



RESEARCH NOTE

Identification of putative thrips vectors of tospovirus on tomato (*Solanum lycopersicum* L.) employing ITS and mtCOI

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ABSTRACT: Tospoviruses (Bunyavirales) which are exclusively transmitted by thrips cause server yield loss on vegetables. In this regard, out of 6000 species of thrips, only 16 species have been reported to be the vectors of different tospoviruses worldwide. In this regard, a member of the tospovirus called groundnut bud necrosis virus (GBNV) is known to infect groundnut has become one of the production constraint on tomato in India since for few years. In this regard there is no definitive information available on the species of thrips involved in transmission of GBNV to tomato in India. Therefore, a survey was carried out to determine different species of thrips available in the tomato ecosystem at the ICAR - IIHR experimental farm, Bengaluru during 2019. Species diagnosis of the thrips specimens was carried out using ITS2 and mtCOI markers. Results revealed the prevalence of four thrips species viz., *Thrips palmi*, *Scirtothrips dorsalis*, *Frankliniella occidentalis* and *T. hawaiiensis* of which *T. palmi* was predominant.

Keywords: Tospovirus, GBNV, Thrips, Mt-COI and ITS2

Thrips are the major factor affecting production of vegetables as direct pests and more importantly vectors of tospoviruses (single stranded, ambisense RNA viruses). Globally there are 6000 species of thrips have been documented of which only 16 species are reported to be vectors of different tospoviruses. With regard to India, only six species viz. *Thrips palmi* (Bhatti 1980), *Ceratothripoides claratris* (Premachandra *et al.*, 2005), *Thrips tabaci* (Gupta *et al.*, 1992), *Scirtothrips dorsalis* (Singh *et al.*, 2005), *Frankliniella schultzei* (Nakahara *et al.*, 1997) and *F. occidentalis* (Suganthi *et al.*, 2016 and Tyagi and kumar 2015) have been implicated in the transmission of tospoviruses. there out of 692 species. In this regard, tomato crop suffers heavily from an important tospovirus known as *Groundnut Bud Necrosis Virus* (GBNV) in the recent past and are transmitted by undetermined species of vector thrips.

Therefore, a survey was conducted during November, 2019 on tomato at the ICAR-IIHR experimental farm in order to determine the relative abundance of thrips species from 45 days old crop and the collected specimens were stored in 70% alcohol at 4°C until further use. These specimens were molecularly identified using Barcoding (mtCOI) and by Internal transcribed spacer (ITS). Modified CTAB method was used to

isolate genomic DNA from individual thrips. PCR amplification was achieved by employing the above two markers viz. Universal mt-COI primers of LCO-1490 - 5' - GGTCACAAATC ATAAAGATATTGG -3'; HCO-2198- 5' - TAAACTTCAGGGTGACCAAAAAATCA -3' (Hebert *et al.*, 2003a & Hebert *et al.*, 2003b) which amplified a 700 bp product. Yet another marker used was F5'-TGTGAACTGCAGGACACATGA-3', R5'-GGTAATCTCACCTGAACTGAGGTC-3' (Toda and Komazaki, 2002) with an amplicon size ranging from 588bp to 600bp. All the PCR amplicons were eluted and further cloned into the general purpose cloning vector, pTZ57R/T (Thermo fisher Scientific, UK) and cloned into chemical competent E. coli cells which were cultivated on antibiotic plates for Blue/White selection. Three positive clones (white colonies) for each specimen were inoculated into LB broth and the harvested plasmids were used for sequencing.

A total of 16 amplicons were sequenced and the sequences were deposited in NCBI GenBank (Table 1). BLAST analysis of mtCOI sequences clearly showed that seven specimens were of *T. palmi*, four of *S. dorsalis*, three of *T. hawaiiensis* and two of *F. occidentalis*. Further analysis revealed that *T. palmi* sequences showed 100% and 99.85% similarities with Pakistan (JF839936) and

Table 1. ITS2 and mtCOI gene sequences submission in NCBI

| S.No. | Thrips Species | mtCOI Amplicon size | NCBI Acc.No. | ITS2 Amplicon size | NCBI Acc.No. |
|-------|------------------------|---------------------|--------------|--------------------|--------------|
| | <i>T. palmi</i> | 655bp | MN893761 | 582bp | MN889880 |
| | <i>F. occidentalis</i> | 656bp | MN893762 | 562bp | MN889879 |
| | <i>S.dorsalis</i> | 655bp | MN893763 | 606bp | MN889881 |
| | <i>T. hawaiiensis</i> | 655bp | MN893764 | 577bp | MN889882 |

Table 2. ITS2 and mt-COI gene sequence similarity index in among the species

| Species | <i>T.palmi</i> | <i>F. occidentalis</i> | <i>S. dorsalis</i> | <i>T.hawaiiensis</i> |
|------------------------|----------------|------------------------|--------------------|----------------------|
| <i>T. palmi</i> | | 0.378 | 0.374 | 0.370 |
| <i>F. occidentalis</i> | 0.804 | | 0.665 | 0.542 |
| <i>S. dorsalis</i> | 0.803 | 0.800 | | 0.541 |
| <i>T. hawaiiensis</i> | 0.841 | 0.805 | 0.806 | |

Mt-COI

ITS2

Bengaluru isolate (MK333270), respectively. Similarly, *S. dorsalis* sequences exhibited 99.85% identity with IHR-LPR-CHI isolate (KT324678) while *F. occidentalis* showed 99.70% identity with Netherlands, Mexico and China isolates (HQ697596, MF993427 & KY775399). The *T. hawaiiensis* sequence had matched 99.85% with an Indian isolate (KX622360). Sequence analysis with ITS2 region showed that *T. palmi* had 99% similarity with an Indian isolate (FM956422); *S. dorsalis* had 99% similarity with an India isolate (GQ343260), while *F. occidentalis* showed 100% similarity with an USA isolate (GQ343257). *T. hawaiiensis* had 96% of similarity with that of a Thailand isolate (AB775426). The sequence similarity index for both ITS2 and mtCOI is given in the table 2.

The molecular identification clearly indicated that there are three known vectors species viz. *T. palmi*, *S. dorsalis* and *F. occidentalis*; and one flower feeder *T. hawaiiensis* (Takahashi, 1936) on tomato. In this regard, *T. palmi* has been reported to be the vector of watermelon bud necrosis virus (WBNV) (Lakshmi *et al.*, 1995). Similarly Amin and Mohammad in 1980 reported that Peanut yellow spot virus (PYSV) and

Tomato spotted wilt virus (TSWV) has been transmitted by *S. dorsalis* (Amin *et al.*, 1981), but later German *et al.*, (1992) proved that it was indeed GBNV. In this regard, *F. occidentalis* is a highly invasive and a viruliferous vector in many agricultural cropping systems spreading different tospoviruses. Nagata *et al.*, in 2004 reported the transmission of *Chrysanthemum stem necrosis virus* (CSNV), *Tomato chlorotic spot virus* (TCSV) and *Groundnut ring spot virus* (GRSV) by *F. occidentalis*. Similarly, Perrings *et al.*, in 2005 reported the trans boundary movement of *F. occidentalis* mainly through International shipments of ornamental crops. In 1995 Wijkamp *et al.*, has reported TSWV and *Impatiens necrotic spot virus* (INSV). Thrips vectors has attracted global attention due to the economic loss on different crops. Hence there are many researchers globally working on different thrips vector species (Reitz 2008, 2009; Salguero Navas *et al.*, 1991a, b; Kirk and Terry 2003; Kirk 2002; Groves *et al.*, 2003). There is no report of *T. hawaiiensis* as vector till date. In the present study we have observed the predominance of *T. palmi* over *S. dorsalis* and *F. occidentalis*. Detailed studies are being carried out the vector status of these

three species of thrips with regard to the transmission of both GBNV and TSWV on tomato. Tyagi and kumar in 2015 reported the prevalence of *F. occidentalis* in bitter melon, watermelon, cucumber, and pumpkin in Karnataka. Similarly Suganthi *et al.*, (2016) reported *F. occidentalis* in chrysanthemum, carnation, dahlia and gerbera in Nilgiris and Salem districts of Tamil Nadu with no information on the thrips vector involved in transmission of the same. Since both GBNV and TSWV has been reported on tomato it is important to find out the thrips vector/s involved in transmission. Our study has indicated three potential thrips vectors and need to be further studied for transmission possibilities of both GBNV and TSWV on tomato.

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