Sequence Analysis of Mitochondrial Cytochrome Oxidase 1 from *Bemisia tabaci* (Hemiptera: Aleyrodidae) Populations in Iran[1]

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J. Entomol. Sci. 56(3): 366–373 (July 2021)

Abstract *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) has been phylogenetically divided into several putative species based on nucleotide sequence of mitochondrial cytochrome oxidase 1 (mtCOI). To confirm the putative species among *B. tabaci* populations in Iran, insects were collected from different regions and plant hosts in Khuzestan province of southwestern Iran, and their mtCOI sequences were determined. DNA polymorphism and phylogenetic analysis of the 10 mtCOI sequences obtained showed four haplotypes among the specimens from Khuzestan province that were identified as the putative species MEAM1-subcladeB. Moreover, specimens previously collected from Iran were found to be members of the MEAM1-subcladeB2. Twelve variable sites were detected throughout the Iran-originated mtCOI sequences contributing to their position on the phylogenetic tree. This is the first study reporting the putative species MEAM1-subcladeB from southwestern Iran.

Key Words whitefly, phylogenetic analysis, putative species, DNA polymorphism

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) is a devastating phloem-feeding pest of many crops (Basu et al. 2019) that transmits several plant viruses (Gilbertson et al. 2015, Pinheiro-Lima et al. 2020). Based on the mitochondrial cytochrome oxidase 1 (mtCO1) sequence, 39–42 cryptic (putative) species have been described within the species (Kanakala and Ghanim 2019). In Iran, it has been showed that the putative species of Africa/Middle East/Asia Minor group and the Middle East/Asia Minor 1 subgroup is the dominant species (Shahbazi et al. 2014). However, there is no information on the putative species within Khuzestan province of southwestern Iran. The objective of this study was to determine the putative species among *B. tabaci* populations from the region. Moreover, the mtCOI sequences of Iranian specimens were subjected to DNA polymorphism and phylogenetic analysis.

Materials and Methods

Bemisia tabaci adults were collected from tobacco, tomato, cucumber, and cowpea fields in six counties of Khuzestan province during 2019–2020 (Table 1).

[1]Received 1 January 2021; accepted for publication 31 January 2021.
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Table 1. *Bemisia tabaci* specimens collected from different regions in Khuzestan province, southwestern Iran.

		Geographical Coordinates		
Sample ID	**City**	**Longitude (λ)**	**Latitude (φ)**	**Host**
MOL1	Mollasani	48.8648334	31.6242601	Cowpea
MOL2		48.8697864	31.5428604	
SHT1	Shushtar	48.8060657	31.9958553	Cucumber
SHT2		48.8502399	32.0788974	
GOT	Gotvand	48.7745395	32.2224402	Cucumber
SHS1	Shush	48.2489124	32.1363965	Tomato
SHS2		48.2695117	32.1061566	
HAM	Hamidieh	48.4394565	31.4889089	Tomato
BEH1	Behbahan	50.3503403	30.5937206	Tobacco
BEH2		50.1409135	30.6987066	

Specimens were preserved individually in microtubes containing 70% alcohol and stored at −20°C. At each sampling, 20–30 whiteflies representing 10 distinct populations were collected. Morphologic characters of pupae from each population were used to confirm species identity (Hodges and Evans 2005).

Total DNA was extracted from the specimens using the Animal DNA Isolation Kit (Denazist, Iran) and stored at −20°C. Polymerase chain reaction (PCR) amplification was performed according to Masood et al. (2017). PCR products were then sequenced at BIONEER Corp. (Daejeon, South Korea).

Sequences were primarily checked using Chromas (ver. 2.6.6) (Technelysium, South Brisbane, Australia) software, and a complete sequence was obtained by assembling the reads of 3′ and 5′ termini using SeqMan Pro software (DNASTAR Lasergene, ver. 8). Nuclear mitochondrial sequences (NUMTs) and PCR-derived pseudogenes were excluded according to Kunz et al. (2019). BLASTn was performed, and the confirmed sequences were then submitted to GenBank under the accession numbers presented in Table 2. DNA polymorphism and haplotype analysis were performed using DnaSP software (ver. 6). Haplotype diversity and sampling variance were conducted according to Nei (1987). Nucleotide diversity (π), mean nucleotide differences per site, sampling variance, and standard deviation of the sequences were determined. Theta (θ) per site from π, S, and η and mean the number of nucleotide differences (k) were also calculated. Diallelic model was applied to analyze insertion/deletion (InDel) polymorphism among the sequences, and possible InDel haplotypes were investigated.

Fifty-six sequences of mtCOI, including 4 sequences obtained here and 52 sequences from GenBank, were used to perform a phylogenetic analysis. The sequences were aligned by CLC Main Workbench software (ver. 7.6.2), and the

Table 2. Mitochondrial cytochrome oxidase 1 sequences of *B. tabaci* specimens from different provinces in Iran.

Isolate	GenBank Accession Number	Province	Host	Reference
1	EU547768	Hormozgan	Tomato	Shoorcheh et al. (2008)
5	EU547769	Kerman	Cucumber	
10	EU547770	Khorasan	Cucumber	
15	EU547771	Yazd	Squash	
20	EU547772	Kerman	Eggplant	
FSA13	JN542543	Fars	Cotton	Shahbazi et al. (2014)
FSA12	JN559742			
FSA2	JN559743			
FSA2	JN559744			
FSA6	JN559745			
FSA8	JN559746			
FSA9	JN559747			
FSA1	JN559748			
KAF10	JN559749		Pepper	
KAF4	JN559750			
KAF5	JN559751			
MOB	JN559753		Cotton	
Iran1	MT038431	Khorasan		Alimirzaee and Karimi (unpubl.)
BEH1_rev2	MW161484	Khuzestan	Tobacco	This study
BEH2_rev2	MW161485			
GOT_rev2	MW161486		Cucumber	
HAM_rev2	MW161487		Tomato	
MOL1_rev2	MW161488		Cowpea	
MOL2_rev2	MW161489			
SHS1_rev2	MW161490		Tomato	
SHS2_rev2	MW161491			
SHT1_rev2	MW161492		Cucumber	
SHT2_rev2	MW161493			

maximum likelihood algorithm using the general time reversible model test with 100 bootstrap replicates was used to construct nucleotide-based phylogenetic trees. A corresponding sequence from *Trialeurodes abutilonea* Haldeman was used as an outgroup.

Results

The examined pupal specimens from the populations were identified as *B. tabaci*. The partial mtCOI gene (780 bp) was amplified using the specific primers, and four haplotypes (H) were identified among the sequences (Table 3). The sequences from Khuzestan province exhibited the least values of polymorphism parameters including the number of polymorphic sites (S), haplotype diversity (HD), Pi, Theta (per site) from Eta ($Theta_{eta}$), Theta (per site) from S (Theta-W), and K. In contrast, the highest values of S, HD, Pi, $Theta_{eta}$, Theta-W, and K were observed in the population from Kerman, Hormozgan, Khorasan, and Yazd provinces. The sequences from Fars province were placed in the second rank relative to the values. No InDel polymorphic event was observed among the sequences.

All sequences obtained herein were highly identical (99.88%) to a sample from Pakistan (LN835385). The second highest identity (99.84%) was found with an isolate of MEAM1-subcladeB from the UAE (DQ133382). Additionally, a 99.69% identity was observed against three Iraqi isolates (HM070413, KX679575, and KX679577) and one isolate from the Kingdom of Saudi Arabia (GU086344). Two Syrian isolates (KP342512 and AB473559) exhibited high levels of identity (99.74 and 99.61%, respectively) to the sequences determined here. Isolates from Yemen and Kuwait (GU086343 and GU086346, respectively) showed a 98.96% identity to Khuzestan-originated sequences. A putative species MEAM1-subcladeB2 from Pakistan (GU977267) differed by 21 nucleotides from the sequences obtained herein. The lowest identity (95.06%) was observed against an isolate of the putative species MEAM2 from Uganda (KX570778). The sequences obtained here were identified as putative species MEAM1-subcladeB.

The phylogenetic tree showed the position of the putative species MEAM1-subcladeB (MW161484, MW161487, MW161488, and MW161490) from the present study (Fig. 1). These sequences, together with the isolate from the UAE (DQ133382), formed a separate cluster on the tree. The Iranian haplotypes reported by Shahbazi et al. (2014) formed two separate clusters on the tree. One cluster containing four isolates (JN559744, JN559748, JN559749, and JN559750) were clustered together with an Iraqi MEAM1-subcladeB2 species (KX679577). The other cluster consisted of two isolates (JN559751 and JN559753), which were placed close to a MEAM1-subcladeB2 species from Syria (AB473559 and KP342512). Another Iranian haplotype reported by Shoorcheh et al. (2008) (EU547771) was clustered with a MEAM1-subcladeB2 species from Pakistan (GU977267). Similarly, one unpublished Iranian specimen (MT038431) was placed close to the MEAM1-subcladeB2 species from Pakistan (GU977267) (Fig. 1).

Multiple alignments of the sequences showed 12 nucleotide positions in which genetic variation had occurred. The sequences from Fars province had seven nucleotide sites (positions: 139, 193, 242, 468, 493, 624, and 643) with genetic variation. The sequences from Khuzestan province demonstrated three variable

Table 3. DNA polymorphism parameters of *B. tabaci* populations examined in this study using DNASP software (ver. 6).

Population Analyzed in	Size	S	H	HD	Var(HD)	SD(HD)	Pi	$Theta_{eta}$	Theta-W	K
Shoorcheh et al. (2008)	5	690	5	1.000	0.016	0.126	0.54779	0.56750	0.43125	420.7
Shahbazi et al. (2014)	12	11	7	0.773	0.016	0.128	0.00258	0.00446	0.00446	2.106
This study	10	4	4	0.733	0.014	0.120	0.00180	0.00248	0.00173	1.467
Total	28	15	10	0.762	0.003	0.058	0.00322	0.00531	0.00531	2.341

S, number of polymorphic (segregating) sites; h, number of haplotypes; HD, haplotype (gene) diversity; Var(HD), variance of haplotype diversity; SD(HD), standard deviation of haplotype diversity; Pi, nucleotide diversity; $Theta_{eta}$, theta (per site) from eta; Theta-W, theta (per site) from S; K, aerage number of nucleotide differences.

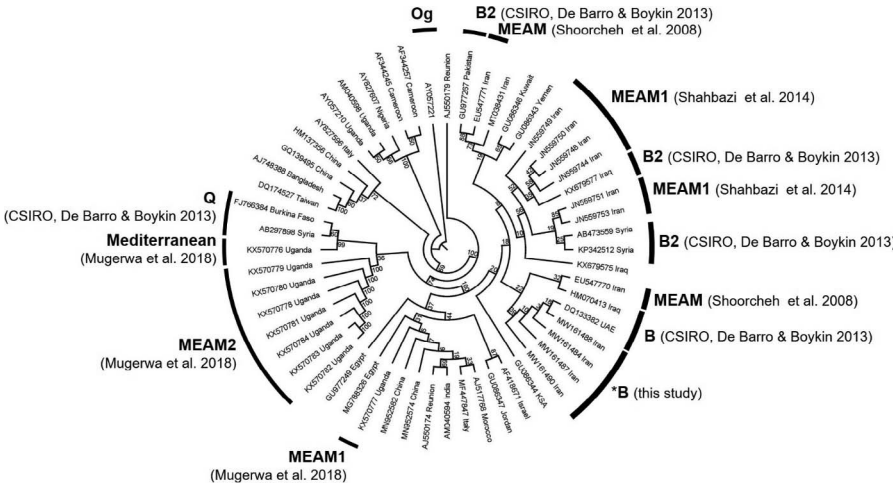

Fig. 1. Circular cladogram created using multiple alignments of 56 mitochondrial cytochrome oxidase 1 sequences from *Bemisia tabaci* specimens all around the world. The phylogenetic tree was constructed using maximum likelihood and bootstrap (100 replicates) by CLC Main Work-bench 7.6.2. The four Iranian *B. tabaci* haplotypes B obtained in this study are marked with an asterisk. The MEAM1 and MEAM2 sequences were obtained from GenBank database. The corresponding sequence of *Trialeurodes abutilonea* were used as outgroups (Og). All other sequences are available at CSIRO (De Barro and Boykin 2013).

nucleotide sites at positions 58, 548, and 606. One sequence from Khorasan province (EU547770) showed the highest number (four) of nucleotide positions (58, 369, 548, 606, and 692) with genetic variation, whereas another sequence from the same province (MT038431) exhibited no variation. Additionally, a specimen from Yazd province (EU547771) showed only one nucleotide position (692) in which genetic variation was observed. The remaining specimens showed no variation throughout the sequence.

Discussion

Here, we report for the first time the mtCOI sequences of *B. tabaci* populations from Khuzestan province, southwestern Iran. We demonstrated that these sequences belong to the putative species MEAM1-subcladeB. Additionally, the sequence analysis of Iranian specimens has led to the determination of their subclade (B and B2), which had not been previously reported.

The Africa/Middle East–Asia Minor genetic group was found to be the main putative species in Iran that also has been reported from neighboring countries including Iraq (Kareem et al. 2020) and Pakistan (Masood et al. 2017). The MEAM1 group that contains two subclades (B and B2) has been observed in tropical and

subtropical regions globally. The species have been considered invasive and cosmopolitan (De Barro et al. 2011). It appears that MEAM1-subcladeB and MEAM1-subcladeB2 are the dominant putative species in Iran. The MEAM1-subcladeB presented here was identical to the previous sequence (EU547770) reported from Khorasan province (Shoorcheh et al. 2008). It had shown a genetic variation among the sequences determined by Shoorcheh et al. (2008) resulting in formation of a separate branch on the phylogenetic tree (Fig. 1). Accordingly, this sequence was the only Iranian isolate with a close phylogenetic relationship to the sequences obtained here (Fig. 1). Although Fars and Khuzestan specimens showed no diversity in their putative species (MEAM1-subcladeB2 and -subcladeB, respectively), no location-dependent distribution of the putative species was observed. Accordingly, a single putative species (MEAM1-subcladeB2) has been reported from different provinces (Yazd and Khorasan) (Shoorcheh et al. 2008). This phenomenon also has been shown in other studies (De Barro et al. 2011). More specimens are required to better conceptualize the distribution of the putative species in the country.

DNA polymorphism analysis showed the highest rate of polymorphism among the sequences reported by Shoorcheh et al. (2008) (Table 2). This might be because of a relatively wider sampling span covering four provinces. In contrast, the sequences from a single province showed a relatively low diversity (Table 2). Furthermore, no host-dependent genetic diversity was found among the specimens collected from Khuzestan as *B. tabaci* individuals on different hosts shared identical nucleotide sequences. These results were consistent with the results of previous studies (Kareem et al. 2020, Masood et al. 2017, Shahbazi et al. 2014, Shoorcheh et al. 2008).

In Iran, control of *B. tabaci* is highly dependent on chemical practices; however, resistance has developed to at least six groups of pesticides (Basij et al. 2017). The alternative control practice, biocontrol, also has been challenged by the symbiont-mediated immune responses of *B. tabaci* individuals to their parasitoid (Mahadav et al. 2008). Considering the presence of subcladeB2 individuals, and possibly its symbionts, this might lead to the development of resistance to chemical compounds and biocontrol agents. Further experiments for detection of the symbionts are required to estimate the risk of pesticide/parasitoid-resistance, virus transmission, and insect fitness.

Acknowledgments

The authors thank the Vice-Consular Office for Research and Technology in Agricultural Sciences and Natural Resources University of Khuzestan. This study was supported by Agricultural Sciences and Natural Resources University of Khuzestan Grant 971.51.

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