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Survey of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers

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Abstract

In 1991, the poinsettia strain, silverleaf whitefly or B biotype of *Bemisia tabaci* was detected in Brazil. This variant is a far more serious agricultural pest than the previously prevalent non-B (BR) biotype. The correct identification of *B. tabaci* is problematic since it is highly polymorphic with extreme plasticity in key morphological characters that vary according to the host. RAPD-PCR was used to survey the B biotype and other biotypes of *B. tabaci* in Brazil. Whiteflies were collected from cultivated plants and weeds from 57 different localities and on 27 distinct crops. RAPD analyses using two selected 10-mer primers reliably identified the BR biotype and the B biotype of *B. tabaci* and also differentiated other whitefly species. The presence of the B biotype was confirmed in 20 Brazilian states. The BR and B biotypes of *B. tabaci* were found to coexist in the whitefly populations of three different localities: Jaboticabal, SP; Rondonópolis and Cuiabá, MT, and Goiânia, GO.

INTRODUCTION

In the last two decades, the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), has become one of the most important pests of agricultural crops worldwide. *B. tabaci* is a vector of numerous plant viruses and also reduces crop production by direct feeding. Infestations of *B. tabaci*, associated with phytotoxic symptoms of squash silverleaf in *Cucurbita* spp., uneven ripening of tomato and white stem disorder in *Brassica* spp., were first described in the US. Similar phytotoxic-like disorders are now widespread throughout cropping systems in many countries (Costa *et al*., 1993; Brown *et al*., 1995; Hilje, 1996), including Brazil.

The heavy use of pesticides, intensive agricultural production, overlapping crop seasons and an insect pest species with the biotic potential to exploit a wide resource base within a permissive environment may have contributed to the aggregation of strains or races which are morphologically indistinguishable but genetically different from each other (Wool *et al*., 1993; Toscano *et al*., 1998). Among the races or biotypes of *B. tabaci*, the B biotype has caused tremendous losses worldwide. The B biotype has a broader host range, and a higher honeydew egg production than the less damaging A biotype (Bethke *et al*., 1991; Costa and Brown, 1991).

B. tabaci was first identified in Brazil in 1928 on *Euphorbia pulcherrima* in Bahia State (Bondar, 1928). From the Northeast, the insect spread to other regions of the country where outbreaks were previously occasional and problems were caused largely due to its properties as a vector. Bean production was affected by the bean golden mosaic virus in many regions and tomato and soybean crops were also occasionally infected with geminiviruses transmitted by *B. tabaci* (Ribeiro *et al*., 1998; Ferreira *et al*., 1999).

In the early 1990's, a heavy infestation of *B. tabaci* on important crops and wild plants was detected in São Paulo State, where there is extensive international trade in ornamental plants. The changes in the behavior of *B. tabaci* populations were similar to those observed for the emergence of the B biotype in other countries (Lourenção and Nagai, 1994). Since its arrival, substantial losses due to *B. tabaci* have been reported by 21 out of 27 Brazilian states.

Important crops such as melons, watermelons, cotton, bean, tomato and cucumber have been damaged. In many areas, losses of 20 to 100% have been reported with the estimates being close to US$ 1 billion, although a precise monetary loss has not been calculated (Ferreira and Avidos, 1998).

Molecular analysis has been used to identify and characterize whitefly populations. Esterase pattern analysis was applied to population studies of *B. tabaci*, which demonstrated the association of the B biotype with silverleaf symptoms (Costa and Brown, 1991). Electrophoretic analysis of esterase isoenzymes has also been used to determine the genetic variation between individuals of the whitefly species, *Trialeurodes vaporariorum* (Westwood) and *B. tabaci* (Liu *et al*., 1992). This approach enabled the differentiation between species and distinguished whitefly males and females (Oliveira and Lima, 1997).

Random amplified polymorphic DNA polymerase chain reaction (RADP-PCR) is a relatively simple, inexpensive and rapid technique, revealing polymorphisms which are useful as genetic and taxonomic markers (Welsh and McClelland, 1990). RAPD has been applied to studies

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of insects (Haymer, 1994) and to differentiate whiteflies, including the identification of the A and B biotypes of *B. tabaci* (Gawel and Bartlett, 1993).

MATERIAL AND METHODS

Adult whiteflies were collected from host plants and fields using a hand-held aspirator and preserved immediately in 100% ethanol for later processing. A total of 61 samples, each of approximately 100 insects, were collected from 53 localities in 15 states and on 27 distinct crops. All insects used in this study were from infestations identified as *B. tabaci* based on the morphology of the pupal stage. Reference colonies of B biotype adults originating from California, USA, and a non-B biotype (biotype A) originating from Arizona, USA (Perring *et al*., 1993), were used as standard.

DNA was extracted from 10 individual insects of each sample, according to a standard protocol (Barro and Driver, 1997) with several modifications. Individual female whiteflies were homogenized in 56 µl lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.30% Triton X-100, 60 µg/ml proteinase K) using a 1.5-ml microcentrifuge tube and micropestle. The homogenate was incubated at 65°C for 15 min and boiled for 6 min to inactivate proteinase K. Processed samples were stored at -20°C.

The two 10mer primers (Operon Technologies Inc., Alameda, CA, USA) used for RAPD-PCR are shown in Table I. PCR was performed in a 30-µl reaction mix containing 6.0 mM Tris-HCl, pH 8.8, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM primer, 2.5 U of *Taq* DNA polymerase and 10-15 ng DNA. Mineral oil (25 µl) was added on the top of the reaction mix. Samples were amplified using a PTC-100 thermocycler (MJ Research) with the following program: one cycle of 3 min at 94°C followed by 45 cycles of 1 min at 93°C, 1 min at 35°C and 2 min at 72°C, followed by a final cycle of 5 min at 72°C. PCR products were separated by electrophoresis on 1.5% agarose gels, run at 5 V/cm for 3 h in 0.5X TBE buffer. A 100-bp ladder (Amersham Pharmacia Biotech) was included on each gel as molecular weight marker. DNA bands were visualized under UV light after staining with a 0.5 µg/ml ethidium bromide solution. Gels were documented using the Eagle-Eye II System (Stratagene). A negative control without DNA was included in all reaction sets.

Table I - Nucleotide sequence of RAPD primers.

Primer name	Sequence (5'-3')
OPA-02	TGCCGAGCTG
OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT
OPA-13	CAGCACCCAC
OPA-15	TTCCGAACCC
OPA-20	GTTGCGATCC
OPR-07	ACTGGCCTGA
OPE-13	CCCGATTCGG

RESULTS AND DISCUSSION

RAPD-PCR was used to identify whiteflies from 61 sampling sites originating from different Brazilian localities and crops. Representative samples of *B. tabaci* biotype A, collected from wild populations in Arizona, USA, and biotype B colonies from California, USA, were included in each reaction set as identification control. Brazilian isolates with profiles resembling biotype A are subsequently referred to as biotype BR. For each site or crop, PCR was conducted with the DNA of 10 individual insects for each RAPD primer (see Figure 1). All 10 primers screened in this study (Table I) produced RAPD patterns that clearly

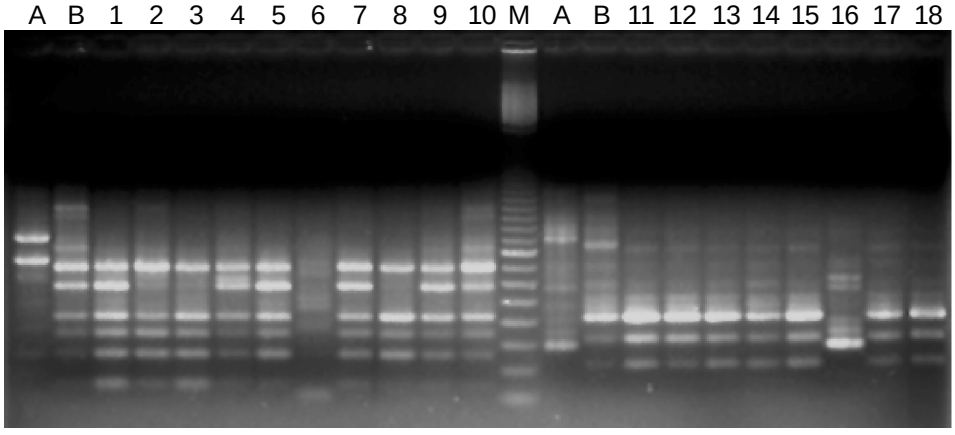

Figure 1 - A representative RAPD reaction set for the differentiation of whitefly biotypes. M: 100-bp DNA ladder (Amersham Pharmacia Biotech); samples on the left of the marker were amplified using primer OPA-04 and samples on the right of the marker using OPA-10. Lanes 1-6: Individual whiteflies from cabbage and lanes 7-10: individual whiteflies from melon. Lanes 11-14: Individual whiteflies from cabbage and lanes 15-18: individual whiteflies from melon. Standard biotypes (biotype A, Arizona, USA, and biotype B, California, USA) used for identification are indicated by the letters A and B, respectively.

Table 2 - Biotype identification using RAPD markers of *Bemisia tabaci* sampled from different crops and localities in Brazil.

Crop	Locality	Identification (biotype)
01 - melon	Arizona - USA	A-type (= BR)
02 - melon	California - USA	B-type
03 - bean	Goiânia - GO	BR-type
04 - melon	Brasília - DF	B-type
05 - bean, eggplant	Jaboticabal - SP	B
06 - tomato, cabbage	Lavras - MG	B
07 - squash	Mançuba - BA	B
08 - cotton	Campina Grande - PB	B
09 - cotton	Guanambi - BA	B
10 - okra, various weeds, bean, soybean	Rondonópolis - MT	BR
11 - cucumber	Guaiuba - CE	B
12 - watermelon	Itaberaba - BA	B
13 - tomato, squash, weeds, peanut	Petrolina - PE	B
14 - cabbage, sweet pepper	Itaiçaba - CE	B
15 - melon, watermelon, tomato	Aracati - CE	B
16 - cabbage	Tianguá - CE	B
17 - soybean, cotton	Miguelópolis - SP	B
18 - tomato	Pacoti - CE	B
19 - squash	Vassouras - RJ	B
20 - melon, soybean	Boa Vista - RR	B
21 - okra, watermelon, tomato, squash	Goiânia - GO	B
22 - bean and cucumber	Goiânia - GO	BR
23 - lettuce, tomato, cabbage	Goianira - GO	B
24 - bean	Limoeiro do Norte - CE	BR
25 - soybean	Piracicaba - SP	B
26 - soybean	Pedra Preta - MT	BR
27 - tomato	Uberlândia - MG	B
28 - melon	Russas - CE	B
29 - squash	Tabuleiro do Norte - CE	B
30 - broccoli, okra, gherkin	Taiobeiras - MG	B
31 - broccoli, cabbage, carrot	Salinas - MG	B
32 - cucumber, eggplant, sweet pepper	Planura - MG	B
33 - cauliflower, cabbage, tomato	Teófilo Otoni - MG	B
34 - cotton	Janaúba - MG	B
35 - soybean	Viçosa - MG	B
36 - soybean	Carolina - MA	B
37 - soybean	Riachão - MA	B
38 - soybean	St. Antônio de Goiás - GO	B
39 - lettuce, broccoli, cabbage	Palmas - TO	B
40 - cabbage	Mogi Mirim - SP	B
41 - grape	Campinas - SP	B
42 - eggplant	Espírito Santo do Pinhal - SP	B
43 - cabbage	Campos Goytacazes - RJ	7 B + 3 BR
44- melon, watermelon	Mossoró - RN	B
45 - tomato	Teresina - PI	B
46 - watermelon	São João - PI	B
47 - eggplant, cucumber, bitter eggplant	Cuiabá - MT	8 B + 2 BR
48 - weed, *leguminosae*, forage	Campo Grande - MS	B
49 - cabbage, squash	Vitória - ES	B
50 - squash, cabbage, soybean, tomato, cauliflower, broccoli	Brasília - DF	B
51 - tomato, watermelon	Paulo Afonso - BA	B
52 - squash, watermelon, bean	Pão-de-açúcar - AL	B
53 - squash	Delmiro Gouveia - AL	B
54 - melon, squash	Iaçu - BA	B
55 - cotton	Display Plot/ Embrapa - DF	BR
56a - cotton, soybean	Display Plot / Embrapa - DF	BR (initial)
56b - cotton, soybean	Display Plot / Embrapa - DF	B (week 2)
57 - bean	Display Plot / Embrapa - DF	B
58 - tomato	Recife - PE	B
59 - cotton	Recife - PE	B
60 - cabbage	Recife - PE	B
61 - tomato	Recife - PE	B
62 - cucumber	Vilhena - RO	B

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Table II - Continued

Crop	Locality	Identification (biotype)
63 - tomato	Santa Cruz do Sul - RS	B
64 - melon	Baraúna - RN	B
65 - tomato	Tibau - RN	B
66 - melon	Assu - RN	B
67 - tomato	Trindade - GO	B
68 - tomato	Tocantins - MG	B
69 - cotton	Lagoa - PB	B
70 - bean	São João do Jaguaribe - CE	B
71 - tomato, melon	Quixeré - CE	B
72 - tangerine	Bonfim - MG	B
73 - soybean	Balsas - MA	B
74 - cabbage	Caruaru - PE	B
75 - tomato	Vitória - PE	B
76 - tomato	Sapucarana - PE	B
77 - bean	Buriti de Goiás - GO	BR
78 - weeds	Nilo Coelho - PE	B
79 - weeds	Feira de Santana - BA	B

distinguished biotype B individuals from non-B individuals (data not shown). The average number of RAPD bands produced in a single reaction was 7.8 per primer and ranged from 300 to 600 bp. Patterns produced within the 10 replicate samples were highly homogenous; however, the relative intensity of some bands was occasionally variable. The produced profiles usually resembled those of the corresponding standard biotypes originating from the US. In some reaction sets we also used the native biotypes from Brazil as control, which induced silverleaf symptoms on squash, and two other whitefly species: *T. vaporariorum* and *Aleurothrixus floccosus* (Maskell). The RAPD profiles of the last two species were always highly distinct from the *B. tabaci* profiles.

Primers OPA-04, OPA-10 and OPA-13 gave the clearest and most unambiguous DNA profiles and were used for most whitefly identifications shown in Table II. These primers also reliably separated *B. tabaci* biotypes from *T. vaporariorum* and *A. floccosus*. No assessed crop was found to be exclusively infested by the BR biotype of *B. tabaci*. The survey rather demonstrated the dominance and broad host range of the B biotype in Brazil.

Figure 2 illustrates the distribution of the BR and B biotypes among the different states of Brazil. Investigations after the first detection of the B biotypes in 1993 suggested that the insect was first introduced in 1991 via poinsettias imported from California to Holambra, São Paulo (Lourenção and Nagai, 1994). Our survey shows that this biotype is now well established and widespread in the northeast, east and midwest of Brazil. However, we did not detect the B biotype in the southern- and westernmost Brazilian states, due to a potential climatic influence on the spread of two *B. tabaci* biotypes. The occurrence of both BR and B biotypes was confirmed in the States of Mato Grosso, Goiás and Rio de Janeiro. We also found both biotypes in the Federal District, at Embrapa (display plot), where a second sampling was made two weeks after the first. *B. tabaci* biotype BR was exclusively found in the

Figure 2 - Distribution of *B. tabaci* collected in Brazil and identified by RAPD markers.

first sample. After two weeks, however, the original population appeared to have been replaced by the B biotype, illustrating the competitive advantage of this insect (Costa and Brown, 1991).

We found the use of RAPD-PCR to identify whiteflies to be relatively inexpensive (approximately US$ 3.00/specimen), requiring just 30 min for sample preparation and 10 h for RAPD analysis. The technique also has the advantage that alcohol can be used to preserve the insects, avoiding the need to keep samples alive or frozen. Effective monitoring of the spread and establishment of *B. tabaci* in Brazil and the ability to reliably distinguish non-B biotypes from the more important B biotype of this pest will assist in the forecasting of potential losses and improve the prospects for adoption of appropriate management strategies.

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RESUMO

Em 1991, um novo biótipo de *Bemisia tabaci* denominado de raça B, mosca branca da poinsétia ou mosca da folha prateada foi detectado no Brasil. Esta praga trouxe muitos prejuízos e danos à agricultura nacional, por ser mais agressiva do que a existente anteriormente, conhecida como *B. tabaci* ou *B. tabaci* biótipo BR (não B). A relação taxonômica entre *B. tabaci* e *B. tabaci* biótipo B não é clara e não existem evidências morfológicas consistentes que possam distinguir esses dois biótipos. RAPD-PCR tem sido utilizada para identificação de biótipos presentes nas populações, utilizando-se, como padrões de referência, adultos de *Bemisia tabaci* das raças A e B provenientes dos Estados Unidos. As coletas de mosca branca foram feitas em 27 culturas e plantas daninhas em 57 localidades do país. As populações foram então analisadas, observando-se que a população predominante em 20 estados brasileiros é de *B. tabaci* biótipo B. Os biótipos BR e B foram encontrados coabitando a mesma região, em três diferentes localidades: Jaboticabal, SP; Rondonópolis e Cuiabá, MT e Goiânia, GO.

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