Identification of three major *Bemisia tabaci* biotypes in China based on morphological and DNA polymorphisms

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**Abstract**

The sweet potato whitefly, *Bemisia tabaci*, is a complex species, and many of its biotypes are important agricultural pests. The B and Q biotypes are invasive and coexist with the native Cv biotype on vegetable and ornamental crops in China. In this study, these three biotypes were identified based on their morphological characteristics, RAPD–PCR analysis, and DNA sequences of the COI gene. The anterior and posterior wax fringes of the B, Q, and Cv biotypes significantly differed from each other. Based on this morphological characteristic, the three biotypes can easily be distinguished in greenhouses and fields. Genomic DNA RAPD–PCR band patterns also revealed differences between these biotypes using the H16 primer. The B biotype has three bright DNA bands between 250 and 600 bp, the Q biotype only has one bright band at ~300 bp, while the Cv biotype has no band between 250 and 500 bp. Both the Cv and Q biotypes have two bright bands at ~750 and 1000 bp while the B biotype has only one band at ~1000 bp. Based on the COI gene, the genetic identity between B and Cv, B and Q, and Q and Cv was 85.8%, 94.7%, and 86.0%, respectively. The MP tree indicated that the phylogenetic relationship between the B and Q biotypes is much closer than that between the B and Cv or the Q and Cv biotypes. The uses of the morphological, RAPD–PCR, and DNA sequencing methods in biotype identification of *B. tabaci* are discussed.

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**Keywords:** *Bemisia tabaci* complex; Biotype; DNA polymorphism; Identification; Morphology

1. **Introduction**

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), has been a severe pest of agriculture and horticulture for the past 20 years [1–3]. This homopteran pest can seriously damage crops by feeding directly on plant sap, excreting honeydew, and transmitting more than 110 plant viruses [4–6]. Based on the differences in abiotic and biotic characteristics, such as geographical distribution, chemical resistance, host specialization, degree of fecundity, variability in geminivirus transmission efficiency, and the ability to induce phytotoxic disorders upon feeding, at least 41 distinct populations including 24 different biotypes have been identified [7]. Among these, some especially damaging biotypes, such as B and Q, have a cosmopolitan distribution [8–10].

In China, *B. tabaci* was not considered a major pest until an outbreak occurred in the mid-1990s, that was the invasion of a new biotype, the B biotype (also known as the silverleaf whitefly *Bemisia argentifolii*) belonging to the Mediterranean/Asia Minor/Africa race [9,11]. In recent years, this biotype has caused serious damage to a wide range of vegetables, fibers, and ornamental crops over China [2]. Meanwhile, a new biotype of *B. tabaci* viz. (the Q biotype) was also recorded on poinsettia in Yunnan
Province, southwest China [12,13]. The Q biotype had already been recognized as a damaging pest in the Mediterranean region [8], and was also found to spread rapidly to many Asian and African countries in the same period [10,14]. Since first recorded in China in 2004 [8,15], the Q biotype has caused substantial damage to crops in Beijing, Jiangsu, Zhejiang, Yunnan, and other areas.

In addition to the invasive B and Q biotypes, some native biotypes or haplotypes of B. tabaci can also cause heavy damage on particular host plants. These native biotypes have been shown to differ from the other B. tabaci biotypes when their mitochondrial cytochrome oxidase I (COI) genes were compared using Bayesian analysis [16]. Among the native biotypes of China, the Cv biotype caused the most losses [17–22]. The Cv biotype not only destroys the Codiaeum variegatum plant, on which it was first discovered [23], but also damages hibiscus and other related ornamental plants, reducing their aesthetic values. Because different biotypes require different management, pest managers should know which biotype is present. Differentiating between the B, Q, and Cv biotypes, however, is difficult.

In the present study, these three major biotypes were identified and distinguished using morphological, RAPD–PCR, and DNA sequencing methods. RAPD–PCR has been widely used to identify biotypes or haplotypes of many insects, plants, and bacteria because it is a simple and rapid technology. In contrast, DNA sequencing is more difficult to perform but more precise because it identifies genetic sequences. The current results increase our understanding of the key differences between these three biotypes and will be useful in identifying damaging biotypes and therefore in selecting efficient management strategies.

2. Materials and methods

2.1. Whitefly populations

Adults of B. tabaci B biotype were collected in 2006 on eggplant Solanum melongena at the training farm of South China Agricultural University (SCAU) in Cencun, Guangzhou. The Cv and Q biotypes were collected in 2007 from the ornamental plant Capsicum frutescens in Guangzhou and Nantong, Jiangsu, respectively. All three populations were raised on the same plant species from which they were collected and were kept in separate greenhouses at SCAU with ambient temperature, photoperiod, and humidity.

2.2. Morphological identification of the three biotypes

The “pupae” (late fourth nymphal stage with red eyes) of the three biotypes of B. tabaci were collected and observed under a binocular stereomicroscope (Leica M10) at 10–90× magnification. For 30 individuals of each biotype, the length and width of the left, right and posterior wax fringes were recorded using a stereo camera.

2.3. DNA extraction and biotype identification by RAPD–PCR

Three to five adults of each biotype were used to extract genomic DNA using a method modified by De Barro and Driver [24] and Qiu et al. [23]. The primer used for RAPD–PCR was H16: 5’TCTCAGCTGG3’, and PCR was performed on a PTC-100 thermocycler (MJ Research Co., Ltd.) with a total volume of 25 μl containing 1 × PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 15 pM PCR primer, 2 U Taq DNA polymerase (Sino-America Biotech, China), and 10–20 ng DNA template. The amplification profile was one cycle of 5 min at 94 °C, 2 min at 40 °C, and 3 min at 72 °C; followed by 39 cycles of 60 s at 94 °C, 90 s at 40 °C, and 120 s at 72 °C. PCR products were electrophoresed on 1% agarose gel at 8 V/cm for 2 h, after which the gel was stained with ethidium bromide for 30 min. Bands on the gels were observed using a UV light and then photographed.

2.4. Biotype identification by DNA sequencing

For further identification of the three biotypes of B. tabaci, the mitochondrial cytochrome oxidase I (COI) gene was selected as a marker gene. The primers used in the COI gene amplification were: C1-J-2195 (5’-TTGATTTTTGTCTCATCCAGAAGT-3’) and L2-N-3014 (5’-TCCAATGCACTAATCTGC CATATTA-3’). PCR was done in a 50 μl volume, and the DNA was first denatured for 5 min at 94 °C; followed by 39 cycles at 94 °C for 90 s, 55 °C for 90 s, and 72 °C for 60 s; and an final extension for 10 min at 72 °C. PCR products were electrophoresed above, and the sequencing was carried out for five adult individuals of each biotype. These sequences were aligned and the mismatches in the same biotype were revised according to the trace files.

2.5. Data analysis

The morphological characteristics and RAPD–PCR bands of the B, Cv, and Q biotypes were photographed in the laboratory. The data for the length and width of the anterior and posterior wax fringes were first arcsine-square root transformed to meet the requirements of normality and homogeneity of variances before they were analyzed in a one-way ANOVA (Tukey HSD for unequal N) with Statistica 8.0 software (Statsoft Inc., USA). The COI sequences of the three biotypes were analyzed and aligned with DNASTar (Lasergene® v5.0), ClustalX1.83, and Mega 4.0. The phylogenetic relationships were determined using maximum parsimony (MP) options available in Phylogenetic Analysis Using Parsimony (PAUP) version 4.0b [25], and a single most parsimonious tree was constructed using the heuristic search method, tree-bisection-reconnection, and random branch swapping options for 1000 bootstrap replicates. Bootstrap values were calculated using the >70% majority role. Sequences of the Texas B
biotype (AF164675), Morocco Q biotype (AJ517769), and China Cv biotype (AY686064) were downloaded from GenBank and compared with the COI sequences of the biotypes. In the MP tree, the COI sequences of *Bemisia afer* (AF48763) and *Trialeurodes vaporariorum* (AF418672) from GenBank were used as species and genus outgroups, respectively.

3. Results

3.1. Morphological identification of the three biotypes

The nymphs of the three biotypes of *B. tabaci* differ from each other in both anterior and posterior wax fringes (Fig. 1 and Table 1). The Cv biotype has the longest fringes while the B biotype has the shortest (Table 1). The width of the anterior wax fringes is greatest in the B biotype, intermediate in the Cv biotype, and smallest in the Q biotype. The lengths of the posterior wax fringes are similar for the Cv and Q biotypes but significantly shorter for the B biotype. The width of the posterior fringes of the Cv and B biotypes are nearly the same and conspicuously wider than that of the Q biotype. Thus, these three biotypes can be roughly distinguished from each other by their morphology.

3.2. Biotype identification by RAPD–PCR

The RAPD–PCR patterns of the B, Q, and Cv biotypes are shown in Fig. 2. The B biotype produced three DNA bands between 250 and 600 bp, and the Q biotype produced one band of ~300 bp. In contrast, the bands between 250 and 500 bp are absent in the Cv biotype. Both the Cv and Q biotypes produced two bright bands at ~750 and

![Fig. 1. The morphological characteristics of *B. tabaci* B (a), Cv (b), and Q biotype (c) "pupae".](image)

![Fig. 2. The RAPD-PCR pattern of the B, Cv, and Q biotypes of *B. tabaci*. M: molecular weight; CK, negative control; 1–2, B biotype; 3–4, Cv biotype; 5–6, Q biotype.](image)

### Table 1

The length and width of anterior and posterior wax fringes of *Bemisia tabaci* Cv, Q, and B biotypes (M ± SE, μm).

<table>
<thead>
<tr>
<th>Biotype</th>
<th>n</th>
<th>Anterior wax fringe (left)</th>
<th>Anterior wax fringe (right)</th>
<th>Posterior wax fringe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length</td>
<td>Width</td>
<td>Length</td>
</tr>
<tr>
<td>Cv</td>
<td>30</td>
<td>120.84 ± 4.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.67 ± 1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>123.83 ± 4.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Q</td>
<td>30</td>
<td>101.50 ± 4.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.63 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.42 ± 4.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>28.96 ± 2.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.88 ± 1.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.54 ± 1.61&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F&lt;sub&gt;S&lt;/sub&gt;,&lt;sub&gt;R&lt;/sub&gt;</td>
<td></td>
<td>151.15</td>
<td>40.07</td>
<td>165.00</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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</table>

*Note: n, the number of individuals observed; within the same column values with different superscripts are significantly different at P < 0.05.*
1000 bp while the B biotype produced only one band at ~1000 bp. Moreover, a band at 1500 bp was found in the Cv biotype, which was absent in both the B and Q biotypes.

### 3.3. Biotype identification by DNA sequence

PCR amplification of the COI gene using genomic DNA from the B, Q, and Cv biotypes produced a similar-sized DNA fragment of ~840 bp. The alignment and comparison of these three sequences with the four reference sequences downloaded from GenBank (~720 bp) are shown in Fig. 3. The genetic identity was 99.6% between the B, Cv, and Q biotypes in this study and those of the B, Q, and Cv reference sequences from GenBank. The identities of B and Cv, B and Q, and Q and Cv were 85.8%, 94.7%, and 86.0%, respectively. The phylogenetic maximum parsimony tree of the B, Cv, and Q biotypes (generated from the COI sequences determined in the present study) as well as those downloaded from GenBank.

![Fig. 3. The nucleotide sequences of the *B. tabaci* COI gene amplified from the B, Q, and Cv biotypes in the present study as well as those downloaded from GenBank.](image)

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![Fig. 4. The phylogenetic maximum parsimony tree of the B, Cv, and Q biotypes in this study as well as those downloaded from GenBank based on their COI gene. *B. afer* (AF418763) and *T. vaporariorum* (AF418672) were used as outgroups in the tree.](image)
present study and those downloaded from the GenBank) is shown in Fig. 4. The B and Q biotypes from China were first clustered into two different subgroups; the B biotype clustered with a Texas population and the Q biotype clustered with a Morocco population. Then these two subgroups were arranged into one group, which formed a sister branch of another group consisting of the two Cv populations.

4. Discussion

*Bemisia tabaci* has been described as a species complex based on widespread evidence for biological and genetic variations among a number of distinct populations [26–29]. Biological, biochemical, and molecular genetic analyses have identified a large number of polymorphic populations of this cosmopolitan pest, among which the B and Q biotypes are considered the most serious and most widely distributed [30–32]. The B biotype invaded China as an exotic pest in the mid-1990s, after which its biological, ecological, and molecular characteristics were widely studied.

To date, the Q biotype has been found in several provinces of China [13,14]. Other severe biotypes such as Cv and ZHJ-2 also caused substantial damage in different crops [19,20,33], although these biotypes sometimes co-exist. No detailed investigation, however, has been undertaken to identify these important biotypes and clarify their phylogenetic relationships [2]. This study focused on identification of the B, Cv, and Q biotypes based on differences in morphology, RAPD–PCR patterns, and COI gene sequences, with the aim of finding a rapid, efficient, and accurate way to distinguish them from each other.

Wax fringes are a characteristic feature of whiteflies and are widely used to identify species [34] and *B. tabaci* biotypes [28,35]. In the present study, the anterior and posterior wax fringes of the B, Cv, and Q biotype pupae were found to be significantly different from each other. Based on this characteristic, the three major biotypes of *B. tabaci* can be easily distinguished in the field with the aid of a hand magnifier. This method will help farmers and local technicians, who lack access to sophisticated laboratories, to identify *B. tabaci* populations and select effective control measures. However, the development of whitefly larva can be affected by the physical morphology of a plant leaf, such as the density and size of hairs on the leaf surface [36], which might confuse those who try to identify the *B. tabaci* biotype using only morphological characteristics [37,38]. Hence, additional technologies such as molecular methods are necessary.

The genomic DNA RAPD–PCR patterns in this study revealed differences between the three biotypes. The differences in RAPD–PCR patterns between the B and Cv biotypes as well as between the B and Q biotypes were also studied by Qiu et al. [23] and Chu et al. [12]. This research when done previously, however, failed to simultaneously compare these three major biotypes, which is important for obtaining the complete pattern of differences between them. The differences between the B and Cv biotypes, and between the B and Q biotypes reported here are quite similar to those reported by Qiu et al. [23] and Chu et al. [12], while differences between Cv and Q biotypes are reported for the first time.

DNA RAPD–PCR patterns can give a rapid and intuitive identification of the three biotypes, and the procedures can be completed in less than half a day. On the other hand, to gain the most accurate differentiation and to clarify the phylogenetic relationships between the three biotypes, DNA sequencing is superior to RAPD–PCR. Therefore, the alignment and comparison of the COI gene sequences of the B, Cv, and Q biotypes performed in the present study not only confirmed our morphological and RAPD–PCR identifications, but also showed the high percentage of genetic identity between our populations of the three biotypes and those from GenBank. The MP tree also indicated that the phylogenetic relationship between the B and Q biotypes is much closer than that between the B and Cv as well as between the Cv and Q biotypes, which is consistent with the studies of Qiu et al. [2,23].

In summary, *B. tabaci* in China is represented by many biotypes, and the invasive B/Q biotypes as well as the native Cv/ZHJ biotypes have already caused substantial damage to agricultural and horticultural ecosystems in many regions. Because the biotypes differ in pesticide resistance, plant specialization, reproduction, and geminivirus transmission, the establishment of integrated control strategies must be based on the accurate identification of the biotypes. The three methods of identifying the B, Q, and Cv biotypes presented in this study can be used in the field (for morphological identification) or in the laboratory (for molecular identification). However, because DNA sequencing is costly, the sequence-characterized amplified region (SCAR) method might be a more economical alternative [39,40].

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