*A. hypogaea*) [7], Other crops: sunflower (*H. annuus*) [9, 31], cotton (*G. hirsutum*) [35].

K. Vemana
Acharya N.G. Ranga Agricultural University, Agricultural
Research Station, Kadiri 515591, Andhra Pradesh, India

R. K. Jain (✉)
Division of Plant Pathology, Indian Agricultural Research
Institute, New Delhi 110012, India
e-mail: rakeshjain56@yahoo.co.in

So far, experimental host range of TSV was reported by authors from different countries [8, 12, 19, 25]. However, host range studies of groundnut TSV isolate was not studied extensively in India as it is a newly emerging virus among oil seed, vegetable and ornamental crops. Hence, present investigation on experimental host range was carried out by taking crop and weed species growing in proximity of groundnut crop.

TSV transmission has been reported from seeds harvested from both naturally and sap inoculated plants in different countries. Thomas and Graham, 1951 [33] reported the natural seed transmission of TSV in pinto bean cultivars for the first time and subsequently from *M. alba* [19], *Raphanus raphanistrum* [8], *Phaseolus vulgaris* cv Black Turtle Soup bean [20]. The seed transmission has also been recorded from seeds harvested from sap inoculated plants, such as, black raspberry [6], *G. max* [13], *Chenopodium quinoa*, *Gomphrena globosa*, *N. clevelandii*, *V. unguiculata* (cv California Black Eye), *G. max* (cv Bragg) [31], *V. unguiculata* (cv Adzuki bean), *P. vulgaris* (cv Black Turtle Soup bean) [20], *Frageria vesca* [16] and *Parthenium hysterophorus* [32]. Seed transmission of TSV was not detected from any crop in India even though it was reported from 13 principle crops. So far, detailed study of TSV seed transmission was not carried out in legume crops in India.

Keeping in view of limited experimental host range study of TSV in India and its seed transmission in different crop and weed species in different countries, present investigation was undertaken to ascertain its experimental host range and seed transmission in groundnut and other legume crops using groundnut TSV isolate.

Materials and Methods

Virus Culture

From different parts of Andhra Pradesh, TSV infected groundnut leaf samples showing necrotic spots on young leaves were collected. The virus presence was confirmed by direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA) [5] and then maintained on cowpea (cv. Pusa Komal) following three successful single lesion transfer by sap inoculations at National Phytotron Facility (NPF), Indian Agricultural Research Institute (IARI), New Delhi. This is the source of TSV inoculum used for both host range and seed transmission tests.

Host Range

Seventy plant species belonging to 18 families growing in and around groundnut crop were selected for host range

studies (Table 1). Three replications were maintained for each plant species. In each replication, 3–5 seedlings were maintained per pot (12.5–22.5 cm) filled with autoclaved sterilized potting mixture containing river sand, clay and coconut peat (1:1:2). The experiment was carried out at NPF, IARI, New Delhi and maintained at temperature of 26–28°C.

Virus sap was prepared using TSV infected cowpea leaves (2–3 days post inoculation) (DPI) and ground (1:1 w/vol) with ice cold 0.01 M potassium phosphate buffer with pH 7.20 and containing 0.2% sodium sulfite and 0.01 M β mercapto ethanol using a chilled pestle and mortar. The virus sap prepared above was used for sap inoculation to the first true leaf of the seedlings by dusting Celite prior to inoculation. Virus sap was applied directly on to the upper surface of the leaves with the help of pestle. After inoculation, the seedlings were washed with tap water.

Inoculated seedlings were observed daily for symptom development and continued up to 4–6 weeks post inoculation. Biological parameters, such as, incubation period, per cent infection and symptoms were recorded for all plant species. Virus titer was also monitored by DAC-ELISA both in localized and systemic infection from 7 to 20 DPI.

Production of TSV Infected Groundnut Pods

Healthy seeds from seven popular groundnut varieties (JL 24, TMV 2, Prasuna, Kadiri 6, Kadiri 9, Anantha and Kadiri 7 Bold) were sown (4–5 seeds/pot) in plastic pots (22.5 cm). Seedlings were sap inoculated with TSV at 25, 30, 35 and 40 days after sowing (DAS) by maintaining 20 pots per age group per variety separately. Similarly, healthy controls (20 pots) were maintained for each age group of variety. Inoculation was made to the first true leaf of the seedlings. Carborundum (320 grit) was used as abrasive due to rough texture of groundnut leaf. The pods were harvested at maturity from both sap inoculated and healthy plants and stored up to 2 months at 4°C after proper drying for 10–15 days under shade to bring the moisture to 9.0%. Above seed material was used for detection of seed infection and seed transmission tests.

In the similar manner, the legume crops, namely, *V. mungo* (cv T9), *V. radiata* (cv PDM 139), *G. max* (cv JS 335), *D. lablab* (cv Pusa Early Prolific), *P. vulgaris* (cv Selection 9) and *Trigonella foenum-graecum* (cv Lam Selection1) were sown and TSV sap inoculated at 10–15 DAS. The collected seed material was used for detection of both seed infection and seed transmission of TSV.

Collection of Naturally TSV Infected Groundnut Pods

Three popular groundnut varieties (JL24, TMV2, Kadiri 6) were tagged prior to flowering under field conditions after

Table 1 Reaction of different plant species against *Tobacco streak virus* (TSV) by sap inoculation

Test plant	Incubation period (DPI)	No of symptomatic plants/ No of plants inoculated	Symptoms ^a		DAC-ELISA ^b A _{405nm} (1 h)	
			Local	Systemic	Local (7 DAS)	Systemic (14-20 DAS)
Family: Fabaceae						
<i>Arachis hypogaea</i> cv JL24(Groundnut)	3	(15/15)	2	2, 7, 8, 9, 10, 11, 12,	3.00 (0.09)	3.0 (0.09)
<i>Cajanus cajan</i> cv. Pusa -2002-2 (Pigeon pea)	5	(15/15)	1, 2, 4	2, 12	0.52 (0.09)	2.53 (0.04)
<i>Cicer arietinum</i> (Chick pea)	7	(15/15)	6	13	2.57 (0.03)	0.07 (0.06)
<i>Dolichos lablab</i> cv. Pusa Early Prolific(Sem)	5	(13/15)	1, 2	3	0.24 (0.07)	0.01 (0.02)
<i>Macrotyloma uniflorum</i> (Horse gram)	3	(15/15)	2, 4	11	3.00 (0.10)	0.40 (0.08)
<i>Glycine max</i> cv. JS-335 (Soybean)	6	(15/15)	1	4, 12	0.37 (0.04)	2.60 (0.04)
<i>Phaseolus vulgaris</i> cv. Selection-9 (Kidney bean)	2	(15/15)	2	5, 12	1.63 (0.13)	0.10 (0.05)
<i>Trigonella foenum-graecum</i> cv Lam Selection 1 (Methi)	5	(15/15)	1	8, 9, 11, 12	3.00 (0.13)	3.00 (0.09)
<i>Vigna mungo</i> cv. T9 (Black gram)	5	(15/15)	2	2, 8, 9, 11	1.25 (0.09)	3.0 (0.09)
<i>Vigna mungo</i> cv. PDM-139 (Green gram)	5	(15/15)	1, 2	2, 8, 9, 11	3.0 (0.13)	3.0 (0.13)
<i>Vigna unguiculata</i> cv. Pusa Komal (Cowpea)	2	(15/15)	2, 4	2, 10, 12	3.00 (0.12)	3.00 (0.08)
<i>Pisum sativam</i> (Pea)	-	0/15	13	13	0.06 (0.05)	0.07 (0.04)
<i>Cyamopsis tetragonoloba</i> (Cluster bean)	-	0/15	13	13	0.09 (0.05)	0.08 (0.06)
<i>Lens culinaris</i> cv TL-O4 (Lentil)	-	0/15	13	13	0.05 (0.05)	0.06 (0.06)
Family: Compositae (Asteraceae)						
<i>Calendula officinalis</i> (Pot marigold)	7	15/15	2	8, 9, 11	0.93 (0.03)	2.50 (0.19)
<i>Carthamus tinctorius</i> (Safflower)	5	13/13	1, 2	8, 9, 11	3.0 (0.02)	3.0 (0.04)
<i>Chrysanthemum indicum</i>	7	13/15	1	8, 9, 11	1.50 (0.08)	0.20 (0.04)
<i>Cosmos sulphurens</i> (Yellow cosmos)	7	10/15	1, 2	8, 9, 11	1.54 (0.11)	3.00 (0.11)
<i>Guizotia abyssinica</i> (Niger)	4	15/15	1, 2	2, 11	0.76 (0.11)	0.72 (0.08)
<i>Helianthus annuus</i> (Sunflower)	3	13/15	2	5, 10, 11	3.00 (0.02)	1.23 (0.01)
<i>Lactuca sativa</i> – (Lettuce)	7	15/15	1	3, 8, 11	3.00 (0.02)	3.0 (0.30)
<i>Parthenium hysterophorus</i> (Rag weed)	7	8/8	13	13	1.20 (0.02)	0.50 (0.03)
<i>Tagetes erecta</i> (Marigold)	3	15/15	2	2, 8, 9, 10, 11, 12	1.33 (0.08)	3.0 (0.14)
<i>Tridax procumbence</i> (Daisy)	7	8/10	13	13	1.07 (0.20)	0.35 (0.00)
Family: Solanaceae						
<i>Nicotiana benthamiana</i> (Tobacco)	7	11/12	2	8	3.00 (0.03)	3.00 (0.05)
<i>Nicotiana glutinosa</i> (Tobacco)	7	11/12	1, 3	13	3.00 (0.15)	0.01 (0.0)
<i>Nicotiana tabacum</i> (Tobacco)	7	10/12	2	13	3.00 (0.06)	0.12 (0.03)
<i>Petunia axillaries</i> (White petunia)	5	15/15	1,3	8, 9, 11	3.00 (0.08)	3.00 (0.10)
<i>Solanum melongena</i> (Brinjal)	7	13/15	2	13	3.00 (0.07)	0.40 (0.04)
<i>Capsicum annuum</i> (Chilli)	-	0/15	13	13	0.04 (0.03)	0.05 (0.04)
<i>Solanum lycopersicon</i> (Tomato)	-	0/15	13	13	0.09 (0.07)	0.03 (0.04)
Family: Cucurbitaceae						
<i>Citrullus lunatus</i> (Watermelon)	7	8/12	13	13	0.42 (0.14)	0.18 (0.15)
<i>Cucumis melo</i> (Muskmelon)	14	8/12	13	13	0.38 (0.11)	0.31 (0.11)
<i>Cucumis melo</i> var <i>momordica</i> (Kachri/Snap melon)	5	10/12	2	5, 8, 12	0.80 (0.12)	0.49 (0.13)
<i>Cucumis melo</i> var <i>utilissimus</i> (Kakri/Serpent melon)	5	11/12	2	12	1.44 (0.12)	0.48 (0.13)
<i>Cucumis sativus</i> (Cucumber)	14	10/12	13	13	0.44 (0.12)	0.27 (0.11)
<i>Cucurbita maxima</i> (Summer squash)	7	10/12	1, 2	13	2.17 (0.13)	0.20 (0.17)
<i>Cucurbita moschata</i> (Pumpkin)	4	12/12	2	5	3.00 (0.13)	0.28 (0.20)
<i>Cucurbita pepo</i> (Zucchini) (Yellow squash)	4	11/12	1, 2	5	3.0 (0.16)	0.14 (0.08)

Table 1 continued

Test plant	Incubation period (DPI)	No of symptomatic plants/ No of plants inoculated	Symptoms ^a		DAC-ELISA ^b A _{405nm} (1 h)	
			Local	Systemic	Local (7 DAS)	Systemic (14-20 DAS)
<i>Lagenaria siceraria</i> (Bottle gourd)	7	9/12	13	13	0.64 (0.11)	0.38 (0.11)
<i>Luffa acutangula</i> (Ridge gourd)	6	12/12	1, 2	5	0.89 (0.08)	0.10 (0.10)
<i>Citrullus vulgaris</i> var <i>fistulosus</i> (Tinda/Indian squash)	-	0/12	13	13	0.06 (0.09)	0.09 (0.09)
<i>Luffa cylindrica</i>	-	0/12	13	13	0.05 (0.05)	0.04 (0.05)
<i>Momordica charantia</i>	-	0/15	13	13	0.05 (0.06)	0.06 (0.06)
Family: Malvaceae						
<i>Abelmoschus esculentus</i> (Lady's finger)	4	15/15	4, 6	4, 5, 8, 11	3.00 (0.13)	3.00 (0.15)
<i>Gossypium herbaceum</i> (Cotton)	7	15/15	2	13	0.82 (0.13)	0.30 (0.10)
<i>Hibiscus subderiffa</i> var. <i>subderiffa</i> (Roselle)	5	15/15	2	13	1.79 (0.14)	0.07 (0.20)
Family: Amaranthaceae						
<i>Amaranthus blitum</i> var. <i>oleraceae</i> –Amaranth (Chaulai sag)	7	10/15	2	13	0.51 (0.11)	0.05 (0.04)
<i>Amaranthus viridis</i> (Pig weed)	5	7/10	2	13	3.00 (0.10)	0.39 (0.11)
<i>Celosia cristata</i> (Cocks comb)	14	10/15	13	13	0.21 (0.10)	0.14 (0.10)
<i>Gomphrena globosa</i> (Globe amaranth)	3	15/15	1	8, 11	3.00 (0.10)	0.13 (0.12)
Family: Chenopodiaceae						
<i>Beta vulgaris</i> (Beet root)	7	10/15	2	13	0.18 (0.06)	0.06 (0.04)
<i>Beta vulgaris</i> var. <i>bengalensis</i> (Palak/Indian spinach)	5	12/15	2	12	2.03 (0.12)	0.13 (0.07)
<i>Chenopodium amaranticolor</i>	4	8/8	1	1, 3, 8, 9, 11	3.0 (0.03)	1.05 (0.21)
Family: Portulacaceae						
<i>Portulaca oleraceae</i> (Little hogweed)	7	15/15	1, 2	8, 9, 11	1.93 (0.20)	0.29 (0.20)
Family: Aizoaceae						
<i>Trianthema portulacastrum</i> (Giant pig weed)	3	8/8	1, 2	1	3.0 (0.19)	0.73 (0.18)
Family: Commelinaceae						
<i>Commelina benghalensis</i> (Day flower)	7	6/8	13	13	0.21 (0.03)	0.21 (0.08)
Family: Umbelliferae (Apiaceae)						
<i>Coriandrum sativum</i> (Coriander)	7	13/15	13	13	0.98 (0.18)	1.81 (0.14)
<i>Daucus carota</i> (Carrot)	–	0/15	13	13	0.08 (0.06)	0.07 (0.07)
Family: Crusiferae (Brassicaceae)						
<i>Brassica nigra</i> (Black mustard)	–	0/15	13	13	0.09 (0.05)	0.07 (0.06)
<i>Brassica oleraceae</i> var. <i>botrytis</i> (Cauliflower)	–	0/15	13	13	0.08 (0.03)	0.07 (0.05)
<i>Brassica oleraceae</i> var. <i>capitata</i> (Cabbage)	–	0/15	13	13	0.05 (0.05)	0.06 (0.06)
<i>Raphanus sativus</i> (Radish)	–	0/15	13	13	0.08 (0.05)	0.08 (0.06)
Family: Alliaceae						
<i>Allium cepa</i> (Onion)	–	0/15	13	13	0.08 (0.05)	0.08 (0.06)
Family: Polygonacea						
<i>Rumex vesicarius</i>	–	0/15	13	13	0.06 (0.04)	0.09 (0.08)
Family: Pedaliaceae						
<i>Sesamum ndicum</i> (Gingelly)	–	0/15	13	13	0.06 (0.04)	0.09 (0.08)
Family: Apocyanaceae						
<i>Vinca rosea</i>	–	0/15	13	13	0.08 (0.06)	0.04 (0.07)
Family: Euphorbiaceae						
<i>Ricinus communis</i> (Castor)	–	0/15	13	13	0.06 (0.06)	0.08 (0.07)

Table 1 continued

Test plant	Incubation period (DPI)	No of symptomatic plants/ No of plants inoculated	Symptoms ^a		DAC-ELISA ^b A _{405nm} (1 h)	
			Local	Systemic	Local (7 DAS)	Systemic (14-20 DAS)
Family: Poaceae						
<i>Zea mays</i> (Maize)	–	0/15	13	13	0.05 (0.05)	0.05 (0.06)
<i>Pennisetum typhoides</i> (Bajra)	–	0/15	13	13	0.06 (0.06)	0.04 (0.03)

^a 1 Chlorotic spot, 2 Necrotic spot, 3 Veinal chlorosis, 4 Veinal necrosis, 5 Mosaic, 6 Leaf necrosis, 7 Bud necrosis, 8 Little leaf, 9 Axillary shoot proliferation, 10 Necrotic streaks on stem, 11 Stunting, 12 Wilting, 13 No symptoms, *DPI* Days post inoculation, ^b Values in parentheses were healthy values

confirmation by DAC-ELISA for TSV. Infected pods were collected from the above varieties at maturity stage and stored at 4°C up to 2 months after drying. The seed material thus collected was used to check the seed infection and seed transmission.

Detection of Seed Infection

Stored groundnut seed material collected from sap inoculated and naturally field infected plants of different varieties were used for testing the seed infection. Whole seed was used to detect the seed infection using DAC-ELISA test by maintaining negative controls using healthy seed collected from un inoculated plants. Similar procedure was followed for other legume crops tested.

Detection of TSV in Different Parts of Pod

Different parts of groundnut pod namely, pod shell, seed coat (testa), cotyledon and embryo were tested using seed material collected from sap inoculated plants under glass-house conditions. Seeds were dissected after soaking in the sterile deionized water for overnight. Different parts of seed were separated with sterilized scalpel and used to detect the presence of virus by DAC-ELISA after washing under tap water for 3–5 min.

TSV Seed Transmission Tests

The seed material collected from sap inoculated seedlings (JL 24, TMV 2, Prasuna, Kadiri 6, Anantha) belonging to different age groups in each variety was bulked and used for grow-out test. Similarly, seed material collected from naturally infected groundnut varieties (JL 24, TMV 2, Kadiri 6) were also used. Ten to twenty pots were maintained for each variety along with their healthy controls. Seed material was sown in plastic pots (5–7 seeds/pot). The

percent germination was recorded in each variety and seedlings grown were tested by DAC-ELISA.

The leaf samples collected randomly at 20, 40 and 70 DAS from grow-out test seedlings were bulked separately and used for bio-assay test by sap inoculating on cowpea (cv Pusa Komal) indicator host. Leaf samples collected randomly from sap inoculated cowpea plants were checked by DAC-ELISA. Followed similar method for other legume crops.

DAC-ELISA

DAC-ELISA, as described by Clark and Bar-Joseph (1984) was followed [5]. The samples (leaf/seed/seed parts) were ground in 0.1 M coating buffer (1:1 w/v; pH 9.2) containing 2% polyvinyl pyrrolidone (PVP, MW 40,000). For each sample, two replications were maintained. Samples were loaded in ELISA plate (Costar, Sigma, USA) and kept at 37°C for 1 h incubation. Followed by three washings using Phosphate buffer saline (PBS) having 0.05% Tween - 20. For blocking, 1% bovine serum albumin (BSA) was used. Later kept at 37°C for 1 h and washed three times as above. Crude antiserum developed against TSV at Unit of Virology, IARI, New Delhi was used after cross adsorption with healthy extract (1:20). Above crude antiserum was loaded after dilution (1:4000) in PBS containing tween-20 (0.05%), polyvinyl pyrrolidone (2%) and egg ova albumin (0.2%) and kept at 37°C for 2 h followed by washings for three times. Goat anti-rabbit immunoglobulin conjugated to alkaline phosphatase (Sigma chemicals, St Louis, USA) was diluted in antibody diluent (PBS-Tween, polyvinyl pyrrolidone ova albumin, PBS-TPO) used (1:30,000). Later kept at 37°C for 2 h and washed the plate three times. The substrate buffer (10% Diethanolamine) containing *p*-nitro phenyl phosphate (0.5 mg/ml) (Sigma, USA) was used. Absorbance was recorded at 405 nm after 1 h of incubation with a substrate at 37°C using a Teacan Sunrise (version 1.2) ELISA reader. The mean absorbance value of the two

wells was compared with the absorbance values of healthy control. Absorbance values more than 2 times of healthy control are considered as positive for TSV.

Results

Host Range of TSV

Seventy plant species belonging to 18 families were tested by TSV sap inoculation and out of them 50 species belonging to Fabaceae (11), Asteraceae (10), Solanaceae (5), Cucurbitaceae (10), Malvaceae (3), Amaranthaceae (4), Chenopodiaceae (3), Umbelliferae (1), Aizoaceae (1), Commelinaceae (1), and Portulacaceae (1) were susceptible to TSV. The remaining 20 plant species, namely, *Allium cepa* (Alliaceae), *Brassica nigra*, *B. oleraceae* var. *botrytis*, *B. oleraceae* var. *capitata*, *Raphanus sativus* (Crusiferae), *C. annuum*, *S. lycopersicon* (Solanaceae), *Citrullus vulgaris* var. *fistulosus*, *Luffa cylindrica*, *Momordica charantia* (Cucurbitaceae), *Cyamopsis tetragonoloba*, *Lens culinaris* (cv TL-O4), *Pisum sativum* (Fabaceae), *Rumex vesicarius* (Polygonaceae), *Sesamum indicum* (Pedaliaceae), *Daucus carota* (Umbelliferae), *Vinca rosea* (Apocyanaceae), *Ricinus communis* (Euphorbiaceae) *Penisetum typhoides*, and *Zea mays* (Poaceae) were insusceptible to TSV as they neither exhibited clear symptoms nor supported virus multiplication.

Necrotic and chlorotic spots as localized symptoms and necrotic spots, axillary shoot proliferation, stunting, total necrosis and wilt of plant as systemic symptoms were observed in majority of plant species, such as, *A. esculentus*, *A. hypogaea*, *Calendula officinalis*, *C. tinctorius*, *C. amaranticolor*, *Cosmos sulphurens*, *Macrotyloma uniflorum*, *G. max*, *G. globosa*, *G. abyssinica*, *H. annuus*, *Petunia axillaries*, *P. vulgaris*, *Portulaca oleraceae*, *Tagetes erecta*, *T. foenum-graecum*, *V. mungo* and *V. unguiculata*. Among different family members tested, majority of cucurbitaceous members did not show systemic infection. Some of the susceptible plant species belonging to different families namely, *Celosia cristata*, *C. benghalensis*, *C. lunatus*, *C. melo*, *C. sativus*, *Coriandrum sativum*, *L. siceraria*, *P. hystrophorus* and *Tridax procumbence* reacted symptomlessly and are likely to serve as symptomless carriers of TSV (Table 1).

The new experimental hosts identified were *Amaranthus blitum* var. *oleracea* (Chaulai sag), *C. cristata* (Cocks comb) (Amaranthaceae), *Beta vulgaris* var. *bengalensis* (Palak/Indian spinach) (Chenopodiaceae), *C. officinalis* (Pot marigold), *Chrysanthemum indicum*, *C. sulphurens* (Yellow cosmos) (Asteraceae), *C. lunatus* (Watermelon), *L. siceraria* (Bottle gourd) (Cucurbitaceae), *C. sativum* (Coriander) (Umbelliferae), *Hibiscus subderiffa* var.

subderiffa (Roselle) (Malvaceae) and *Portulaca oleraceae* (Little hogweed) (Portulacaceae) (Table 1; Fig. 1).

Production of TSV Infected Pods and Symptoms

Produced both localized and systemic symptoms upon sap inoculation with TSV to the different aged group of groundnut seedlings (25, 30, 35, 40 DAS) belonging to different varieties. Localized symptoms were observed at 1–2 DPI. Initial symptoms included pale necrotic spots which turned to necrotic spots within a day followed by yellowing and wilting of inoculated leaf. Systemic symptoms included necrotic spots on young leaves, necrosis of top growing bud, necrotic streaks on stem, axillary shoot proliferation, peg necrosis, reduction of pod and kernel size, necrotic streaks on pod and discoloration of seed. Younger age group of seedlings (25 DAS) in all the varieties of groundnut tested were severely infected and majority of them produced small size pods with shriveled kernels. Localized and systemic symptoms observed were similar among different varieties of groundnut tested.

Detection of Seed Infection

Of 505 seeds collected from different groundnut varieties sap inoculated with TSV under glasshouse conditions, 130 seeds showed TSV seed infection in DAC-ELISA test. Maximum seed infection observed in cultivar TMV 2 (28.9%) followed by Prasuna, JL 24 (26.5%), Kadiri 9 (25.0%), Anantha (23.4%), Kadiri 6 (22.2%) and Kadiri 7 Bold (18.9%). Virus titer ranged from 0.20 to 3.00 (A_{405nm} , 1 h) in all the varieties tested. Of 407 seeds collected from naturally infected groundnut cultivars, 84 seeds were positive for TSV seed infection. Maximum seed infection observed with the variety JL 24 (21.9%), followed by TMV 2 (20.9%) and Kadiri 6 (19.1%). The virus titer ranged from 0.23 to 1.80 (A_{405nm} , 1 h) in all the varieties tested. The absorbance values (A_{405nm} , 1 h) of 400 healthy seeds collected from glasshouse and field samples belonging to different varieties ranged from 0.04 to 0.09 in DAC-ELISA (Table 2).

Detection of TSV in Different Parts of Pod

To know the virus distribution in different parts of seeds, 460 pods were collected from seven TSV sap inoculated groundnut varieties. TSV was detected both in pod shell and seed coat (testa). In DAC-ELISA, absorbance values (A_{405nm} , 1 h) ranged from 0.38 to 3.00 and 0.19 to 1.81 in pod shell and seed coat, respectively. No virus was detected in either cotyledons or embryos obtained from infected seeds from all the varieties tested. The absorbance values (A_{405nm} , 1 h) of different healthy parts of pod (225)

Fig. 1 New experimental hosts of *Tobacco streak virus*: **a–c**: *Amarantus blitum* var. *oleracea* (Chaulai sag) (**a** healthy, **b** necrotic spots, **c** Symptom less); **d–f** *Beta vulgaris* var. *bengalensis* (Palak) (**d** healthy, **e** necrotic spots, **f** wilting); **g–i** *Calendula officinalis* (**g** healthy, **h** Necrotic spots, **i** auxillary shoots proliferation); **j–l** *Chrysanthemum indicum* (**j** healthy, **k** chlorotic spots, **l** auxillary shoots proliferation); **m–o** *Cosmos sulphurens* (**m** healthy, **n** chlorotic and necrotic spots, **o** auxillary shoots proliferation); **p–r**: *Hibiscus subderiffa* var. *subderiffa* (**p** healthy, **q** necrotic spots, **r** Symptom less); **s–u** *Portulaca oleraceae* (**s** healthy, **t** chlorotic spots, **u** auxillary shoots proliferation)

collected from different groundnut varieties ranged from 0.04 to 0.09 in DAC-ELISA (Table 3).

TSV Seed Transmission

Grow-out test was conducted using pods harvested from TSV sap inoculated (275 seeds) and naturally TSV infected (132 seeds) plants. The seed germination ranged from 88.0 to 91.2%

against 100% germination using 407 healthy seeds collected from glasshouse and field from different varieties. None of the seedlings raised from infected seeds showed symptoms of TSV and were ELISA negative. Bio-assay test using cowpea (cv Pusa Komal) indicator host did not show any TSV symptoms by sap inoculation and were ELISA negative. The absorbance values (A_{405nm} , 1 h) of seedlings in grow-out and bio-assay tests were similar to healthy controls in DAC-ELISA (Table 4).

Table 2 Detection of seed infection of *Tobacco streak virus* in seeds collected from sap inoculated and naturally infected groundnut varieties by DAC-ELISA

Method of seed collection	Variety	Seed infection		A_{405nm} (1 h) ^b (Range)
		No. ^a	Percent	
I. Sap inoculated	JL-24	40/151	26.5	0.20–3.00 (0.04–0.09)
	TMV2	42/145	28.9	0.22–2.00 (0.03–0.05)
	Prasuna	9/34	26.5	0.23–1.50 (0.04–0.09)
	Kadiri 6	14/63	22.2	0.20–1.70 (0.02–0.06)
	Kadiri 9	7/28	25.0	0.31–0.50 (0.06–0.08)
	Anantha	11/47	23.4	0.48–3.00 (0.03–0.09)
	Kadiri 7 Bold	7/37	18.9	0.55–1.37 (0.03–0.05)
II. Naturally infected	Total	130/505	25.7	
	JL-24	27/123	21.9	0.23–1.80 (0.04–0.06)
	TMV2	31/148	20.9	0.71–1.51 (0.02–0.06)
	Kadiri 6	26/136	19.1	0.23–1.20 (0.04–0.06)
	Total	84/407	20.6	

^a Number of seeds positive in DAC-ELISA/total number of seeds tested

^b DAC-ELISA absorbance values and figures in parentheses were healthy values

Table 3 Detection of *Tobacco streak virus* by DAC- ELISA in different parts of groundnut pod collected from TSV sap inoculated groundnut varieties

Variety	No. of samples tested	DAC-ELISA (A_{405nm} , 1 h)- [#] Range			
		Pod shell	Seed coat (Testa)	Cotyledon	Embryo
JL 24	86	0.76–3.00 (0.02–0.09)	0.19–1.72 (0.04–0.05)	0.04–0.05 (0.03–0.05)	0.04–0.05 (0.03–0.06)
Prasuna	45	0.89–3.00 (0.03–0.08)	0.20–1.40 (0.02–0.06)	0.03–0.04 (0.03–0.05)	0.04–0.06 (0.03–0.06)
TMV2	73	0.38–2.50 (0.04–0.09)	0.23–1.20 (0.03–0.06)	0.08–0.09 (0.04–0.06)	0.08–0.10 (0.05–0.08)
Kadiri 6	80	1.80–2.26 (0.03–0.07)	1.20–1.81 (0.02–0.06)	0.03–0.05 (0.04–0.05)	0.02–0.05 (0.02–0.05)
Anantha	48	0.85–2.10 (0.02–0.05)	0.69–0.91 (0.02–0.07)	0.05–0.09 (0.03–0.08)	0.05–0.09 (0.04–0.09)
Kadiri 9	87	0.65–1.30 (0.03–0.08)	0.71–0.91 (0.03–0.08)	0.04–0.08 (0.04–0.08)	0.05–0.09 (0.04–0.07)
Kadiri 7 Bold	41	0.81–1.21 (0.02–0.05)	0.69–1.12 (0.03–0.07)	0.04–0.05 (0.04–0.08)	0.05–0.06 (0.04–0.05)
Total	460				

[#] Figures in parentheses were healthy values

Table 4 Detection of *Tobacco streak virus* by grow-out and bio-assay tests with seedlings raised from virus infected seeds collected from sap inoculated and naturally infected groundnut varieties

Method of seed collection	Variety	Grow-out tests			Bio-assay tests	
		No. ^a	Germination Percent	A_{405nm} (1 h) ^b (Range)	No. ^c	A_{405nm} (1 h) ^b (Range)
I. Seeds collected from sap inoculated plants	JL-24	71/80	88.8	0.03–0.08 (0.02–0.05)	0/20	0.05–0.09 (0.04–0.05)
	TMV2	73/80	91.2	0.05–0.08 (0.04–0.09)	0/20	0.03–0.05 (0.04–0.06)
	Prasuna	32/37	86.5	0.06–0.11 (0.05–0.08)	0/10	0.05–0.16 (0.03–0.09)
	Kadiri 6	40/45	88.9	0.05–0.06 (0.03–0.05)	0/20	0.04–0.08 (0.03–0.06)
	Anantha	29/33	87.9	0.05–0.09 (0.03–0.06)	0/13	0.05–0.11 (0.03–0.07)
II. Naturally infected seeds	JL-24	40/45	88.9	0.02–0.07 (0.02–0.04)	0/20	0.04–0.08 (0.03–0.05)
	TMV2	41/45	91.1	0.03–0.07 (0.02–0.07)	0/23	0.05–0.08 (0.03–0.07)
	Kadiri 6	37/42	88.0	0.05–0.09 (0.03–0.05)	0/13	0.05–0.12 (0.02–0.08)

^a Number of seeds germinated/total number of infected seeds sown

^b DAC-ELISA absorbance values and figures in parentheses were healthy values

^c Number of samples positive by indexing on cowpea/total number of samples

Detection of Seed Infection and Seed Transmission in Legumes

Of 59 seeds of *V. mungo* (cv T9) tested, 15 seeds (25.0%) were positive for seed infection by DAC-ELISA and absorbance values ranged from 0.21 to 0.28 ($A_{405\text{nm}}$, 1 h). In the similar way, of 47 seeds tested, 11 seeds (23%) were infected in *P. vulgaris* (cv Selection 9). From 45 seeds tested, 9 seeds were positive (20.0%) in the crop *T. foenum-graecum* (cv. Lam Selection 1). No seed infection was detected in other legume crops, namely, *V. radiata* (cv PDM 139), *G. max* (cv JS335), *D. lablab* (cv. Pusa Early prolific). Absorbance values ($A_{405\text{nm}}$, 1 h) ranged from 0.02 to 0.08 using 60 healthy seeds belonging to different varieties. In grow-out tests, none of the seedlings raised from infected seed of different legumes showed symptoms of TSV and they were ELISA negative as absorbance values were similar to healthy seedlings. Bio-assay test also showed none of the TSV symptoms by sap inoculation on cowpea (cv Pusa Komal) and reacted negatively in DAC-ELISA (Table 5).

Discussion

Present study on the experimental host range of groundnut TSV isolate was led to the identification of 11 new experimental hosts in addition to presently known host range of TSV from different countries [8, 12, 19, 25]. Among different plant species tested, the hosts, such as, cowpea (*V. unguiculata*), groundnut (*A. hypogaea*), horse gram (*M. uniflorum*), methi (*T. foenum-graecum*), green gram

(*V. radiata*) (Fabaceae), sunflower (*H. annuus*), safflower (*C. tinctorius*), marigold (*T. erecta*), lettuce (*Lactuca sativa*), cosmos (*C. sulphurens*), tobacco (*N. benthamiana*), white petunia (*P. axillaries*) and okra (*A. esculentus*) were supported high virus titer both in localized and systemic infection and could be used as excellent propagating hosts for virus maintenance. Similarly, *V. unguiculata*, *M. uniflorum*, *C. amaranticolor*, *N. tabacum*, *p. axillaries* and *G. globosa* were identified as excellent local lesion assay hosts. Of 70 plant species tested, 20 plant species were insusceptible to TSV. However, some of the insusceptible hosts identified in the present study such as, *B. campestris*, *P. sativum*, *L. culinaris*, *C. tetragonoloba*, *S. lycopersicum* and *V. rosea* were reported as well known experimental hosts for TSV [8, 12, 19]. This discrepancy may be due to the difference in the TSV isolates used. This study clearly indicates that isolate specific experimental host range of TSV can't be ruled out.

In the present study, seed infection was recorded both in sap inoculated and naturally infected seeds from different varieties of groundnut tested (Table 2). This is the first report of seed infection of TSV in groundnut crop under natural and glasshouse conditions. Further, to ascertain exact location of TSV, the virus was checked in different parts, such as, pod shell, seed testa, cotyledon and embryo using seeds collected under glasshouse experiment from different varieties of groundnut. The virus was detected in pod shell and seed testa only but not in either cotyledon or embryo (Table 3). In the similar manner, none of the cotyledons and embryos showed presence of TSV in naturally infected groundnut seeds (data not showed). Hence, present study clearly suggests that TSV restricts to seed

Table 5 Detection of seed infection of *Tobacco streak virus* in seeds of legume crop produced by sap inoculation with TSV and confirmation of seed transmission by grow-out and bio-assay tests

Crop	Seed infection			Grow-out test		Bio-assay test		
	No. ^a	Percent	$A_{405\text{nm}}$ ^b (Range)-1 h	No. ^c	$A_{405\text{nm}}$ (1 h) ^b	No. ^d	$A_{405\text{nm}}$ ^b (1 h)	
<i>Vigna mungo</i> cv T9 (Black gram)	15/59	25.0	0.21–0.28 (0.02–0.04)	0/35	0.19–0.20 (0.02–0.10)	0/20	0.06–0.09 (0.02–0.05)	
<i>Vigna radiata</i> cv PDM 139 (Green gram)	0/62	0.00	0.04–0.08 (0.03–0.05)	0/42	0.03–0.08 (0.03–0.05)	0/24	0.04–0.06 (0.03–0.04)	
<i>Glycine max</i> cv JS335 (Soybean)	0/51	0.00	0.02–0.08 (0.02–0.06)	0/37	0.02–0.08 (0.02–0.05)	0/15	0.06–0.11 (0.04–0.09)	
<i>Dolichos lablab</i> cv Pusa early prolific (Sem)	0/42	0.00	0.03–0.11 (0.02–0.08)	0/38	0.06–0.12 (0.04–0.08)	0/12	0.05–0.08 (0.02–0.08)	
<i>Phaseolus vulgaris</i> cv Selection 9 (French bean)	11/47	23.0	0.22–0.33 (0.03–0.06)	0/39	0.05–0.08 (0.03–0.06)	0/15	0.06–0.10 (0.04–0.06)	
<i>Trigonella foenum-graecum</i> cv Lam selection 1 (Methi)	9/45	20.0	0.13–0.18 (0.03–0.06)	0/31	0.05–0.11 (0.03–0.07)	0/18	0.04–0.10 (0.03–0.07)	

^a Number of seeds positive in DAC-ELISA/total seeds

^b Range of absorbance values ($A_{405\text{nm}}$) in DAC-ELISA and figures in parentheses were healthy control values

^c Number of seedlings positive in DAC-ELISA/total number of seedlings

^d Number of samples positive by indexing on cowpea/total number of samples

testa in groundnut kernel even though TSV was sap inoculated at flowering period. Further tests are needed to ascertain barriers which prevent entry of virus into the cotyledons and embryo of kernel.

To ascertain seed transmission of TSV in groundnut, grow-out test was conducted using seeds collected from ELISA positive sap-inoculated and naturally infected plants separately. Germination varied from 88.0 to 91.2% and none of the seedlings showed symptoms of TSV and were ELISA negative. The loss of germination per cent might be due to non viability of severely infected seeds (Table 4). In grow-out tests, some of groundnut seedlings showed mild chlorotic symptoms immediately after germination. However, plants recovered well in a short span of time and produced normal flowers and pods. Bio-assay tests using cowpea (cv Pusa Komal) as indicator host were also negative. The present study suggests that TSV fails to enter into seedlings raised from infected seeds, in spite of presence of virus in the seed coat. Hence, it is concluded that seed transmission is negative in groundnut host. In the similar manner, we are reporting TSV seed infection for the first time in the seeds collected from *V. mungo* (cv T9) and *T. foenum-graecum* (cv Lam Selection1) under glass-house experiments. However, seed transmission tests were negative in all the legume crops tested based on the limited studies conducted by sap inoculation (Table 5).

Seed transmission was very well proved in different TSV isolates worldwide since seed transmission nature of TSV has been documented both in naturally infected and artificially sap inoculated plants belonging to different families. The percent seed transmission rate was less in naturally infected plants when compared to artificially sap inoculated plants. Ghanekar and Schwenk, [13] revealed that seed transmission was obtained with soybean TSV isolate (*Glycine max*) but not with tobacco TSV isolate. In the similar manner, Walter et al. [34] observed highly seed transmitting (Mel 40) and non or less seed transmitting (Mel F) strains of TSV infecting *M. alba* (Biennial wild legume). Hence, existence of seed and non-seed transmissible strains of TSV might be the reason for presence or absence of seed transmission.

So far, TSV seed transmission was not proved from any crop or weed hosts in India. Hence, it is concluded that non-seed transmissible strain of TSV might be existing in India. Absence of seed transmission of TSV was also observed by Prasada Rao et al. [26] in crop and weed hosts belonging to Fabaceae and Asteraceae family members in India. Moreover, complete genome sequence is lacking for Indian TSV isolate and comparison has to be made with seed transmissible strain of TSV from different countries as Walter et al. [34] observed extra minor RNAs in non-seed transmissible strain (Mel F) of TSV. So, differentiation has to be made between seed and non seed transmissible strains

of TSV with respect to their host range, physiochemical properties and genetic diversity.

Acknowledgments Greatly indebted to the Indian Agricultural Research Institute, New Delhi and Acharya N.G. Ranga Agricultural University, Andhra Pradesh for financial support and to Dr. R.D.V.J Prasada Rao, Principal Scientist and Dr. M. Johnson, Senior Scientist for their generous help. I thank to Dr. K. Sankar Reddy, Associate Director of Research, and Dr. D. Lokanadha Reddy, Principal Scientist (Groundnut) and Head for critical reading of the manuscript and encouragement.

References

1. Arun Kumar N, Lakshmi narasu M, Zehr UB, Ravi KS. First report of *Tobacco streak virus* infecting *Guizotia abyssinica* from India. *Plant Dis.* 2007;91:330.
2. Arun Kumar N, Lakshmi Narasu M, Zehr UB, Ravi KS. Molecular characterization of *Tobacco streak virus* causing soybean necrosis in India. *Indian J Biotechnol.* 2008;7:214–7.
3. Bhat AI, Jain RK, Chaudhary V, Krishna Reddy M, Ramaiah M, Chattannavar SN, Varma A. Sequence conservation in the coat protein gene of *Tobacco streak virus* isolates causing necrosis disease in cotton, mungbean, sunflower and sunn-hemp in India. *Indian J Biotechnol.* 2002;1:350–6.
4. Chander Rao S, Prasada Rao RDVJ, Manoj Kumar V, Raman DS, Raoof MA, Prasad RD. First report of *Tobacco streak virus* infecting safflower (*Carthamus tinctorius*) in Maharashtra, India. *Plant Dis.* 2003;87:1396.
5. Clark MF, Bar-Joseph. Enzyme-linked immunosorbent assay in plant virology. In: Marmorash K, Korowski H, editors. *Methods in virology*, vol. VII. New York: Academic press; 1984. p. 51–85.
6. Converse RH. *Tobacco streak virus* in black rasp berry. *Phytopathology.* 1972;62:1001–4.
7. Cook G, Miranda RD, Roossinck MJ, Pietersen G. Tobacco streak ilarvirus detected on groundnut in South Africa. *Afr Plant Protect.* 1999;5:13–9.
8. Cupertino FP, Grogan RG, Petersen LJ, Kimble KA. *Tobacco streak virus* infection of tomato and some natural weed hosts in California. *Plant Dis.* 1984;68:331–3.
9. Dijkstra J. *Tobacco streak virus* in sunflower (*Helianthus annuus*). *Neth J Plant Pathol.* 1983;89:153.
10. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. *Virus taxonomy: VIIIth Report of International Committee on Taxonomy of Viruses.* New York, USA: Academic Press; 2005.
11. Finlay JR. *Tobacco streak virus* in tobacco. *Aust Plant Pathol Soc News Let.* 1974;3:71.
12. Fulton RW. *Tobacco streak virus*, CMI/AAB descriptions of plant viruses. No. 307. Wellesbourne, UK: Association of Applied Biologists; 1985.
13. Ghanekar AM, Schwenk FW. Seed transmission of *Tobacco streak virus* in six cultivars of soybeans. *Phytopathology.* 1974;64: 112–4.
14. Gracia O, Feldman JM. *Tobacco streak virus* in pepper. *Phytopath Z.* 1974;80:313–23.
15. Jain RK, Bag S, Awasthi LP. First report of natural infection of *Capsicum annuum* by *Tobacco streak virus* in India. *Plant Pathol.* 2005;54:257.
16. Johnson H A Jr, Converse RH, Amorao A, Espejo JI, Frazier NW. Seed transmission of *Tobacco streak virus* in strawberry. *Plant Dis.* 1984;68:390–2.
17. Jones AT, Mc Gavin WJ, Dolan A. Detection of *Tobacco streak virus* isolates in North American Cranberry (*Vaccinium macrocarpon*). *Online Plant Health Prog.* 2001. doi:10.1094/PHP-2001-0717-01-RS

18. Jones AT, Mayo MA. Further properties of black raspberry latent virus and evidence for its relationship to *Tobacco streak virus*. *Ann Appl Biol*. 1975;79:297–306.
19. Kaiser WJ, Wyatt SD, Pesho GR. Natural hosts and vectors of *Tobacco streak virus* in Eastern Washington. *Phytopathology*. 1982;72:1508–12.
20. Kaiser WJ, Wyatt SD, Klein RE. Epidemiology and seed transmission of two *Tobacco streak virus* pathotypes associated with seed increases of legumes germplasm in Eastern Washington. *Plant Dis*. 1991;75:258–64.
21. Krishna Reddy M, Devaraj Lakshmi R, Salil J, Samuel DK. Outbreak of *Tobacco streak virus* causing necrosis of cucumber (*Cucumis sativus*) and gherkin (*Cucumis anguria*) in India. *Plant Dis*. 2003;87:1264.
22. Krishna Reddy M, Salil J, Samuel DK. Fruit distortion mosaic diseases of okra in India. *Plant Dis*. 2003;87:1395.
23. Ladhakshmi D, Ramaiah M, Ganapathy T, Krishna Reddy M, Khabbaz SE, Babu M, Kamalakannan A. First report of the natural occurrence of *Tobacco streak virus* on blackgram (*Vigna mungo*). *Plant Pathol*. 2006;12:55.
24. Pappu HR, Hammett KRW, Draffell KI. *Dahlia mosaic virus* and *Tobacco streak virus* in dahlia (*Dahlia variabilis*). *Plant Dis*. 2008;92:1138.
25. Prasada Rao RDVJ, Reddy AS, Reddy SV, Thirumala Devi K, Chander Rao S, Manoj Kumar V, Subramaniam K, Yellamanda Reddy T, Nigam SN, Reddy DVR. The host range of *Tobacco streak virus* in India and transmission by thrips. *Ann Appl Biol*. 2003;142:365–8.
26. Prasada Rao RDVJ, Jyothirmai Madhavi K, Reddy AS, Varaprasad KS, Nigam SN, Sharma KK, Lava Kumar P, Waliyar F. Non transmission of *Tobacco streak virus* isolate occurring in India through the seeds of some crop and weed hosts. *Indian J Plant Prot*. 2009;37:92–6.
27. Prsada Rao RDVJ, Reddy AS, Chander Rao S, Varaprasad KS, Tirumaladevi K, Nagaraju, Muniyappa V, Reddy DVR. *Tobacco streak virus ilarvirus* as a causal agent of sunflower necrosis disease in India. *J Oilseeds Res*. 2000;17:400–1.
28. Ramaiah M, Bhat AI, Jain RK, Pant RP, Ahlawat YS, Prabhakar K, Varma A. Isolation of an isometric virus causing sunflower necrosis disease in India. *Plant Dis*. 2001;85:443.
29. Reddy AS, Prasad rao RDVJ, Thirimala Devi K, Reddy SV, Maya MA, Roberts I, Satyanarayana T, Subramaniam K, Reddy DVR. Occurrence of *Tobacco streak virus* on peanut (*Arachis hypogaea*) in India. *Plant Dis*. 2002;86:173–8.
30. Salazar LE, Abad JA, Hooker WJ. Host range and properties of a strain of *Tobacco streak virus* from potatoes. *Phytopathology*. 1982;72:1550–4.
31. Sharman M, Thomas JE, Persley DM. First report of *Tobacco streak virus* in sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), chickpea (*Cicer arietinum*), mungbean (*Vigna radiata*) in Australia. *Aust Plant Dis Notes*. 2008;3:27–9.
32. Sharman M, Persley DM, Thomas JE. Distribution in Australia and seed transmission of *Tobacco streak virus* in *Parthenium hysterophorus*. *Plant Dis*. 2009;93:708–12.
33. Thomas WD Jr, Graham RW. Seed transmission of red node virus in pinto beans. *Phytopathology*. 1951;41:959–62.
34. Walter MH, Wyatt SD, Kaiser WJ. Comparison of the RNAs and some physiochemical properties of the seed transmitted *Tobacco streak virus* isolate Mel 40 and the infrequently seed-transmitted isolate Mel F. *Phytopathology*. 1995;85:1394–9.
35. Waqar A, Butt TB, Insan J, Rehman A. Natural occurrence of *Tobacco streak virus* in cotton in Pakistan and screening for its resistance sources. *Pak J Bot*. 2003;35:401–8.