DISTRIBUTION AND MITOTYPE DIVERSITY OF BEMISIA TABACI

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ABSTRACT

Comparative assessment of the mitochondrial cytochrome oxidase I (mtCOI) gene sequence uncovers the Bemisia tabaci mitotypes. The current study aims to define B. tabaci mitotypes found in various agro-ecological zones across India. Phylogenetic analysis of 13 samples from various agroclimatic regions revealed presence of four mitotypes: Asia 1, Asia II 1, Asia II 5 and Asia II 7. Among, Asia II 1 was found to be abundant and widespread distribution in the thirteen locations studied. From the 13 sequences examined, 7 haplotypes viz., three of Asia II 1, two of Asia II 5, each one of Asia 1 and Asia II 7 were identified. Nucleotide diversity was observed to be greater in Asia II 5 (0.00388) than in Asia II 1 (0.00258), and haplotype diversity was observed to be greater in Asia II 5 (1.000) than in Asia II 1 (0.600).

Key words: Bemisia tabaci, mitotypes, Asia 1, Asia II 1, Asia II 5, Asia II 7, haplotypes, mtCOI, nucleotide diversity

Bemisia tabaci (Gennadius), (Hemiptera: Aleyrodidae) is a cosmopolitan and polyphagous insect pest and among the most devastating insect pests of the twentieth century. B. tabaci is known to attack over 700 plant species and to vector around 100 plant viruses causing huge economic loss in many crop plants. It is designated as a complex of morphologically similar but physiologically, biochemically and genetically dissimilar species (Rehman et al., 2021; Khamis et al., 2021). Currently, there are 45 cryptic species of B. tabaci reported globally and 9 have been identified in India based on 4% genetic divergence in the mitochondrial cytochrome oxidase subunit I (mtCOI) sequences (Rehman et al., 2021). Bayesian inference analysis recognizes 45 well established cryptic species which were grouped into 11 major clades viz., Asia, Australia, China, Italy, Japan 1, Japan 2, Middle East Asia, New world, Sub-Saharan, Sub-Saharan Africa 7, Sub-Saharan Africa 10 and Uganda. Commonly these clades are recognized as genetic groups (Jiu et al., 2017; Mugerwa et al., 2018; Rehman et al., 2021). Similarly, the Bayesian inference analysis reveals 9 established cryptic species of B. tabaci in India namely Asia I, Asia II 1, Asia II 5, Asia II 7, Asia II 8, Asia II 11, Asia IV, MEAM1, and China 3. The cryptic species viz., Asia 1, Asia II 5, and Asia II 8 are predominant in southern part of India whereas Asia II 1 and Asia I was found prevalent in northern and central parts of India. Western India has the highest number of B. tabaci Asia I and Asia II 7 records. In Eastern India, B. tabaci Asia I, Asia II 1, and Asia II 5 were most common. The most recent sequence data showed a rise in B. tabaci China 3 occurrence in eastern India with its wider host range (Rehman et al., 2021; Chowda-Reddy et al., 2012; Ellango et al., 2015; Prasanna et al., 2015; Rekha et al., 2005; Singh et al., 2012).

The genetic divergence among cryptic species and B. tabaci genetic groups was 4.1–23.4% and 11.7–21.2%, respectively (Rehman et al., 2021). The intraspecific diversity B. tabaci has been characterized as biotypes before being identified as a cryptic species based on mitochondrial cytochrome oxidase subunit I (mtCOI) (De Barro et al., 2011). The genetic difference between these cryptic species was determined to be 3.5% but with far more sequence divergence between individuals of the same species, the cryptic species categorization cut-off value was reset to 4% (Dinsdale et al., 2010; Lee et al., 2013). Asia II 1 genotype was found to be more prevalent, with widespread distribution over tropical, subtropical, and temperate zones of Indian subcontinent and showed the highest haplotype diversity, whereas Asia 1 genotype indicated the highest nucleotide diversity (Prasanna et al., 2015). The most common genetic groups in Delhi are Asia II 1 and Asia II 7 (Hashmi et al., 2018; Ahmed et al., 2010; Prabhulinga et al., 2020; Hameed et al., 2012). A deeper understanding of the distribution patterns of genetic variation, species diversity, haplotype variability is very much essential to design or formulate effective management approaches for the continually evolving B. tabaci cryptic species
complex. The primary goal of this research was to document the identity, spread, and diversity of *B. tabaci* species on Cotton from various agro-climatic regions in India using the mitochondrial subunit.

**MATERIALS AND METHODS**

Whiteflies were collected from 13 agroclimatic regions viz., Banswara: Rajasthan, Sri Ganganagar: Rajasthan, Amravati: Maharashtra, Hisar: Haryana, Indore: Madhya Pradesh, Bathinda: Punjab, Meerut: Uttar Pradesh, Guntur: Andhra Pradesh, Raipur: Chhattisgarh, Jalandhar: Punjab, Coimbatore: Tamil Nadu, Pusa: New Delhi. A hand-held aspirator was used to capture adult whiteflies from cotton plants. Thereafter, the collected whiteflies shifted to 1.5 ml eppendorf tubes containing 70% ethanol. The collected samples were preserved in -20°C until further analysis. A single whitefly was used to isolate DNA. Each whitefly was thoroughly cleaned in autoclaved water. The washed whiteflies were then homogenized with a hand-held homogenizer (Sigma Aldrich) and DNA was extracted using the DNASure Tissue mini kit (Qiagen® NP-61305) according to the manufacturer’s instructions. The extracted DNA treated with RNase (0.1g/L) for 45 min at 37°C. The DNA was then be examined on a 0.8 % agarose gel containing ethidium bromide (0.5g/ml). The quantified DNA was used for further PCR analysis. The universal primers C1-J-2195 (5’-TGTATTTTGGTCATCCAGAAGT-3’) and TL2-N-3014 (5’ - TCCAATGCACTAATCTG C1-J-2195 (5’-TTGATTTTGGTCATCCAGAAGT-3’)) were used to amplify a partial mtCOI gene fragment of nearly 820 bp (Simon et al., 1994). A 25 µl reaction mixture comprising 12.5 µl of Ready to use PCR master mix (Promega M750A), 5.5 µl of nuclease free water, 1 µl each of forward and reverse primer, and 5 µl of DNA template was used for PCR amplification. A 3 µl amplified PCR product was run for 45 min on a 1.2% agarose gel in 1X TAE at 100V (Jordan Scientific). The amplified PCR products were sent to AgriGenome (Kochi, India) for purification and sequencing.

All whitefly sequences from the study were aligned using ClustalW, which was implemented in BioEdit v7.2.5, before being analyzed with Mega X for phylogeny (Kimura 1980; Kumar et al., 2018). The phylogenetic tree was constructed using maximum likelihood approach and the Kimura 2-parameter model. A bootstrap replication of 1000 was run to test the phylogenies (Felsenstein, 1985). The whitefly sequences were assigned to species based on pair-wise sequence divergence greater than 3.5% upon clade formation (Dinsdale et al., 2010). All the whitefly sequences were submitted to the NCBI GenBank to obtain accession numbers. DnaSP v5.10 was used to define the sequence polymorphism, singleton variable sites, average nucleotide differences, G+C content, and number of haplotypes, haplotype diversity and nucleotide diversity of the *B. tabaci* COI sequences (Librado and Rozas, 2009). The popART software was used to analyze the haplotype network of *B. tabaci* sequences by constructing a minimum spanning network relationship among the cryptic species.

**RESULTS AND DISCUSSION**

Based on the maximum likelihood approach, the phylogenetic analysis of 13 samples from different agro climatic regions showed a total of 4 different mitotypes namely Asia 1, Asia II 1, Asia II 5 and Asia II 7. Asia II 1 was found prevalent and distributed in 6 out of thirteen regions viz., Banswara: Rajasthan, Sri Ganganagar: Rajasthan, Amravati: Maharashtra, Hisar: Haryana, Indore: Madhya Pradesh and Bathinda: Punjab. Asia 1 mitotype detected in three agroclimatic regions namely Meerut: Uttar Pradesh, Guntur: Andhra Pradesh, Hisar: Haryana, Indore: Madhya Pradesh and Bathinda: Punjab. Asia II 5 mitotype detected in three agroclimatic regions namely Meerut: Uttar Pradesh, Guntur: Andhra Pradesh and Raipur: Chhattisgarh. The samples from Jalandhar: Punjab and Coimbatore: Tamil Nadu identified as Asia II 5 whereas samples collected from two different farmscapes within Pusa: New Delhi were grouped as Asia II 7 (Fig. 1). Colour coded pair wise identity matrix for similarity scores of mitotypes of *B. tabaci* was carried out using Sequence Demarcation tool Version 1.2 (SDTv1.2) and was represented in Fig. 2. The distribution and frequency of different mitochondrial haplotypes of *B. tabaci* cryptic species belongs to India grouped as H1 (MN830428_Meerut_Asia1; MN830436_Guntur_Asia1; MN830440_Raipur_Asia1), H2(MN830429_Banswara_Asia1), H3(MN830430_Sri_Ganganagar_Asia1; MN830431_Amaravati_Asia1; MN830432_Hisar_Asia1; MN830433_Indore_Asia1), H4(MN830437Batinda_Asia1), H5(MN830434_Jalandhar_Asia1), H6(MN830438_Coimbatore_Asia1), H7(MN830435_Delhi_Asia1; MN830439_New_Delhi_Asia1). The number of haplotypes, total number of variable sites, haplotype diversity, nucleotide diversity, average number of nucleotide differences, G+C content and total number of mutations are represented in Table 1. Nucleotide diversity observed higher in Asia II 5 (0.00388) followed by AsiaII 1 (0.00258) and in the same way, haplotype diversity observed higher in Asia II 5 (1.000) followed by AsiaII 1 (0.600).

In total, seven haplotypes were identified from 13 analyzed sequences among them; Asia II 1 observed with three haplotypes, Asia II 5 had two whereas
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Fig. 1. Phylogenetic tree of mtCOI mitotypes of *B. tabaci* including other cryptic species as out groups using maximum likelihood Approach in MEGA X. Samples from study indicated in black colored text: all other sequences obtained from GenBank

Asia I and Asia II 7 had one haplotype each. The relationship among the haplotypes of each mitotype was determined by minimum spanning network analysis (Fig. 3). The four mitotypes were diversified into seven haplotypes that were highly distant from each other. Among the three haplotypes of Asia II 1, the H3 haplotype occupied the center between H2

Fig. 2. Color coded pair wise identity matrix for similarity scores of mitotypes of *B. tabaci* (using Sequence Demarcation tool Version 1.2- SDTv1.2)

Fig. 3. Minimum spanning network from mitotypes of *B. tabaci* (using PopART-Population analysis with reticulate trees software)
and H4 in the network. Reports of mitotypes from our results coincide with previous reports in categorization of mitotypes in collected localities. Asia II 1 mitotype found in 6 out of 13 locations and proved prevalent from our study. In the same way, Asia II 1 was observed to be the most abundant mitotype with a countrywide distribution and the highest haplotype diversity, and was also found to be closely linked by its outbreak in endemic cotton leaf curl virus prone regions. One such plausible threat posed by the Asia III genetic group is that it has the ability to expand its range by displacing previously established mitotypes in cotton and other Agro ecosystems (Prabhulinga et al., 2020; Ashfaq et al., 2014; Ahmed et al., 2011). The presence of Asia III1 genetic group in a northern cotton-growing region and Asia I in a south-central cotton-growing region has been confirmed by maximum likelihood phylogenetic analysis, which was supported by previous reports on different whitefly host crops in India (Chowda-Reddy et al., 2012; Ellango et al., 2015).

Samples from two different farmscapes within Pusa: New Delhi location are grouped and detected Asia II7 and the results are in support by Hashmi et al. (2018) who previously reported that Asia II 1 and Asia II 7 are the leading genetic groups occurring in Delhi. The Asia II 7 crotic species have been reported in a majority of Asian countries (Kanakala and Ghanim, 2019). It has been found in Pakistan (Ashfaq et al., 2014; Islam et al., 2018), India (Ellango et al., 2015), Indonesia (Firdaus et al., 2013), China (Qiu et al., 2011), Malaysia (Shadmany et al., 2019) and Taiwan (Shadmany et al., 2019). As on date 13 B. tabaci genetic groups viz., Asia I, Asia I India, Asia II 1, Asia II 5, Asia II 6, Asia II 7, Asia II 8, Asia II 11, Asia II 13, Middle East Asia Minor (MEAM) 1, MEAM K, China 3 and China 7 have been recorded from India (Rehman et al., 2021; Kanakala et al., 2019; Tamilnayagan et al., 2018; Naveen et al., 2017; Ellango et al., 2015; Singh et al., 2012; Rekha et al., 2005). The successful control of whiteflies by knowing their identity, biology and biocontrol strategies has the potential to save a million tonnes of pesticides from being used in farmer fields. Population genetics of many insect species is influenced by a variety of factors, such as environmental and ecological factors, alternative host plants, natural barriers, migration and human interference. These factors also have an impact on species diversity and distribution thus, studying diversity, distribution, and changes in population structure is essential. Similar studies should be carried out by using population genetics and phylogenetic analysis of important pests like, B. tabaci.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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