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DETECTION AND PHYLOGENETIC ANALYSIS OF *WOLBACHIA* IN ONION THRIPS (*THRIPS TABACI* LINDEMAN)

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ABSTRACT

***Wolbachia* is a group of bacteria that is known to infect many arthropods and nematodes. It is one of the most common parasitic microbes and is possibly the most common reproductive parasite in the biosphere. The bacterium is best known for its ability to manipulate host reproductive biology where it can induce cytoplasmic incompatibility, parthenogenesis, feminization and male-killing. In the present study, onion thrips (*Thrips tabaci* Lindeman) were collected from 9 locations in India along with melon thrips (*Thrips palmi* Karny) and chilli thrips (*Scirtothrips dorsalis*). From the molecular level detection by using 16s rDNA, the Wolbachia infection has been detected in the onion thrips collected from 6 locations out of 9. Melon thrips and chilli thrips samples were also found to be infected by *Wolbachia*. The phylogenetic analysis revealed that all detected *Wolbachia* samples showed that all were distantly related to the previously known *Wolbachia* samples.**

Key words: *Wolbachia pipientis*, *Thrips tabaci*, 16s rDNA, phylogeny, melon thrips, chilli thrips, reproductive parasite, *Allium cepa*, geographical distributions, male-killing

The *Wolbachia* is a group of bacteria that are known to infect a wide range of arthropods (Gomes et al. 2022). Earlier the infection of *Wolbachia* in insects was estimated to be approximately up to only 20% (Werren and Windsor, 2000; Jiggins et al., 2001), but the later progression in the study has shown that *Wolbachia* is very commonly known to infect a majority of arthropods (up to 70% of all insects) (Stouthamer et al., 1999). *Wolbachia* was first described in the year 1924 when M. Hertig and S. B. Wolbach observed certain bacteria in the cells of a mosquito (*Culex pipientis*) which were later named *Wolbachia pipientis* in 1936 by Hertig. The actual study of *Wolbachia* started gaining momentum when Yen and Barr (1971) discovered that due to the presence of *W. pipientis* in the maternal cytoplasm of mosquitoes, it is transmitted from the parent to the offspring. This association had caused cytoplasmic incompatibility in certain intraspecific crosses within Culex mosquitos which lead to the cause of the death of mosquito eggs when sperm of infected males fertilized the eggs of *Wolbachia*-free females. The major fascination of *Wolbachia* infection is that it is been thought to cause unusual manipulations in the reproductive system of its host organism by inducing parthenogenesis, cytoplasmic incompatibility, feminization of genetic males, and male-killing (Werren, 1997). The different modes of reproduction in thrips are thelytoky (females produced from unfertilized eggs), arrhenotoky (males produced from unfertilized eggs and females produced from fertilized eggs) and deuterotoky (females and males produced from unfertilized eggs) (Nault et al., 2006). The infection of *Wolbachia* has been reported in both thelytokous and arrhenotokous populations of thrips species (Kumm and Moritz., 2008). In India, *Wolbachia* has been reported from *Thrips palmi* (Saurav et al., 2016), *Sciothrips cardamomi* (Jacob et al., 2015), *Plicothrips apicalis* (Ambika and Rajagopal, 2018) and onion thrips (*Thrips tabaci* Lindeman) (Gawande et al., 2019). Many species of thrips infest a variety of crops (Ullman et al., 2002). *T. tabaci* has been first described in 1888 by Lindeman. *T. tabaci* has become a global pest of increasing concern in onion (*Allium cepa* L.) (Diaz-Montano et al., 2011). Thrips ability to develop resistance to insecticides, ability to transmit plant pathogens and frequency of producing more generations at high temperatures makes it more concerning. *T. tabaci* feeds directly on leaves, causing blotches and premature senescence as well as distorted and undersized bulbs. The yield loss due to *T. tabaci* can go up to 50% and it can be even more problematic when it transmits Iris yellow spot virus (family *Bunyaviridae*, genus *Tospovirus*, IYSV) (Diaz-Montano et al., 2011).

T. tabaci feeding can reduce onion bulb weight (Kendall and Capinera 1987; Fournier et al., 1995; Rueda et al., 2007; Diaz-Montano et al., 2010). In thrips, *Wolbachia* was discovered by Pintureau et al. (1999) in *Heliothrips hemorrhoidalis* and *Hercinothrips femoralis* (Reuter), by Arakaki et al. (2001) in *Franklinothrips vespiformis* (Crawford) and Cano-Calle et al. (2021) in *Frankliniella sp*. and *Scirtothrips hansoni* found in avocado (*Persea americana*). It has been observed that *Wolbachia* induce thelytokous reproduction in *F. vespiformis* (Arakaki et al., 2001).

To study the phylogenetic relationship between different *Wolbachia*, in various host organisms in which *Wolbachia* has been proven to infect, the 16S ribosomal DNA sequence is used since the initial times and is still being used even in recent times. Later Baldo et al., (2006) developed a standard Multilocus Sequence Typing (MLST) system for characterization of different *Wolbachia* strains. The MLST system uses a combination of five housekeeping conserved genes (ftsZ, gatB, coxA, hcpA, and fbpA) for more efficient detection of *Wolbachia* and analysis of its diversity in various hosts. Gawande et al. (2019) conducted a study on microbiome profiling of the *T. tabaci*, to study the bacterial communities associated with *T. tabaci* in India. It was first time the presence of the genus *Wolbachia* in *T. tabaci* was reported. Hence, to carry forward this study further, we tested the presence of *Wolbachia* infections in *T. tabaci* that were collected from several locations across the country. By using the molecular approach, particularly PCR amplification of the targeted 16S rRNA gene, the presence of *Wolbachia* was confirmed. Further, phylogenetic analysis was also conducted based on the sequences obtained by PCR amplification of the 16S rRNA gene.

MATERIALS AND METHODS

Onion thrips were obtained from nine locations across India; 1. Junagadh Agricultural University (JAU); 2. Indian Agricultural Research Institute (IARI), New Delhi; 3. CSK Himachal Pradesh Agricultural University, Palampur; 4. Punjab Agricultural University (PAU), Ludhiana; 5. Kotputli, Rajasthan; 6. Tamil Nadu Agricultural University, Tamil Nadu; 7. National Horticultural Research and Development Foundation (NHRDF), Karnal; 8. Srinagar; and 9. ICAR-DOGR, Rajgurunagar, Pune. In addition to that chilli thrips (*Scirtothrips dorsalis*) and melon thrips (*Thrips palmi* Karny) were collected from ICAR- Indian Agricultural Research Institute, Regional Station, Pune. The collected samples were preserved in absolute ethanol and kept at 4°C until further use. Fifty individual thrips from a population (location-wise) were used for DNA isolation. Extraction of the genomic DNA was done by using the DNeasy Blood and Tissue Kit (Qiagen, cat. no. 69504) from thrips by following the manufacturer's protocol and the DNA was then stored at −20 °C until further use. In this study, PCR screening was done for testing the presence of *Wolbachia* infections in thrips. For this, we targeted the 16S rRNA gene (Forward primer 5' TTGTAGCCTGCTATGGTATAACT 3' and Reverse primer 5' GAATAGGTATGATTTTCATGT 3') which was amplified under the following conditions: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 7 min. The PCR products were electrophoresed on 1% agarose gels and stained by Ethidium Bromide. Thrips DNA yielding amplicons of the expected size (900bp) were scored as positive for *Wolbachia*.

Extraction of the PCR product from the agarose gel was performed using Mini Elute Gel Extraction Kit (Qiagen) by following the recommendations of the manufacturer. The eluted PCR product was further subjected to ligation reaction which was performed by using CloneJET PCR Cloning Kit (Thermofisher) by following the manufacturer's protocol. After ligation of the PCR product with pJET 1.2/ blunt Cloning Vector (Thermofisher), the ligated product was then transformed and cloned into DH10β competent *E. coli* cells. Antibiotic marker selection technique was used for screening and selection of positive colonies. Further, colony PCR was done for confirming the colonies that were containing the plasmid along with the desired sequence. Bacterial colonies containing the positive plasmid were transferred to Luria Bertani broth medium and were grown overnight. Finally, the plasmid DNA was isolated from an overnight bacterial culture by using the GeneJET™ Plasmid Miniprep Kit (Thermofisher). This plasmid DNA was subjected to Sanger sequencing and the resulting sequences were compared with the sequence database at the National Centre for Biotechnology Information using BLAST program. 16S rRNA gene sequences (900bp) representative of *Wolbachia* strains from different host organisms were selected from the NCBI database (https://www.ncbi.nlm.nih.gov) and used to classify *Wolbachia* strains detected in our thrips samples. Sequence alignments were carried out using ClustalX 1.8344. Maximum likelihood (ML) and Bayesian inference (BI) were used for phylogenetic analysis.

RESULTS AND DISCUSSION

Wolbachia has been first discovered by M Hertig and S B Wolbach from the reproductive tissues of mosquitoes. In thrips, *Wolbachia* was discovered by Pintureau et al. (1999) in *Hercinothrips*. Gawande et al. (2019) reported the presence of the genus *Wolbachia* for the first time in *T. tabaci*. In this study, the presence of *Wolbachia* infection in *T. tabaci* from different locations throughout India has been shown along with the phylogenetic analysis. Previous studies in insects and arthropods have shown a high level of *Wolbachia* infection ranging from 40 to 90% depending on their geographical distributions (Jeyaprakash and Hoy 2000; Chai et al., 2011). Recent microbiome profiling experiment on fungivorous thrips *Hoplothrips carpathicus* showed 69.95% *Wolbachia* population out of a total bacterial population (Kaczmarczyk et al., 2018). In the present study, PCR amplification reactions were carried out by *Wolbachia* 16S *rDNA* specific primers. The 16S rDNA gene amplified fragment of 900 bp was obtained on 1% Agarose gel (Fig. 1A). Out of nine locations (Fig. 2A), thrips from 6 locations (DOGR, Tamil Nadu, Karnal, Gujarat, Punjab, Himachal Pradesh) were found to have *Wolbachia* in them while remaining three were found to be negative for *Wolbachia* infection. In addition, *S. dorsalis* and *T. palmi* collected from Pune were also found to be positive for *Wolbachia* infection (Fig. 1B). The level of *Wolbachia* infection in *T. tabaci* was found to be 66%. In addition, *Wolbachia* infection was also found in *T. palmi* as previously reported by Saurav et al. (2016). The *S. dorsalis* samples collected from Pune were also found to be positive for *Wolbachia* infection. This is the first report of *Wolbachia* infection in *S. dorsalis*. In conclusion, the results showed high infection rates of *Wolbachia* in *T. tabaci* populations from different geographical locations in India.

The amplified fragments were sequenced by 16S rDNA specific primers and then compared with the 16S ribosomal RNA sequence of *W. pipientis* (Sequence ID: AF501664.1) that exists in the GenBank. As a result, the amplified sequences of 16S rDNA from DOGR, Karnal, Punjab, Gujarat, Tamil Nadu, TP 1 (*T. palmi*), ST 1 (*S. dorsalis*) showed 98.89, 99.44, 98.67, 99.55, 99.55, 97.88, and 97.27% nucleotide identity, respectively. While thrips samples collected from Himachal Pradesh 16S rDNA showed 98.66% nucleotide identity with *Wolbachia* sp. MSebKT1 gene for 16S ribosomal RNA (Sequence ID: AB795345.1). The obtained sequences of samples from all locations in India were submitted to NCBI database (GenBank accession number: OL702843, OL702844, OL702845, OL702846, OL702847, OL702848, OL702849, OL702850)

Phylogenetic analysis between the *Wolbachia* samples collected from a different locations in present study showed that the *Wolbachia* sample from ICAR-DOGR and *S. dorsalis* were closely related to each other (Fig. 2). Similarly, the *Wolbachia* sample from *T. palmi* showed homology with *T. palmi* (KM393213) sequence in GenBank. The rest of the *Wolbachia* samples showed to have a common ancestor but were distantly related. Phylogenetic analysis of *Wolbachia* sequence from present study along with *Wolbachia* from other hosts showed that samples from present study were distantly related to the ones which were previously discovered (Fig. 3). Moreover, *Wolbachia* samples from Tamil Nadu, Gujarat, Karnal, Himachal Pradesh Punjab, *T. palmi* from a clade, separating *S. dorsalis* and DOGR sample from them. The phylogenetic analysis showed that all the *Wolbachia* sequences from thrips collected from different locations in India showed no similarity with the previously known *Wolbachia* from other hosts. This indicated the horizontal transfer between

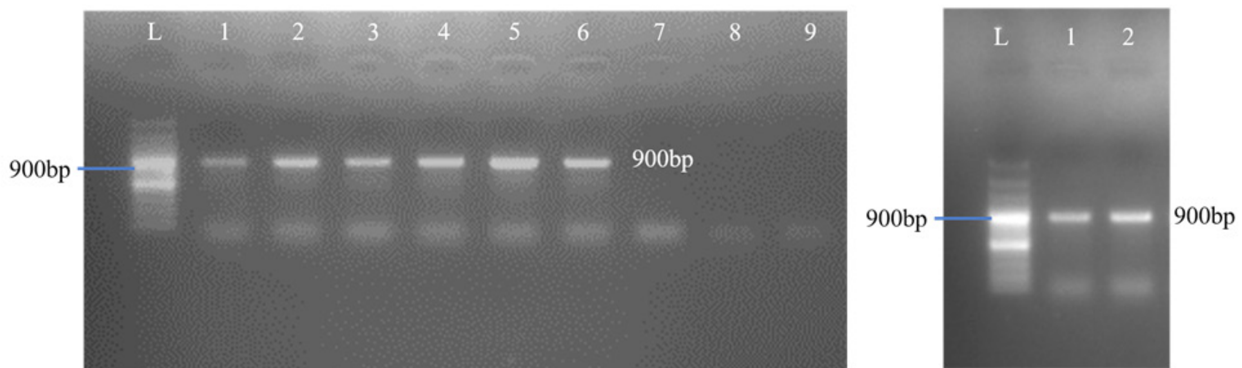

Fig. 1. Detection of *Wolbachia* via amplification of 16S rDNA gene fragment from thrips collected from locations in India. *Wolbachia* detection with forward and reverse primers for a fragment (900 bp) of the 16S rDNA gene. (A) L: 100bp Ladder, 1: DOGR, 2: Tamil Nadu, Lane 3: Karnal, Lane 4: Gujarat, Lane 5: Punjab, Lane 6: Himachal Pradesh. 7: Jammu Kashmir, 8: Rajasthan, 9: New Delhi, (B) L: 100bp Ladder, 1: *Thrips palmi* (melon thrips), 2: *Scirtothrips dorsalis* chilli thrips

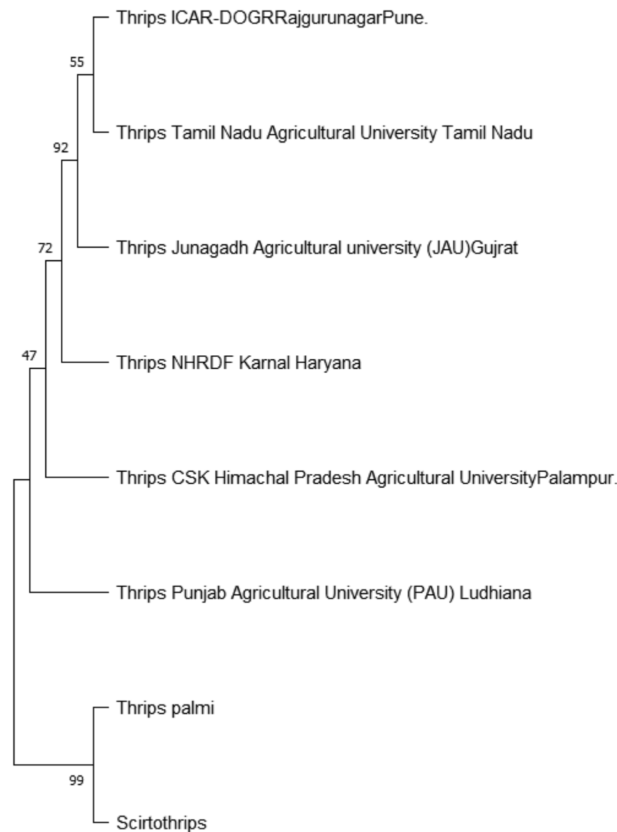

Fig. 2. The phylogenetic tree of *Wolbachia* were detected in onion, chilli and melon thrips based on their 16S rDNA sequences. Trees inferred from Maximum likelihood and Bayesian inference (BI) methods using MEGA 11.0 program

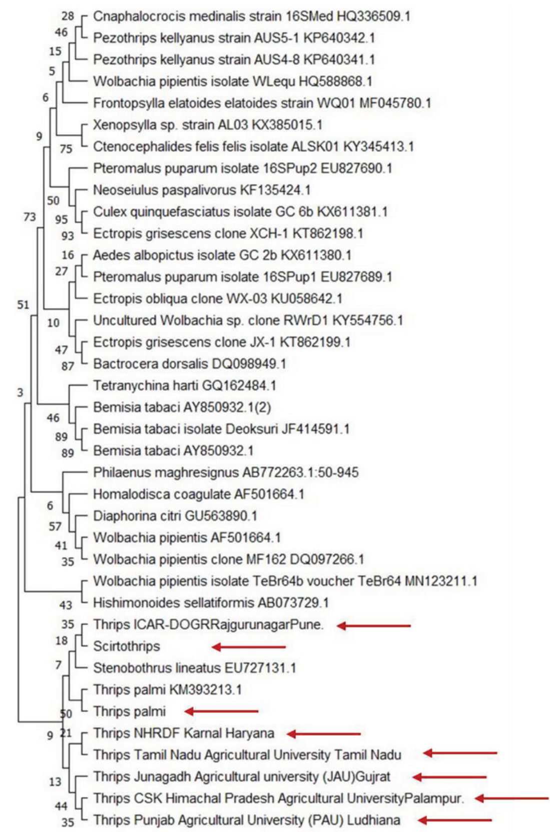

Fig 3. The phylogenetic tree of *Wolbachia* from different arthropods. Tree inferred from Maximum likelihood and Bayesian inference (BI) methods using MEGA 11.0 program. The arrows indicate the thrips samples used in this study

anthropods species as reported previously (O'Neill et al., 1992; Werren et al., 1995; Huigens et al., 2004; Klasson et al., 2009; White et al., 2017). For instance, closely related strains of *Wolbachia* are found in diverse hosts as flies, beetles, and wasps (Werren et al., 1995). Moreover, the horizontal transmission of *Wolbachia* has been proven to occur in spiders (Rowley et al., 2004). However, the mechanism of horizontal transmission of *Wolbachia* in nature is poorly understood. These findings can help us to gain the basic information related to *Wolbachia's* evolution in various hosts as well as to analyse its diversity and its behavioural pattern with its host in the prior stages of the study. In the advanced stage as thrips are known as potential vectors for transmission of viral diseases in plants, the study of *Wolbachia* can unravel novel methods for controlling pest populations, by modifying the insect's ability to transmit disease as well as enhancing the mass production of beneficial insects used for biological control.

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AUTHOR CONTRIBUTION STATEMENT

SG conceived and supervised research, PR, PK, IB conducted experiment, KC provided chilli and melon thrips, KK, PK and PR wrote draft of MS and all authors reviewed and approved the manuscript

CONFLICT OF INTEREST

No conflict of interest.

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