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THRIPS VECTORS OF TOSPOVIRUSES ON TOMATO IN SOUTH INDIA

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ABSTRACT

The present study was undertaken to determine distribution of thrips species from major tomato growing regions in South India (Tamil Nadu, Karnataka, Maharashtra, Telangana, Andhra Pradesh). The thrips species were identified using molecular techniques employing universal barcoding primer (mt-COI). These revealed the presence of six species viz., *Thrips palmi* Karny, *Scirtothrips dorsalis* Hood, *Thrips tabaci* Lindeman, *Frankliniella schultzei* (Trybom), *Thrips apicatus* Priesner and *Tusothrips sumatrensis* (Karny). Of these, *T. palmi* was found to be predominant in all the states surveyed followed by *S. dorsalis*, *F. schultzei* and *T. tabaci*.

Key words: *Thrips palmi*, *Thrips tabaci*, *Thrips apicatus*, *Scirtothrips dorsalis*, *Frankliniella schultzei*, *Tusothrips sumatrensis*, species identity, mtCOI, phylogenetic analysis, tomato

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop in India, and in 2017 its production was 19.76 mt and a rise of 2% in production is expected in coming years (APEDA, 2017). Tomato cultivation is affected by biotic factors like insect pests, bacteria, fungus and viral diseases. Among the insect pests, thrips (Thysanoptera: Thripidae) cause significant damage and globally about 15 species are known, as pests or vectors (German et al., 1992). Thrips alone can transmit tospoviruses to 15 monocotyledonous and 69 dicotyledonous plants (Pappu et al., 2009). Tospoviruses are the major constraints in tomato production too, and of these *Groundnut Bud Necrosis Virus* (GBNV) has been found to be major constraint causing up to 100% damage (Kunkalikar et al., 2011). Other tospoviruses such as *Capsicum Chlorosis Virus* (CaCV), *Groundnut Bud Necrosis Virus* (GBNV) and *Watermelon Bud Necrosis Virus* (WBNV) have also been reported on tomato from the Indian subcontinent. These viruses can infect crops alone or as mixed infections (Kunkalikar et al., 2011). Of the 15 species that transmit tospovirus, only six species, *Frankliniella occidentalis* (Pergande), *F. schultzei* Moulton, *Ceratothripoides claratris* Shumsher, *Scirtothrips dorsalis* Hood, *Thrips palmi* Karny, and *T. tabaci* (Lindeman) are reported from India (Ullman 1997; Jones 2005; Pappu et al., 2009; Ciuffo et al., 2010; Hassani-Mehraban et al., 2010; Riley et al., 2011; Zhou and Tzanetakis 2013). Information on prevalence and species composition of thrips transmitting virus on tomato is still inadequate. Hence, the present study to determine the thrips species complex in tomato using DNA barcodes.

MATERIALS AND METHODS

Thrips were collected from Andhra Pradesh, Karnataka, Telangana, Maharashtra and Tamil Nadu during November 2018 to August 2019 in 70% ethanol and stored at 4^{O}C for further studies. Permanent slide mounts were prepared with digestion process using methodology of Mound and Kibby (1998). Photographs were taken in a compound microscope (Leica DM-1000) using Leica software application suite (LAS EZ 2.1.0). For molecular identification, DNA was isolated from single thrips employing CTAB method with little modification (Asokan et al., 2011). PCR was performed in 25 µl total reaction volume containing 5µl of isolated genomic DNA (50-100 ng/ µl) followed by 1.0 µl of forward primer and 1.0 µl of reverse primer of 20 p moles of each primer, 2.5 µl of 10 mMTris Buffer (pH-8.3), 0.5µl of 2.5 mM $MgCl_2$, 1.0 µl of dNTP mixture (0.25 mM of each dNTP) and 0.5 µl of (0.5 U concentration) Taq DNA polymerase (GeNei^{TM,} Genei laboratories Pvt ltd). PCR amplification performed in a thermal cycler (TECHNC,TC-512) with the following cycles: 94^{O}C for 2 min as initial denaturation followed by 35 cycles of 94^{O}C for 45 sec, 47^{O}C for 45 sec, 72^{O}C for 45 sec and final extension for 72^{O}C for 10 min. Universal Mitochondrial cytochrome oxidase I (CO-I) primers used as follows (LCO-1490- 5' - GGTCAACAAATC ATAAAGATATTGG -3'; HCO-2198- 5' - TAAACTTCAGGGTGACCAAAAAATCA

-3') (Hebert et al., 2003a and Hebert et al., 2003b) and resolved in 1.5% agarose gel, stained with 0.5µg/ ml ethidium bromide and documented in a gel documentation system (UVP). Amplified PCR Products were purified using gel extraction kit (Nucleospin® Extract II, Macherey Nagel, Germany) and cloned in to PTZ57R/T vector (Thermo Fisher Scientific, UK). Competent *Escherichia coli* (DH5α) cells were used to clone the PCR products and further blue-white selection carried out. Plasmids were isolated using GenJET™ plasmid MiniPrep kit (Fermentas Life Sciences, UK), as per manufacturers protocol. Three independent clones selected for each sample were sequenced using M13 forward and reverse primers (Ms. Medauxin, Bengaluru, India). NCBI-BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov/) tool was used for analysing the sequence homology. Sequence alignment was done in BioEdit (version 7.0.9.0) (Hall et al., 1999) and phylogenetic tree was constructed in MEGA.7.0 software (Kumar et al., 1993).

RESULTS AND DISCUSSION

The genomic DNA of all six thrips were isolated. All the samples were amplified with amplicon size of ~ 700bp. Not all six species of thrips were present in a single location/ region on tomato. Five species (*T. palmi, S.dorsalis, F.schultzei, T.apicatus* and *T. sumatrensis*) were observed in Karnataka; only *T. palmi* was present in all the sampled locations of Telangana (Table 1) *Thrips palmi* mtCOI sequence analyzed via BLAST showed an identification of 99.85% with USA *T. Palmi* sequences (KX233569). *Scirtothrips dorsalis* was present in all the states except Telangana, and revealed 99.85% identity with KM355512 Indian isolate. *Thrips tabaci* was present only in Tamil Nadu and Maharashtra (Tamil Nadu-2 and Maharashtra-1), and KF036290 USA isolate was found to have 100% identity. *Frankliniella schultzei* occurs in Karnataka and Tamil Nadu (Karnatak-2; Tamil Nadu-4) and has 100% identity with Pakistan sequence (HQ990721); and 99.85% identity with Indian isolates (MK333280). *Thrips apicatus* and *Tusothrips sumatrensis* were observed only in Karnataka, and corresponding identity was of 100% (KX622321) and 97.37% (KX622448).

Phylogenetic tree was constructed by maximum likelihood (ML) tree method using mt-COI sequence obtained from all *Thrips* spp (Fig. 1). All thrips sequences are precisely grouped in three clades corresponding to three genera of Thysanoptera (*Thrips* spp., *Scirtothrips* spp. and *Frankliniella* spp.). The present results agree with earlier ones of Boykin et al. (2007). In all locations *T. palmi* stood out as single subgroup; in that five samples (Devarayapuram, Hosur, Oddanchatram, Shoolagiri and Mecheri of Tamil Nadu) along with one from Maharashtra (Narayanagaon) formed a separate clade; and *T. tabaci* and *T. apicatus* were also found associated in the same group; while *F. schultzei* went in separate group- in that two subgroups were formed (samples from Manaparai and Surandai locations of Tamil Nadu falls in one), while those from Bandigere, Kavalande, Mussenalu and ICAR-IIHR fell in another subgroup. Samples of *S. dorsalis* irrespective of locaztions formed a single group. *Tusothrips sumtrensis* (from Sivakotai) was in separate group as it was only found from Karnataka.

Earlier reports suggested that thrips like *S. dorsalis* (German et al., 1992; Meena et al., 2005), *T. palmi* (Lakshmi et al., 1995; Meena et al., 2005; Reddy et al., 1992) and *F. schultzei* (Meena et al., 2005) were observed as putative vectors for GBNV, whereas *T. tabaci* could transmit *Tomato spotted wilt virus* (TSWV) (Wijkamp et al., 1995). In NCBI few entries were available related to *Tusothrips sumatrensis and T. apicatus*. Tyagi and Kumar (2014) reported *T. apicatus* from Himachal Pradesh, India. In the present study majority were GBNV vectors like *T.palmi* followed by *S. dorsalis and F. schultzei*. Thus, all the five states surveyed stand a great risk of GBNV infection; whereas Tamil Nadu and Maharashtra need to be cautious about TSWV infection due to prevalence of *T. tabaci*.

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Table 1. Thrips spp. collected from major tomato regions in South India

S. No.	Location	Latitude and longitude	Thrips spp.	NCBI Acc. No
1.	Hosur, Krishnagiri, Tamil Nadu, India	12.4621.3°N 77.5200.5°E	*Thrips palmi*	MN972622
2.	Shoolagiri, Krishnagiri, Tamil Nadu, India	12.4003.4°N 78.0037.1°E	*Thrips palmi*	MN972623
3.	Devarayapuram, Coimbatore, Tamil Nadu, India	10.5948.8°N 76.4907.1°E	*Thrips palmi*	MN972624
4.	Kinathukadavu, Coimbatore, Tamil Nadu, India	10.4852.6°N 77.0133.0°E	*Thrips tabaci*	MN972625
5.	Mecheri, Salem, Tamil Nadu, India	11.4806.3°N 77.5836.6°E	*Thrips palmi*	MN972626
6.	Manapari, Trichy, Tamil Nadu, India	10.3707.9°N 78.2515.1°E	*Frankliniella schultzei*	MN972627
7.	Sembatti, Dindigul, Tamil Nadu, India	10.1818.6°N 77.5151.6°E	Nil	
8.	Oddanchatram, Dindigul, Tamil Nadu, India	10.3041.8°N 77.4442.8°E	*Thrips palmi*	MN972628
9.	Pavoorchatram, Thirunelveli, Tamil Nadu, India	8.5523.7°N 77.2256.5°E	*Scirtothrips dorsalis*	MN972629
10.	Kilapavoor, Thirunelveli, Tamil Nadu, India	8.5418.5°N 77.2356.3°E	*Thrips tabaci*	MN972630
11.	Surandai, Thirunelveli, Tamil Nadu, India	8.5850.9°N 77.2443.7°E	*Frankliniella schultzei*	MN972631
12.	Nyamathi, Shimogga ,Karnataka, India	14.0405.1°N 75.3617.3°E	*Thrips palmi*	MN972632
13.	Mussenalu, Shimogga, Karnataka, India	14.0353.2°N 75.3626.7°E	*Frankliniella schultzei*	MN972633
14.	Kadgamdoddi, Raichur, Karnataka, India	16.1354.5°N 77.2557.4°E	*Thrips palmi*	MN972634
15.	UAS-Raichur, Karnataka, India	16.1146.1°N 77.1850.7°E	*Scirtothrips dorsalis*	MN972635
16.	Mangasandra, Kolar, Karnataka, India	13.0606.3°N 78.0451.3°E	*Thrips palmi*	MN972636
17.	Chintamani, Kolar, Karnataka, India	13.2534.5°N 78.0426.7°E	*Scirtothrips dorsalis*	MN972637
18.	Budumanahalli, Bengaluru, Karnataka, India	13.1134.7°N 77.3144.7°E	*Thrips palmi*	MN972638
19.	Thambarsnhalli, Bengaluru, Karnataka, India	13.0717.8°N, 77.29392°E	Nil	
20.	Sivakottai, Bengaluru, Karnataka, India	13.0735.9°N 77.3058.2°E	*Tusothrips sumatrensis*	MN972639
21.	ICAR-IIHR, Bengaluru, Karnataka, India	13.0811.0°N 77.2952.6°E	*Frankliniella schultzei*	MN972640
22.	Shanthigrama, Hassan, Karnataka, India	12.5843.9°N 76.1245.2°E	Nil	
23.	Malali, Hassan, Karnataka, India	16.2057.1°N 75.1201.0°E	*Thrips palmi*	MN972641
24.	Kavalande, Mysuru, Karnataka, India	12.0150.6°N 76.4805.8°E	*Frankliniella schultzei*	MN972642
25.	Mulluru, Mysuru, Karnataka, India	12.1438.4°N 76.3321.2°E	Nil	
26.	Thagadooru, Mysuru, Karnataka, India	12.0554.8°N 76.4745.5°E	*Thrips palmi*	MN972643
27.	Chikkarasikere, Mandya, Karnataka, India	12.3041.8°N 77.0241.0°E	*Thrips apicatus*	MN972644

(contd.)

(Table 1 contd.)

28.	Doddegowdanakoppalu, Mandya, Karnataka, India	12.2617.6°N 76.3933.8°E	*Scirtothrips dorsalis*	MN972645
29.	Magala, Chamrajnagar, Karnataka, India	14.5950.4°N 75.4834.4°E	Nil	
30.	Bandigere, Chamrajnagar, Karnataka, India	11.5214.9°N 76.5721.1°E	*Frankliniella schultzei*	MN972646
31.	Narayanagov, Pune, Maharashtra, India	19.0801.3°N 73.5738.6°E	*Thrips palmi*	MN972647
32.	Saswad, Pune, Maharashtra, India	18.2113.0°N 74.0241.7°E	*Scirtothrips dorsalis*	MN972648
33.	Chondoli, Pune, Maharashtra, India	18.5049.6°N 73.5136.0°E	*Thrips palmi*	MN972649
34.	Rajgurunagar, Pune, Maharashtra, India	18.5102.8°N 73.5348.2°E	*Thrips tabaci*	MN972650
35.	Kowdipally, Medak, Telengana, India	17.5230.7°N 78.1214.6°E	Nil	
36.	Polepalle, Mahbubnagar, Telengana, India	16.4841.7°N 78.0830.6°E	*Thrips palmi*	MN972651
37.	Nuthimadugu, Anantapur, Andhra Pradesh, India	14.2914.9°N 77.1941.0°E	*Scirtothrips dorsalis*	MN972652
38.	Tekmal, Kurnool, Andhra Pradesh, India	17.5848.2°N 78.0146.0°E	*Thrips palmi*	MN972653

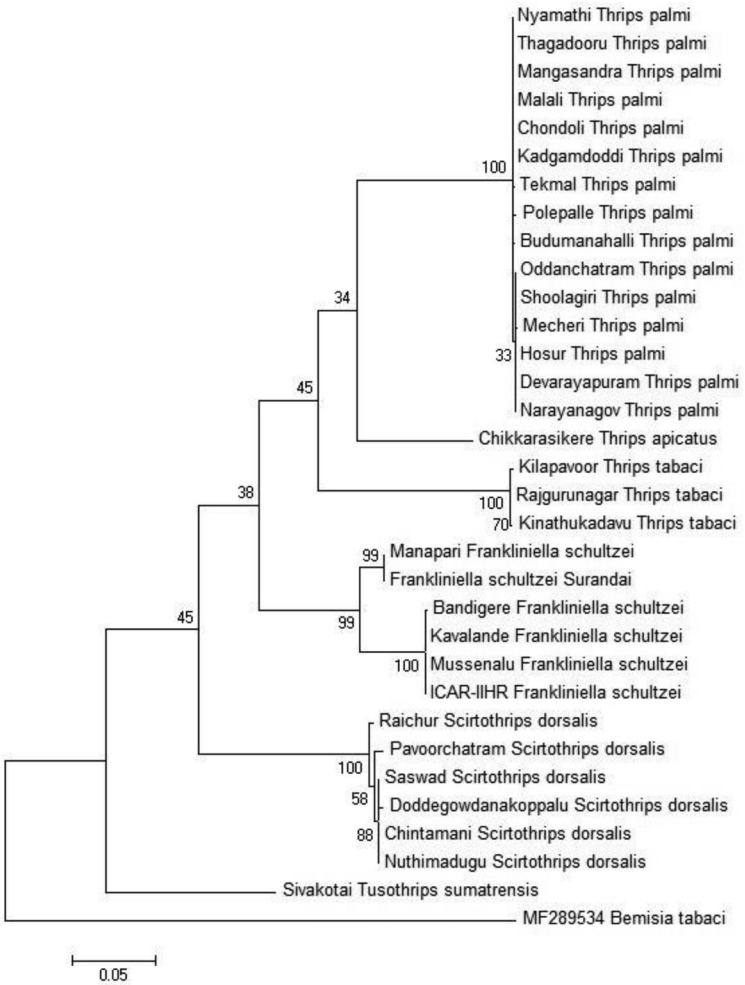

Fig. 1. Maximum-likelihood tree for *Thrips* spp., based on 655bp length fragment of the mt-COI gene. The tree was obtained by using the Kimura's 2 parameter (K2P) distance with 1000 bootstrap replicates. *Bemisia tabaci* is used as out-group

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