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Experimental and natural host range studies of *Tobacco streak* virus (TSV) isolates of Blackgram and Greengram of Telangana using DAC-ELISA

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Abstract
In the present study, experimental and natural host range of Tobacco streak virus (TSV) isolates of Blackgram (BG) and Greengram (GG) of Andhra Pradesh was studied. Upon mechanical inoculations, TSV isolates of Blackgram and Greengram infected 25 out of 36 plant species tested. Both TSV-BG and TSV-GG isolates showed similar symptoms on the test plants. *A. hypozaea* cv. JL-24, *C. cajan*, *H. annuus* cv.PAC-36, *P. vulgaris* cv. Top crop, *V. unguiculata* cv. C-152, *G. max* cv. Bragg, *C. quinoa*, *C. amaranticolor* were manifested with local and systemic symptoms. *V. radiata*. cv. K-851, *V. mungo* developed both local and systemic symptoms while *N. glutinosa*, *N. rustica*, *N. tabacum* cv. Samsun, recorded with both local and systemic symptoms. *S. melongena* L. developed both local and systemic symptoms. Both local and systemic symptoms were observed in *D. stramonium*. *Vicia faba* L. developed both local and systemic symptoms. *G. herbaceum* developed only local symptoms, and the ornamental crops, *C. infundibularis* and *T. patuala* Cav. showed only systemic symptoms. TSV isolates of Blackgram and Greengram symptomlessly infected *Amaranthus viridis*, *Commelina bengalensis*, *P. hysterophorus* in the present study. *T. procumbense* also recorded with symptomless infection of TSV. No TSV infection was found in *Cucumis sativus, C. tetragonaloba, Lagenaria cineraria, L. esculentum.* Out of 44 suspected as well as random plant species collected and tested by DAC-ELISA, TSV was detected in 19 samples, comprising economically important crops and several weeds. TSV was detected in *H. annuus*, *A. hypogaea*, *G. herbaceum, A. esculentum*., *V. unguiculata, T. patula, P. hysterophorus, A. aspera, Acanthospermum hispidum, V. radiata, C. infundibuliformis*., *C. bengalensis, T. procumbense* and *C. sparsiflorus* L.). TSV was found in *V. mungo* and in the weed species *Digera arvensis*. No TSV infection was found in weed species *viz*., *Argemone mexicana, Cassia auriculata, Cleome viscosa, Amaranthus viridis, Eclipta alba, E. hirta, L. cramera, T. zylanicum* and *T. terrestris* and crop plants, *C. annuum, H. annuus* and *L. esculentum*.

Keywords: Host range, *Tobacco streak virus* (TSV), Blackgram, Greengram

Introduction
Among pulses, Blackgram (urdbean - *Vigna mungo* L. Hepper) and Greengram (mungbean - *Vigna radiata* L. Wilczek) are major grain legumes and are cultivated in both upland and rice fallows in India. *Tobacco streak virus* (TSV) has been reported to be a cause of leaf curl symptoms on Blackgram (Prasada Rao *et al.*, 2003; Ladhalakshmi *et al.*, 2005) [4, 5] and Greengram (Bhat *et al.*, 2002; Prasada Rao *et al.*, 2003) [2, 5] paving confusion in field diagnosis to assess the disease incidence among other viral diseases attacking. In the present study, the TSVV isolates of Blackgram and Greengram were tested on a different crop and weed species to determine experimental and natural host range as knowledge of the sources of the virus inoculum plays a crucial role in plant virus disease management.

Materials and Methods
Experimental host range under glasshouse conditions
Crop and weed species belonging to different families were tested under glasshouse conditions by mechanical inoculation with the four isolates *viz.*, TSV-BG and TSVV-GG. Ten plants (2 plants/pot) of each species were sap inoculated. Cucurbits and legumes were inoculated at the primary leaf stage and others at 2-4 leaf stage.

The plants were kept under observation for symptom expression. Development of local and systemic symptoms was recorded up to 30 days. Irrespective of symptoms produced, all inoculated and newly emerged leaves were indexed back on local lesion assay host (cowpea cv. C-152) and tested also by DAC-ELISA with respective antiserum.

Natural host range under field conditions

Crop and weed species samples were collected randomly from the fields of Anantapur and experimental fields of NBPGR-RS, Hyderabad. The samples collected were tested by DAC-ELISA using both PBNV antiserum.

Results

Experimental host range

The studies on experimental host range of TSV-BG and TSV-GG isolates under glasshouse conditions by mechanical inoculation revealed that 25 plant as well as weed species could be infected out of 36 tested (Table 1). Symptoms expressed on the experimental host plants ranged from chlorotic, necrotic lesions/rings, chlorotic, necrotic concentric rings, leaf and stem necrosis, chlorotic spots, leaf curl, smalling of leaves, stunting, mosaic mottling, apical necrosis to death with both the TSV isolates inoculations (Fig.1 & 2).

Leaf necrosis, veinal necrosis, stem and apical necrosis leading to death was observed on groundnut test plants inoculated with TSV isolates while no infection was recorded on tomato with TSV-BG as well as TSV-GG isolates. Both the, TSV isolates produced localized necrotic lesions on the inoculated leaves of *Gossypium* spp. followed by no systemic infection. TSV isolates could infect the plant species like *Abelmoschus esculentum.*, *Helianthus annuus* cv.PAC-36, *Trigonella foenum-graecum*, *Gossypium herbaceum*, *Crossandra infundibuliformis*, *Tagetes patula*, *Amaranthus* spp., *Croton sparsiflorus* with varied symptoms. TSV-BG and TSV-GG isolates produced only localized necrotic lesions on the inoculated leaves of *G. herbaceum* followed by no systemic infection while *C. infundibuliformis* L. and *Tagetes patula* developed only systemic symptoms.

Table 1: Experimental host range of TSV-BG and TSVV-GG isolates under glasshouse conditions

Host species	Symptoms			
	TSV-BG		TSV-GG	
	Local	Systemic	Local	Systemic
Arachis hypogaea cv. JL-24	NR, N	VN, LN, SN, AN, D	NR, N	VN, LN, SN, AN, D
Lycopersicon esculentum cv. Marutham	-	-	-	-
Capsicum annuum cv.G-4	-	-	-	-
Gossypium herbaceum	LN, NL	-	LN, NL	-
Abelmoschus esculentum.	NL	LN, D	NL	LN, D
Cajanus cajan	NL	LN, VN, LC, AN, D	NL	LN, VN, LC, AN, D
Solanum melongena L.	CR, CCR, NR	CR, CCR, NR	CR, CCR, NR	CR, CCR, NR
Nicotiana glutinosa	CL, NL	CL, NL, MM, LD	CL, NL	CL, NL, MM, D
N. rustica	CCR	NS, CL, LD	CCR	NS, CL, LD
N. tabacum cv. Samsun	CCR	NCR, MM, LD	CCR	NCR, MM, LD
Cucumis sativus	-	-	-	-
Cucurbita moschata	-	-	-	-
Lagenaria siceraria	-	-	-	-
Mimordica charantia	CL, NL	-	CL, NL	-
Helianthus annuus cv.PAC-36	CL	C, CL, NL, SN, D	CL	C, CL, NL, SN, D
Trigonella foenum-graecum	NL	VN	NL	VN
Phaseolous vulgaris cv. Top crop	NL, VN	VN, SN	NL, VN	VN, SN
Vigna unguiculata cv. C-152	NR, VN, NL	LC, LN, SN	NR, VN, NL	LC, LN, SN
Cyamopsis tetragonaloba	-	-	-	-
Vigna radiata	CL, NL, VN	LN, AN, SL, S, D	CL, NL, VN	LN, AN, SL, S, D
Vigna mungo	CL, NL	LN, AN, SL, S, D	CL, NL	LN, AN, SL, S, D
Vicia faba	NL, VN	N, VN, SL, S	NL, VN	N, VN, SL, S
Crossandra infundibuliformis	-	LN, MM	-	LN, MM
Tagetes patula	-	LN, SN, D	-	LN, SN, D
Amaranthus spp.	-	-	-	-
Datura stramonium	CR, CCR	MM, CR	CR, CCR	MM, CR
Parthenium hysterophorus	-	-	-	-
Corchorus sps.	-	-	-	-
Glycine max	CL	NL, SN, CN	CL	NL, SN, CN
Tridax procumbens	-	-	-	-
Croton sparsiflorus	NL	LN, AN	NL	LN, AN
Commilina bengalensis	-	-	-	-
Chenopodium quinoa.	NL,	NL, LN, AN, D	NL,	NL, LN, AN, D
C. amaranticolor	CL	CL, VC, S	CL	CL, VC, S
Euphorbia hirta	-	-	-	-
E. geniculata	NL	NL, AN	NL	NL, AN

In *Gomphrena globosa*, both the TSV isolates induced necrotic lesions on the inoculated leaves followed by mosaic mottling symptoms of the newly emerging leaves. Infection by TSV-BG and TSV-GG isolates in *H. annuus* cv. PAC-36 produced chlorotic and necrotic lesions on the inoculated leaves followed by systemic mosaic mottling and leaf deformation whereas the necrotic rings were produced on the inoculated leaves of *A. esculentum* gradually converted into concentric chlorotic rings

followed by mosaic mottling and deformation of the inoculated leaves. No systemic infection was observed with TSV isolates in case of okra.

No symptoms were produced on *Parthenium hysterophorus* and *Tridax procumbens* either by TSV-BG or TSV-GG isolates where the plants were reacted positive to TSV by DAC-ELISA. TSV infection with both the isolates showed numerous necrotic lesions with systemic infection showing leaf necrosis, veinal necrosis and apical necrosis on *Cajanus cajan*. *Solanum melongena* developed chlorotic rings, concentric chlorotic rings and necrotic rings on inoculated well as on newly emerged leaves upon inoculations with TSV-BG and TSV-GG.

Different species of *Nicotiana* spp. tested in experimental host range showed varied symptoms. *N. tabacum* cv. Samsun produced necrotic rings with TSV-BG and TSV-GG on the inoculated leaves which gradually enlarged in size and developed as concentric necrotic lesions. The necrotic lesions coalesced and produced large necrotic patches or complete necrosis of the inoculated leaves. Sometimes the newly emerged leaves produced necrotic rings, which merge or enlarge to form an oak leaf like pattern on the leaf lamina. Subsequently, the newly emerged young leaves either showed with no visible symptoms or with dentate leaf margins or necrotic rings. Both the isolates, TSV-BG & TSV-GG showed chlorotic and necrotic lesions and veinal necrosis on inoculated leaves of *N. glutinosa.* Chlorotic and necrotic lesions, mosaic and deformed newly emerged leaves were observed with TSV isolates. TSV-BG and TSV-GG produced concentric chlorotic rings locally and chlorotic lesions, necrotic spots and leaf distortion systemically on *N. rustica*.

TSV-BG and TSV-GG induced venial, petiole and complete leaf necrosis and lead to death of *Vicia faba* test plant where stem necrosis was prominently observed. *Chenopodium quinoa* developed necrotic lesions, apical necrosis with TSV-BG & TSV-GG while *C. amaranticolor* produced chlorotic lesions, veinal chlorosis and stunting with both the TSV isolates. *Euphorbia geniculata* showed leaf necrosis and apical necrosis with TSV isolates. *Datura stramonium* expressed mild chlorotic lesions on inoculated leaves and numerous mild chlorotic rings on newly emerged leaves with TSV-BG and TSV-GG infection.

In the present study, *Cyamopsis tetragonoloba*, *Luffa acutangula*., *Cucumis sativus*, *Cucurbita moschata*, *Lagenaria siceraria*, *Mimordica charantia*, *Corchorus* spp. and *Euphorbia hirta* were not found to be infected (neither produced any visible symptom nor tested positive either to TSV-BG or TSV-GG) upon sap inoculations of TSV isolates of Blackgram and Greengram under glasshouse conditions.

CR-chlorotic rings, NR-necrotic rings, NCR-necrotic concentric rings, CCR-chlorotic concentric rings, NL-necrotic lesions, CL-chlorotic lesions, SL-smalling of leaves, M-mosaic, MM-mosaic mottling, SN-stem necrosis, LD-leaf distortion, AN-apical necrosis, LC-leaf curl, CS-chlorotic spots, NS-necrotic spots, VN- veinal necrosis, VC-veinal chlorosis, S-stunting, WSL-water-soaked lesions

Natural host range

Out of 44 suspected as well as random plant species collected and tested by DAC-ELISA, TSV was detected in 19, comprising economically important crops and several weeds (Table 3). The symptoms were ranging from chlorosis to necrosis of different plant parts. TSV was found in *Arachis hypozaea, Vigna unguiculata, Abelmoschus esculentum., Helianthus annuus, Gossypium herbaceum, Cyamopsis tetragonaloba, Vigna mungo* and *Vigna radiata.* Among ornamental weed species, *Crossandra infundibuliformis, Tagetes patula, Digera arvensis, Croton sparsiflorus, Corchorus trilocularis, Ageratum conyzoides, Commilina bengalensis, Datura stramonium, Parthenium hysterophorus, Achiranthus aspera, Tridax procumbens* reacted positive to TSV under natural conditions.

Arachis hypozaea plants showed leaf and stem necrosis and apical necrosis in TSV infected plants while no TSV infection was found in *Lycopersicon esculentum*. Chlorotic spots, veinal chlorosis, necrosis and leaf curl was observed in *V. unguiculata* due to TSV infections under natural conditions. All samples collected from *T. patula* showing leaf and stem necrosis as well as stunting reacted positive to TSV while *Croton sparsiflorus* showed leaf, stem and apical necrosis with TSV infection.

The following species were not infected by TSV, *Lycopersicon esculentum, Cucumis melo, Carica papaya, Capsicum annuum, Cleome viscosa, Acalypha indica, Amaranthus* spp., *Boerhavia erecta, Datura stramonium, Tephrosia hirta, Vinca minor, Phyllanthus madraspatensis, Euphorbia hirta, Tribulus terrestris, Euphorbia geniculata, Phyllanthus neruri, Calotropis* sp., *Carthamus tinctorius, Eclipta alba, Citrullus lanatus, Argemone mexicana, Lantana cramera, Trichodesma zylanicum and Cassia auriculata.*

Table 2: Natural host range of TSV in crop and weed samples determined by DAC-ELISA.

Crop/weed species	TSV	
	ELISA	Symptoms
Arachis hypogaea	27/57	LN, SN, AN
Lycopersicon esculentum	0/14	Nil
Vigna unguiculata	1/2	VN, C, LC
Cucumis melo	0/5	Nil
Abelmoschus esculentum	4/5	C, S
Helianthus annuus	2/3	N, S
Gossypium herbaceum	2/3	NL, VN
Carica papaya	0/5	Nil
Cyamopsis tetragonoloba	2/5	C
Crossandra infundibularis	2/5	LN
Tagetes patula	34/35	LN, SL, S
Ageratum conyzoides	12/32	Nil
Citrullus lanatus	0/3	Nil
Cleome viscose	0/54	Nil
Digera arvensis	2/45	Nil
Acalypha indica	0/15	Nil
Croton sparsiflorus	2/19	LN, SN, AN
Amaranthus spp.	0/44	Nil

Boerhavia erecta	0/26	Nil
Datura stramonium	0/29	Nil
Parthenium hysterophorus	217/454	Nil
Chorchorus trilocularis	5/49	Nil
Achiranthus aspera	4/27	Nil
Tephrosia hirta	0/20	Nil
Vinca minor	0/17	Nil
Tridax procumbens	11/123	Nil
Acanthospermum hispidum	1/11	Nil
Phyllanthus madraspatensis	0/30	Nil
Euphorbia hirta	0/25	Nil
Tribulus terrestris	0/29	Nil
Euphorbia geniculata	0/38	Nil
Phyllanthus neruri	0/35	Nil
Calotropis sp.	0/22	Nil
Carthamus tinctorius	0/10	Nil
Commilina bengalensis	7/35	Nil
Vigna mungo	2/82	VN, C, LC, AN
Vigna radiata	7/92	VN, C, LC, AN
Solanum melongena	0/39	Nil
Eclipta alba	0/14	Nil
Argemone mexicana	0/12	Nil
Lantana camera	0/15	Nil
Capsicum annuum	0/11	Nil
Trichodesma zylanicum	0/12	Nil
Cassia auriculata	0/9	Nil

C-chlorosis, CL-chlorotic lesions, NL-necrotic lesions, LN- leaf necrosis, SL-smalling of leaves, SN-stem necrosis, AN-apical necrosis, LC-leaf curl, NS-necrotic spots, VC-veinal chlorosis, VN- veinal necrosis, M-mosaic, WL-water-soaked lesions, PL-purple lesions, N-necrosis.

Discussion

Experimental host range

Upon mechanical inoculations, TSV isolates of Blackgram and Greengram infected 25 out of 36 plant species tested. Both TSV-BG and TSV-GG isolates showed similar symptoms on the test plants. In the present study, upon mechanical inoculations with TSV-BG and TSV-GG, *A. hypozaea* cv. JL-24, *C. cajan*, *H. annuus* cv.PAC-36, *P.vulgaris* cv. Top crop, *V. unguiculata* cv. C-152, *G. max* cv. Bragg, *C. quinoa*, *C. amaranticolor* were manifested with local and systemic symptoms which in agreement with the findings of Reddy *et al.* (2002) [6], Prasada Rao *et al.* (2003) [5] and Arun Kumar (2006) [1]. Both local and systemic symptoms observed on *A. esculentum* confirming the result of Prasada Rao *et al.* (2003b) [5], whereas Arunkumar (2006) [1] reported only local symptoms. *V. radiata*. cv. K-851, *V. mungo* developed both local and systemic symptoms in the present study in contrast to the findings of Prasada Rao *et al.* (2003) [5], whereas local and systemic symptoms reported in *V. radiata*. by Reddy *et al.* (2002) [6] and Arun Kumar (2006) [1] and in *V. mungo* by Reddy *et al.* (2002) [6]. *N. glutinosa*, *N. rustica*, *N. tabacum* cv. Samsun, recorded with both local and systemic symptoms confirming the findings of Reddy *et al.* (2002) [6] and Arunkumar (2006) [1], whereas no TSV infection found by Prasada Rao *et al.* (2003) [5]. *S. melongena* L. developed both local and systemic symptoms in agreement with the finding of Arunkumar (2006) [1]. Both local and systemic symptoms were observed in *D. stramonium* in contrast to the finding of Arunkumar (2006) [1] where only local symptoms reported, whereas no TSV infection was found by Prasada Rao *et al.* (2003) [5]. *Vicia faba* L. developed both local and systemic symptoms strengthening the results of Reddy *et al.* (2002) [6] and Arunkumar (2006) [1]. *G. herbaceum* developed only local symptoms, and the ornamental crops, *C. infundibularis* and *T. patuala* Cav. showed only systemic symptoms in agreement with Arunkumar (2006) [1], whereas local and systemic symptoms were reported in *G. hirsutum* by Reddy *et al.* (2002) [6].

TSV isolates of Blackgram and Greengram symptomlessly infected *Amaranthus viridis*, *Commelina bengalensis*, *P. hysterophorus* in the present study which is strengthening the findings of Prasada Rao *et al.* (2003) [5]. *T. procumbense* also recorded with symptomless infection of TSV in the present study which is confirming the finding of Arun Kumar (2006) [1], whereas no TSV infection was found by Prasada Rao *et al.* (2003) [5]. No infection was found in *Cucumis sativus*, *C. tetragonaloba*, *Lagenaria cineraria*, *L. esculentum* which is in accordance with findings of Prasada Rao *et al.* (2003) [5] and Arun Kumar (2006) [1].

Natural host range

Out of 44 suspected as well as random plant species collected and tested by DAC-ELISA, TSV was detected in 19 samples, comprising economically important crops and several weeds. In the present study, TSV was detected in *H. annuus*, *A. hypogaea*, *G. herbaceum*, *A. esculentum*., *V. unguiculata*, *T. patula*, *P. hysterophorus*, *A. aspera*, *Acanthospermum hispidum*, *V. radiata*, *C. infundibuliformis*., *C. bengalensis*, *T. procumbense* and *C. sparsiflorus* L. confirming the results of Arun Kumar (2006) [1], whereas TSV was not detected in *T. procumbens* and *C. sparsiflorus* by Prasada Rao *et al*. (2003) [5]. TSV was found in *V. mungo* which is in agreement with Ladhalakshmi *et al.* (2006) [4]. In addition, TSV found in the weed species *Digera arvensis*, in the present study.

No TSV infection was found in following weed species, *Argemone mexicana*, *Cassia auriculata*, *Cleome viscosa*, *Amaranthus viridis*, *Eclipta alba*, *E. hirta*, *L. cramera*, *T. zylanicum* and *T. terrestris* and crop plants, *C. annuum*, *H. annuus* and *L. esculentum* in agreement with Prasada Rao *et al*. (2003) [5]. However, TSV infection was found in *C. annuum* by Jain *et al.* (2005) [3].

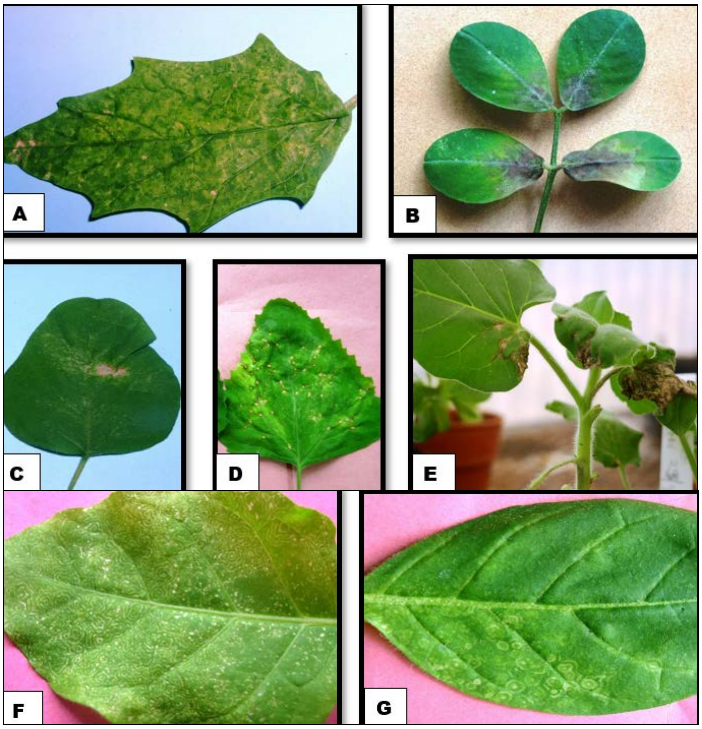

Fig 1: Symptoms produced by TSV BG and GG isolates upon mechanical inoculations under glasshouse conditions.
A. *Datura stramonium*, B. *Arachis hypogea*, C. *Glycine max*, D. *Chenopodium amaranticolor*, E. *Nicotiana glutinosa*, F. *Nicotiana tabacum* cv. Samsun and G. *Nicotiana rustica*

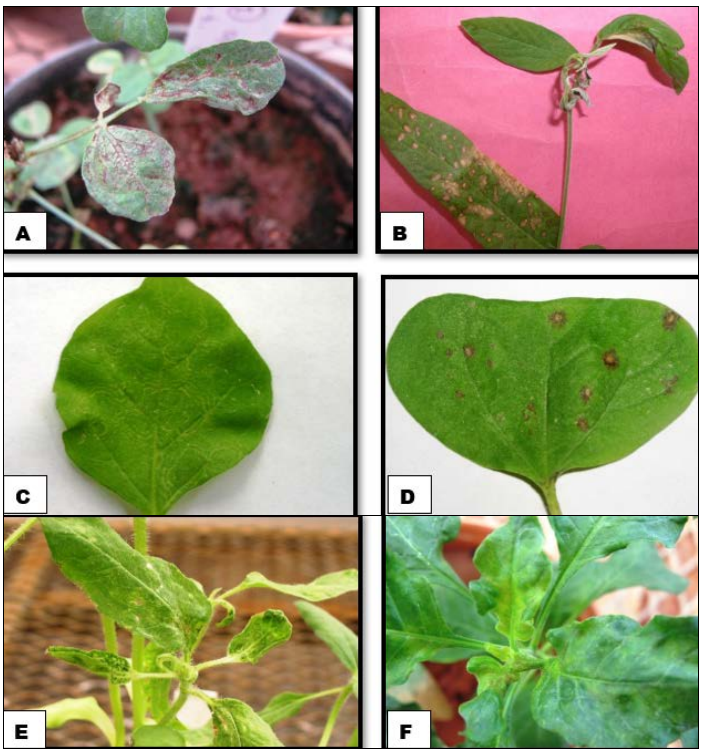

Fig 2: Symptoms produced by TSV BG and GG isolates upon mechanical inoculations under glasshouse conditions.
A. *Vicia faba*, B. *Cajanus cajan*, C. *Solanum melongina*, D. *Gossypium herbaceum*, E. *Helianthus annuus cv.* PAC-36 *and* F. *Crossandra infundibuliformis*

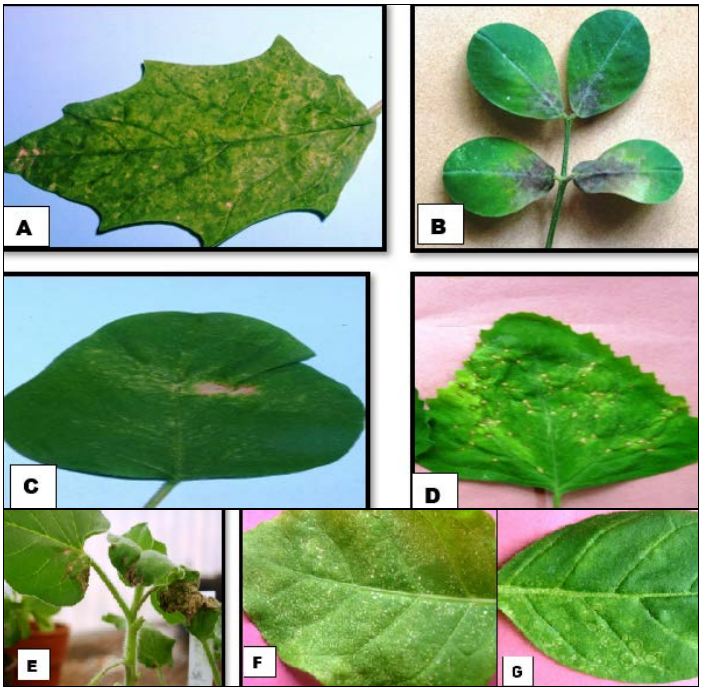

Fig 1: Symptoms produced by TSV BG and GG isolates upon mechanical inoculations under glasshouse conditions.
A. Datura stramonium, B. Arachis hypogea, C. Glycine max, D. Chenopodium amaranticolor, E. Nicotiana glutinosa, F. Nicotiana tabacum cv. Samsun and *G. Nicotiana rustica*

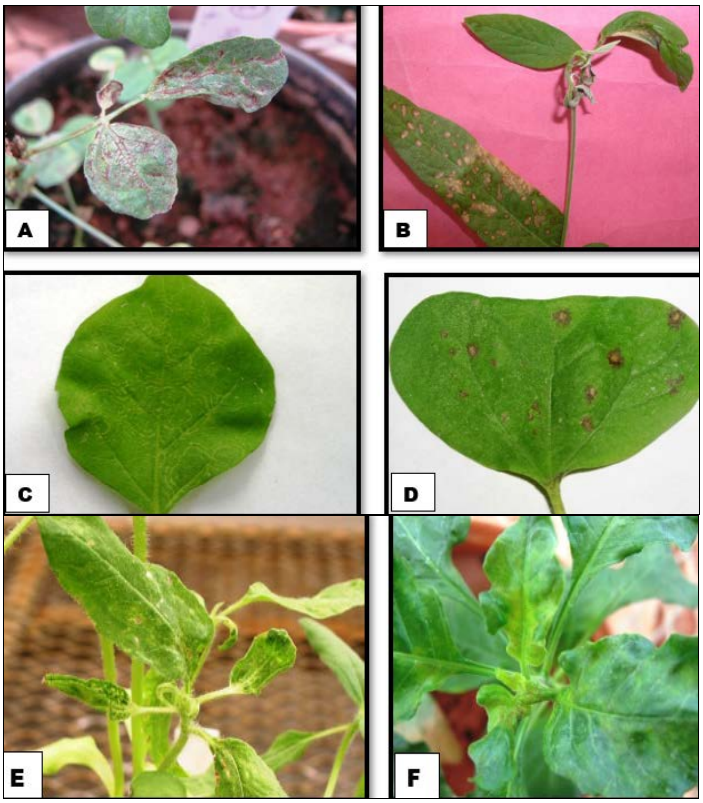

Fig 2: Symptoms produced by TSV BG and GG isolates upon mechanical inoculations under glasshouse conditions.
A. *Vicia faba*, B. *Cajanus cajan*, C. *Solanum melongina*, D. *Gossypium herbaceum*, E. *Helianthus annuus cv.* PAC-36 *and* F. *Crossandra infundibuliformis*

Conclusion

This study demonstrated that Tobacco streak virus (TSV) isolates from Blackgram and Greengram successfully infected 25 of 36 tested plant species, displaying both local and systemic symptoms. Notable hosts included *Arachis hypogaea*, *Cajanus cajan*, and *Vigna unguiculata*, aligning with previous research. Some species exhibited symptomless infections, reinforcing earlier findings. Furthermore, TSV was detected in 19 out of 44 plant species tested via DAC-ELISA, confirming its presence in economically significant crops and various weeds. This highlights the complex host range of TSV and the necessity for ongoing monitoring in agricultural settings.

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