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Non-systemic Infection of *Tobacco streak virus* on Cotton in Warangal District, Andhra Pradesh

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During 2005, cotton was sown on 3.67 lakh acres in Warangal district of Andhra Pradesh, 62 per cent of it being Bt seeds. Hybrid RCH - 2 Bt alone was sown on 1.20 lakh acres. During late August 2005, several farmers complained about a disease causing chlorotic/necrotic lesions on leaves accompanied occasionally with leaf purpling, necrotic buds and drying up of young bolls in their cotton fields. It was also noticed that the incidence of the disease was more on Bt cultivars, compared to non Bt cultivars. A survey was conducted in some of the cotton fields to ascertain the extent of incidence and also to collect disease samples for the identification of causal agent. Samples collected during the survey were tested by Enzyme linked immuno-sorbent assay (ELISA) and studies carried out subsequently with cotton *Tobacco streak virus* (TSV) isolate gave interesting results, which we report hereunder.

Collection of samples. The entire plant/ twig of cotton leaves exhibiting chlorotic/ necrotic lesions were collected in polythene bags, along with plants showing, purple coloration, necrotic growing points/drying of bolls with or with out any chlorotic/ necrotic lesions on leaves separately. The samples were kept in refrigerator until ELISA test/ virus assays were conducted.

Virus culture and maintenance. Extracts of cotton leaves showing chlorotic/ nectoric lesions prepared in 0.05M phosphate buffer pH 7.0 containing 0.075 per cent thioglycerol were inoculated on to primary leaves of cowpea cv C-152. The virus from a single lesion, following three successive transfers was maintained on cowpea cv. C-152 and french bean cv. Top Crop which served as inoculum source for all mechanical inoculations. Mechanical sap inoculations were carried out on groundnut cv. JL-24, Sunflower cv. PAC-36 and cotton cv. Narasimha.

Graft transmission. Stem grafting was done on healthy cotton cv. Narasimha plants with the scion obtained from infected cotton plants (cv. RCH-2) by tightly securing with parafilm and covering the plants with polythene bag to maintain humidity until graft establishment. The grafted plants were kept at 25° C to 28 ° C in a glasshouse for observations.

Thrip transmission. The pollen in which virus presence was confirmed by ELISA, collected from *Parthenium* plants that were mechanically inoculated with TSV cotton isolate earlier, was dusted onto the leaves of seedlings of cowpea cv. C-152, groundnut cv. JL-24, sunflower PAC-36 and cotton cv. Narasimha. At least 10 adult thrips of *Frankliniella schultzei* were released onto each seedling and covered with polystyrene cage. After one day exposure, thrips were killed by spraying 'dimethoate'. Exposed plants were maintained in a glass house at 25-28° C. Both exposed and subsequently produced leaves were assayed for virus presence by ELISA.

In another experiment, 30-day old plants of cotton cv. RCH-2 were dusted with TSV infected *Parthenium* pollen and caged with at least 100 thrips per plant. After 48 h of exposure, thrips were killed by spraying dimethoate and the plants were thoroughly washed by sprinkling water to wash away the pollen grains. The numbers of chlorotic lesions on the leaves were counted 10 days after exposure. Both exposed and subsequently produced leaves were assayed for virus presence by ELISA.

Screening. Cotton hybrids/ varieties used in this study were obtained from Regional Agricultural Research Station. Acharya NG Ranga Agricultural University, Guntur and Central Institute for Cotton Research, Nagpur. Seeds were grown in 8" plastic pots (5 seeds/pot) filled with sterilized potting mixture in a glasshouse. At 4-leaf stage the plants were inoculated with TSV by mechanical sap inoculation. TSV cotton isolate maintained on cowpea cv. C-152 was used as source of inoculum. The plants were monitored for symptoms up to 6 weeks after inoculation and both the inoculated and subsequently produced leaves were tested

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by ELISA.

The symptoms on cotton cv. RCH-2 Bt included chlorotic lesions on leaf covering either partially or the entire leaf surface. On some plants such chlorotic lesions were accompanied by purple coloration of leaf and in some plants necrosis of buds. Some infected leaves showed both chlorotic and necrotic lesions, perhaps these were the early infected leaves, in which chlorotic lesions turned necrotic. Necrosis of stem or leaf or veins, usually associated with TSV infection on groundnut (Reddy *et al.*, 2002), sunflower (Prasada Rao *et al.*, 2000) and urd bean (Ladalakshmi *et al.*, 2006) were not found in cotton infected with TSV.

The disease incidence varied form 20-80% in cotton cv. RCH-2 Bt and 0-15% in Bunny. In the initial ELISA tests, 7 out of 37 samples collected from the field, were positive to TSV and none to *Peanut bud necrosis virus* (PBNV) (Table 1). The ELISA conducted on infected plants showing different symptoms indicated that the leaf area showing chlorotic/ necrotic lesions either alone or in combination with purple coloration or necrotic buds were positive to TSV whereas plants showing purple color alone or necrotic buds alone were negative to TSV (Table 2). Thus retesting of infected plants by obtaining leaf tissue from the chlorotic / necrotic lesions, resulted in 24 samples out of 37 being positive to TSV. Non-symptomatic leaves on a plant with leaves showing chlorotic lesions were negative to TSV in ELISA and the same was the case with the non-symptomatic area of a leaf showing chlorotic lesions on part of the leaf. This gives an indication of localized infection and lack of systemic movement of the virus in cotton from the area of its infection.

Table 1. ELISA result of cotton samples collected from fields of Warangal

Name of the village	No. of samples tested	No. of TSV positive samples	No. of PBNV positive samples
Kamareddypalli	6	0	0
Keshavapuram	5	2	0
Krushidham	4	0	0
Nagaram	6	1	0
Gunuparthy	6	2	0
Vangapahad	6	1	0
Muchherla	4	1	0
Total	37	7	0

In cotton fields in Warangal, the disease caused a lot of anxiety among the farmers until 1st week of September 2005.

Table 2. ELISA results of cotton samples tested symptom wise collected from fields of Warangal

Symptom type	No. of samples tested	No. of TSV positive samples
Chlorotic lesion area	13	13
Chlorotic/ necrotic lesion area	6	6
Purple colored leaf	4	0
Purple colored leaf with chlorotic lesions	5	5
Plant showing necrosis of buds	3	0
Non-symptomatic leaf from a plant showing leaves with chlorotic lesions	5	0
Non-symptomatic area of a leaf showing partial chlorotic lesions	2	0

However, with the heavy down pour received during the 2nd week of September 2005, the fresh growth put forth by the infected cotton plants was free from the infection and the crop did not suffer much from the TSV infection.

During 2005, Warangal district received heavy rains in July and again in September with a prolonged dry spell during August. The dry spell during August encouraged production of pollen on TSV infected parthenium plants and also building up and movement of thrips to cotton, thus facilitating TSV infection causing large number of infection sites i.e., chlorotic lesions giving an impression of systemic infection. With the heavy rains during September, both the infected pollen and thrips were washed away giving little scope for the fresh infection, as rains continued till September end.

TSV cotton isolate was easily sap transmitted to groundnut, sunflower and cowpea. In case of groundnut, sunflower and cowpea, the virus was detected not only on the inoculated leaves, but also in subsequently produced fresh growth indicating systematic spread, whereas in cotton chlorotic lesions were produced on inoculated leaves, and fresh growth was free from the virus.

To mimic (simulate) the field infection, cotton plants were sprinkled with TSV infected parthenium pollen and caged with large number of thrips for 2 days. After 10 days, the number of chlorotic lesions on 5 exposed cotton plants ranged from 83 to 137, which did not increase significantly 30 days after exposure and the fresh growth produced on such plants completely remained free from chlorotic lesions, and also negative to TSV in ELISA tests.

The scion obtained from cv. RCH-2 Bt infected with TSV,

grafted on to root stock of cotton cv. Narasimha, did not show symptoms on the fresh growth produced on the stock until 6 weeks and was negative to TSV in ELISA. Graft transmission of TSV in cotton producing typical mosaic on leaves after 4-5 weeks of grafting has been reported in literature (Ahmed *et al.,* 2003).

All the 31 cotton varieties / hybrids mechanically inoculated with TSV in the present study produced chlorotic/ necrotic local lesions on inoculated leaves and none produced systematic symptoms (Table 3). No virus was detected in freshly produced leaves in ELISA tests, indicating non systemic infection. Cotton varieties screened under natural infection conditions against TSV, CIM-70, S-12, B-622, B-630, B-496, BH-4, BH-89, BH-94, BH-95 and Krishma were reported to be resistant in Pakistan (Ahmed *et al.,* 2003). The TSV isolate infecting cotton in Warangal resembled cotton mosaic disease caused by TSV in Pakistan (Ahmed *et al.,* 2003) in symptomatology and positive to TSV in ELISA tests, but differed from it, being sap transmitted, thrips transmitted and non transmission through graft. Although TSV cotton isolate from Warangal was sap and thrips transmissible, the infection was confined to the inoculation sites without systemic movement from the site of inoculation to other parts. Because of the non-systemic movement of the virus in cotton, the virus has not moved from the infected scion to the stock and hence no graft transmission occurred.

Based on sap transmission, thrips transmission, non graft transmission and localized infection on the inoculated leaves without becoming systemic in 31 cotton varieties/ hybrids mechanically inoculated with the virus, it is concluded that the TSV infection in cotton in Warangal district during 2005 was localized infection. The severity of such localized infection depends on the abundance of TSV infected pollen, which in turn depends on the parthenium population, thrips multiplication and movement to cotton crop, which usually occurs during prolonged dry spells in rainy seasons. Although none of the cotton cultivars tested in the present study was systemically infected by TSV, the continuous exposure of commonly grown cotton cultivars to TSV in endemic areas may lead to systemic infection in near future.

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