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, Clustal W, distribution

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'-TTGATTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5' - TCCAATGCACTAATCTG CCATATTA-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 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_AsiaII1), H3(MN830430_Sri_Ganganagar_AsiaII1; MN830431_ Amaravati_AsiaII1; MN830432_Hisar_AsiaII1; MN830433_Indore_AsiaII1), H4(MN830437Batinda_ AsiaII1), H5(MN830434_Jalandhar_AsiaII5), H6(MN830438_Coimbatore_AsiaII5), H7(MN830435_ Delhi_AsiaII7; MN830439_New_Delhi_AsiaII7). 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 Asia II 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
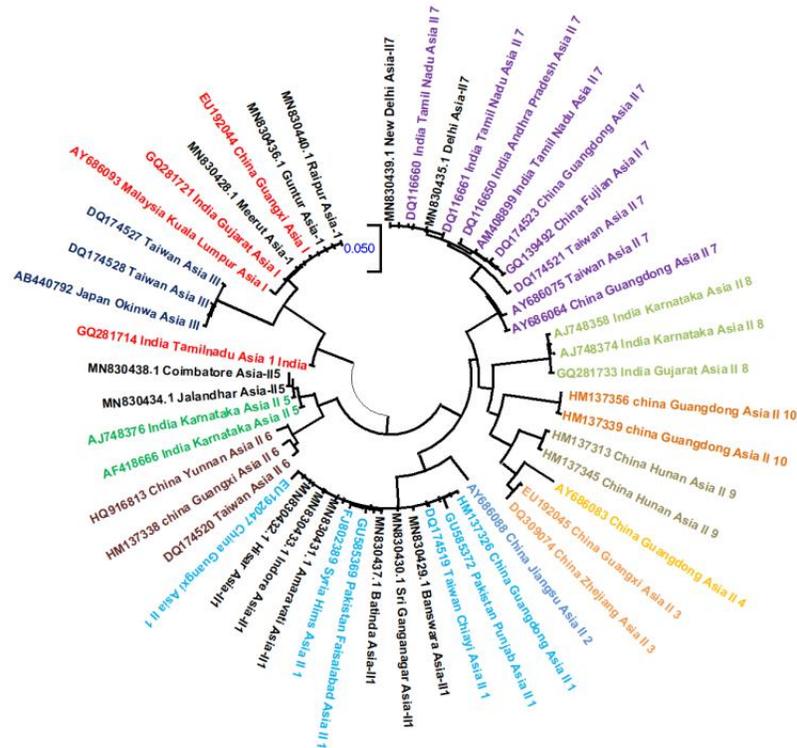
Fig. 1. Phylogenetic tree of mtCOI mitotypes of *B. tabaci* including other cryptic species as out groups using maximum likelihood Approach in MEGA X. Samples from study indicated in black colored text: all other sequences obtained from GenBank
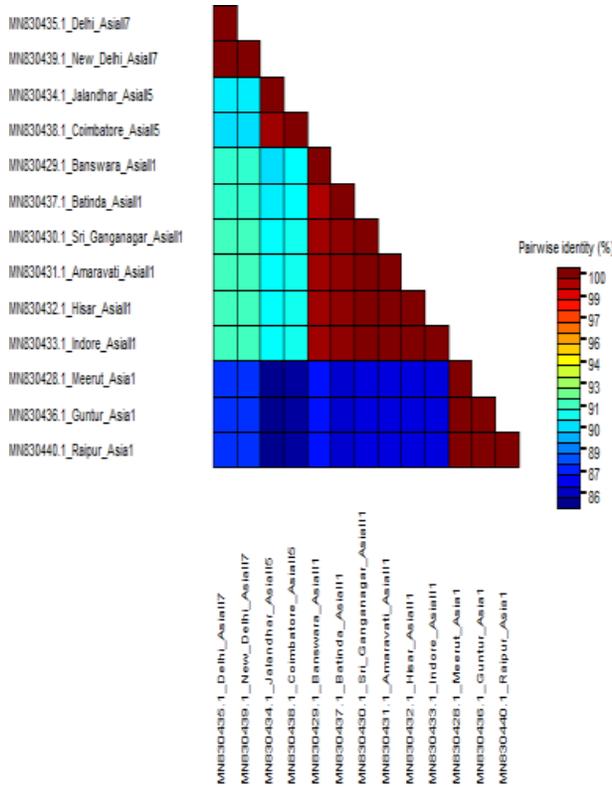

Fig. 2. Color coded pair wise identity matrix for similarity scores of mitotypes of *B. tabaci* (using Sequence Demarcation tool Version 1.2- SDTv1.2)

Asia1 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
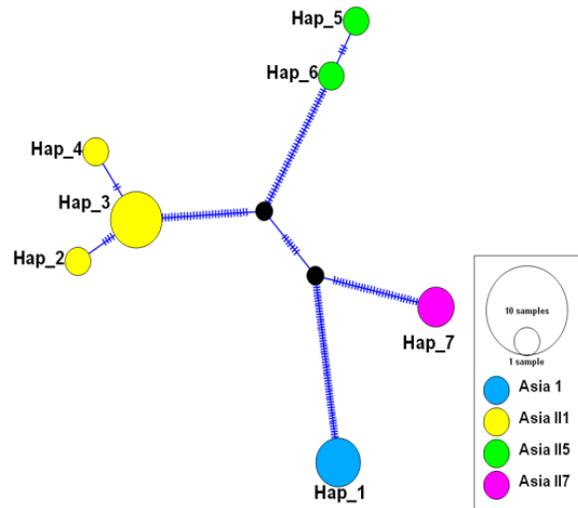

Fig. 3. Minimum spanning network from mitotypes of *B. tabaci* (using PopART-Population analysis with reticulate trees software)

Table 1. Genetic diversity analysis in mitotypes of *B. tabaci* India

Mitotypes	No. Sequences	No. haplotypes	Number of variable sites, S	Haplotype diversity	Nucleotide diversity Pi	Average number of nucleotide differences, k	G+C content	Total number of mutations, Eta:
Asia1	3	1	0	0.000	0.00000	0.000	0.316	0
AsiaII1	6	3	6	0.600	0.00258	2.000	0.339	6
AsiaII5	2	2	3	1.000	0.00388	3.000	0.348	3
AsiaII7	2	1	0	0.000	0.00000	0.000	0.335	0

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 II1 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 II1 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 cryptic 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.

REFERENCES

Ahmed M Z, De Barro P J, Greeff J M, Ren S X, Naveed M, Qiu B L. 2011. Genetic identity of the *Bemisia tabaci* species complex and association with high cotton leaf curl disease (CLCuD) incidence in Pakistan. Pest Management Science 67(3): 307-317.

Ashfaq M, Hebert P D, Mirza M S, Khan A M, Mansoor S, Shah G S, Zafar Y. 2014. DNA barcoding of *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) reveals southerly expansion of the dominant whitefly species on cotton in Pakistan. PLoS One 9(8): p.e104485.

Chowda-Reddy R V, Kirankumar M, Seal S E, Muniyappa V, Valand G B, Govindappa M R, Colvin J. 2012. *Bemisia tabaci* phylogenetic groups in India and the relative transmission efficacy of Tomato leaf curl Bangalore virus by an indigenous and an exotic population. Journal of Integrative Agriculture 11(2): 235-248.

Dinsdale A, Cook L, Riginos C, Buckley Y M, De Barro P. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Annals of the Entomological Society of America 103(2): 196-208.

Ellango R, Singh S T, Rana V S, Gayatri Priya N, Raina H, Chaubey R, Naveen N C, Mahmood R, Ramamurthy V V, Asokan R,

Rajagopal R. 2015. Distribution of *Bemisia tabaci* genetic groups in India. Environmental Entomology 44(4): 1258-1264.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Firdaus S, Vosman B, Hidayati N, Jaya Supena E D, Visser R, van Heusden A W. 2013. The *Bemisia tabaci* species complex: additions from different parts of the world. Insect Science 20(6): 723-733.

Hameed S, Hameed S, Sadia M, Malik S A. 2012. Genetic diversity analysis of *Bemisia tabaci* populations in Pakistan using RAPD markers. Electronic Journal of Biotechnology 15(6): 6-6.

Hashmi T R, Dey D, Prasad R. 2018. Distribution frequency of endosymbionts and genetic characterization of *Bemisia tabaci* (Hemiptera: Aleyrodidae) on fabaceous host plants in India. Oriental Insects 52(2): 128-142.

Islam W, Lin W, Qasim M, Islam S U, Ali H, Adnan M, Arif M, Du Z, Wu Z. 2018. A nation-wide genetic survey revealed a complex population structure of *Bemisia tabaci* in Pakistan. Acta Tropica 183: 119-125.

Jiu M, Hu J, Wang L J, Dong J F, Song Y Q, Sun, H Z. 2017. Cryptic species identification and composition of *Bemisia tabaci* (Hemiptera: Aleyrodidae) complex in Henan Province, China. Journal of Insect Science 17(3): 78.

Kanakala S, Ghanim M. 2019. Global genetic diversity and geographical distribution of *Bemisia tabaci* and its bacterial endosymbionts. PLoS One 14(3): p.e0213946.

Khamis F M, Ombura F L, Ajene I J, Akutse K S, Subramanian S, Mohamed S A, Dubois T, Tanga C M, Ekesi S. 2021. Mitogenomic analysis of diversity of key whitefly pests in Kenya and its implication to their sustainable management. Scientific reports 11(1): 1-11.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111-120.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 5(6): 1547.

Lee W, Park J, Lee G S, Lee S, Akimoto S I. 2013. Taxonomic status of the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) and reassessment of the number of its constituent species. PLoS One 8(5): p.e63817.

Librado P, Rozas J. 2009. DnaSP v5: software for comprehensive analysis of DNA polymorphism data. Bioinformatics Applications Note 25: 1451-1452.

Mugerwa H, Seal S., Wang H L, Patel, M V, Kabaalu R, Omongo C A, Alicai T, Tairo F, Ndunguru J, Sseruwagi P, Colvin J. 2018. African ancestry of New World, *Bemisia tabaci*-whitefly species. Scientific reports 8(1): 1-11.

Naveen N C, Chaubey R, Kumar D, Rebijith K B, Rajagopal R, Subramanian B, Subramanian S. 2017. Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I, Asia-II-1 and Asia-II-7 on the Indian subcontinent. Scientific Reports *7*(1): 1-15.

Prabhulinga T, Kranthi S, Raghavendra K P, Kumar R, Suke R, Chawla S, Kranthi K R. 2021. Mitochondrial COI based genetic diversity and phylogeographic structure of whitefly *Bemisia tabaci* (Gennadius) on cotton in India. International Journal of Tropical Insect Science 41: 1543-1554.

Prasanna H C, Kanakala S, Archana K, Jyothsna P, Varma R K, Malathi V G. 2015. Cryptic species composition and genetic diversity within *Bemisia tabaci* complex in soybean in India revealed by mtCOI DNA sequence. Journal of Integrative Agriculture 14(9): 1786-1795.

Qiu B L, Dang F, Li S J, Ahmed M Z, Jin F L, Ren S X, Cuthbertson A G. 2011. Comparison of biological parameters between the invasive B biotype and a new defined Cv biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in China. Journal of Pest Science 84 (4): 419-427.

Rehman M, Chakraborty P, Tanti B, Mandal B, Ghosh A. 2021. Occurrence of a new cryptic species of *Bemisia tabaci* (Hemiptera: Aleyrodidae): an updated record of cryptic diversity in India. Phytoparasitica 49: 869-882.

Rekha A R, Maruthi M N, Muniyappa V, Colvin J. 2005. Occurrence of three genotypic clusters of *Bemisia tabaci* and the rapid spread of the B biotype in south India. Entomologia Experimentalis et Applicata 117(3): 221-233.

Shadmany M, Boykin L M, Muhamad R, Omar D. 2019. Genetic diversity of *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex across Malaysia. Journal of Economic Entomology 112(1): 75-84.

Singh S T, Priya N G, Kumar J, Rana V S, Ellango R, Joshi A, Priyadarshini G, Asokan R, Rajagopal R. 2012. Diversity and phylogenetic analysis of endosymbiotic bacteria from field caught *Bemisia tabaci* from different locations of North India based on 16S rDNA library screening. Infection, Genetics and Evolution 12(2): 411-419.

Tamilnayagan T, Suganthy M, Renukadevi P, Malathi V G. 2018. First report of Asia II 6 cryptic species from India and Asia I cryptic species of whiteflies, *Bemisia tabaci* (Gennadius), in ash gourd. Unpublished conference paper. https://www. Research gate Net publication/323004766.