

## Note

### Only the B biotype of *Bemisia tabaci* is present on vegetables in São Paulo State, Brazil

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**ABSTRACT:** *Bemisia tabaci* (Genn.) is one of the most important pests in cultivated areas of vegetables and ornamental crops around the world. Based on the mitochondrial cytochrome oxidase I (mtCOI) sequence, there is evidence that *B. tabaci* should be considered a cryptic species complex of 11 groups containing 24 species. Two of the groups, Middle East-Asia Minor 1 and Mediterranean include biotypes B and Q, respectively. In this study we evaluated the mtCOI sequence of *B. tabaci* populations collected in sites of the state of São Paulo, Brazil. Using PCR-RFLP with *Taq I*, a typical biotype B profile was obtained for all specimens. Based on the comparison with mtCOI reference sequences we found four haplotypes all belonging to the Middle East-Asia Minor 1. They occurred in the hosts pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.) and cucurbitaceae plants.

Key words: RFLP, cytochrome oxidase I gene, sequencing, whiteflies

### Somente o biótipo B de *Bemisia tabaci* ocorre em hortaliças no Estado de São Paulo, Brasil

**RESUMO:** *Bemisia tabaci* (Genn.) é considerada uma das mais importantes pragas em cultivos de hortaliças e ornamentais em todo o mundo. Baseado na análise da sequência mitocondrial (citocromo oxidase I - mtCOI) foi proposto recentemente que *B. tabaci* deva ser considerado um complexo críptico de espécies, contendo 11 grupos e 24 espécies. Dois destes grupos: Middle East-Asia Minor e Mediterranean englobam os biótipos B e Q, respectivamente. Avaliou-se a sequência mtCOI de espécimes de *B. tabaci* coletados em regiões do estado de São Paulo, Brasil. Por PCR-RFLP utilizando-se a enzima *Taq I*, pôde-se observar somente o padrão típico de clivagem para o biótipo B. Comparando-se com sequências consenso, todas as moscas brancas foram classificadas no grupo Middle East-Asia Minor e puderam ser separadas em quatro haplótipos, indicando prevalência do biótipo B em áreas de pimentão (*Capsicum annuum* L.), tomate (*Solanum lycopersicum* L.), cucurbitáceas e berinjela (*Solanum melongena* L.) do Estado de São Paulo.

Palavras-chave: RFLP, gene do citocromo oxidase I, sequenciamento, moscas-brancas

#### Introduction

The whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) is one of the most important pest of vegetables and ornamental crops in the world and is a vector of more than 100 plant viruses (Jones et al., 2003). Most of these viruses belong to the genus *Begomovirus*, one of the most important emerging groups of plant viruses (Brown, 2000). *B. tabaci* has a high intraspecific biological and genetic variability (De Barro et al., 2000). No morphological character, neither juvenile nor adult can distinguish different *B. tabaci* (Rosell et al., 1997; Calvert et al., 2001). Dinsdale et al. (2010) have recently proposed that *B. tabaci* should be considered as a cryptic species complex comprising 11 groups

containing 24 species. The more reproducible and informative method available to determine the genetic affiliation of a *B. tabaci* population is sequencing the mitochondrial cytochrome oxidase I (mtCOI) gene (De Barro et al., 2005) and use consensus sequences to assign group affiliation (Dinsdale et al., 2010).

The Middle East-Asia Minor 1 group contains the B and B2 biotypes and the Mediterranean the Q, J, L, SubSaharan Africa Silverleaf biotypes (Dinsdale et al., 2010). The authors provide strong evidence that B and Q are different species not biotypes, but in this paper we will still consider them as different biotypes. The biotype B was introduced in Brazil in the beginning of the 1990's (Lourenção and Nagai, 1994) and in the last few years has spread to several states causing losses in

many crops (Lima et al., 2002). Beside these biotype another were detected under Brazilian condition: biotype A or subsequently referred to as biotype BR by (Lima et al. 2000). The Q biotype has not yet been reported from Brazil (Rabello et al., 2008) and represents a threat due to its resistance to pyriproxyfen (Horowitz et al., 2003), buprofezin and reduced susceptibility to the neonicotinoid insecticides imidacloprid, acetamiprid and thimethoxam (Hodges, 2009), currently used to control *B. tabaci*.

The association of techniques like PCR-RFLP has also been used to successfully distinguish biotypes populations of *B. tabaci* (Bosco et al., 2006; Sartor et al., 2008). To determine whether the Q biotype is present in areas where tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) are grown in the state of São Paulo, *B. tabaci* samples were collected from these plants and the mtCOI gene was analyzed by PCR-RFLP and sequencing.

## Material and Methods

Adults of *B. tabaci* were collected from 27 localities in the state of São Paulo, Brazil, during 2007/2008, using a hand-held aspirator and preserved immediately in 95% ethanol at -20°C until processing. Samples were collected mostly from pepper and tomato crops but also from other vegetables (Table 1). Colonies of B biotype from Brazil and the Q biotype from Spain were used as controls in the analysis.

Each individual sample was macerate directly in sterile Whatman 3 MM paper (2 mm<sup>2</sup> pieces) which was placed on a 0.2 mL PCR tube. Part of the mtCOI gene was amplified with primers C1-J-2195-FW (5'-TTGATTTT'TGGTCATCCAGAAGT-3') and C1-J-2195-RV (5'-TCCAATGCACTAATCTGCCATATTA-3') (Simon et al., 1994; Frohlich et al., 1999). Analysis of PCR were performed in a final volume of 50 µL (MgCl<sub>2</sub> 50 mmol L<sup>-1</sup>, dNTP 2.5 µM, C1-J-2195 and MA483-RV

Table 1 – Biotype determination of *Bemisia tabaci* collected from different hosts and localities of the state of São Paulo compared to a B biotype from Florida, USA (accession numbers FJ188506). Year base: 2007/2008.

Samples	Site of collection	Geographical coordinates	Altitude m	Collect	Host Plant	PCR/ RFLP	Biotype	% Identity	Haplotype
1	Lins	21°40' S / 49°44' W	437	October/07	Pepper	+	B	-	
2	Lins	21°40' S / 49°44' W	437	October/07	Pepper	+	B	98	1
3	Lins	21°40' S / 49°44' W	437	October/07	Pepper	+	B	-	
4	Lins	21°40' S / 49°44' W	437	October/07	Pepper	+	B	-	
5	Lins	21°40' S / 49°44' W	437	April/08	Tomato	+	B	99	1
6	Lins	21°40' S / 49°44' W	437	April/08	Amarathus sp.	+	B	92	3
7	Pirajuí	21°59' S / 49°27' W	468	April/08	Pepper	+	B	99	1
8	Lins	21°40' S / 49°44' W	437	August/08	Eggplant	+	B	99	1
9	Avaí	22°08' S / 49°19' W	481	August/08	Pepper	+	B	91	2
10	Itapetininga	23°35' S / 48°03' W	656	March/08	Pepper	-	-	-	
11	Itapetininga	23°35' S / 48°03' W	656	March/08	Eggplant	+	B	87	1
12	Itapetininga	23°35' S / 48°03' W	656	March/08	Squash	+	B	98	1
13	São Miguel Arcanjo	23°52' S / 47°59' W	659	March/08	Tomato	-	-	-	
14	São Miguel Arcanjo	23°52' S / 47°59' W	659	May/08	Cucumber	+	B	99	1
15	Tejupá	23°20' S / 49°22' W	765	May/08	Tomato	-	-	-	
16	Tejupá	23°20' S / 49°22' W	765	January/08	Tomato	+	B	99	1
17	Tejupá	23°20' S / 49°22' W	765	January/08	Cabbage	+	B	97	1
18	Tejupá	23°20' S / 49°22' W	765	January/08	Pepper	+	B	89	1
19	Fartura	23°23' S / 49°30' W	516	April/08	Tomato	-	-	-	
20	Tejupá	23°20' S / 49°22' W	765	April/08	Pepper	+	B	99	1
21	Pirajú	23°04' S / 49°25' W	646	April/08	Pepper	-	-	-	
22	Itapetininga	23°35' S / 48°03' W	656	September/08	Pepper	+	B	99	1
23	Itapetininga	23°35' S / 48°03' W	656	September/08	Bean	-	-	-	
24	Santa Cruz do Rio Pardo	22°53' S / 49°37' W	467	January/08	Pepper	+	B	99	1
25	São João da Boa Vista	21°58' S / 46°47' W	767	September/08	Squash	+	B	98	4
26	Insectaria - Virology	22°53' S / 48°26' W	804	September/08	Pepper	+	B	97	1
27	Insectaria - Entomology	22°53' S / 48°26' W	804	September/08	Cabbage	+	B	-	

10  $\mu$ M) using 0.5 Unit of *Taq* polymerase (Bioline). The amplification was carried out in a PCR Express Thermal Cycler (Bio-Rad) with the following steps: 5 min. at 94°C (one cycle), followed by 35 cycles of 30 s at 94°C, 45 s at 45°C and 1 min. at 72°C and a final extension step of 10 min. at 72°C. The presence of mtCOI amplicons was visualized by electrophoresis in 1% agarose gel stained with ethidium bromide.

Five microliters of each amplified product (880bp) were digested with one unit of *TaqI* at 65°C for 2h in a final volume of 15  $\mu$ L, according to Bosco et al. (2006). The digested DNA was visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. Five individuals of each population from each region were analyzed.

The mtCOI gene of seventeen representative populations was sequenced. Sequences were assembled and analyzed using BioEdit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and compared against the consensus sequences of Dinsdale et al. (2010). The alignment of the sequences was performed using the Clustal W program (<http://www.ebi.ac.uk/clustaw/#>) (Thompson et al., 1994), and the phylogenetic tree was constructed using the MEGA 4.0 program (Kumar et al., 2004), using the Neighbor-joining method with a "bootstrap" value of 2000.

## Results and Discussion

Analysis of PCR with mtCOI primers provided amplicons of the expected size of 880bp (Figure 1-A); five samples did not amplify with the mtCOI primers. These whiteflies were morphologically indistinguishable from those collected in other localities. These samples also failed to amplify when primed with the ribosomal ITS primers 5'-GTTTCCGTAGGTGAACCTGC-3' (Brust et al., 1998) and 5'-TCCGCGAGCCGAGTGATCC-3' (De Barro et al., 2000); it is therefore likely that the DNA was degraded. The digestion of the PCR product with *TaqI* showed the expected restriction patterns of the B

biotype (Figure 1-B). The PCR-RFLP method used in this work easily distinguishes the B and Q biotypes (Bosco et al., 2006). The controls used in these experiments, a B biotype colony from Brazil, and a Q biotype colony from Spain also showed the expected restriction patterns.

The sequence of the mtCOI gene of some specimens representing the studied populations was determined. In the analysis by CLUSTAL W all the sequences had very high nucleotide (98% to 99%) identity with the mtCOI of the previously characterized B biotype presented in Argentina (accession numbers AF340215) and they could be assigned to one of four haplotypes (Table 1, Figure 2). A phylogenetic tree (Figure 2) was constructed using the mtCOI gene sequences obtained and consensus sequences for the different groups proposed by Dinsdale et al. (2010). All samples analyzed were classified in the Middle East-Asia Minor 1 genetic group that comprises specimens previously classified as biotype B (Figure 2). These results indicate that the Q biotype has not been established in Brazil. In addition, the high infestations of *B. tabaci* reported by Scarpellini et al. (2002) and Yuki (2001) in economically important annual crops and several vegetable crops in producing regions of the São Paulo state are probably of the B biotype. The BR biotype (Lima et al., 2000) was not found in this work.

Accurate identification of insects that are pests and/or virus vectors is a prerequisite for their effective management to reduce crop damage (Brown, 2000). Analysis of the mitochondrial cytochrome oxidase I (mtCOI) gene of worldwide collections of the whitefly have greatly improved the understanding of the genetic diversity of the *B. tabaci* species complex (Bosco et al., 2006; Dinsdale et al., 2010). *B. tabaci* B biotype was the only biotype found in the main tomato and pepper cultivation areas of the state of São Paulo; this biotype also infested other hosts, such as cucumber (*Cucumis sativus* L.), squash (*Cucurbita pepo* L.), cabbage (*Brassica oleracea* L. var. *acephala*), eggplant (*Solanum melongena* L.) and *Amaranthus* spp. in this area. The knowledge gained

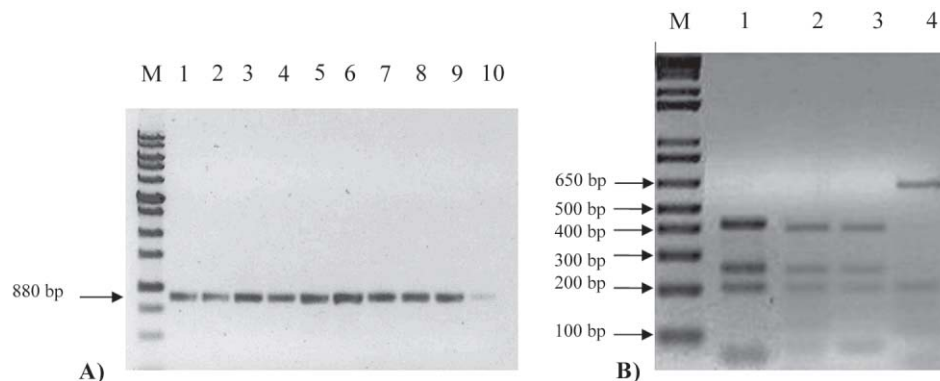


Figure 1 – A) PCR amplification of mtCOI (~880nt) from whitefly samples collected in the state of São Paulo from different crops. M: DNA marker 1Kb DNA Ladder (Invitrogen). Samples: 1- Pepper, 2- Tomato, 3- *Amaranthus* sp., 4- Pepper, 5- Eggplant, 6- Pepper, 7- Eggplant, 8- Squash, 9- Cucumber and 10- Tomato. B) RFLP patterns of mtCOI amplicons cleaved with *TaqI*, samples 1 – 3 B Biotype and 4 Q biotype controls. M: DNA marker 1Kb DNA Plus Ladder (Invitrogen).

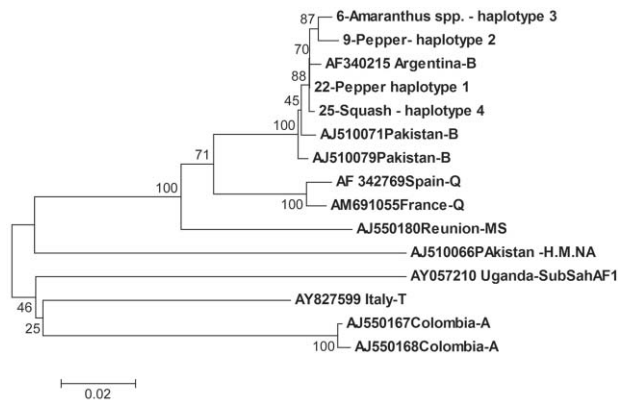


Figure 2 - Phylogenetic tree using the Neighbor-joining method of the partial mitochondrial COI gene of *Bemisia tabaci* samples collected in the state of São Paulo compared with some sequences of GenBank. *Bemisia tabaci* sequences: B biotype Argentina (AF340215), Pakistan (AJ510079) and Pakistan (AJ510071), Q biotype Spain (AF342769) and France (AM691055), MS biotype Reunion (AJ550180), H.M.NA biotype Pakistan (AJ510066), SubSahAF1 biotype Uganda (AY057210), T biotype Italy (AY827599), A biotype Colombia (AJ550167 and AJ550168) was utilized as outgroup.

from mtCO1 is an important tool that can help to prevent the introduction of exotic *B. tabaci* biotypes such as Q into Brazil, the invasion of alien species can cause serious economic, environmental and social impact on agricultural crops.

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