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PHYLOGENETIC PROBE OF *WOLBACHIA* IN *BEMISIA TABACI*

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ABSTRACT

The isolated DNA of *Wolbachia* present in whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleurodidae) collected from six different agroclimatic zones of Assam, India were amplified through polymerase chain reaction (PCR) using *Wolbachia* specific primer. A band in the range of 518-528 bp proved the presence of *Wolbachia* in the samples, which was further confirmed through BLAST analysis using the NCBI database. A phylogenetic analysis of the DNA sequences showed two subgroups of *Wolbachia* in the collected sample of *B. tabaci.* The W2 cluster formed with four samples of *Wolbachia* showed 91% similarity with *Wolbachia* from *Drosophila simulans.*

Key words: *Bemisia tabaci*, endosymbiont, *Wolbachia*, wsp primer, BLAST, phylogenetic analysis

The whitefly is a species complex holding over 44 morphologically indistinguishable cryptic species (Venkataravanappa et al., 2023), which acts as vector of more than 111 numbers of plant viruses, namely begomoviruses (Czosnek et al., 2001). Over 1400 species of whitefly were found all over the world, out of which 290 species are recognized from India (Sunderaraj, 2006). *Bemisia tabaci* (Gennadius) (Hemiptera : Aleurodidae) is a cosmopolitan insect species, which feeds on the phloem sap of a variety of agriculturally important crops. As the phloem of plants transmits carbohydrate rich materials, *B. tabaci* obtains the requisite amount of amino acid with the support of the secondary bacterial endosymbionts horboured intercellularly on the gut. *Wolbachia* is an intracellular facultative endosymbiont and known to be present in all major classes of arthropods estimating around 52% of the known insect species (Weinert et al., 2015), including Hemipterans, which helps the insect in supplementing the nutrient-poor plant sap diets with essential amino acids and carotenoids. *Wolbachia* exhibits a parasitic relationship with *B. tabaci* and acts as a reproductive manipulator either by feminization, parthenogenesis, male killing, and cytoplasmic incompatibility (Weeks et al., 2002). Phylogenetic study of the genus *Wolbachia* had shown 17 major super-groups (Ma et al., 2017). The molecular analysis of *Wolbachia* infection in *B. tabaci* has been proven well through polymerase chain reaction (PCR) and molecular marker from the *Wolbachia* surface protein (*wsp*) gene (Shi et al., 2016). The study of the *wsp* gene had significantly improved the knowledge of the genetic diversity of the whitefly parasitoid species complex in recent times (Francis et al., 2016). Scientific research on the presence of *Wolbachia* on *B. tabaci* population from Assam, India and their possible role in disease transmission are scanty. Hence, our present investigation aimed at determination of distribution pattern and diversity of *Wolbachia* on *B. tabaci* attacking vegetable ecosystem of Assam, India along with their phylogenetic status for better understanding of their role in host biology and disease transmission.

MATERIALS AND METHODS

Based on the dominance in vegetable cultivation, 3 agroclimatic zones viz., Upper Brahmaputra Valley Zone (UBVZ), Central Brahmaputra Valley Zone (CBVZ) and North Bank Plain Zone (NBPZ) were selected for collection of *B. tabaci* samples. From each of the agroclimatic zone, 2 villages were selected and a total of 50 numbers of adult *B. tabaci* were randomly collected from 10 numbers of chilli plant for molecular analysis following a common square grid. The insect samples were then stored in PCR tubes (Make: Tarson, Size: 0.2 ml) having 99% ethyl-alcohol (Make: Himedia), marked and preserved separately at -20°C for further molecular analysis at Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat. The CTAB method of DNA extraction proposed by Doyle and Doyle (1987) was followed for isolation of DNA from *B. tabaci*. The presence of *Wolbachia* on *B. tabaci* was confirmed through the PCR method based on the *Wolbachia* surface protein (*wsp*) gene. The *wsp* gene of *Wolbachia* was amplified using the

Wolbachia specific primer set (forward: 52-CAT ACC TAT TCG AAG GGA TAG-32; reverse: 52-AGA TTC GAG TGAAAC CAA TTC-32) (Murthy et al., 2011). The PCR reaction was performed in a Thermocycler (Make: GeneAmp, Model: PCR System 9700) using a total reaction volume of 12 µl with different reaction mixtures and primers. The temperature profile for *Wolbachia* specific PCR was pre denaturing of 94°C for 2 minutes followed by 35 cycles of denaturation at 94°C, annealing at 48°C, and extension at 72°C for 30 seconds each; and the final extension at 72°C for 10 minutes. The amplified PCR product was visualized, examined, and documented by using a gel documentation unit (Make: BioRad, Model: EZ imager, Gel Doc XR). The PCR samples were sequenced at Bioserve Biotechnologies India Pvt. Ltd, Hyderabad, India. The sequenced results of *Wolbachia* samples were then compared with the sequencing data present in National Centre for Biotechnology Information, San Diego, CA, USA (NCBI) sequence data bank (http://www.ncbi.nlm.nih.gov/ nucleotide) using BLAST program. The consensus sequences of all the DNA samples were sent to GenBank to get the accession number and the phylogenetic analysis was performed using MEGA (Ver. X) software. The sequences were aligned using Clustal W implemented in the MEGAX software (Kumar et al., 2018) and the phylogenetic analysis was performed with the Kimura two-parameter model and Neighbor- Joining method with 1000 bootstrap. The genetic distances were calculated using Maximum Composite Likelihood Option of MEGA X.

RESULTS AND DISCUSSION

Symbiotic relationship of bacteria and insects are proverbial (Chiel et al., 2009) manipulating the host physiology, growth, and development to a large extent for mutual benefit. *Bemisia tabaci* is a cryptic species complex comprising over 1550 species that have been described worldwide till date (Ahmed et al., 2010) and were found infected with endosymbionts (Brumin et al., 2011). The first report of whitefly was from China in the year 1949 as B biotype (Weeks et al., 2002) causing considerable damage to a wide range of crops (Qiu et al., 2008). Altogether six secondary endosymbionts, *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Rickettsia* and *Wolbachia* were found present intercellularly in *B. tabaci* population (Li et al., 2017). *Wolbachia* is the most widely prevalent endosymbiont, infecting over 70% of the described insect species (Chiel et al., 2009) helping in provisioning of essential nutrients (Hosokawa et al., 2010), increasing fitness, reproductive manipulations including feminization, sex ratio, etc. apart from protecting from adverse environmental conditions. Horizontal transmission of *Wolbachia* within whitefly species has been an area of interest for ecological and evolutionary biologists (Li et al., 2017) revealing their interaction. Lv et al. (2023) explained the vertical transmission mechanism of bacterial endosymbionts in Asia II 1 biotype of *B. tabaci* along with the spatio-temporal distribution in host tissues.

Wolbachia has received increased interest in recent years because of its high rates of distribution in many insects, but no information available about diversity and presence of *Wolbachia* in *B. tabaci* of Assam, India. The existence of *Wolbachia* was confirmed on the adult *B. tabaci* in our present investigation using the *Wolbachia* specific primers for the *wsp* gene. The PCR amplification of the *Wolbachia* specific primer shows 518 bp size of the band in gel electrophoresis (Fig. 1). The BLAST result of sequenced samples shows 99% similarity with known sequences available in the NCBI GeneBank. The sequences of *Wolbachia* were submitted to GenBank for the accession number viz., MZ031554, MZ031918, MZ031919, MZ031920, MZ031921, and MZ031922. The six sequenced samples of *Wolbachia* were aligned with the reference sequences (40 Nos.) taken from the NCBI database and were edited and aligned manually using Clustal W in MEGA-X. The *Wolbachia* samples could be clustered into two subgroups, W1 and W2 (Fig. 2a, b). The W2 cluster formed with four samples of *Wolbachia* showed 91% similarity with *Wolbachia* from *Drosophila simulans*. The other two samples of *Wolbachia* show almost 90% similarity with most of the selected NCBI database samples. The *wsp* gene of *Wolbachia* that are considered as highly variable codes for the outer membrane protein found present in *Wolbachia* (Braig et al., 1998) obtained from different parts of Assam, India. The samples of

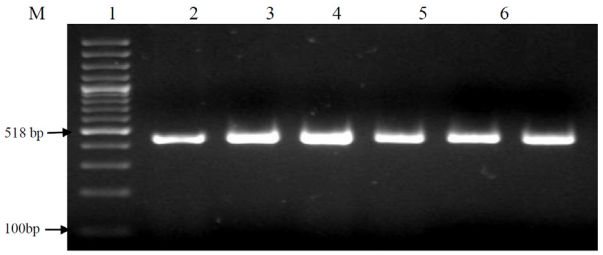

Fig. 1. Agarose gel electrophoresis of genomic DNA isolated from *B. tabaci* upon amplification with *wolbachia* specific primer (M: 1kb ladder, 1-6 *B. tabaci* DNA (NB: 1-CBZ1, 2-CBZ2, 3-NBZ1, 4- NBZ2, 5-UBZ1, 6- UBZ2)

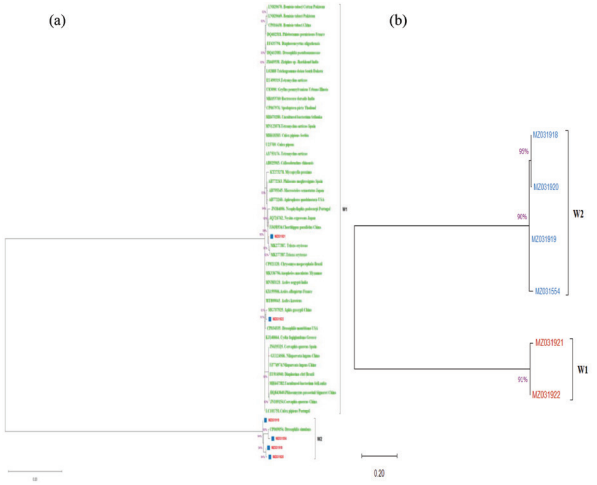

Fig. 2. Phylogenetic tree of *Wolbachia* (a). The maximum likelihood phylogenetic tree of *Wolbachia* based on the *wsp* sequences. The tree has been constructed by maximum-likelihood analysis based on Tamura-nei model with 1000bootstrap value. Red highlighted samples are collected one and green are reference sequences from the National Center for Biotechnology Information. The numbers placed at each node indicate the percentage similarity. (b). The phylogenetic tree of *wolbachia* based on *wsp gene* sequences. The tree was constructed using the maximum likelihood method with 1000 bootstrap values. W1 and W2 show two subgroups of *Wolbachia.*

Wolbachia that were obtained from *B. tabaci* samples collected from UBVZ of Assam formed W1 cluster and the W2 sub was obtained from samples collected from CBVZ and NBZ of Assam. This confirmed that W1 sub-group of *Wolbachia* was found in Asia-II 1 of *B. tabaci* and W2 sub-group of *Wolbachia* was found in *B. tabaci* biotype Asia-II 5. Venkataravanappa et al. (2023) reported presence of 4 groups of whiteflies viz., Asia I, China 3, Asia II 5 and Asia II-1 from of Uttar Pradesh, India and corroborates our present investigation.

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AUTHOR CONTRIBUTION STATEMENT

JG carried out research, data recording and analysis and writing original draft; DKS conceptualized the topic and reviewing of manuscript, PD finalized the methodology and provided logistic support, and SK involved in data analysis, writing of manuscript and reviewing.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Ahmed M Z, Ren S X, Mandour N S, Greeff J M, Qiu B L. 2010. Prevalence of *Wolbachia* supergroups A and B in *Bemisia tabaci* and some of its natural enemies. Journal of Economic Entomology 103: 1848-1059.

Braig H R, Zhou W, Dobson S, O'Neill S L. 1998. Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia*. Journal of Bacteriology 180(9): 2373-8.

Brumin M, Kontsedalov S, Ghanim M. 2011. *Rickettsia* influences thermo-tolerance in the whitefly *Bemisia tabaci* B biotype. Insect Science 18: 57-66.

Chiel E, Zchori-Fein E, Inbar M, Gottlieb Y, Adachi-Hagimori T, Kelly S E, Asplen M K, Hunter M S. 2009.Almost there: transmission routes of bacterial symbionts between trophic levels. PLoS ONE 4(3): e4767. https://doi.org/10.1371/journal.pone.0004767.

Czosnek H, Ghanim M, Morin S, Rubinstein G, Fridman V, Zeidan M. 2001.Whiteflies: vectors, and victims (?), of geminiviruses. Advances in Virus Research 56: 291-322.

Doyle J J, Doyle J L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin 19: 11-15.

Francis A W, Stocks I C, Smith T R, Boughton A J, Mannion C M, Osborne L S. 2016. Host plants and natural enemies of Rugose Spiraling Whitefly (Hemiptera: Aleyrodidae) in Florida. Florida Entomology 99(1): 150-153.

Hosokawa T, Koga R, Kikuchi Y, Meng X Y, Fukatsu T. 2010. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. Proceedings of National Academy of Sciences USA 107: 769-774.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549.

Li S J, Ahmed M Z, Lv N, Shi P Q, Wang X M, Huang J L, Qiu B L. 2017. Plant mediated horizontal transmission of *Wolbachia* between whiteflies. ISME Journal 11(4): 1019-1028.

Lv N, Peng J, He Z Q, Wen Q, Su Z Q, Ali S, Liu C Z, Qiu B L. 2023. The Dynamic Distribution of *Wolbachia* and *Rickettsia* in AsiaII1 *Bemisia tabaci*. Insects 14(4): 401.

Ma Y, Chen W J, Li Z H, Zhang F, Gao Y, Luan Y X. 2017. Revisiting the phylogeny of *Wolbachia* in Collembola. Ecology and Evolution 7(7): 2009-2017.

Murthy K S, Rajeshwari R, Venkatesan T, Baby N L. 2011. Detection and characterization of *Wolbachia* in *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), a parasitoid of the diamond back moth *Plutella xylostella* (Linn.). Journal of Biological Control 25(3): 213-216.

Qiu B L, Mandour N S, Xu C X, Ren S X. 2008. Evaluation of entomopathogenic nematode, *Steinernema feltiae* as a biological control agent against the whitefly *Bemisia tabaci*. International Journal of Pest Management 54: 247-253.

Shi P, He Z, Li S, An X, Lv N, Ghanim M, Cuthbertson A G S, Ren S X, Qiu B L. 2016. *Wolbachia* has two different localization patterns in Whitefly *Bemisia tabaci* AsiaII7 species. PLoS One 11(9): e0162558. https://doi.org/10.1371/journal.pone.0162558.

Sunderaraj R. 2006. Species diversity of whiteflies (*Aleyrodidae: Homoptera*). National Biodiversity Authority India, India, pp. 1-12. http://nbaindia.org/uploaded/docs/ncb_jan_06_23.pdf.

Venkataravanappa V, Kodandaram M H, Prasanna H C, Krishna Reddy M, Lakshminarayana Reddy C N. 2023. Unraveling different begomoviruses, DNA satellites and cryptic species of *Bemisia tabaci* and their endosymbionts in vegetable ecosystem. Microbial Pathogen 174: 105892. https://doi.org/10.1016/j.micpath.2022.105892.

Weeks A R, Reynolds K T, Hoffmann A A, Mann H. 2002.*Wolbachia* dynamics and host effects. Trends in Ecology and Evolution 17: 257-262.

Weinert L A, Araujo-Jnr E V, Ahmed M Z, Welch J J. (2015) The incidence of bacterial endosymbionts in terrestrial arthropods. Proceedings of Royal Society of London B282:20150249.20150249. http://doi.org/10.1098/rspb.2015.0249.

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