Genetic Diversity of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Ash Gourd in India[1]

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Abstract *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a species complex that is one of the most devastating agricultural pests worldwide and affects a broad range of food, fiber, and ornamental crops. It is multivoltine and polyphagous, and vectors economically important plant viruses including those belonging to the family *Geminiviridae*. Therefore, understanding genetic variation among vector populations is important for its management. In order to gain insight into whitefly genotypes occurring on ash gourd, *Benincasa hispida* (Thunberg) Cogniaux, whitefly samples were collected from 18 locations in Tamil Nadu, and their mitochondrial cytochrome oxidase subunit I (mtCOI) genes were sequenced. Sequences generated in this study, when compared with sequences from the mtCOI data set, revealed that the whitefly populations TN1 Ja, TN2 Po, TN3 Ud, TN4 Ma, TN5 Ga, TN6 Co, TN9 Td, TN10 Gp, and TN11 El belong to Asia I genotype. Contrastingly, the populations TN7 On and TN8 Er were identified as Asia II 6 genotype. Interestingly, more than one genetic group was found coexisting in the same field.

Key Words *Bemisia tabaci*, mtCOI, cryptic species, ash gourd

Ash gourd, *Benincasa hispida* (Thunberg) Cogniaux (Cucurbitaceae), is widely cultivated throughout humid tropical and subtropical climates and is used as a vegetable in India and China. It is an excellent source of vitamin B1 (thiamine), a good source of vitamin B3 (niacin) and vitamin C, and also rich in many minerals like calcium. Its high potassium content makes this a preferred vegetable for maintaining a nutrient-rich diet.

The whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), is an agricultural pest in tropical and subtropical regions and has colonized plants in the greenhouses of semitemperate countries. *Bemisia tabaci* has increased in importance globally since the 1990s, as a serious pest of vegetable, fiber, and root crops (Duffus 1987, Otim-Nape et al. 1997, Polston and Anderson 1997, Varma and Malathi 2003). In addition to direct damage, it also vectors a number of plant viruses belonging to the family *Geminiviridae* (de Barro et al. 2011, Dinsdale et al. 2010). The whitefly was first collected and described *as Aleyrodes tabaci*

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(Gennadius) from tobacco, *Nicotiana tabacum* L., in Greece in 1889. It was subsequently renamed (Russell 1957) as *B. tabaci* and was found across the globe in the United States, Africa, Middle East, the Orient, Russia, China, Southeast Asia, and South America (Brown et al. 1995). Its geographical diversity and broad host range gave rise to several common names associated with host plants such as sweet potato whitefly, cotton whitefly, etc. Though whiteflies damage the crop through direct feeding, their major impact on agriculture is through the transmission of plant viruses (Brown et al. 1995). This problem is exacerbated by its polyphagous nature. It has a broad host range, with more than 600 different host plant species that include representatives from Solanaceae, Cucurbitaceae, Malvaceae, Fabaceae, Euphorbiaceae, and Asteraceae (Brown et al. 1995, Oliveira et al. 2001). Different populations of *B. tabaci* are morphologically indistinguishable but display distinctive biological, physiological, and genetic variation, and thus are deemed a cryptic species complex (Boykin et al. 2007, 2012; de Barro et al. 2011; Dinsdale et al. 2010; Tay et al. 2012). The *B. tabaci* complex consists of cryptic species that need to be differentiated and identified. As these cryptic species are morphologically indistinguishable, various molecular markers have been employed such as RAPD PCR (Gawel and Bartlett 1993, de Barro and Driver 1997), AFLP (Cervera et al. 2000), mitochondrial cytochrome oxidase gene subunit I (mtCOI) (Frohlich et al. 1999, Brown et al. 2000), and ribosomal ITS1 nucleotide sequence (de Barro et al. 2000). The most widely accepted method is differentiation on the basis of nucleotide sequence of mtCOI. Using mtCOI-based Bayesian phylogenetic analysis, Dinsdale et al. (2010) and de Barro et al. (2011) proposed a speciation system keeping 3.5% sequence divergence as threshold. Following these criteria, 34 species have been delimited at global level (Boykin 2014; Boykin et al. 2012, 2013). Whether these cryptic species could be differentiated on the basis of mating behavior, insecticide resistance, and transmission characteristics is being studied. In the present study, mtCOI gene nucleotide sequence was used to determine the genetic affiliation of *B. tabaci* populations (de Barro et al. 2011) occurring on ash gourd in Tamil Nadu, India.

Materials and Methods

Sample collection. Adults of *B. tabaci* were collected from 18 different locations covering four districts during a field survey conducted in major ash gourd–growing belts of Tamil Nadu. At each location, individual insect samples were collected in separate Eppendorf tubes containing 70% ethanol and stored in a freezer at −80°C until used.

DNA isolation. To extract the DNA from a single whitefly, the lysis method of DNA extraction was followed as per Zeidan and Czosnek (1991). A petri dish lid was covered with aluminum foil and Parafilm one on another. Then 5 µl of lysis buffer (5 µl of Tris HCl, 1 µl of EDTA, 5 µl of Triton X 100, 50 µl of proteinase K 20 mg/ml, and 939 µl of ddH_2O) was spotted on the surface of the lid covered with Parafilm. A single whitefly was placed on the buffer spot using a brush and crushed with the edge of polymerase chain reaction (PCR) tube. After crushing, the entire content was transferred into fresh PCR collection tube. In addition to this, the surface of the PCR tube that was used to crush the whitefly was washed with 35 µl

of lysis buffer. The entire content was kept on ice for 5 min and incubated at 65°C for 15 min followed by 95°C for 10 min and placed immediately on ice. The contents were spun for 5 s, and the supernatant was processed for PCR.

mtCOI subunit I amplification. The mtCOI gene sequence was analyzed for whitefly samples collected from 18 locations for genetic identification. Approximately 800 bp of the mtCOI gene fragment was amplified using LCO1 1490 forward primer 5′ GGTCAACAAATCATAAAGATATTGG 3′ and HCO1 2198 reverse primer 5′ TAAACTTCAGGGTGACCAAAAAATCA 3′ (Folmer et al. 1994). The 25-μl reaction volume containing 12.5 μl of 2× smART master mix, Cat. No. 280311 (ready-made mix of Taq polymerase, dNTPs, and PCR buffer), 5 μl of template DNA (approximately 50 ng), 3.5 μl of sterile distilled water, and 2 μl of each forward and reverse primer (15 pg each). The PCR was performed with initial denaturation at 94°C for 3 min, followed by 40 cycles each consisting of denaturation for 30 s at 94°C, annealing for 40 s at 53°C with final extension for 1 min at 72°C, followed by final extension for 20 min at 72°C. The PCR products were gel purified and sequenced availing the commercial facility.

Sequence analysis. All mtCOI sequences corresponding to 34 different genetic groups of *B. tabaci* were downloaded from the National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/Blast.cgi). Sequence alignment was performed employing MUSCLE implemented in Seaview (Thompson et al. 1994). Genetic divergence was calculated employing MEGA 7 using ClustalW (Tamura et al. 2013). The mtCOI DNA sequences generated in this study were submitted to the NCBI database.

Results

Genetic identification based on mtCOI. The genotype of the whitefly population collected from ash gourd in different locations of Tamil Nadu was identified by applying the taxonomic criteria of identity in the mtCOI. Of the 18 populations in the study, PCR amplicons were generated for 14 isolates using LCO1 and HCO1 primers. Amplicons of 800 bp were obtained for all the isolates that were sequenced (Fig. 1A, B). After confirming the whitefly origin of the sequence in the BLAST (Basic Local Alignment Search Tool) search, the sequences were trimmed and aligned with the sequences available for 34 genotypes of whitefly in the database (https://www.ncbi.nlm.nih.gov/Blast.cgi). The details on the sequences used in the analysis to identify the species are given in Table 2. When the sequences of about 605 bp (the maximum sequence which aligned) were compared, the whitefly populations TN1 Ja, TN2 Po, TN3 Ud, TN4 Ma, TN5 Ga, TN6 Co, TN9 Td, TN10 Gp, and TN11 El showed 99% identity with the Asia I genotype. The isolate TN12 Ap was slightly different though exhibited 99% identity with the Asia I genotype. Since all these whitefly populations revealed 99% identity with Asia I and the divergence being less than 3.5%, the threshold value kept for demarcation of the species (de Barro et al. 2011), these whitefly populations were recognized as belonging to the genotype Asia I. Contrastingly, the populations TN7 On and TN8 Er exhibited 92–99% identity with the Asia II 6 genotype. Again, according to de Barro et al. (2011), TN7 On and TN8 Er were recognized as belonging to Asia II 6 genotype (Table 3).

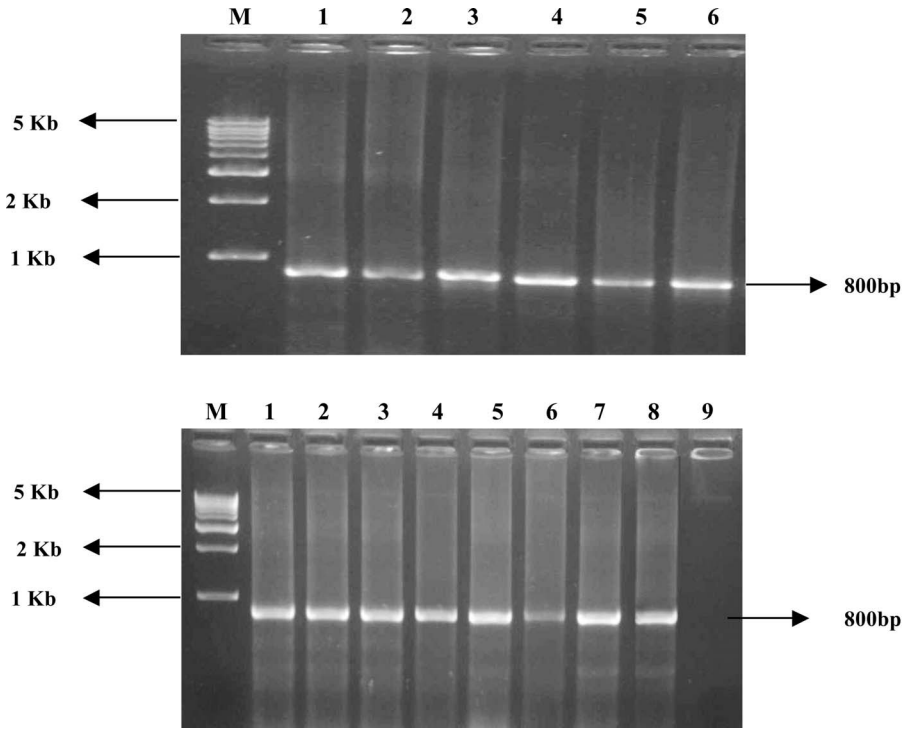

Fig. 1. Agarose gel electrophoresis of polymerase chain reaction amplicon generated using LCO1 and HCO1 primers in whiteflies samples collected from different locations. (A) Amplicon obtained from the DNA extracted from single whitefly collected from ash gourd from different locations: Lane 1, Jakkarpalayam; Lane 2, Pollachi; Lane 3, Udumalpet; Lane 4, Mangarai; Lane 5, Ganapathypalayam; Lane 6, Ongur; Lane M, 1-kb ladder. (B) Amplicon obtained from the DNA extracted from single whitefly collected from ash gourd from different locations: Lane 1, Coimbatore; Lane 2, Eraiyur; Lane 3, Tindivanam; Lane 4, Govindhapuram; Lane 5, Ellaigramam; Lane 6, Pillur; Lane 7, Andapattu; Lane 8, Kuvadur; Lane M, 1-kb ladder.

Phylogenetic analysis. Grouping of the whitefly populations collected in the present study with Asia I genotype is further supported by the phylogenetic relationship analysis. A phylogenetic tree constructed on the basis of mtCOI nucleotide sequence (Fig. 2) clearly shows that the populations TN 14 Th, TN13 Cu, TN11 Eg, TN4 Ma, TN1 Ja, TN6 Co, TN2 Po, TN10 Gp, TN 3 Ud, TN5 Ga, and TN9 Td clustered with Asia I genotype. Although the population TN12 Ap belonged to Asia I, it branched off from Asia I due to substitutions in nucleotide sequence. In the tree, the two populations TN 7 On and TN8 Er grouped with the genotype Asia II 6. It is noteworthy that the Asia II 6 genotype has not yet been recorded in India. Presence of both genotypes within proximity of 2.8 km in the ash gourd fields

Table 1. Diversity of whitefly genotypes reported from different host plants in India.

Sequence No.	Host Plant—Scientific Name	Whitefly Genotypes	References
1.	Brinjal—*Solanum melongena* L. Soybean—*Glycine max* (L.) Merrill Cotton—*Gossypium hirsutum* L. Cucumber—*Cucumis sativus* L. Watermelon—*Citrullus lanatus* Thunberg Bottle gourd—*Lagenaria siceraria* (Molina) Standley Sponge gourd—*Luffa acutangula* (L.) Roxburgh Pumpkin—*Cucurbita pepo* L. Musk melon—*Cucumis melo* L. Bhendi—*Abelmoschus esculentus* (L.) Moench Tomato—*Solanum lycopersicum* L. Chili—*Capsicum annuum* L. Tobacco—*Nicotiana tabacum* L. Sweet potato—*Manihot esculenta* Crantz Pointed gourd—*Trichosanthes dioica* Roxburgh Mung bean—*Vigna radiata* (L.) R. Wilczek Cabbage—*Brassica oleracea* L.	Asia I Asia II 1 Asia II 2 Asia II 5 Asia II 7 Asia II 8 MEAM 1 China 3	Chowda-Reddy et al. (2012) Ellango et al. (2015) Prasanna et al. (2015) Rekha et al. (2005)

indicates the presence of mixed population of whitefly genotypes in Tamil Nadu, even within limited geographic areas (Table 3).

Discussion

The status of *B. tabaci* as a pest dates to 1889, but it received greater attention only from the 1980s as severe crop losses occurred in Israel and the United States. *Bemisia tabaci* is responsible for huge crop losses in both countries, and high levels of insecticide resistance and induced physiological changes in squash (Cucurbitaceae), named silver leafing, were observed. This population of whitefly showed an esterase pattern different from the population occurring in the United States. To differentiate these two populations, the indigenous population was designated as "A" and the one that caused severe damage as "B." Since then, numerous studies were conducted to identify variants within and among whitefly populations. It is now accepted that *B. tabaci* consists of cryptic species that need to be differentiated and identified. Using mtCOI sequences, efforts have been made in India to identify the

Table 2. GenBank accession numbers of whitefly genotype sequences retrieved from database for comparison.

Sequence No.	Genotype	GenBank Accession No.	Sequence No.	Genotype	GenBank Accession No.
1	Asia I	AJ 748359	18	China 2	AY686072
2	Asia II 1	AJ 867557	19	China 3	EU 109050
3	Asia II 2	AJ 51006	20	Indian Ocean	AJ 550171
4	Asia II 3	AY 686088	21	Mediterranean	GU 086329
5	Asia II 4	DQ 309075	22	Italy	AY 827596
6	Asia II 5	AY 686083	23	Middle East 1	AJ 748368
7	Asia II 6	AJ 748376	24	Middle East 2	AJ 550177
8	Asia II 7	AJ 784261	25	New World 1	DQ 130060
9	Asia II 8	DQ 174523	26	New World 2	AF 340212
10	Asia II 9	DQ 116662	27	South America 1	AY837591
11	Asia II 10	AJ 748358	28	South America 2	GU086361
12	Asia II 11	HM 137345	29	South America 3	AF 344257
13	Asia II 12	HM 137356	30	South America 4	AF 344245
14	Asia III	DQ 174527	31	Uganda	AF 418665
15	Australia	GU 086328	32	*Bemisia atriplex*	GU 086362
16	Australia–Indonesia	HQ 457045	33	*Bemisia atriplex*	GU 220055
17	China 1	AY 686085	34	*Bemisia* subspecies	GU 220056

species of whitefly population (Ramappa et al. 1998). The diversity of whitefly genotypes, that is, Asia I, Asia II, Asia II 2, Asia II 5, Asia II 7, Asia II 8, MEAM 1, and China 3, is reported from different host plants in India (Table 1). It is interesting to note that so far Asia II 6 has not been reported in India, which has been recorded in the present study in two locations at Ongur and Eraiyur (TN7 On and TN8 Er; Table 3). Whether Asia II 6 is confined only to these two locations or is present throughout South India needs to be further investigated based on the record of whitefly populations in different crops in India, limited information is available on the cryptic species of whiteflies on cucurbit hosts. Prasanna et al. (2015) recorded Asia II 7 in cucumber, *Cucumis sativus* L., in Madhyapradesh, and Ellango et al. (2015) observed Asia II 1 in cucurbits in Jammu and Kashmir region. Roopa et al. (2015) recorded the whitefly cryptic species Asia I, Asia II 7, and Asia II 8 in the cucurbit crops, for example, *Cucumis sativus*, *Citrullus lanatus* (Thunberg) Matsumura and

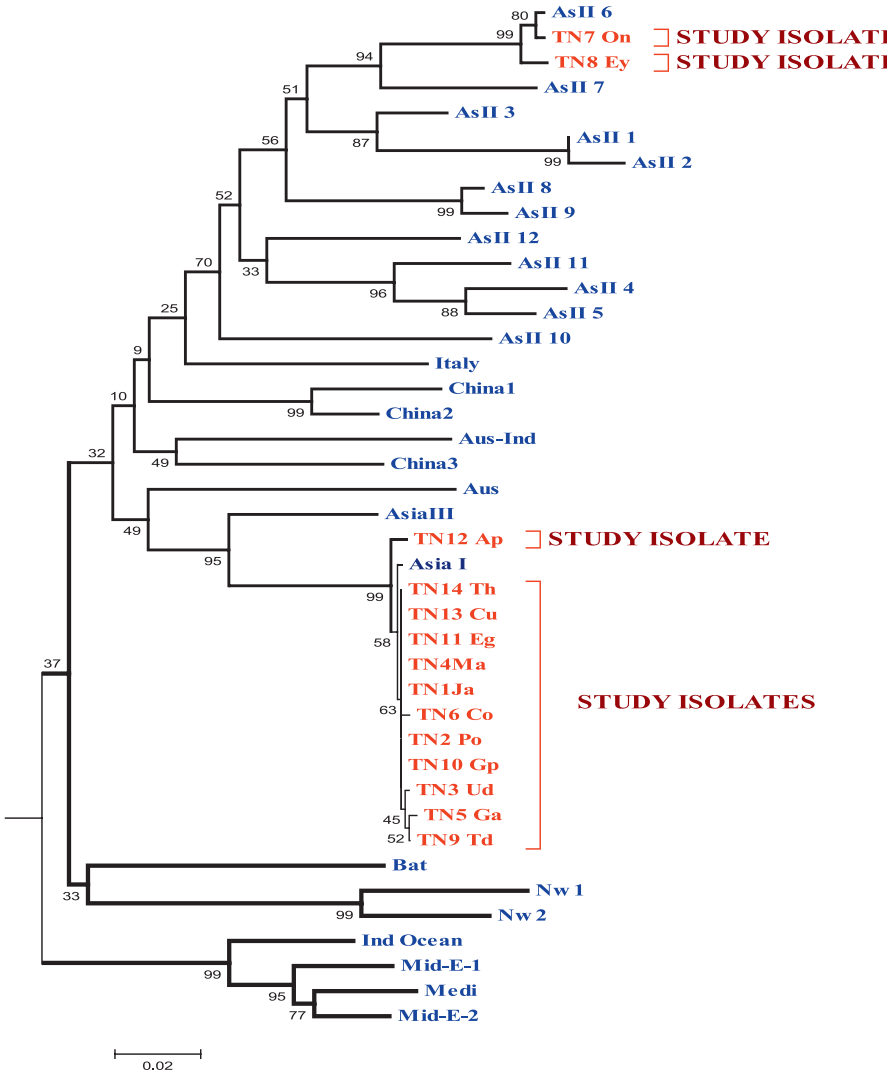

Fig. 2. Phylogenetic dendrogram based on alignment of partial nucleotide sequences of mitochondrial cytochrome oxidase subunit I (mtCOI) of *Bemisia tabaci* genotypes. Phylogenetic tree generated from aligned partial mtCOI nucleotide sequences of *Bemisia tabaci* genotypes with other selected whitefly genotypes. Tree was generated by neighbor-joining method by aligning the sequences in MEGA 7 using ClustalW. Vertical branches are arbitrary; horizontal branches are proportional to calculated mutation distances; values at nodes indicate percentage bootstrap values (1,000 replicates).

Table 3. Diversity of whitefly genotypes in ash gourd in Tamil Nadu.*

Sequence No.	Location	GIS Coordinates	Sample I.D.	Whitefly Biotype	GenBank Accession No.
1	Jakkarpalayam	N11°47′15.76″ E77°08′28.77″	TN1 Ja	Asia I	KX361119
2	Pollachi	N11°47′15.76″ E77°08′28.77″	TN2 Po	Asia I	KX361120
3	Udumalpet	N10°35′04.45″ E77°15′05.20″	TN3 Ud	Asia I	KX361121
4	Mangarai	N12°15′12.23″ E78°11′51.08″	TN4 Ma	Asia I	KX361124
5	Ganapathypalayam	N11°01′55.95″ E77°19′46.45″	TN5 Ga	Asia I	KX361126
6	Coimbatore	N9°11′28.71″ E77°52′49.10″	TN6 Co	Asia I	KX361125
7	Ongur	N12°19′37.78″ E79°46′40.21″	TN7 On	Asia II 6	KX361130
8	Eraiyur	N11°46′50.94″ E79°11′51.9″	TN8 Er	Asia II 6	KX361131
9	Tindivanam	N12°14′12.78″ E79°38′59.82″	TN9 Td	Asia I	KX361127
10	Govindhapuram	N11°39.28′96″ E79°14′42.13″	TN10 Gp	Asia I	KX361128
11	Ellaigramam	N12°15′15.17″ E79°45′14.43″	TN11 Eg	Asia I	KX361129
12	Andapattu	N12°15′15.17″ E79°45′14.43″	TN12 Ap	Asia I	KX361132
13	Thondamuthur	N10°57′73.29″ E77°04′81.09″	TN13Cu	Asia I	KX361122
14	Coimbatore	N9°11′28.71″ E77°52′49.10″	TN14 Th	Asia I	KX361123

* GIS, geographic information system; I.D., identification.

Nakai, *Lagenaria siceraria* (Molina) Standl, *Luffa acutangula* (L.) Roxburgh, *Cucurbita pepo* L., and *Cucumis melo* L., in Karnataka. It is surprising that, in contrast to the above observations, the Asia II 6 population of whiteflies was recorded on ash gourd in the present study for the first time in India. The transmission characteristics and insecticide resistance properties of this population need to be studied to contain the active spread of the disease vectored by this whitefly population.

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