



Incidence of *Groundnut bud necrosis virus* (Bunyaviridae: *Tospovirus*) and associated vector (*Frankliniella schultzei* Trybom) in major tomato growing regions of Tamil Nadu and Karnataka

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ABSTRACT: A field survey was conducted during 2016-17 to know the extent of incidence of Groundnut bud necrosis virus (GBNV) and prevalence of thrips species in tomato at different locations of Tamil Nadu and Karnataka states. The vector population and GBNV disease were observed from 20 randomly selected plants at the time of flowering. The plant (shoot) was gently tapped against black cloth which was wrapped with thermo coal sheet and a 10x hand lens was used for counting. The mean population of thrips ranged from 0.35 to 1.39 no. / shoot and mean GBNV incidence was ranged from 20 to 48% in different regions. Thrips sample collected from 30 locations showed that only *Frankliniella schultzei* Trybom was associated in all the places invariably. The identified thrips species was confirmed by taxonomical keys, DNA analysis and SEM analysis. This kind of association may help in deep understanding of vector-virus-host interactions.

Keywords: *Frankliniella schultzei*, *Ground nut bud necrosis virus*, tomato, vector-virus-host interactions

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important vegetable grown all over the world and in India (NHB, 2017-18). India is the second largest tomato producing country after China and it is being cultivated in 7.89 lakh ha with annual production of 19,759 thousand metric tons and productivity of 20t/ha (Indiastat, 2017- 18). Though, the area under tomato cultivation is high, the productivity is very low. One of the major limiting factor in production is *Ground nut bud necrosis virus* (family *Bunyaviridae*, genus *Tospovirus*) cause more than 80 per cent yield loss (Das Gupta *et al.*, 2003) and it is a serious problem in tomato cultivation (Kunkalikar *et al.*, 2011; Singh and Krishnareddy, 1995).

Tospovirus on tomato was first reported from Nilgiris in India during 1964 and later from Andhra Pradesh (Prasadarao *et al.*, 1980), Karnataka (Sastri, 1982), Maharashtra (Joi and Summanvar, 1986) and Tamil Nadu (Doraiswamy *et al.*, 1984). Earlier it was noted as *Tomato spotted wilt virus* (TSWV) by Prasadarao *et al.* (1980) then reconsidered as PBNV (Prasadarao *et al.*, 2003; Venkataramana *et al.*, 2004) and as GBNV by

Umamaheswaran *et al.* (2003). The severity of disease incidence depends on age at vector intervention where early infection would cause 100 per cent yield loss (Ruth *et al.*, 2013). GBNV infected tomato plant showed necrosis on growing bud, bronzing of leaves with brown necrotic lesion, wilting, drying of plants and concentric rings with red and yellow markings on the ripened fruit (Todd *et al.*, 1975, Manjunatha, 2008).

GBNV is primarily transmitted by thrips in a persistent and circulative manner (Jones, 2005). Chilli thrips, *Scirtothrips dorsalis* (Hood) in Groundnut (Amin *et al.*, 1981) and melon thrips *Thrips palmi* (Karny) in tomato (German *et al.*, 1992; Meena *et al.*, 2005) and *F.schultzei* in tamato (Rabeena *et al.*, 2019) were reported as vectors of GBNV transmission. *Frankliniella schultzei* Trybom was also documented as vector of GBNV in groundnut (Amin *et al.*, 1981; Vijayalakshmi *et al.*, 1995; Ananthkrishnan and Annadurai, 2007). Since thrips are very cryptic and polymorphic in nature (Sakimura, 1969; Sakurai, 2004; Millne *et al.*, 2007), traditional methods had created misleading confusion on the species identification (Ullman, 1996; Tyagi *et al.*, 2017). Therefore, identifying thrips species associated with disease hotspot region is

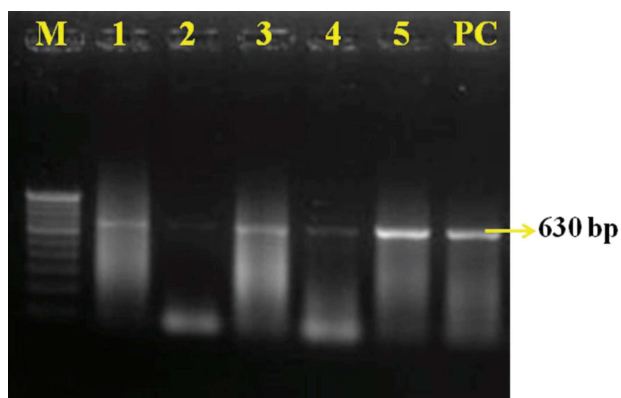


Fig. 1. PCR amplification of mtCOI gene from *F. schultzei* sample collected from GBNV infected tomato plants; M–100 bp DNA ladder ; PC- Positive control; Lane 1- Coimbatore; 2- Dharmapuri; 3- Krishnagiri; 4- Madurai; 5- Dindigul

very important step in understanding vector-virus-host interactions in order to develop an effective management strategy. With this background, a survey was conducted in GBNV hotspot regions of Tamil Nadu and Karnataka to identify the predominant thrips species associated.

MATERIALS AND METHODS

A field survey was conducted to know the per cent disease incidence of GBNV disease on tomato and prevalence of thrips populations in different locations *viz.*, Coimbatore, Krishnagiri, Dharmapuri and Madurai in Tamil Nadu and Mandya, Mysuru, Bengaluru, Channapatna and Kolar districts of Karnataka states (Table 1). In each location, the per cent of disease incidence was assessed by recording the number of plants showing necrosis, chlorosis, wilting and purplish leaf symptoms (Todd *et al.*, 1975) (Fig 4.a & b) the per cent infestation has been worked out.

The thrips population was observed from 20 randomly selected plants during flowering stage. The plant was gently tapped against black cloth which was wrapped with thermocoal sheet and a 10x hand lens was used for counting. The thrips were collected by using aspirator and then transferred to 1.5 ml eppendorf tubes either containing 95 per cent ethanol or in AGA solution (Alcohol 60%: Glycerol: Acetic Acid-6:1:1) with the help of camel hair brush (000 size) and stored in refrigerated condition for molecular confirmation (Asokan *et al.*, 2007) and taxonomical identification (Hoddle *et al.*, 2008., Moritz *et al.*, 2013). The various life stages were taken through a Leica Trinocular Microscope (Leica M205C) and using a Leica software application suite (LAS X). The selected fields were not

received any pesticide sprays ten days prior to sampling and GBNV infected tomato plant parts were collected for PCR analysis (Mandal *et al.*, 2008).

RESULTS AND DISCUSSION

The results showed that the GBNV incidence was ranged 20-48.75%, maximum observed in Coimbatore (48.75%) and minimum (20%) was in Dindigul, Mandya, Mysuru and Ramanagara (Table 1). The thrips population ranged from 0.35 to 1.39 No./ shoot where the incidence was maximum (1.39) in Coimbatore and minimum (0.35) in Dindigul (Table. 1). In all the surveyed locations, the associated thrips species was identified as *F. schultzei*, by observing taxonomical characteristics of eight segmented antennae; head with three pairs of ocellar setae; veins of forewing presenting continuous and equally spaced setae; five and two pairs of pronotal and metanotal setae and absence of comb at eighth abdominal segment (Fig. 1) and it is confirmed with earlier morphological description for *F.schultzei* (Hoddle *et al.*, 2008; Moritz *et al.*, 2013).

The PCR amplification with the primers LCO-1490 F and HCO-2198 R (Hebert *et al.*, 2003) showed a single band of approximately 638 bp (Fig. 2) which was 90-99% similarity between the sequences of the mtCOI gene of *F. schultzei* when analysed using BLAST. The identified nucleotide sequences of *F. schultzei* were deposited in Gen Bank (Accession No MK333277; MK333278; MK333279; MK333280; MK113913; MK113914; MK113915). Tomato plant leaves showing the typical symptoms of GBNV (Fig. 3 & b) was sap inoculated to cowpea (variety Co. 5) plant. After four days, the inoculated plants were exhibited concentric circular chlorotic rings (Fig. 4a) which later turned into necrotic spots. Total RNA was extracted from symptomatic leaves and subjected to RT-PCR with *Tospovirus* universal primers, gl3617/F and gl4435c/R (Chu *et al.*, 2001) resulted in a single product with a length of 730 bp. There was no amplification from healthy leaves, which confirmed the presence of GBNV in the inoculated cowpea leaves (Fig. 5b).

It was observed, a wide distribution of *F. schultzei* species in all the GBNV infected tomato fields. It represents an association between *F. schultzei* and GBNV incidence in the tomato hosts. *F. schultzei* is one of the serious polyphagous pests among the genus *Frankliniella*. It causes economic damage to various ornamental and vegetable crops (Kakkar *et al.*, 2012; Seal *et al.*, 2014). It was also reported as potential vector of GBNV in India (Amin *et al.*, 1981). About 15 species from six genera (Ceratothripoides, Dictyothrips,

Table 1. Incidence of *F. schultzei* and GBNV in tomato hotspot regions of Tamil Nadu and Karnataka

District	GIS-Co-ordinate	Village	Variety/ Hybrid	Crop Stage	Species	No of thrips/ 5 tapping/ plant		PDI*	
						Total	Mean	Total	Mean
Tamil Nadu									
Coimbatore	10.9899° N, 76.8409° E	Thondamuthur	Meenakshi	Flowering	<i>F. schultzei</i>	1.05	30		
	11.2915° N, 77.4214° E	Valayapalayam	Sagar	Flowering	<i>F. schultzei</i>	1.15	45	48.75	
Krishnagiri	11.0152° N, 76.9326° E	TNAU	PKM1	Flowering	<i>F. schultzei</i>	1.05	45	1.39	
	10.9989° N, 76.7813° E	Viraliyur	Sivam	Flowering	<i>F. schultzei</i>	2.30	75		
	12.3696° N, 78.2191° E	Paiyur	Paiyur	Flowering	<i>F. schultzei</i>	1.10	40		
	12.5186° N, 78.2137° E	Kodaiyur	Sivam	Flowering	<i>F. schultzei</i>	0.85	35	43.75	
	12.5166° N, 78.0304° E	Rayakotai	Sivam	Flowering	<i>F. schultzei</i>	1.55	50	1.10	
	12.6634° N, 78.0124° E	Soolagiri	Sivam	Flowering	<i>F. schultzei</i>	0.80	50		
Dharmapuri	12.2088° N, 78.0552° E	Papparapatti	Sivam	Flowering	<i>F. schultzei</i>	1.00	55		
		Gundalapatti	Sivam	Flowering	<i>F. schultzei</i>	0.05	-	0.73	45
		Gittampatti	Sagar	Flowering	<i>F. schultzei</i>	0.80	60		
Madurai		Marandahalli	Sivam	Flowering	<i>F. schultzei</i>	1.10	65		
	10.0333° N, 78.3359° E	Kodikulam	Sivam	Flowering	<i>F. schultzei</i>	0.20	30		
		Melur	Sivam	Flowering	<i>F. schultzei</i>	0.40	20	0.45	25
		Sendangulam	Sivam	Flowering	<i>F. schultzei</i>	0.70	30		
	9.9699° N, 78.2040° E	AC&RI, Orchard	Sivam	Flowering	<i>F. schultzei</i>	0.50	20		

Dindigul	10.5912° N, 77.6864° E	Kallimanthaiyam	Sivam	Flowering	<i>F. schultzei</i>	0.35	25
	10.5149° N, 77.8767° E	Sullerumbu	Sivam	Flowering	<i>F. schultzei</i>	0.25	10 20
	10.4858° N, 77.7464° E	Ottanchathiram	Sagar	Flowering	<i>F. schultzei</i>	0.15	20
	10.5240° N, 77.7373° E	Thangachiyamma patti	Sagar	Flowering	<i>F. schultzei</i>	0.25	15
Karnataka							
Bengaluru	13.039° N, 77.474° E	Thirumalpura	Meenakshi	Flowering	<i>F. schultzei</i>	0.65	25 25
Mandya	12.428° N, 77.020° E	Thalagavaadi	Paiyur	Flowering	<i>F. schultzei</i>	0.55	15 20
	12.506° N, 77.093° E	Bannahalli	Sivam	Flowering	<i>F. schultzei</i>	0.70	25
	12.996° N, 78.214° E	Nertekere	Sivam	Flowering	<i>F. schultzei</i>	0.45	20
Mysuru	13.056° N, 77.402° E	Yelegowdanahalli	Sivam	Flowering	<i>F. schultzei</i>	0.55	20 20
	12.306° N, 76.830° E	Rangasamudra	Sivam	Flowering	<i>F. schultzei</i>	0.60	20
Kolar	13.127° N, 77.99° E	Naraspura	Sivam	Flowering	<i>F. schultzei</i>	0.85	25 25
	13.152° N, 77.976° E	Dinnehosahalli	Sivam	Flowering	<i>F. schultzei</i>	0.50	25
Ramanagara	12.610° N, 77.6864° E	Chakkere	Sivam	Flowering	<i>F. schultzei</i>	0.70	20 20

* Percent Disease Index

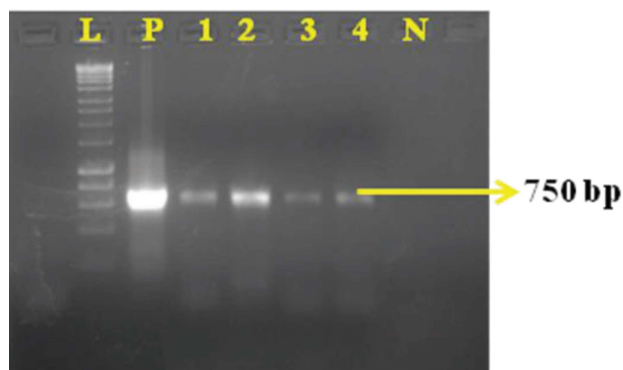
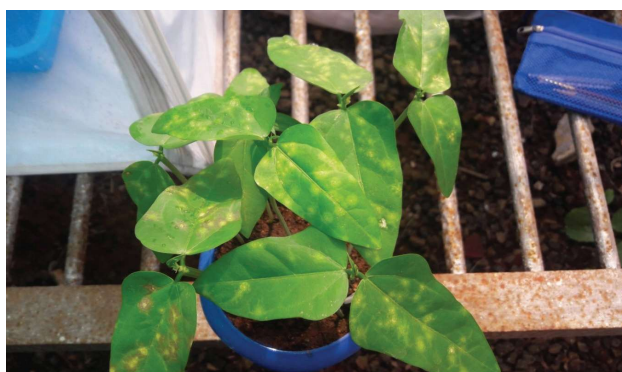


Fig. 2. a. GBNV inoculated cowpea (cv. Co 5) leaves with circular chlorotic rings at 5 days after inoculation; **b.** PCR amplification with the primer pair gl3617/F, gl4435c/R from GBNV infected cowpea leaves (Lane 1-5); M –1kb DNA ladder ; PC- Positive control;

Frankliniella, Neohydatothrips, Scirtothrips and Thrips) were reported as vector of tospovirus and six thrips species such as *Ceratothrips clataris*, *Thrips palmi*, *Thrips tabaci*, *Thrips flavus*, *Frankliniella schultzei* and *Scirtothrips dorsalis* were recorded as vector of tospovirus in India (Pappu *et al.*, 2009) and currently *Frankliniella schultzei* is predominantly distributed all over India (Tyagi *et al.*, 2017).

Chilli thrips, *Scirtothrips dorsalis* (Hood) in groundnut (Amin *et al.*, 1981; Vijayalakshmi, 2017) and *T. palmi* in tomato (German *et al.*, 1992; Meena *et al.*, 2005) were reported as efficient vectors of GBNV transmission. *F. schultzei* had also been documented as vector of GBNV in groundnut (Amin *et al.*, 1981; Vijayalakshmi *et al.*, 1995; Ananthakrishnan and Annadurai, 2007) and the species is present all the parts of India (Tyagi *et al.*, 2017). Within *F. schultzei* two forms (pale and dark) were reported (Sakimura, 1969), the pale form distributed in north of equator and dark form found in south of the Equator where as both are present in Indian subcontinent (Mound 1968). The two forms are differing in host preference, interbreeding and distribution which could be different species (Gikonyo *et al.*, 2016). However, we have observed only pale form in all the surveyed locations. Since thrips are very cryptic and polymorphic in nature (Sakimura, 1964; Sakurai, 2004; Millne *et al.*, 2007), traditional methods had created misleading confusion on the species identification (Ullman, 1996; Tyagi *et al.*, 2017).

Initially *S. dorsalis* and *F. schultzei* were considered as vectors of GBNV in groundnut (Amin *et al.*, 1981) but Lakshmi *et al.*, (1995), reported *T. palmi* as efficient vector of GBNV in groundnut. Thereafter *T. palmi* was presumed as vector of GBNV in tomato (Manjunatha, 2008; Daimi *et al.*, 2017) where clear description on the thrips species identification was not available. At present,

modern techniques like PCR assay and SEM analysis are a valuable addition to traditional phenotypic methods (Hebert *et al.*, 2003; Asokan *et al.*, 2007; Mound *et al.*, 2010). Taxonomical keys (Palmer *et al.*, 1989; Bhatti, 1990, Moritz *et al.*, 2013), molecular based techniques (Hebert *et al.*, 2003; Asokan *et al.*, 2007; Glover *et al.*, 2010) and integrated approach (Kumar *et al.*, 2011; Tyagi *et al.*, 2015; Tyagi *et al.*, 2017) could be used for successful species identification to confirm to study the vector-virus-host interactions.

This association may be due to virus manipulation on the vector fitness and behaviour in order to enhance the virus spread and vector survival (Ingwell *et al.*, 2012; Rajabaskar *et al.*, 2014; Eigenbrode *et al.*, 2018; Mauck *et al.*, 2019). The reason for this kind of vector virus interaction has evolutionary significance it should be explored further.

ACKNOWLEDGEMENTS

We thank the funding agency DBT- GOI (No. BT/PR11996/BPA/118/44/2014) to carry out the research, Lawrence. A. Mound (CSIRO, Australia) and S. Subramanian, ICIPE, Kenya for thrips species identification and K. Gayathiri for survey.

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MS Received 16 October 2019
MS Accepted 9 November 2019