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Survey for the Incidence of *Tobacco streak virus* (TSV) in Field and Horticultural Crops

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ABSTRACT

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Survey conducted for disease incidence of *Tobacco streak virus* (TSV) during *Kharif* 2014-15 and 2015-16 in Andhra Pradesh and Karnataka revealed disease incidence of 9-28 per cent in groundnut, 6-18 per cent in sunflower and 5-22 per cent in cucumber. In Andhra Pradesh during *Kharif* 2014-15 and 2015-16, the incidence of TSV in groundnut ranged from 9.8 (Bathalapalli) to 28.2 (Obuldevarayacheruvu) per cent and 9.2 (Kothacheruvu) to 23.2 (Gorantla) per cent respectively; similarly on sunflower the incidence ranged from 6.4 (Aluru) to 18.7 (Bethamcherla) per cent and 5.2 (Adoni) to 14.2 (Bethamcherla) per cent respectively. In Karnataka the incidence of TSV during *Kharif* 2014-15 and 2015-16 ranged from 10.9 (Herapanahalli) to 22.8 (Tumkur) per cent and 5.2 (Pavagada) to 14.2 (Tumkur) per cent respectively. The collected samples were tested by Enzyme Linked Immunosorbent Assay (ELISA) using TSV specific antisera and through electron microscopy and positive samples were maintained on cowpea for further studies.

Introduction

TSV is the type member of *Ilarvirus* genus of the *Bromoviridae* family. In recent years, *Ilarviruses* (TSV) had received national and international attention because they cause major crop losses. TSV had caused severe epidemics in India on several important crops such as groundnut, sunflower, okra, cotton, cucurbits and gherkins. However, in recent years, vegetable crop cultivation (okra,

gherkin, cucumber, pumpkin) has been seriously hampered due to TSV infection. Due to wide range of host plants, transmission through pollen and seed, this virus was placed among the most economically important virus. TSV infection at seedling stage results in premature death of the plant, Infection during mid – stage of the plant growth may result in necrosis of the leaves and severe reduction in yield. Infection at late stage of the plant growth results in mild chlorotic symptoms

with little effect on plant growth and yield. In several weed hosts, such as parthenium, TSV causes asymptomatic infection. Keeping in view the economic importance of virus, a survey was conducted in Andhra Pradesh and Karnataka to assess the disease incidence of TSV in groundnut, sunflower and cucumber.

Materials and Methods

Field surveys

Roving survey was conducted to note the incidence of *Tobacco Streak Virus* (TSV) in different crop plants viz., groundnut, sunflower and Cucumber from different locations of Andhra Pradesh and Karnataka. The Suspected TSV infected samples exhibiting characteristic symptoms of severe necrosis of leaf lamina, petioles and other plant parts were collected and identified by Enzyme Linked Immunosorbent Assay (ELISA) using TSV specific antisera or through electron microscopy by leaf dip method. The TSV identified samples either stored in -80°C for further virus characterization or mechanically inoculated to test hosts for culture maintenance in an insect proof glass house.

Electron microscopy

The suspected plant sample was ground in Sodium phosphate buffer pH 7.5 at 1:5 (w/v) proportion filtered through double layered cheese cloth and observed under transmission electron microscope for determining the shape and size of the virus particles causing mosaic symptoms on diseased sample. To examine virus particles in suspected leaf, a drop of filtered leaf preparation was placed on the carbon-coated grids and allowed to settle for 2-3 minutes. The excess of sample was removed by using blotting paper. A small droplet of dye (uranyl acetate or phosphotungstic acid) was placed on it and

allowed to stay for 2-3 minutes. The excess stain was drained by touching the blotting paper strip to the edge of the grid. The grids were dried for 15-30 min in dessicator and examined under JOEL 100 S transmission electron microscope at various magnifications. The photographs of the virus particles were taken.

$$\frac{\text{Actual size of a Particle in nm}}{\text{Size of the particle in electronmicrograph(mm)}} = \text{Magnification}$$

Bioassay

Suspected TSV infected samples from groundnut, sunflower and cucumber were collected from different locations of Andhra Pradesh and Karnataka, were used for sap inoculation on cowpea (*Vigna unguiculata* cv. C-152), a diagnostic host for TSV. Test plants were raised in insect proof glasshouse conditions. Five to eight seeds were planted in each plastic pot (4" diameter) and cotyledonary stage with uniform growth were selected for inoculation. Infected as well as healthy tissues from field samples macerated separately using sterilized chilled pestle and motor in 0.1M phosphate buffer (pH 7.2, 1:1 w/v) containing 0.1% mercaptoethanol as extraction buffer (EB) (Appendix I). The sap was kept on ice till the inoculation was completed. The test plant seedlings were dusted with celite (diatomaceous earth) or carborandum powder, which served as an abrasive. The inoculum was applied directly on to the upper surface of the leaves. After inoculations, the seedlings were sprayed with distilled water and kept in the insect-proof glass house. Inoculations were preferably carried out in the evening. The inoculated seedlings were observed for symptoms development. Local lesions developed 4-5 days post-inoculation were used for further inoculation by taking single lesion and subsequently maintained pure virus cultures of

groundnut, sunflower and cucumber TSV isolates on cowpea.

Results and Discussion

Intensive roving survey was conducted during *Kharif* 2014-15 and 2015-16 to record the disease incidence of TSV in groundnut, sunflower and cucumber at different places in Andhra Pradesh and Karnataka. The incidence of TSV in Andhra Pradesh ranged from 9-28 per cent in groundnut and 6-18 per cent in sunflower samples. The incidence of TSV in cucumber ranged from 5-22 percent in Tumkur and Davanagere districts of Karnataka. In Andhra Pradesh during *Kharif* 2014-15 the incidence of TSV in groundnut was highest in Obuldevaraya cheruvu (28.2 per cent) and lowest in Bathalapalli (9.8 per cent). Similarly Gorantla recorded highest disease incidence of 23.2 per cent and Kothacheruvu recorded lowest disease incidence of 9.2 per cent during *Kharif* 2015-16 (Table 1 and Fig. 1).

In sunflower, Bethamcherla recorded highest disease incidence of 18.7 and 14.2 per cent during *Kharif* 2014-15 and 2015-16 respectively and lowest disease incidence was recorded in Aluru (6.4 per cent) and Adoni (5.2 per cent) during *Kharif* 2014-15 and 2015-16 respectively (Table 2 and Fig. 2). In Karnataka, cucumber growing areas of Tumkur district, Sira recorded highest disease incidence of 22.8 (*Kharif*, 2014-15) and 14.2 (*Kharif*, 2015-16) per cent, while Herapanahalli (10.9 per cent) and Pavagada (5.2 per cent) recorded lowest disease incidence during *Kharif* 2014-15 and 2015-16 respectively (Table 3 and Fig. 3).

TSV incidence was first observed on groundnut during the year 2000 (Reddy *et al.*, 2002) in Anantapur district of Andhra Pradesh. Since then its incidence on groundnut crop has been recorded regularly (16.5 per

cent-2004; 5-80 per cent-2006 and 2-30 per cent-2007) in Andhra Pradesh (Anonymous 2004b, 2006 and 2007). TSV incidence has also been reported on groundnut in Raichur district of Karnataka (Prasada Rao *et al.*, 2003). Sunflower Necrosis Disease (SND) caused by *Tobacco streak virus* was reported as an epidemic consecutively for the three years (1997-99), with the incidence ranging from 10 to 80 percent and causing yield losses up to 90 per cent in most of the sunflower growing regions of Southern India (DOR Annual Report, 2001). Halakeri (1999) recorded incidence of sunflower necrosis disease ranged from 3 to 70 per cent around Dharwad, Gadag, Bagalkot, Haveri and Bijapur districts. Five to thirty per cent necrosis disease was recorded at Sira, Chitradurga, Bellary, Bijapur and Gulbarga districts, 50 to 70 per cent around Nagalapur and Lingsur of Raichur district (Anonymous, 2002). Krishna Reddy *et al.*, (2003) reported severe outbreak of *Tobacco streak virus* (TSV) in cucumber and gherkin causing yield losses of 31 to 75 per cent in Bangalore, Bellary, Davanagiree, and Tumkur districts of Karnataka state during 2000-2002.

Identification of TSV isolates

Suspected *Tobacco streak virus* (TSV) infected groundnut samples showing necrotic symptoms on leaves, top bud and stem collected from farmer's fields of different mandals in Anantapur district during *Kharif*, 2014-15 and 2015-16 were tested by bio assay and ELISA for the TSV identification. Out of 44 samples collected, 30 samples reacted positively with polyclonal antiserum to TSV by DAC-ELISA. The absorbance values (A_{405} nm) ranged from 0.19-3.16 (Table 1). TSV suspected symptomatic groundnut samples were sap transmitted to the cowpea seedlings (*Vigna unguiculata* cv. 132) and presence of TSV was confirmed by DAC-ELISA (A_{405} nm: 2.45-3.12) (Table 4).

Table.1 Per cent disease incidence of *Tobacco streak virus* (TSV) in groundnut samples and absorbance values (A_{405} nm) of samples collected from different mandals of Andhra Pradesh

District	Place (Mandal)	Per cent disease incidence		Sample code	(A_{405} nm)	Range of absorbance values (A_{405} nm)	Range of disease incidence (%)
		<i>Kharif</i> 2014	<i>Kharif</i> 2015				
Ananthapur (Andhra Pradesh)	Nallamada	14.2	22.2	GNAP1	2.44	0.19-3.16	9-28
				GNAP2	2.89		
				GNAP3	2.42		
				GNAP4	1.22		
	N.P. Kunta	21.1	19.8	GNAP5	3.02		
				GNAP6	1.32		
				GNAP7	3.16		
				GNAP8	0.42		
	Obuldevaraya cheruvu	28.2	11.2	GNAP9	1.76		
				GNAP10	0.39		
				GNAP11	3.02		
				GNAP12	0.22		
	Kothacheruvu	19.2	9.2	GNAP13	1.43		
				GNAP14	2.92		
				GNAP15	0.62		
				GNAP16	3.00		
	Rapthadu	10.2	16.6	GNAP17	1.64		
				GNAP18	0.82		
				GNAP19	0.19		
				GNAP20	0.23		

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District	Place (Mandal)	Per cent disease incidence		Sample code	(A ₄₀₅ nm)	Range of absorbance values (A ₄₀₅ nm)	Range of disease incidence (%)
		<i>Kharif 2014</i>	<i>Kharif 2015</i>				
Ananthapur (Andhra Pradesh)	Kalluru	14.6	14.2	GNAP21	0.71	0.19-3.16	9-28
				GNAP22	2.65		
				GNAP23	1.12		
				GNAP24	2.72		
	Penukonda	22.4	19.8	GNAP25	0.55		
				GNAP26	2.82		
				GNAP27	1.84		
				GNAP28	2.96		
	Gorantla	25.2	23.2	GNAP29	1.24		
				GNAP30	2.62		
				GNAP31	1.92		
				GNAP32	2.02		
	Dharmavaram	11.2	10.2	GNAP33	2.22		
				GNAP34	1.54		
				GNAP35	1.72		
				GNAP36	2.14		
	Bathalapalli	9.8	9.4	GNAP37	0.56		
				GNAP38	1.92		
				GNAP39	0.62		
				GNAP40	0.45		
	Kothacheruvu	13.2	15.2	GNAP41	1.67		
				GNAP42	2.01		
				GNAP43	0.22		
				GNAP44	0.12		

Table.2 Per cent disease incidence of *Tobacco streak virus* (TSV) in sunflower samples and absorbance values (A_{405} nm) of samples collected from different mandals of Andhra Pradesh

District	Place (Mandal)	Per cent disease incidence		Sample code	(A_{405} nm)	Range of Absorbance values (A_{405} nm)	Range of disease incidence (%)
		<i>Kharif</i> 2014	<i>Kharif</i> 2015				
Kurnool (Andhra Pradesh)	Bethamcherla	18.7	14.2	SFAP1	2.62	0.11-2.76	6-18
				SFAP2	1.28		
				SFAP3	0.52		
				SFAP4	1.43		
	Atmakur	11.2	10.2	SFAP5	0.86		
				SFAP6	1.91		
				SFAP7	0.37		
				SFAP8	2.44		
	Aluru	6.4	7.4	SFAP9	0.28		
				SFAP10	1.82		
				SFAP11	2.10		
				SFAP12	0.67		
	Nandikotkur	9.2	8.9	SFAP13	1.55		
				SFAP14	0.32		
				SFAP15	1.62		
				SFAP16	0.28		
	Aspari	17.2	9.2	SFAP17	2.76		
				SFAP18	1.37		
				SFAP19	1.42		
				SFAP20	2.44		

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District	Place (Mandal)	Per cent disease incidence		Sample code	(A ₄₀₅ nm)	Range of Absorbance values (A ₄₀₅ nm)	Range of disease incidence (%)
		<i>Kharif 2014</i>	<i>Kharif 2015</i>				
Kurnool (Andhra Pradesh)	Paparli	12.9	11.6	SFAP21	1.28	0.11-2.76	6-18
				SFAP22	2.62		
				SFAP23	1.62		
				SFAP24	2.46		
	Veldthurthi	4.2	13.2	SFAP25	1.42		
				SFAP26	2.29		
				SFAP27	1.32		
				SFAP28	1.92		
	Adoni	6.7	5.2	SFAP29	0.42		
				SFAP30	2.42		
				SFAP31	0.91		
				SFAP32	2.68		
	Gudur	14.2	8.8	SFAP33	0.62		
				SFAP34	2.52		
				SFAP35	0.45		
				SFAP36	1.34		
	Kodimur	10.3	9.4	SFAP37	2.01		
				SFAP38	0.11		
				SFAP39	1.87		
				SFAP40	2.10		

Table.3 Per cent disease incidence of *Tobacco streak virus* (TSV) in cucumber samples and absorbance values ($A_{405\text{ nm}}$) of samples collected from different mandals of Karnataka

District	Place (Mandal)	Per cent disease incidence		Sample code	($A_{405\text{ nm}}$)	Range of Absorbance values ($A_{405\text{ nm}}$)	Range of disease incidence (%)
		<i>Kharif</i> 2014	<i>Kharif</i> 2015				
Tumkur (Karnataka)	Sira	22.8	14.2	CUKA1	2.62	0.28-3.18	5-22
				CUKA2	1.28		
				CUKA3	0.52		
				CUKA4	1.43		
	Madhugiri	13.2	13.2	CUKA5	0.86		
				CUKA6	1.91		
				CUKA7	0.37		
				CUKA8	2.44		
	Tiptur	14.8	9.8	CUKA9	0.28		
				CUKA10	1.82		
				CUKA11	2.10		
				CUKA12	0.67		
	Gubbi	21.2	11.2	CUKA13	3.18		
				CUKA14	0.32		
				CUKA15	1.62		
				CUKA16	0.28		

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District	Place (Mandal)	Per cent disease incidence		Sample code	($A_{405\text{ nm}}$)	Range of Absorbance values ($A_{405\text{ nm}}$)	Range of disease incidence (%)
		<i>Kharif</i> 2014	<i>Kharif</i> 2015				
Tumkur (Karnataka)	Pavagada	19.8	5.2	CUKA17	1.55	0.28-3.18	5-22
				CUKA18	1.37		
				CUKA19	1.42		
				CUKA20	2.44		
	Davanagere	14.2	7.8	CUKA21	1.28		
				CUKA22	2.62		
				CUKA23	1.62		
				CUKA24	2.46		
	Harapanahalli	10.9	9.2	CUKA25	1.42		
				CUKA26	2.29		
				CUKA27	1.32		
				CUKA28	1.92		
				CUKA29	0.42		
				CUKA30	2.42		

Table.4 Detection of *Tobacco streak virus* (TSV) in sap inoculated cowpea seedlings (*Vigna unguiculata* cv. 132) by DAC-ELISA

Virus isolates	(A ₄₀₅ nm)
GNAP7	2.45 – 3.12
SFAP17	2.01 – 2.82
CUKA13	2.58 – 2.96
PHAP15	2.34 – 2.67
Positive control	3.02
Negative control	0.12

Fig.1 Symptoms exhibited by *Tobacco streak virus* infected groundnut plants

Fig.2 Symptoms exhibited by *Tobacco streak virus* infected sunflower plants

Fig.3 Symptoms exhibited by *Tobacco streak virus* infected cucumber plants

Fig.4 Maintenance of TSV isolates on test host, cowpea

Fig.5 Manifestation of TSV symptoms on cowpea

Localised symptoms were observed on cowpea after 1-2 Days Post Inoculation (DPI). Initial symptoms included pale necrotic spots followed by yellowing and wilting of inoculated leaves (Fig. 4 and 5). Similar symptoms were produced on healthy groundnut plants upon re inoculation using cowpea samples. Groundnut TSV isolate, GNAP7 maintained on cowpea was used for biological and molecular characterization.

Suspected TSV infected sunflower samples showing typical symptoms of necrotic streaks on stem and petioles, bending of terminal portion of stem, petiole elongation and drying of terminal portion of leaves were collected from farmer's fields of different mandals in Kurnool district. The collected samples were subjected to immuno-assay and bio-assay separately. Out of 40 samples collected, 25 samples reacted with polyclonal antiserum of TSV by DAC-ELISA. The absorbance values (A_{405} nm) ranged from 0.11-2.76 (Table 2). ELISA positive sunflower TSV samples were used for sap inoculation on cowpea seedlings (*Vigna unguiculata* cv. 132), a diagnostic host for TSV and inoculated on first true leaf stage seedlings (5 days old) and presence of virus was confirmed by DAC-ELISA (A_{405} nm: 2.01-2.82) (Table 4). Pale necrotic symptoms were observed after 3 Days Post Inoculation (DPI). Later necrosis spread to petiole and lead to defoliation of cowpea leaves (Fig. 4 and 5). Similar symptoms were produced on healthy sunflower plants upon re inoculation using cowpea samples. Sunflower TSV isolate, SFAP17 maintained on cowpea was used further for biological and molecular characterization.

Cucumber plants showing symptoms of tip necrosis characterized by necrotic lesions on leaves and general leaf and stem necrosis extending to mid veins, petioles, flower buds and tip, were collected from farmer's fields of different mandals in Tumkur district of

Karnataka state. The samples were subjected to Immuno- and bio-assays separately. Out of 30 samples collected 24 samples reacted with polyclonal antiserum of TSV by DAC-ELISA. The absorbance values (A_{405} nm) ranged from 0.28-3.18 (Table 3). TSV from symptomatic cucumber samples were sap transmitted to the cowpea seedlings (*Vigna unguiculata* cv.132) and presence of virus was confirmed by DAC-ELISA (A_{405} nm: 2.58-2.96) (Table 4). Initially necrotic spots developed on leaves which later lead to drying and defoliation of leaves (Fig. 4 and 5). Similar symptoms were produced on healthy cucumber plants upon re inoculation using cowpea samples. Cucumber TSV isolate, CUKA13 maintained on cowpea was used for biological and molecular characterization.

In the present study TSV produced various symptoms on cowpea seedlings which included necrotic lesions, systemic veinal necrosis, necrotic streaks on stem, finally leading to complete drying of leaves. These results are supported by Ramaiah *et al.*, (2001), Ladhakshmi *et al.*, (2006) and Arun Kumar *et al.*, (2008) (circular necrotic lesions on cowpea are the characteristic symptoms of TSV by mechanical inoculation).

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