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Host range studies of *Tobacco Streak Virus* infecting Cotton

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ABSTRACT

Tobacco streak virus (TSV) is an emerging menace in cotton growing belts of Tamil Nadu, found to be mechanically transmissible to 27 hosts with varying symptoms of chlorotic local lesions, necrotic lesions and systemic symptoms. Necrotic lesions and chlorotic lesions were observed on *Gossypium barbadense* within 5 to 8 days after inoculation. The highest virus titre was achieved with the host plants *Vigna unguiculata* and *Chenopodium amaranticolor* which was performed by DAS-ELISA.

Key words: *Gossypium hirsutum, Tobacco streak virus, Vigna unguiculate,* DAS ELISA.

Cotton is one of the most important fibre and cash crop of India and plays a dominant role in the industrial and agricultural economy of the country. It provides the basic raw material (cotton fibre) to cotton textile industry. Cotton necrosis disease caused by tobacco streak virus (TSV) is an emerging threat in India (Rageshwari *et al.,* 2016). Tobacco streak virus infecting various crops have been reported to be transmitted through mechanical means, infected seeds and through thrips species.

So far, natural occurrence of TSV in India has been reported from sunflower (*Helianthus annuus*) (Bhat *et al.,* 2001). About 19 plant species belonging to five different families *viz.,* malvaceae, chenopodiaceae, compositeae, leguminaceae and solanaceae were tested for host range and virus isolate causing cotton mosaic disease (Jagtap *et al.,* 2012). Hence, present investigation was carried out by taking crop and weed species growing in/ proximity of cotton crop.

Materials and Methods

Totally 27 hosts species belonging to 9 families were tested under insect proof net house conditions to know about the suitable indicator host for TSV on cotton (Table 1). The TSV inoculums of cotton (*Gossypium barbadense*) was maintained on cowpea plants (CO 7) by mechanically inoculating cowpea leaves (2 generation) from cotton. At least minimum of 5 to maximum of 10 plants of each species of experimental hosts were inoculated mechanically with sap extracts in their respective growth stage from young infected (lesion produced upon inoculation from cotton) primary leaves of cowpea. The crude sap of infected plant was extracted using sodium phosphate buffer pH 7.2 with 0.01 M mercaptoethanol, 1% sodium sulphite and 1-2% sodium EDTA. The plants were monitored daily for the expression of symptoms; number of days for the symptom expression and the type of symptoms. Both symptomatic and asymptomatic plants were tested in ELISA for the presence of virus titre.

Double antibody Sandwich ELISA (DAS-ELISA) was performed in all the assay hosts as described by Clark and Adams (1977). The TSV antiserum (from DSMZ, Germany) at 1:500 dilution and goat anti-rabbit IgG conjugated with alkaline

phosphatase at 1:500 dilution were used for the test. For each sample three replications were maintained and the mean value was calculated by taking the average of all the replications. pNPP (p-Nitrophenyl phosphatase) substrate (Sigma Aldrich) was used in a concentration of 1 mg/ml and the absorbance was recorded at 405nm 1 hr after incubation.

Results and Discussion

Among 27 host plants tested for host range studies, seven host belongs to Malvaceae family, four host belongs to Leguminaceae family, six host belongs to Cucurbitaceae family, three host belongs to Amaranthaceae family, two host belongs to Solanaceae family, two host belongs to Asteraceae family and each one host belongs to Fabaceae, Apocynaceae. Among 27 host plants tested, plant species *viz., Nicotiana tabaccum, N. rustica, Vigna mungo, V. radiata, Glycine max* and all the species belonging to family Cucurbitaceae exhibited

Table 1. Host range of TSV infecting Cotton under insect proof net house

Test host	Days for symptom	Type of symptom	DAS-ELISAA 405nm (1hr)
G. b. (Suvin)	5-8	Chlorotic lesions & Veinal necrosis	2.436 (0.06)
G. b. (MRC 7918)	5-8	Chlorotic lesions & Veinal necrosis	2.120 (0.05)
G. b. (ICB 25)	5-8	Chlorotic lesions & Veinal necrosis	2.036 (0.05)
G. b. (CCB 11)	5-8	Chlorotic lesions & Veinal necrosis	2.005 (0.05)
G. b. (CCB 29)	5-8	Chlorotic lesions & Veinal necrosis	2.325 (0.06)
G. h. (Suraj)	5-8	Chlorotic lesions & Veinal necrosis	1.724 (0.04)
G. h. (Mallika Bt)	5-8	Chlorotic lesions & Veinal necrosis	1.635 (0.04)
V. unguiculata (CO 7)	3-5	Chlorotic lesions, Veinal necrosis, Necrotic lesions, systemic symptom, necrosis on petioles, total necrosis	2.956 (0.06)
V. radiata	3-5	Chlorotic lesions, Veinal necrosis, Necrotic lesions, systemic symptom, necrosis on petioles, total necrosis	2.876 (0.06)
V. mungo	3-5	Chlorotic lesions, Veinal necrosis, Necrotic lesions, systemic symptom, necrosis on petioles, total necrosis	2.653 (0.05)
G. max	3-5	Chlorotic lesions, Veinal necrosis, Necrotic lesions, systemic symptom, necrosis on petioles, total necrosis	1.923 (0.04)
L. sicerarea	8-10	Mosaic symptom, necrotic lesions	1.586 (0.04)
T. cucumerina	8-10	Mosaic symptom, necrotic lesions	1.002 (0.04)
C. sativus	8-10	Mosaic symptom, necrotic lesions	1.345 (0.04)
C. melo	8-10	Mosaic symptom, necrotic lesions	1.125 (0.04)
L. acutangula	8-10	Mosaic symptom, necrotic lesions	1.258 (0.04)
C. moscheta	8-10	Mosaic symptom, necrotic lesions	1.423 (0.04)
C. amaranticolor	3-5	Chlorotic lesions	2.893 (0.05)
C. quinoa	3-5	Chlorotic lesions	2.145 (0.05)
G. globose	3-5	Chlorotic lesions	0.253 (0.04)
T. patula	3-5	Chlorotic lesions, necrotic lesions	0.586 (0.03)
C. ternatea	3-5	Chlorotic lesions, necrotic lesions	1.086 (0.05)
C. roseus	8-10	Chlorotic lesions, necrotic lesions	0.384 (0.04)
B. diffusa	3-5	Chlorotic lesions, necrotic lesions	1.832 (0.05)
P. hysterophorus	3-5	Chlorotic lesions, necrotic lesions	1.586 (0.05)
N. rustica	8-17	Curling, necrotic lesions	2.184 (0.06)
N. tabacum	8-17	Curling, necrotic lesions	2.025 (0.05)
Healthy Cotton (field)	–	–	0.23 (0.04)
Healthy Cowpea	–	–	0.33 (0.05)
Buffer	–	–	0.06

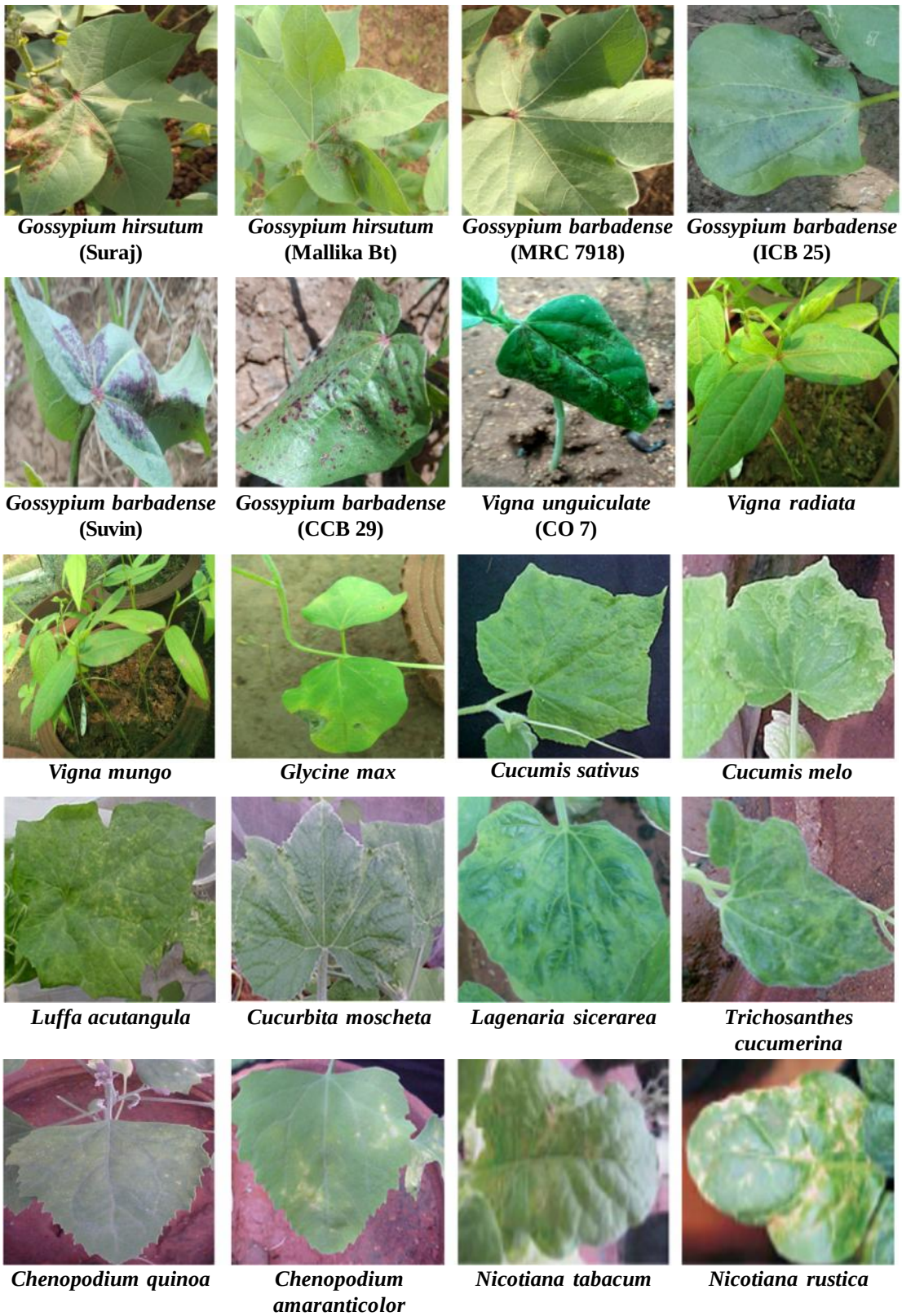

Fig 1. Sap inoculation studies from different hosts of TSV from cotton (*Gossypium barbadense*)

systemic symptoms while remaining all the host produce only local lesions (Fig. 1; Table 1). Duhan *et al*. (2020) reported 3 isolates were member of cotton leaf curl multan virus.

Symptoms like chlorotic lesions, necrotic lesions, necrotic spots, veinal necrosis, systemic symptoms, necrosis on petioles, stem necrosis and total necrosis were observed on cowpea (*Vigna unguiculata*) seedlings at 3 to 7 days after inoculation. Viral inoculum was maintained artificially in different hosts under insect proof net house conditions. Kumari *et al*. (2018) detected mix infection of poly virus and tobacco virus in bitter gourd in Karnataka state. However, Negi and Vishunavat (2004) observed the seed borne inocula of leaf crinkle virus in urd bean and thus affected yield. Rajabaskar *et al.* (2019) also reported similar results on tospovirus. *Catharanthus roseus* showed only few necrotic rings. In order to produce local lesion symptom, three days taken by the host to produce necrotic lesions wherein in systemic symptoms, it took 5 to 15 days to express symptoms in the host. DAS-ELISA was preformed for all the hosts and virus titre for different hosts were given in the Table 1. The virus titre found in the host *Chenopodium amaranticolor* was 2.893 and *Chenopodium quinoa* was 2.145. The virus titre found in the host *Nicotiana rustica* was 2.184 and *Nicotiana tabacum* was 2.025.

Among 27 host species tested, *Vigna unguiculata* and *Chenopodium amaranticolor* expressed local lesions within 2 to 3 days with higher OD value of 2.956 and 2.893 when compared to healthy control. Since, cotton leaves have high phenol content there may be inhibition in successful transmission. 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 (Vemana & Jain 2010). Lava Kumar *et al.* (2014) reported that *V. unguiculata* and *Phaseolus vulgaris* can be used as diagnostic crop for TSV as it produces necrotic local lesions and veinal necrosis upon mechanical inoculation. Similarly, Rageshwari *et al*. (2016) reported that *V. unguiculata* and *C. amaranticolor* can be used as the best host for maintaining the TSV inoculum for cotton crop. TSV was trans-inoculated into various indicator hosts in order to assess their efficiency in multiplication and establishment. *C. amaranticlor, C. quinoa,* and *V. unguiculata* were found identified as best study plants with higher titre values.

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