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Source: Florida Entomologist, 96(1) : 280-282

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.096.0147>

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FIRST REPORT OF *BEMISIA TABACI*
(HEMIPTERA: ALEYRODIDAE) BIOTYPE Q IN MALAYSIA

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Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is one of the most destructive pests of mainly vegetables and ornamental crops around the world. Certain qualities of this pest including but not limited to its vast host range, polyvoltinism and short generation time, cosmopolitan nature, ability to transmit important plant viruses and capability of quickly developing resistance against almost all groups of insecticides have contributed to its enormous damage potential.

Bemisia tabaci is a cryptic species complex with at least 32 species having been discovered so far based on the 3.5 % divergence limit of the partial mitochondrial cytochrome oxidase subunit 1 (mtCO1) sequence (Dinsdale et al. 2010; Hu et al. 2011; Alemandri et al. 2012; Chowda-Reddy et al. 2012; Esterhuizen et al. 2012; Parrella et al. 2012). Previously, members of this complex were regarded as biotypes. However, based on our current knowledge of divergence limit and mating interactions, application of the term, species, to members of this complex is accepted (De Barro et al. 2011). Nevertheless, for the purpose of connecting this study to the previous literature the term biotype is retained. Among all biotypes, biotypes B and Q are the most invasive, and the huge losses caused by this pest are almost always associated with these biotypes. Invasive biotypes have usually shown superiorities over their endemic counterparts in qualities like virus transmission efficiency (Li et al. 2010), life history traits (DeLatte et al. 2009), and ability to resist insecticides (Luan et al. 2012). In many cases upon introduction to a region, they establish and partially or completely displace indigenous biotypes. For instance, Q biotype was introduced into China in 2003 and since then it spread to other parts of the country and displaced non-Q biotypes in most regions (Pan et al. 2011; Rao et al. 2011). Both invasive biotypes have already invaded and

continue to invade many other countries around the world. Timely identification of these biotypes can help prevent or reduce huge economic losses. In fact, once they are identified it might be possible to slow down their further spread and also modify management plans to effectively control them. Invasion of this pest into Malaysia was suspected (Syed et al. 2000); however no studies had tested this probability thus far. This study aims to assess probable introduction of the invasive *B. tabaci* biotypes in Malaysia by examining biotype status of some populations using mtCO1 genetic markers (Frohlich et al. 1999).

In this study, samples were collected from important vegetable and flower producing areas. Sampling was conducted in Pahang, Peninsular Malaysia and Sabah East Malaysia. All samples came from highlands where the crops were cultured under a protected agriculture system (Table 1). Whiteflies collected using mouth aspirators were transferred to 95% ethanol and stored in -20 °C to preserve their quality for further analysis (Hsieh et al. 2006).

With the exception of population 2, at least 3 individual whiteflies from each population were processed for biotype identification (Chu et al. 2006). Genomic DNA was extracted from a single adult whitefly using the ANDE™ Insect Kit (Xytogen, Perth, Australia) (Castalanelli et al. 2010). Partial mtCO1 gene was amplified by polymerase chain reaction (PCR) using primers C1-J-2195 (5'-TTGATTTTTTGGTCATC-CAGAAGT-3'), and L2-N-3014 (5'-TCCAATG-CACTAATCTGCCATATTA-3') (Frohlich et al. 1999). The reaction volume was set to 25 µL, which consisted of .06 µM of each primer, 12.5 µL DreamTaq™ Green PCR Master Mix (2X) (Fermentas, ThermoFisher Scientific, Waltham, Massachusetts, USA), 7.5 µL PCR-grade water, and 2 µL of template DNA. The PCR reaction

TABLE 1. LOCATIONS, HOSTS, WHEN COLLECTED, AND GENBANK ACCESSION NUMBERS OF POPULATIONS SAMPLED IN THIS STUDY.

Population	Location	Host	When Collected	GenBank accession number
1	Cameron Highlands, Pahang	Ornamental chili	11 Jul 2011	JX393088
2	Cameron Highlands, Pahang	Poinsettia	11 Jul 2011	JX393092
3	Cameron Highlands, Pahang	Hibiscus	11 Jul 2011	JX393093
4	Kundasang, Sabah	Poinsettia	19 Sep 2011	JX393096
5	Kundasang, Sabah	Ornamental chili	19 Sep 2011	JX393098
6	Kundasang, Sabah	Tomato	19 Sep 2011	JX393102

program was started at 94 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 52 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 5 min (Hsieh et al. 2006). PCR products (≈800 bps) were run on 1.5% agarose gel and ethidium bromide-stained bands were visualized under UV light. PCR products were purified using MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology, South Korea). Unidirectional sequencing with either C1-J-2195 or L2-N-3014 primers was performed on Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, California, USA) using BigDye® Terminator Cycle Sequencing Kits (Life Technologies).

Sequences were checked for the presence of stop codons in the coding region and those with stop codons interrupting the open reading frame were discarded. In addition, sequence containing gaps and/or > 1% ambiguous bases were not included in the analysis. Sequences of this study together with some consensus sequences of the Dinsdale et al. (2010) study were aligned manually in BioEdit (Hall 1999). *Bemisia afer* was selected as an out-group. Phylogenetic analysis of aligned sequences was done using MEGA4 (Tamura et al. 2007). The method of neighbor-joining (NJ) with the Kimura two-parameter model (Kimura 1980) was selected to build the phylogenetic tree. To assess the robustness of the tree 1000 bootstrap replicates were selected.

Phylogenetic tree for sequences of this study is shown in Fig. 1. As the figure indicates all sampled individuals from Malaysia belong to Mediterranean genetic group (Q biotype), and their position in the tree is supported with a high bootstrap value. To our knowledge there has been no comprehensive study of the biotype status of this pest in Malaysia. However, some studies with very limited number of samples have identified only the Asia I genetic group in this country so far (Boykin et al. 2007; De Barro et al. 2008). Thus, the invasive Q biotype has been found in this study for the first time.

The invasion of Q biotype to Malaysia is of great concern especially because of its reputation for developing high levels of insecticide resistance. The insecticide resistance problem in *B. tabaci* is further complicated as the control of the pest is achieved mainly by insecticides. In fact, recent cases of strong resistance to pyriproxyfen were associated with the Q rather than the B biotype. Moreover, more cases of resistance to neonicotinoids have been recorded for the Q biotype than for the B biotype (Horowitz et al. 2011).

Another serious concern with the invasive biotypes relates to the possibility of serious virus disease outbreaks with huge economic losses following their invasion and establishment. This phenomenon has happened many times at different geographical locations (Varma & Malathi 2003; Li et al. 2004; McKenzie et al. 2004). Finding of this noxious pest in the Cameron Highlands and Kundasang

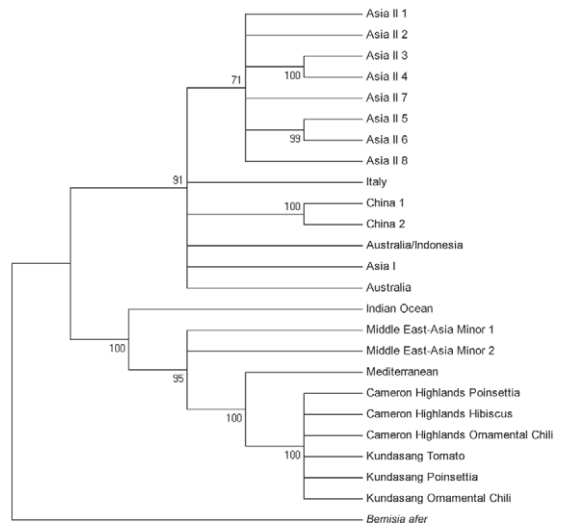


Fig. 1. Condensed neighbor joining tree of *B. tabaci* biotypes from Malaysia and around the world (cutoff value is 65%).

may have critical consequences as both regions are important vegetable and flower producing centers in the country, Cameron Highlands ranks as the second most important vegetable growing region in Malaysia (Mazlan & Mumford 2005). The invasive Q biotype upon introduction can spread rapidly (Grille et al. 2011), and may eventually partially or completely displace benign endemic biotypes of a region. Currently we are not aware of the breadth and extent of the Q biotype invasion in Malaysia and it is highly recommended that further studies be done to delimit its distribution. If the Q biotype is still in an early stage of invasion, then by using appropriate quarantine measures, its further spread into other states may be hindered or delayed. Finally, the early development of emergency management plans must be sought if huge economic losses are to be avoided.

SUMMARY

The invasive Q biotype of *Bemisia tabaci* has been found for the first time in 2 highland regions in Peninsular and East Malaysia, respectively. This invasive pest can adversely affect vegetable and ornamental production industry in the country. Further studies are recommended to more comprehensively delimit its presence in Malaysia.

Key Words: mitochondrial cytochrome oxidase subunit 1, PCR, phylogenetic analysis, competitive displacement, invasive species

ACKNOWLEDGMENTS

We express appreciation to Mr. Abu Zarim Bin Ujang from MARDI Cameron Highlands and Mr. Jamaludin

Bin Jaya from MARDI Negeri Sabah for their assistance in sampling. This work was funded under RUGS (Research University Grant Scheme) with vote number 9199727 from Universiti Putra Malaysia.

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