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Thrip transmission patterns of peanut bud necrosis virus (PBNV) and Tobacco streak virus (TSV) isolates of blackgram and greengram of Andhra Pradesh

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Abstract
In the present study, thrip transmission patterns of *Peanut bud necrosis virus* (PBNV) and *Tobacco streak virus* (TSV) isolates of Blackgram and greengram were studied since both the viruses cause similar necrotic symptoms and are transmitted by thrips, the method of transmission and the virus vector relationship vary and hence need different approaches of management practices. Transmission tests with laboratory reared cultures of *T. palmi*, *F. schultzei* and *S. dorsalis* indicated that only *T. palmi* could transmit PBNV-BG and PBNV-GG to an extent 66.6% and 70% in blackgram cv. LBG-20 50% and 66.6%in greengram cv. K-851 and 60% and 87.5% in cowpea cv. C-152, respectively. *F. schultzei* and *S. dorsalis* did not transmit the virus to the test plants. Pollen from both DAC-ELISA tested TSV positive parthenium plants, collected from field (natural infection) and glasshouse (mechanically inoculated with TSV-BG and TSV-GG) produced symptoms typical of TSV on assay host, cowpea cv.C-152 indicating the presence of the virus in the pollen. The results of the thrip assisted pollen transmission experiments with TSV infective pollen showed that TSV-BG, TSV-GG were transmitted by all the four thrip species tested. All 'thrips only' which were fed on TSV infected leaves and 'pollen only' control seedlings remained healthy. Varied percent transmission was observed in blackgram cv. LBG-20, greengram cv. K-851, cowpea cv.C-152, groundnut cv. JL-24, and sunflower cv.PAC-36 with TSV-BG and TSV-GG. All the four thrip species have showed transmissions to all test plants. All test plants that expressed symptoms were positive in DAC-ELISA tests confirming the transmission of TSV.

Keywords: Vector transmission, Thrips, *Tobacco streak virus* (TSV), *Peanut bud necrosis virus* (PBNV) isolates, blackgram, greengram

Introduction
Blackgram and greengram have been subjected to the attack of several biotic stresses such as fungi, bacteria and viruses, affecting the productivity and among them, viral diseases became great menace and are the great yield reducers. Necrosis caused by viruses is posing a serious threat to *Vigna* species in Andhra Pradesh. Nearly two decades back *i.e.,* during *Kharif* of 1990-91, low incidence of leaf curl/necrosis was recorded on these crops of Andhra Pradesh. Over the years, the incidence of the disease gradually increased to about 70-100 per cent. In the recent years, viruses causing necrosis, transmitted by thrips has assumed epidemic proportion and became a serious production constraint in blackgram and greengram especially in upland areas during all the seasons *i.e*, *kharif, rabi* and summer in Andhra Pradesh.
Of several viral diseases attacking greengram and blackgram, leafcurl disease caused by *Peanut bud necrosis virus* (PBNV) (= *Groundnut bud necrosis virus* – GBNV) (Amin *et al*., 1985) [1] transmitted by *Thrips palmi* (Karny) in a propagative manner (Sreekanth *et al.*, 2002) [32] was considered to be a major threat, causing 40% yield loss (Nene, 1972) [16]. Recently, *Tobacco streak virus* (TSV) has also been reported to be a cause of leaf curl symptoms on blackgram (Prasada Rao *et al.*, 2003a, b; Ladhalakshmi *et al.*, 2005) [19, 11] and greengram (Bhat *et al.*, 2002; Prasada Rao *et al.*, 2003a, b) [6, 19, 23] paving confusion in field diagnosis to assess the disease incidence. Although both the viruses cause necrotic symptoms and are transmitted by thrips, the method of transmission and the virus vector relationship vary and hence need different approaches of management practices. It is necessary to understand virus vector relationships and

differentiate necrosis-causing viruses and their incidence on blackgram and greengram to follow the appropriate management practices.

Materials and Methods

Collection and identification of thrips

The colonies of thrips species *viz.*, *Thrips palmi* (Karny), *Frankliniella schultzei* (Trybom) and *Scirtothrips dorsalis* (Hood) were initiated with the thrips collected from apparently healthy blackgram and greengram plants in the fields at NBPGR-RS, Rajendranagar, Hyderabad. Large number of terminal leaves and flowers were collected during early morning hours. They were placed in a glass beaker and covered with a funnel attached with a small homeopathic glass vial (Fig. 1). The adult thrips crawled along the walls of the funnel and gathered in the glass vial. The glass vial was changed at 2 h interval (Sakimura *et al*., 1961) [29].

Thrips were immobilized by placing the vials in a refrigerator at 4 °C for 15 min and were dislodged onto an ice tray (Lewis, 1973) [12]. After immobilization by the cold treatment, thrips were sorted to different species (within 15 min) using a stereo-binocular microscope, as per the key characters (Table 1) given by Amin and Palmer (1985) [1], Palmer *et al*. (1989) [17], Reddy *et al*. (1991) [28] and Vijayalakshmi (1994) [33]. The suspected vectors *viz., S. dorsalis* (Amin *et al*., 1978; 1981; Ghanekar *et al.*, 1979) [4, 5, 8], *F. schultzei* (Amin *et al*., 1981) [5] and *Thrips palmi* (Palmer *et al*., 1990; Vijayalakshmi, 1994; Lipa, 1999 and Sreekanth, 2002) [18, 33, 13, 32] were reared on detached cowpea/groundnut leaflets using the method (Fig. 2) developed by Amin *et al*. (1981) [5]. Small homeopathic glass vials were washed with water and sterilized at 160 °C for 1 h in hot air oven. After sex differentiation (Lewis, 1973) [12], five females and two males were released gently into each glass vial, held in an inverted position. The thrips moved upward and gathered in the upper portion of the inverted vial. A young cowpea cv. C-152/ groundnut cv. JL-24 leaf let was then introduced and the vial was closed with cork. The vials were kept in an incubator adjusted to 12 h light period at 25 °C and 12 h dark period at 22 °C. After allowing a 24h oviposition access, thrips dislodged were collected into a separate vial. A fresh leaflet was introduced into the vial for further feeding and egg laying by thrips. This process was continued for about 10 days during which 90 per cent of total fecundity was completed. The leaflets with eggs were transferred to a new vial for the incubation of eggs. Thrips from these cultures were frequently released onto susceptible blackgram and greengram plants and the plants were assayed onto cowpea.

PBNV - Thrip transmission

PBNV infected portions of young leaflets of blackgram and greengram were separated and kept floating on water in separate Petri dishes and fifteen freshly emerged first instar larvae (Fig. 3), from the lab cultures of *T. palmi, F. schultzei* and *S. dorsalis*, were released separately onto these diseased leaflet portions with the help of a fine camel hairbrush and allowed for two days to acquire the virus. Then ten larvae from the infected source were transferred to individual glass vials each containing healthy blackgram/greengram leaflets. The vials were kept in an incubator (22-26 °C) for adult emergence. After adult emergence, ten thrips of each species were released per plant. A vial containing ten adult thrips of same species, which were given PBNV acquisition at larval stage, was fixed in such a way that the open end of the vial was touching the stem of the seedling. Since the thrips have the tendency of moving upwards, they move to the stem and then leaves. After releasing the thrips, they were restrained on the test plants by placing a cage over the plants (Fig. 4). The thrips were given inoculation access period (IAP) on the test plants for two days, then the insect cages were removed and sprayed with dimethoate @ 1.5ml/l to kill the insects and repeated at weekly intervals to keep the plants free from insect pests, particularly thrips. The seedlings were kept in greenhouse for a maximum period of 20 days to observe for symptoms and to record the frequency of transmission.

TSV – Thrip transmission

All the thrip species reared in the laboratory were used for the transmission of TSV *viz., F. schultzei, S. dorsalis* and *T. palmi*.

Collection of infective pollen from mechanically inoculated plants

Young parthenium plants collected from the field and tested negative to TSV by DAC-ELISA were transplanted in glasshouse. The transplanted healthy plants were inoculated by TSV-BG and TSV-GG isolates and subjected to DAC-ELISA seven days post inoculation. Pollen was collected from tested TSV positive plants and inoculated on cowpea cv. C-152 for the confirmation of presence of TSV in pollen and used as source of infective pollen for the transmission studies.

Pollen associated thrip transmission

Each ten plants (2 plants/pot) of susceptible checks (Blackgram cv. LBG-20 and greengram cv.K-851), groundnut cv. JL-24, sunflower cv. PAC-36 and cowpea cv. C-152 grown in glasshouse were used to perform thrip transmission. Infective pollen from parthenium plants was collected in a Petri plate by gently tapping the flowers. The infective pollen collected was dusted on the leaves of test plants by using a camel brush. Ten thrips of each species were released per plant. A vial containing ten adult thrips of same species was fixed in such a way that the open end of the vial was touching the stem of the seedling. Since the thrips have the tendency of moving upwards, they move to the stem and then leaves. After releasing the thrips, they were restrained on the test plants by placing a cage over the plants (Fig. 8). The thrips were allowed for feeding on the test plants for 24 hours, then the insect cages were removed and sprayed with dimethoate @ 1.5ml/l to kill the insects and repeated at weekly intervals to keep the plants free from insect pests, particularly thrips. Transmission experiments with TSV infective pollen were also carried out with mixed populations of thrip species. Control seedlings were exposed to pollen only or to thrips only, fed on infected leaves. The plants were observed for symptom development and indexed for TSV four to five days post-inoculation by both DAC-ELISA and transmission to cowpea cv.C-152.

Results

PBNV - Thrip transmission

Transmission tests with laboratory reared cultures of *T. palmi, F. schultzei* and *S. dorsalis* indicated that only *T. palmi* could transmit PBNV-BG and PBNV-GG to an extent 66.6% and 70% in blackgram cv. LBG-20 (Fig. 5), 50% and 66.6% in greengram cv. K-851 (Fig. 6) and 60% and 87.5% in cowpea cv. C-152 (Fig. 7), respectively. *F. schultzei* and *S. dorsalis* did not transmit the virus to the test plants (Table 2).

TSV - Thrip transmission

Pollen from both DAC-ELISA tested TSV positive parthenium plants, collected from field (natural infection) and glasshouse

(mechanically inoculated with TSV-BG and TSV-GG) produced symptoms typical of TSV on assay host, cowpea cv.C-152 indicating the presence of the virus in the pollen. The results of the thrip assisted pollen transmission experiments with TSV infective pollen (Table 3) showed that TSV-BG, TSV-GG were transmitted by all the four thrip species tested. All 'thrips only' which were fed on TSV infected leaves and 'pollen only' control seedlings remained healthy. Varied percent transmission was observed in blackgram cv. LBG-20 (Fig. 8), greengram cv. K-851 (Fig. 9), cowpea cv.C-152 (Fig. 10), groundnut cv. JL-24 (Fig. 11), and sunflower cv.PAC-36 (Fig. 12) with TSV-BG and TSV-GG. All the four thrip species have showed transmissions to all test plants. All test plants that expressed symptoms were positive in DAC-ELISA tests confirming the transmission of TSV.

Discussion

PBNV - Thrip transmission

Different authors reported different thrips to be vectors of PBNV. Amin *et al.* (1978, 1981) [4-5] and Ghanekar *et al.* (1979) [8] reported *S. dorsalis* as the vector of the disease on groundnut, while Prasada Rao *et al.* (1980) [21] reported in tomato. Amin *et al*. (1981) [5] indicated that *F. schultzei* was responsible for the transmission. *F. schultzei* was reported as vector of TSWV (considered to be PBNV) in pea (Prasada Rao *et al.*, 1985) [22]. Involvement of *T. palmi* as a vector of peanut bud necrosis disease (PBND) was also suspected by Palmer *et al*. (1990) [18] and later confirmed by Vijayalakshmi (1994) [33]. Findings of the present investigation provided unequivocal evidence that *T. palmi*, as the vector of PBNV on blackgram and greengram. It was also shown that *F. schultzei* and *S. dorsalis* failed to transmit the virus. This finding was in accordance with the reports PBNV transmission by *T. palmi* in groundnut (Vijayalakshmi, 1994; Lipa, 1999) [33, 13], in greengram Sreekanth (2002a) [32] and in blackgram (Rajkumar, 2004) [25]. Obviously, some degree of specificity existed with regard to transmission of viruses belonging to tospovirus group. Experimental evidence to this extent stems from the observations that TSWV was transmitted by *F. schultzei* (Sakimura, 1961; Amin, 1985a; Mulder *et al*., 1991) [29, 1, 15] and *Peanut yellow spot virus* (PYSY) by *S. dorsalis* but not by *T. palmi* (Cho *et al*., 1991; Mau *et al*., 1991) [7, 14]. Under natural conditions, the stationary tendency of larval thrips, non-feeding pre-pupal and pupal stages were not likely to play a role in the spread of PBNV. Thus, the adult thrips only play major role in the spread of the virus (Sreekanth, 2002a) [32].

TSV – Thrip transmission

In the present study, all the four thrips species *viz*., *Frankliniella schultzei, Scirtothrips dorsalis, Megalurothrips usitatus* and *Thrips palmi* transmitted TSV-BG and TSV-GG in presence of infected pollen. In laboratory tests, transmission was achieved with pollen from parthenium and sunflower deposited on leaves of blackgram cv. LBG-20, greengram cv. K-851, groundnut cv. JL-24 and cowpea cv.C-152 and sunflower cv.PAC-36 and colonized by any of the four vector thrip species. The results of the present study confirm the findings of the previous reports from India and elsewhere in the world, on the crucial role played by pollen in thrip transmission. Costa and Lima (1976) reported the transmission of TSV by thrips (*Frankliniella* sp.) from *A. polystachya* to tobacco and soybean in Brazil while, Kaiser *et al*. (1982) [10] reported the transmission of TSV by a mixture of thrips (*T. tabaci* and *F. occidentals*) from naturally infected *M. alba* to *C. quinoa* and *M. alba* in Washington, USA. Rana *et al*. (1987) [26] reported that *C. quiona* plants inoculated with the pollen collected from TSV infected *Clematis vitalba* (TSV-Cle) became infected and Sdoodee and Teakle (1988) [30] also reported that transmission of TSV by thrips might be through their wounding of plants during feeding and the presence of infected plant pollen in the vicinity rather than a specific virus-vector interaction. In India, Prasada Rao *et al.* (2003b) [23] reported that more than one thrips species can act as a vector of TSV. Adults of all the four thrip species *(Frankliniella schultzei, Scirtothrips dorsalis, Megalurothrips usitatus* and *Thrips palmi)* were shown to transmit TSV in groundnut, sunflower and cowpea in the presence of pollen from TSV infected parthenium, sunflower or marigold. The thrips fed on infected leaves alone did not transmit the virus. As infective pollen is required for TSV transmission under field conditions it occur in two ways *viz.*, pollen carrying thrips from flowers of TSV infected weeds or crop plants when colonize groundnut plants transmit the virus, and windblown pollen from TSV infected weeds or crop plants settle on groundnut plants and when these plants are colonized by thrips transmit the virus. Thrips, during their feeding, cause injury to both leaf tissue and deposited pollen and thus facilitate virus infection of the plant. The mechanism of TSV transmission by thrips is mechanical rather than a specific virus-vector interaction as observed in peanut bud necrosis tospovirus (Prasada Rao *et al.*, 2003b, 2003c, 2005; Shukla *et al.*, 2005) [23, 24, 20, 31]. In the present study, thrips free of pollen did not transmit the virus confirming the findings by Sdoode and Teakle (1988) [30], Greber *et al*. (1991) [9], Reddy *et al.* (2002) [27] and Prasada Rao *et al*. (2003b, 2003c) [23, 24].

Table 1: Identification of characters of different thrip species given by Amin and Palmer (1985) [1], Palmer *et al*. (1989) [17], Reddy *et al*. (1991a) [28] and Vijayalakshmi (1994) [33].

Characteristics	*F. schultzei*	*T. palmi*	*S. dorsalis*
Adult female colour and length	Adult female pale in colour, 1 mm long	Straw yellow to pale brown 0.9 mm long	Relatively small, yellow in colour, 0.7 mm long
Antennae	Eight segmented	Seven segmented	Eight segmented
Pronotum	Pronotum with 2 pairs of setae on the anterolateral margin and 2 pair on the posterolateral margin	Pronotum having 2 pairs of setae on the posterolateral margin no setae on the anterolateral margin	No setae on the pronotum. Dark patches on the dorsal side of abdominal tergites
Wings	Fore wings with two complete rows of wing vein setae	Fore wings with broken rows of wing vein setae	Fore wings with few small setae on the veins, hind wings with 2 setae

Table 2: Transmission studies of PBNV-BG and PBNV-GG with thrip species reared under laboratory conditions.

Thrip species [a]	Test plant	PBNV-BG		PBNV-GG	
		Plants infected/ tested [b]	Transmission %	Plants infected/tested	Transmission %
*Thrips palm*i	Blackgram cv.LBG-20	6/9	66.66	7/10	70.00
	Greengram cv.K-851	5/10	50.00	6/9	66.66
	Cowpea cv.C-152	6/10	60.00	7/8	87.50
Frankliniella schultzei	Blackgram cv.LBG-20	0/10	0.00	0/10	0.00
	Greengram cv.K-851	0/9	0.00	0/10	0.00
	Cowpea cv.C-152	0/9	0.00	0/9	0.00
Scirtothrips dorsalis	Blackgram cv.LBG-20	0/10	0.00	0/7	0.00
	Greengram cv.K-851	0/7	0.00	0/9	0.00
	Cowpea cv.C-152	0/9	0.00	0/10	0.00

a- 1st/2nd instar larvae were given 2 days acquisition access period (AAP) and allowed for adult emergence in the vials incerted with healthy groundnut/cowpea leaf.
b- Ten adults were released per seedling allowing for 2 days Inoculation access period (IAP).

Table 3: Pollen transmission studies of TSV-BG and TSV-GG with different thrip species reared under laboratory conditions.

Thrip species [a]	Test plant [b]	TSV-BG		TSV-GG	
		Infected/ tested	Transmission %	Infected/ tested	Transmission %
*Thrips palm*i	Blackgram cv.LBG-20	9/9	100.00	9/10	90.00
	Greengram cv.K 851	8/9	88.88	9/9	100.00
	Cowpea cv.C-152	9/10	90.00	8/9	88.88
Frankliniella schultzei	Blackgram cv.LBG-20	9/10	90.00	7/8	87.50
	Greengram cv.K 851	9/9	100.00	8/8	100.00
	Cowpea cv.C-152	9/9	100.00	8/9	88.88
Scirtothrips dorsalis	Blackgram cv.LBG-20	7/7	100.00	7/8	87.50
	Greengram cv.K 851	8/9	88.88	9/9	100.00
	Cowpea cv.C-152	9/9	100.00	9/9	100.00
Megalurothrips usitatus [c]	Blackgram cv.LBG-20	9/10	90.00	7/7	100.00
	Greengram cv.K 851	8/10	80.00	8/8	100.00
	Cowpea cv.C-152	9/9	100.00	9/10	90.00
Species mixed	Blackgram cv.LBG-20	8/9	88.88	8/9	88.88
	Greengram cv.K 851	9/10	90.00	9/9	100.00
	Groundnut cv.JL-24	7/9	77.77	6/8	75.00
	Sunflower cv.PAC-36	6/9	66.66	7/9	77.77
	Cowpea cv.C-152	9/9	100.00	9/10	90.00
Thrips only	Blackgram cv.LBG-20	0/9	0.00	0/10	0.00
	Greengram cv.K 851	0/8	0.00	0/9	0.00
	Cowpea cv.C-152	0/10	0.00	0/7	0.00
Pollen only	Blackgram cv.LBG-20	0/7	0.00	0/10	0.00
	Greengram cv.K 851	0/10	0.00	0/8	0.00
	Cowpea cv.C-152	0/9	0.00	0/9	0.00

a- Ten adults were released per seedling.
b- Test plants were dusted with TSV infected pollen of parthenium and tridax.
c- *Megalurothrips usitatus* adults were collected from field.

Fig 1: Method followed for thrip collection from terminal leaves of blackgram and greengram using beaker, funnel and a collection vial.

Fig 2: Rearing the thrips on detached leaf lets of healthy groundnut in glass vials

Fig 3: Larval stages of *Thrips palmi* given acquisition access to virus on PBNV infected leaves floating on water

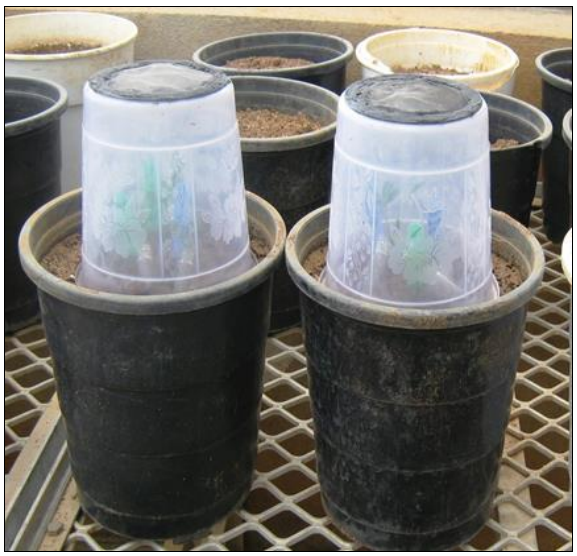

Fig 4: Method of caging the thrips on test plants during the inoculation feeding

Fig 5: Symptoms of T. palmi transmitted PBNV on blackgram cv. LBG-20

Fig 8: Pollen associated thrip transmission of TSV in *Vigna mungo* cv. LBG-20

Fig 9: Pollen associated thrip transmission of TSV in *Vigna radiata* cv. K-851

Fig 10: Pollen associated thrip transmission of TSV in *Vigna unguiculata* cv. C-152

Fig 11: Pollen associated thrip transmission of TSV in *Arachis hypogaea* cv. JL-24

Fig 12: Pollen associated thrip transmission of TSV in *Helianthus annuus* cv. PAC-36

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