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Serological assay of *Tobacco streak virus* (TSV) in the different plant parts of germplasm and advance generations of *Gossypium barbadense*

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Abstract

Tobacco streak virus (TSV), the causal agent of cotton necrosis, is an emerging disease of national significance in India. Tobacco streak virus (TSV), inciting cotton necrosis, exhibits multifarious symptoms which includes purplish brown, necrotic lesions in the leaves, squares, and petioles. Development of diagnostic tools with rapidity will have immense role to play in detection and management of the emerging virus. A simple and rapid procedure of enzyme immunoassay (ELISA) was used to detect and identify viruses in individual plants. Serological assay by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) revealed the presence of TSV in leaf, square, stem, root, petiole and pollen grains of germplasm and advance generations of Gossypium barbadense.

Keywords: *Tobacco Streak Virus* (TSV); *Gossypium barbadense;* DAS-ELISA.

Introduction

At world level, India leads currently in cotton acreage and production. Indian cotton crop is the most diverse one in the world both in terms of its botanical status and range of fibre quality. It has the potential to cultivate all the four cultivated species of cotton. Three of the species of cotton contributing to cotton trade and industrial consumption *viz*., *hirsutum, arboreum* and *herbaceum* are commercially grown in the country. The fourth, *barbadense* which includes the highest fibre quality with extra long staple length fibers as parent of many hybrids and is cultivated in very small scale. This group ELS cotton (34.9 mm and above) is highly demanded by the industries and mostly imported from other countries. The common end uses of ELS cotton are sewing threads, loom yarns, blend with polyester and high quality fabric. As it has high length, strength and micronaire, it generally used to manufacture high quality ring-spun yarns. However, unlike other three cultivated species, *Gossypium barbadense* is highly susceptible to disease and pests.

Various bacterial, fungal and viral diseases hamper cotton productivity. Among the viral diseases infecting cotton, *Cotton leaf curl virus* and *Tobacco streak virus* (TSV) are important. Cotton necrosis disease caused by *tobacco streak virus* (TSV) is an emerging threat in India (Rageshwari *et al.,* 2016). In India, TSV was initially identified in sunflower (Prasada Rao *et al.,* 2000) and peanut (Reddy *et al.,* 2002) causing necrosis disease. In Tamil Nadu, Nakkeeran (AICRP report 2010) first reported the association of TSV in cotton. The germplasm of *G. barbadense* was surveyed for the presence of TSV during the year 2017-2020. The presence of disease affected plants was observed at 90 DAS (Days after sowing). The per cent disease incidence varies from 1.61% (CCB 140) to 26.60 % (ICB 71). The symptoms were very distinct with necrotic spots, dark purple in colour and also drying of squares. Other symptoms include necrotic streaks on petiole and necrosis on crown region (Valarmathi, 2018). At a time cotton was sown in 50 per cent of the total sown area (18,61 lakh hectares) in Telangana, a serious threat to cotton farmers in the country and especially in the state has emerged in the form of TSV by the year 2017. Telangana being the third largest cotton producing state in country is now reported to be the worst affected by TSV among the cotton producing states.

Several variations of the ELISA are currently in use. In the double antibody sandwich ELISA, usually referred to as direct ELISA, the wells (capacity 0.4 ml) of a polystyrene microtiter plate are first half-filled with and then emptied of, sequentially, (a) antibodies to the virus, (b) virus preparation or sap from an infected plant, (c) antibodies to the virus to which molecules of a particular enzyme have been attached, and (d) a substrate for the enzyme, i.e., a substance that the enzyme can break down and cause change in its color (Lavakumar, 2016).

Tobacco streak virus (TSV) belongs to the genus *Ilarvirus* of the family *Bromoviridae* an emerging pathogen posing threat to the crop species worldwide. Identification of symptoms due to TSV infection by visual observation of plants often results in misdiagnosis as symptoms produced by this virus can match with those reflecting physiological and nutritional disorders affecting cotton. Development of diagnostic tools with rapidity will have immense role to play in detection and management of the emerging virus. The protocol for rapid diagnosis of TSV infected samples by using Double antibody sandwich DAS-ELISA was optimised and this is the first report of its use for diagnosis of TSV on cotton. The DAS-ELISA diagnostic tool can be utilized not only for laboratory research but also for quarantine and field diagnosis of this important emerging disease affecting cotton. Keeping in view of the above advantages of serological assay, detection of TSV in the different plant parts of germplasm and advance generations of *G. barbadense* by DAS-ELISA was performed in the present study.

Serological assay

Double antibody Sandwich ELISA (DAS-ELISA) was performed in different plant parts like root, stem, petiole, leaf, squares and pollen grains 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 1mg/ml and the absorbance was recorded at 405nm 1h after incubation. Totally 300 germplasm and 30 advance generations (45 days old) were used for the study. Suvin was used as positive control.

Serological detection of TSV in the different plant parts of germplasm and advance generations of *G.barbadense* by DAS-ELISA was performed. Different plant parts like root, stem, petiole, leaf, squares and pollen grains were used for the study. It was shown the absorbance values in the symptomatic plant root, leaf and squares were several-fold greater than the healthy control. Among the different plant parts tested for ELISA, leaf recorded maximum absorbance followed by petiole and squares for TSV.

Among the germplasm ICB 1 shows maximum absorbance value at A405nm in leaf (3.235), petiole (2.145) and squares (2.119) followed by stem (1.322), root (1.202) and pollen grains (1.119). Among the germplasm ICB 36 and 37 shows absorbance value in leaf (3.878), petiole (2.189) and squares (2.226) followed by stem (1.665), root (1.205) and pollen grains (1.118). Among the germplasm ICB 38 shows absorbance value in leaf (3.332), petiole (2.189) and squares (2.352) followed by stem (1.678), root (1.206) and pollen grains (1.119) (Table 1).

Among the advance generations CCB 129 shows maximum absorbance value in leaf (3.237), petiole (2.287) and squares (2.220) followed by stem (1.789), root (1.236) and pollen grains (1.119). Among the advance generations CCB 141 shows maximum absorbance value in leaf (2.879), petiole (2.279) and squares (2.114) followed by stem (1.785), root (1.238) and pollen grains (1.115). In the control Suvin the absorbance recorded in leaf (3.865), Petiole (2.285) and squares (2.221) followed by stem (1.880), root (1.238) and pollen grains (1.118) (Table 2).

Serological confirmation of TSV in root, stem, leaf, square and pollen grains of asymptomatic and symptomatic cotton variety CO 14. The presence of TSV was confirmed in root, stem, leaf, flower, square and pollen grains of asymptomatic samples of cotton variety CO 14 through DAS-ELISA. However, among the various plant parts assessed for the presence of TSV, the maximum absorbance value of 2.37 was noticed in squares, followed by leaf (2.21), root (1.36) and pollen grains (1.25) at A405 nm, which was several-fold higher when compared with healthy control (0.23), whereas in stem the absorbance value was 1.33. These results confirm the latent nature of TSV in asymptomatic plants of cotton variety (CO 14). Similarly, confirmation of TSV infection from the symptomatic plants of variety CO 14, in root, stem, leaf, flower, square and pollen grains, through DAC-ELISA showed the absorbance value at 405 nm was comparatively higher than the asymptomatic samples. Besides, the absorbance values in the symptomatic plant root (2.27), leaf (2.91) and squares (3.05) were several-fold greater than the healthy control (0.23). It confirmed the systemic nature of TSV in various parts of symptomatic plants of cotton variety CO 14 (Rageshwari *et al.,* 2017). Serological assay through DAS-ELISA confirmed the presence of TSV in cotton samples expressing necrosis symptoms (Vinodkumar *et al.,* 2017). The blackgram necrosis diseased plant parts showed positive reaction with the TSV antiserum (groundnut isolate) and the virus was confirmed as TSV. The TSV was detected in the leaves, stem and seeds using the (raised) TSV antiserum by DAC-ELISA technique. A positive reaction was observed and among the infected parts, the concentration of the virus was more in the stem portion than the leaves and seeds (Ladhalakshmi *et al.,* 2005). The advantage of DAS ELISA assay is that the virus particles are concentrated

Table 1. Serological detection of TSV in the different plant parts of germplasm of *Gossypium barbadense* by DAS-ELISA {A405nm (1 h)}

S. No.	Germplasm	Root	Stem	Petiole	Leaf	Squares	Pollen grains
1.	ICB 1	1.202 (0.08)	1.322 (0.08)	2.145 (0.08)	3.235 (0.09)	2.119 (0.03)	1.119 (0.03)
2.	ICB 2	1.114 (0.05)	1.586(0.05)	2.114 (0.05)	2.124 (0.05)	2.113 (0.09)	1.112 (0.02)
3.	ICB 3	1.115 (0.06)	1.520 (0.06)	2.125 (0.02)	3.131 (0.04)	2.118 (0.05)	0.998 (0.06)
4.	ICB 4	1.113 (0.04)	1.452 (0.08)	2.142 (0.06)	2.521 (0.03)	2.119 (0.08)	0.875 (0.05)
5.	ICB 6, ICB 11	1.118 (0.06)	1.326 (0.09)	2.365 (0.08)	2.124 (0.07)	2.002 (0.02)	0.897 (0.06)
6.	ICB 13, ICB 16	1.119 (0.09)	1.258 (0.06)	2.114 (0.07)	2.312 (0.09)	2.006 (0.04)	0.568 (0.04)
7.	ICB 18, ICB 23	1.089 (0.08)	1.652 (0.06)	2.045 (0.07)	2.325 (0.09)	2.007 (0.05)	0.789 (0.05)
8.	ICB 24	1.023 (0.07)	1.456 (0.07)	2.140 (0.08)	2.452 (0.08)	2.008 (0.06)	0.226 (0.06)
9.	ICB 26	1.112 (0.05)	1.325 (0.08)	2.012 (0.05)	2.178 (0.06)	2.006 (0.07)	1.025 (0.05)
10.	ICB 27	1.115 (0.08)	1.234 (0.09)	2.031 (0.08)	2.856 (0.05)	2.003 (0.09)	1.042 (0.04)
11.	ICB 28	1.117 (0.06)	1.785 (0.05)	2.023 (0.05)	2.879 (0.07)	2.114 (0.08)	1.115 (0.05)
12.	ICB 30	1.115 (0.07)	1.256 (0.06)	2.014 (0.08)	2.213 (0.03)	2.115 (0.07)	1.032 (0.07)
13.	ICB 34	1.113 (0.04)	1.236 (0.04)	2.112 (0.08)	2.102 (0.04)	2.114 (0.06)	1.045 (0.05)
14.	ICB 36, ICB 37	1.205 (0.05)	1.665 (0.05)	2.189 (0.08)	3.878 (0.09)	2.226 (0.04)	1.118 (0.08)
15.	ICB 38	1.206 (0.08)	1.678 (0.08)	2.195 (0.08)	3.332 (0.09)	2.352 (0.05)	1.119 (0.05)

Table 2. Serological detection of TSV in the different plant parts of advance generations of *Gossypium barbadense* by DAS-ELISA {A405nm (1 h)}

S. No.	Germplasm	Root	Stem	Petiole	Leaf	Squares	Pollen grains
1.	CCB 129	1.236 (0.08)	1.789 (0.08)	2.287 (0.08)	3.237 (0.09)	2.220 (0.03)	1.119 (0.03)
2.	CCB 143	1.114 (0.05)	1.685 (0.05)	2.145 (0.05)	2.156 (0.05)	2.113 (0.09)	1.112 (0.02)
3.	CCB 64	1.115 (0.06)	1.520 (0.06)	2.210 (0.02)	3.193 (0.04)	2.118 (0.05)	0.998 (0.06)
4.	CCB 11	1.113 (0.04)	1.452 (0.08)	2.145 (0.06)	2.552 (0.03)	2.119 (0.08)	0.875 (0.05)
5.	CCB 11a	1.118 (0.06)	1.326 (0.09)	2.365 (0.08)	2.112 (0.07)	2.002 (0.02)	0.897 (0.06)
6.	CCB 26	1.119 (0.09)	1.258 (0.06)	2.114 (0.07)	2.345 (0.09)	2.006 (0.04)	0.568 (0.04)
7.	CCB 28	1.201 (0.08)	1.652 (0.06)	2.213 (0.07)	2.356 (0.09)	2.007 (0.05)	0.789 (0.05)
8.	CCB 29	1.203 (0.07)	1.456 (0.07)	2.140 (0.08)	2.564 (0.08)	2.008 (0.06)	0.226 (0.06)
9.	CCB 51	1.206 (0.05)	1.325 (0.08)	2.012 (0.05)	2.542 (0.06)	2.006 (0.07)	1.025 (0.05)
10.	CCB 51-2	1.205 (0.08)	1.234 (0.09)	2.031 (0.08)	2.411 (0.05)	2.003 (0.09)	1.042 (0.04)
11.	CCB 141	1.238 (0.06)	1.785 (0.05)	2.270 (0.05)	2.879 (0.07)	2.114 (0.08)	1.115 (0.05)
12.	CCB 142	1.115 (0.07)	1.256 (0.06)	2.014 (0.08)	2.213 (0.03)	2.115 (0.07)	1.032 (0.07)
13.	S X P	1.113 (0.04)	1.236 (0.04)	2.112 (0.08)	2.102 (0.04)	2.114 (0.06)	1.045 (0.05)
14.	Suvin (Control)	1.238 (0.05)	1.880 (0.05)	2.285 (0.08)	3.865 (0.09)	2.221 (0.04)	1.118 (0.08)

from extracts by the coating antibody, and inhibitory components of extracts are removed by rinsing before addition of detecting antibody and enzyme substrate. This is the first time detection of TSV in the germplasm and advance generations of ELS cotton *G.barbadense* by DAS- ELISA.

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