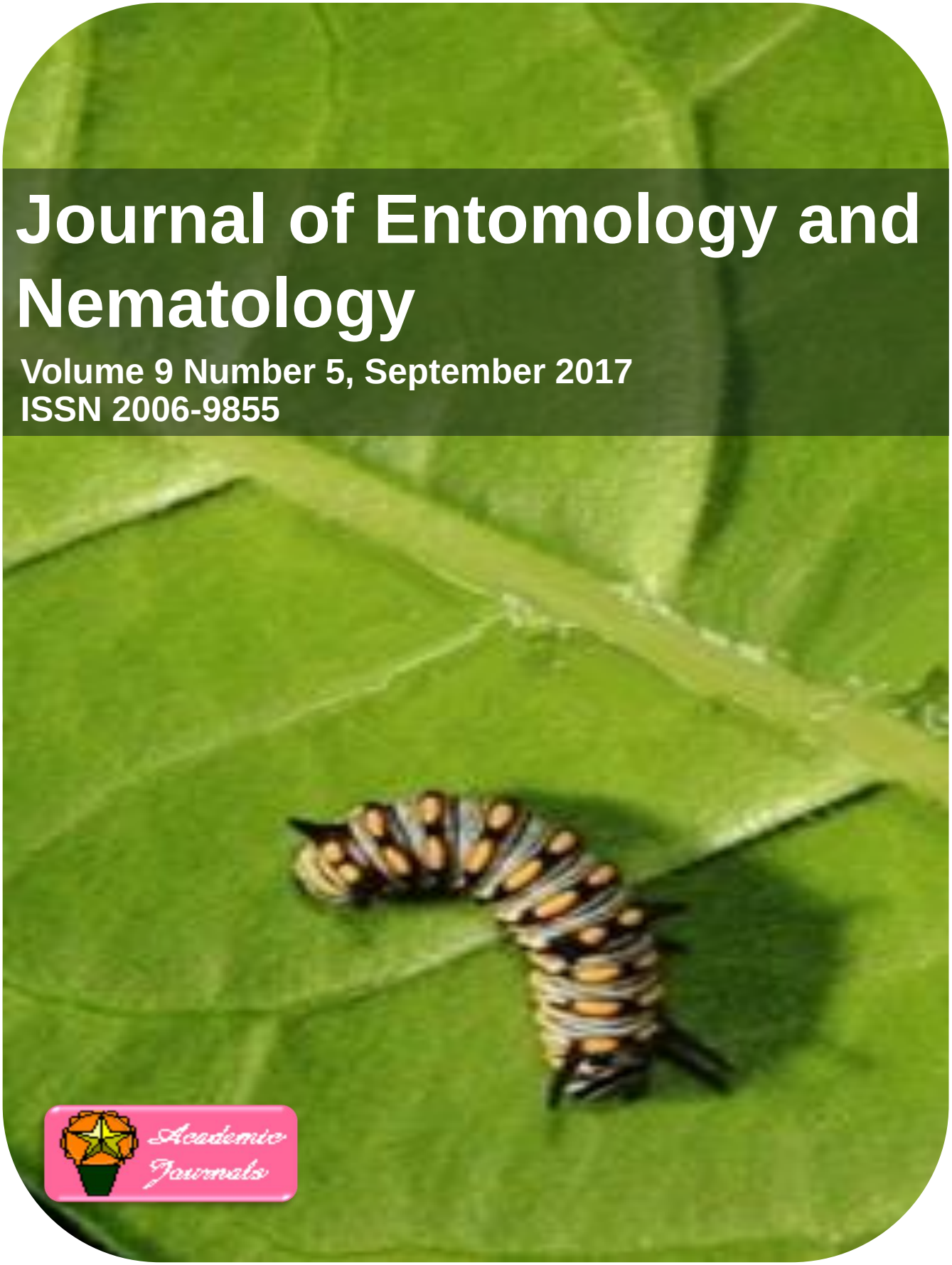
Journal of Entomology and Nematology

Volume 9 Number 5, September 2017
ISSN 2006-9855



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Journal of Entomology and Nematology

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ARTICLE

academicJournals

Vol. 9(5),pp.36-45, September 2017
DOI: 10.5897/JEN2017.0170
Article Number: 217684F66011
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Description of the molecular profiles of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in different crops and locations in Brazil

Paulo Roberto Queiroz[1*], Luzia Helena Lima[2], Érica Soares Martins[1], Edison Ryoiti Sujii[2] and Rose Gomes Monnerat[2]

[1]Instituto Mato-Grossense do Algodão (IMAmt), Rua Eng Edgard Prado Arze, 1777 Ed. Cloves Vettorato - 2 Andar Quadra 03, Setor A - Centro Político Administrativo, CEP: 78049-015, Brazil.
[2]Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica - PqEB s/nº, Brasília, DF - Brasil - CEP 70770-901, Brazil.

Received 17 January, 2017; Accepted 21 August 2017

The Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) causes damages to crops by feeding and/or transmitting various plant pathogenic viruses. Morphologically indistinguishable populations of *B. tabaci* can exhibit variable molecular features and biological behavior. The objective of this work was to present the molecular profile and genetic variability of *B. tabac*i collected from several crops and localities in Brazil. The molecular identification of 100 whiteflies samples collected from these crops and localities in Brazil revealed 14% of non B biotype of *B. tabaci*. About the biotypes of *B. tabaci,* 2% indigenous Brazilian whitefly was found, 1% A biotype, 2% Q biotype, 1% African cassava and 80% B biotype. The dendrogram obtained by molecular markers showed that biotype A of *B. tabaci* from the United States differed from the other biotypes as it showed around 8% of genetic similarity. For the remaining populations previously identified as biotype B, separation was observed among them based on location and crop of collection. The *B. tabaci* native Brazilian biotype did not form a separate grouping from the B biotype. This information may serve as a basis for studies aiming to identify which factors influence the population distribution in a given area.

Key words: Genetic markers, whitefly, oligonucleotides, b biotype, host plants association.

INTRODUCTION

The tobacco whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), also known as the cotton, sweet potato, or silverleaf whitefly, is a pest of several plants including ornamental plants and agricultural crops grown around the world. Whiteflies damage crops through feeding and transmitting various plant pathogenic

*Corresponding author. E-mail: pqsilva@uol.com.br.

viruses (Liu et al., 2014). *B. tabaci* is currently known as a complex composed of several cryptic species. Morphologically indistinguishable populations of *B. tabaci* can exhibit variable molecular features and biological behavior. The host plant ranging from efficiency in plant virus transmission, ability to cause phytotoxicity, fecundity and insecticide resistance are traits that have been used to distinguish the so-called biotypes of *B. tabaci* (Barbosa et al., 2014).

The protein and DNA polymorphisms of *B. tabaci* can be combined with studies of biological characteristics to distinguish their biotypes by using experimental or technological approaches like electrophoresis of allozymes, analysis of randomly amplified polymorphic DNA (RAPDs) and nucleic acid sequence comparisons of nuclear or mitochondrial DNA markers (Calvert et al., 2001).

Nowadays, the more reproducible and informative method available to determine the genetic affiliation of a *B. tabaci* individual is based on molecular data, using markers such as Random Amplified Polymorphic DNA (RAPD) and Internal Transcribed Sequence (ITS) (Rabello et al., 2008), Sequence Characterized from Amplified Regions (SCAR) (Queiroz et al., 2016) and mitochondrial genes such as cytochrome oxidase 1 (De Barro, 2005; Marubayashi et al., 2012).

Molecular markers of *B. tabaci* have been recognized as a useful tool to understand the genetic dynamics of this species in the field and may correlate some molecular profiles with biological features of populations established in agricultural areas. However, since this tool has not yet been used on a large regional scale to evaluate local populations of *B. tabaci* on different crops, the objective of this study was to present the molecular profile and genetic variability of *B. tabac*i collected in several crops and localities in Brazil.

MATERIALS AND METHODS

Obtaining DNA

Adults *B. tabaci* collected from crops in several locations in Brazil were identified according to morphological criteria (Gregory and Gregory, 2005) and maintained in 70% ethanol at -20°C. DNA for molecular analysis was obtained by macerating individual female adults in 60 µL extraction buffer (Tris-HCl 10 mM pH 8, EDTA 1 mM, Triton X-100 0.3%, proteinase K $\mu g.mL^{-1}$). The mash was incubated for 30 min at 65°C followed by boiling for 10 min. The final homogenate was stored at -20°C until use.

Insect molecular identification

OPA-10 and OPA-13 oligonucleotides were used for molecular identification of whitefly biotypes and other whitefly species according to the methodology established by Lima et al. (2000). The default molecular weight profile of the fragments observed by RAPD was established for the B biotypes and the Brazilian native biotype (BR). Any sample whose fragment profile was different from those previously established was subjected to a new morphological identification to identify the biotype of *Bemisia* or other whitefly species.

Analysis of genetic variability of whitefly populations occurring in different crops in Brazil

For the purpose of analysis of genetic variability, five individuals in populations of *B. tabaci* were collected on crops in the US, but also on crops established in several locations in Brazil (Table 1), with the oligonucleotides OPA-02, OPA-03, OPA-05, OPA-10, OPA-11, OPA-13, OPA-15 and OPA-20.

A set of 17 samples (samples 74-93) (Table 1) from 10 Brazilian states and 10 different crops, and two samples from California, USA (cotton and tomato) were selected for analysis of genetic variability. Prior molecular identification had determined that 16 samples collected in Brazil and one sample from California belonged to biotype.

A sample identified as biotype A from California and a sample identified as the native biotype of *B. tabaci* occurring in Brazil were used as external samples to the group.

Comparison of the fragment profiles of the various whitefly biotypes was performed using five individuals of *B. tabaci* collected on various crops distributed in several Brazilian regions, and compared with biotypes coming from other regions of the world (Table 2) using the oligonucleotides OPA-04, OPA-10, OPA-11, OPA-13 and OPA-15.

RAPD reaction

Amplification reactions were carried out in 30 µL of a mixture containing 24.9 µL milliQ autoclaved water, 3.0 µL 10X buffer (60 mM Tris-HCl pH 8.8, 500 mM KCl and 20 mM $MgCl_2$, Amersham, CA, USA), 1.2 µL of an oligonucleotide of random sequence (OPA-02, OPA-03, OPA-04, OPA-05, OPA-10, OPA-11, OPA-13, OPA-15 or OPA-20) (Operon Technologies, Inc.) at a concentration of 10 µM, 0.6 µL of 10 mM dNTP and 1 U of *Taq* DNA polymerase (Amersham, CA, USA) and 4 µL DNA (20 ng).

Genomic DNA amplification conditions

Amplifications were carried out in a thermal cycler (MJ Research PTC-100) having an initial denaturation step of 3 min at 94°C, programmed to 45 cycles of 1 min at 93°C denaturation, annealing for 1 min at 35°C and extension for 2 min at 72°C, and a final extension step of 5 min at 72 °C.

Visualization of DNA fragments by electrophoresis

The amplification products from the RAPD reactions were visualized on 1.5% agarose gel submerged in 1X TBE buffer (90 mM Tris-borate and 1 mM EDTA) for 3 h at 160 V, photographed and stored in the Eagle eye system (Stratagene).

In all gels, molecular weight markers (100 bp Ladder - Invitrogen) were used to determine molecular weight of the fragments amplified by the RAPD technique.

Statistical analysis

Five whiteflies were selected, at random, from the pool of individuals of each sample used in each experiment for molecular

Table 1. Whitefly samples used in molecular identification procedures and studies of genetic variability.

Sample	Locality	State	Crop	Molecular identification
1	Cenargen	Distrito Federal	Cotton	*Bemisia tabaci* BR1
2	Tibau	Rio Grande do Norte	Cassava	*B. tabaci* B2
3	Lavras	Minas Gerais	Broccoli	*B. tabaci* B
4	Floriano	Piauí	Broccoli	*Aleurodicus cocois*
5	Baraúna	Rio Grande do Norte	Melon	*B. tabaci* B
6	Baraúna	Rio Grande do Norte	Melon	*B. tabaci* B
7	Baraúna	Rio Grande do Norte	Melon	*B. tabaci* B
8	Baraúna	Rio Grande do Norte	Melon	*B. tabaci* B
9	Planaltina	Distrito Federal	Eggplant	*B. tabaci* B
10	Planaltina	Distrito Federal	Tomato	*B. tabaci* B
11	Planaltina	Distrito Federal	Cucumber	*B. tabaci* B
12	Planaltina	Distrito Federal	Pumpkin	*B. tabaci* B
13	Petrolina	Pernambuco	Invasive plants	*B. tabaci* B
14	Nova Porteirinha	Minas Gerais	Batata	*B. tabaci* B
15	Nova Porteirinha	Minas Gerais	Tomato	*B. tabaci* B
16	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
17	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
18	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
19	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
20	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
21	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
22	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
23	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
24	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
25	Lavras	Minas Gerais	Cucumber	*B. tabaci* B
26	Águas Mornas	Santa Catarina	Cucumber	*Trialeurodes vaporariorum*
27	Cenargen	Distrito Federal	Tobacco	*T. vaporariorum*
28	Pau Branco	Rio Grande do Norte	Melon	*B. tabaci* B
29	Cajazeiras	Ceará	Melon	*B. tabaci* B
30	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
31	Baraúna	Rio Grande do Norte	Melon	*B. tabaci* B
32	Chapecó	Santa Catarina	Bean	NI
33	Cenargen	Distrito Federal	Tomato	*B. tabaci* B
34	Cenargen	Distrito Federal	Pumpkin	*B. tabaci* B
35	Cenargen	Distrito Federal	Bean	*B. tabaci* B
36	Jaboticabal	São Paulo	Bean	NI
37	Cenargen	Distrito Federal	Tomato	*B.tabaci* B
38	Cenargen	Distrito Federal	Pumpkin	*B. tabaci* B
39	Cenargen	Distrito Federal	Bean	*B. tabaci* B
40	Sinop	Mato Grosso	Weed	NI
41	Várzea Grande	Grande Mato Grosso	Cotton	NI
42	Várzea Grande	Grande Mato Grosso	Weed	NI
43	Várzea Grande	Grande Mato Grosso	Cotton	NI
44	Várzea Grande	Grande Mato Grosso	Cotton	NI
45	Várzea Grande	Grande Mato Grosso	Soy	NI
46	Pau Branco	Rio Grande do Norte	Bean	*B. tabaci* B
47	Pau Branco	Rio Grande do Norte	Tomato	*B. tabaci* B
48	Pau Branco	Rio Grande do Norte	Cassava	*B. tabaci* B
49	Cajazeiras	Paraíba	Melon	*B. tabaci* B
50	Cajazeiras	Paraíba	Melon	*B. tabaci* B

Table 1. Contd.

51	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
52	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
53	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
54	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
55	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
56	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
57	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
58	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
59	Planaltina	Distrito Federal	Tomato	*B. tabaci* B
60	Planaltina	Distrito Federal	Cucumber	*B. tabaci* B
61	Planaltina	Distrito Federal	Eggplant	*B. tabaci* B
62	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
63	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
64	Mossoró	Rio Grande do Norte	Melon	*B. tabaci* B
65	Baraúna	Rio Grande do Norte	Melon	*B. tabaci* B
66	Tibau	Rio Grande do Norte	Melon	*B. tabaci* B
67	*Toca da raposa*	Rio Grande do Norte	Melon	*B. tabaci* B
68	PADF	Distrito Federal	Cabbage	*B. tabaci* B
69	PADF	Distrito Federal	Cabbage	*B. tabaci* B
70	PADF	Distrito Federal	Cabbage	NI
71	PADF	Distrito Federal	Cabbage	NI
72	Tibau	Rio Grande do Norte	Melon	*B. tabaci* B
73	Baraúna	Rio Grande do Norte	Melon	*B. tabaci* B
74	Riverside	Califórnia (USA)	Cotton	*B. tabaci* A
75	Riverside	Califórnia (USA)	Tomato	*B. tabaci* B
76	Janaúba	Minas Gerais	Cotton	*B. tabaci* B
77	Recife	Pernambuco	Mallow	*B. tabaci* B
78	Campo Grande	Mato Grosso do Sul	Clover	*B. tabaci* B
79	Miguelópolis	São Paulo	Soy	*B. tabaci* B
80	Limoeiro do Norte	Ceará	Bean	*B. tabaci* B
81	Tibau	Rio Grande do Norte	Tomato	*B. tabaci* B
82	Cambucí	Rio de Janeiro	Cucumber	*B. tabaci* B
83	Aracati	Ceará	Melon	*B. tabaci* B
84	Boa Vista	Roraima	Melon	*B. tabaci* B
85	Viçosa	Minas Gerais	Soy	*B. tabaci* B
86	Campos de Goytacazes	Rio de Janeiro	Cabbage	*B. tabaci* B
87	Recife	Pernambuco	Cabbage	*B. tabaci* B
88	Park Branco	Rio Grande do Norte	Melon	*B. tabaci* B
89	Juazeiro	Bahia	Melancia	*B. tabaci* B
90	Mogi Mirim	São Paulo	Cotton	*B. tabaci* B
91	Itiquira	Mato Grosso	Cotton	*B. tabaci* BR
92	Park Branco	Rio Grande do Norte	Melon	*B. tabaci* B
93	Embrapa soja	Londrina	Soy	*B. tabaci* B
94	Múrcia	Espanha	Tomato	*B. tabaci* Q
95	Universidade de Al Akhawayn	Marrocos	Pumpkin	*B. tabaci* Q
96	Universidade de Ibadan	Nigéria	Cassava	*B. tabaci*
97	Cenargen	Distrito Federal	Tomato	*B. tabaci* B
98	Campo	Mato Grosso do Sul	Forage legume	NI

Bemisia tabaci BR, biotype used as standard; *B. tabaci* B, biotype used as standard; NI, not yet identified sample.

Table 2. RAPD fragments generated by each sample of *B. tabaci* collected on different types of crops in Brazil.

Sample	RAPD fragment	
	Polymorphic	Polymorphism (%)
74	82	79.6
75	73	70.9
76	73	70.9
77	75	72.8
78	81	78.6
79	73	70.9
80	71	68.9
81	68	66.0
82	71	68.9
83	84	81.6
84	77	74.8
85	72	69.9
86	66	64.1
87	76	73.8
88	86	83.5
89	78	75.7
90	77	74.8
91	72	69.9
92	75	72.8
93	85	82.5

studies. The procedure described previously was repeated three times to confirm the data obtained.

The polymorphism among individuals in the population was determined from the fragments present in the electrophoretic gels. Then, a presence/absence matrix (1 or 0) was generated for each fragment. In case of doubt, the number 9 was used as standard. The similarity matrix was calculated using the Dice coefficient and UPGMA analysis produced a dendrogram which showed a grouping of individuals using the NTSYS program version 2.2 pc (Rohlf, 1993).

After that, the values obtained and tabulated in a spreadsheet were subjected to analysis of molecular variance (AMOVA) for statistical determination of the possible sources of variability found in populations by applying specific algorithms using Arlequin program version 2000 (Schneider et al., 2000).

RESULTS AND DISCUSSION

Molecular identification

Insect samples from different crops originating from diverse locations had their molecular profiles determined using the OPA-13 oligonucleotide according to Lima et al. (2000). The fragment patterns produced by five individuals from each location were compared with the B. *tabaci* whitefly standard previously determined to be biotype B or the native biotype found in Brazil, referred as BR (Figure 1).

Using the oligonucleotide OPA-13 characteristic, RAPD fragment profiles for both biotype B and for the native biotype found in Brazil were identified and used as standard. For the biotype B, two fragments of 1000 and 1700 bp were found biotype B and for the native biotype (BR), 500, 1000 and 1500 bp fragments were found. The B. tabaci sample classification shown in Table 1 was made based on the standards for these biotypes. An RAPD fragment profile of 800 and 1400 bp was observed in two samples. After further investigation, it was observed that the molecular profile corresponded to *Trialeurodes vaporariorum*. This profile was identified in samples from Águas Mornas (SC) and Brasilia (DF).

This strategy allowed the analysis of 100 samples in a short period of time, noting that there was a predominance of individuals belonging to the B biotype of *B. tabaci*, found in 78% of samples, followed by the Brazilian native biotype (BR) of *B. tabaci* (4%). Other whiteflies were also identified using this methodology corresponding to 3% of the samples (1% *Aleurodicus cocois* and 2% *T. vaporariorum*). Some samples (11%), however, could not be identified using the established standards.

In addition, two samples previously identified as biotype Q of *B. tabaci* from Spain and Morocco had their electrophoretic profiles determined. The same was done with a sample from Nigeria containing the *B. tabaci* cassava biotype (Figure 2).

The Q biotype individuals from Spain and Morocco

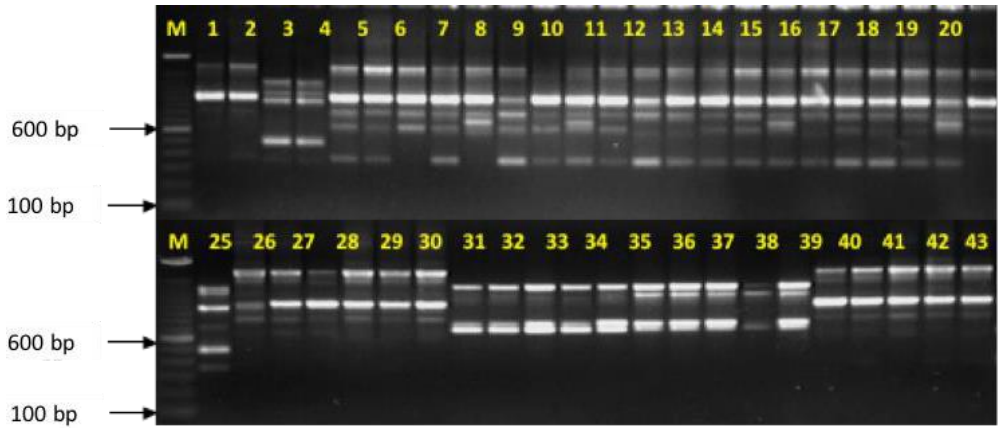

Figure 1. Gel representative of the molecular identification of whitefly populations using the OPA-13 oligonucleotide. The letter M indicates the 100 bp ladder (Invitrogen) marker. The numbers indicate: 1, 2 and 26 biotype B of *B. tabaci* used as standard; 3, 4 and 25 native biotype found in Brazil used as standard; 5-9, whitefly sample collected on broccoli crop (Lavras - MG); 10-24, whitefly sample from melon crop (Baraúna- RN); 27-31, sample from collard green crop (Planaltina - DF); 32-36, whitefly collected on cucumber (Aguaa mornaa - SC); 37-41, whitefly collected on tobacco crop (Brasília - DF); 42-46, whitefly collected on melon (Cajazeiras - PB).

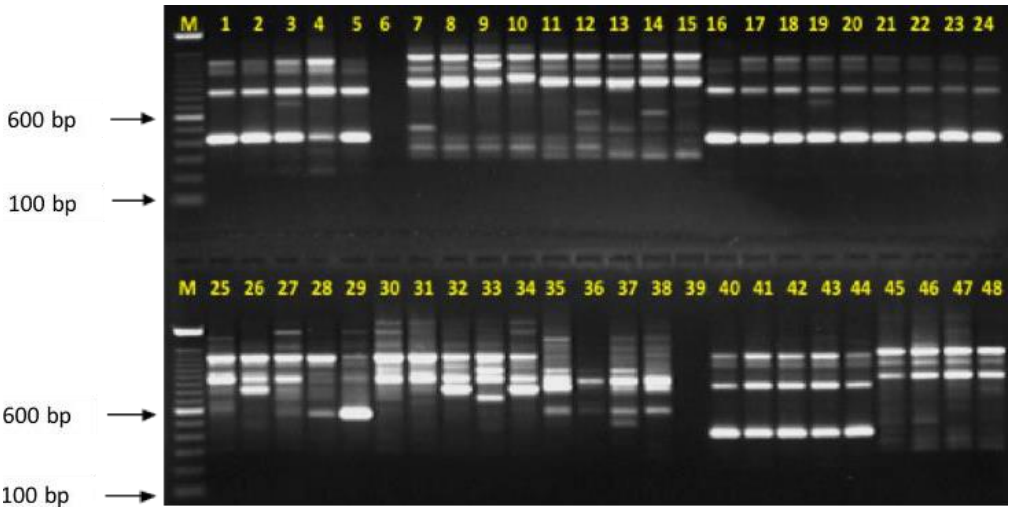

Figure 2. Representative gel of RAPD fragments patterns of whitefly biotypes obtained by amplification of the OPA-10 oligonucleotide. Letter M indicates the 100 bp ladder marker (Invitrogen). The numbers indicate the *B. tabaci* biotypes: 1-5, B biotype collected on tomato (Riverside - California - USA); 6-10, A biotype collected on cotton (Riverside - California - USA); 11-15, Brazilian native biotype found on cotton (Brasília - DF); 16-20, B biotype found on melon (Parque Braco- RN); 21-24, B biotype collected on soybean (Carolina - MA); 25-29, Q biotype collected on tomato (Spain); 30 to 34, Q biotype found on cucumber crop (Morocco); 35-39, cassava biotype collected on cassava (Nigeria); 40-44, B biotype found on tomato crop (Brasília - DF); 45-48, unidentified sample of *B*.

presented a characteristic RAPD fragment of 1350 bp. For the cassava biotype originated from Nigeria, there was a 950 bp fragment. Differentiation of the biotypes Q and cassava is based on the absence of the 1350 bp fragment in the latter. Regarding the established standards, it was observed that the whitefly individuals from the United States and previously identified as biotype B presented the same profile as the other whitefly individuals from Brazil, also identified as biotype B. This was confirmed by the presence of three DNA fragments in all samples (420, 850 and 1300 bp). Analyzing the profile of whitefly individuals previously

Table 3. Average fragments produced by the oligonucleotides used in the study of genetic variability of populations of *B. tabaci* collected in various locations and crops from Brazil.

Oligonucleotide	Fragment		
	Total	Average polymorphic	Polymorphism (%)
OPA-02	20	15.4 ± 1.8	77.0
OPA-03	13	9.6 ± 0.8	73.8
OPA-05	9	6.3 ± 1.0	70.0
OPA-10	14	10.9 ± 1.2	77.9
OPA-11	13	9.6 ± 1.2	73.8
OPA-13	11	8.5 ± 1.0	77.3
OPA-15	12	8.5 ± 0.9	70.8
OPA-20	12	8.0 ± 2.0	66.7

identified as biotype A from the United States, a very similar pattern of DNA fragments was observed when compared to the native Brazilian biotype. The samples analyzed in this study show that, currently, the B biotype of *B. tabaci* predominates in all regions of Brazil. Previous identification results, described by Lima et al. (2002) showed that the Brazilian native biotype was present in several states such as Mato Grosso, Minas Gerais, Rio de Janeiro, Goiás, and the Federal District. However, this biotype currently appears to be restricted to certain regions and crops. These results demonstrate the high capacity for dispersion and invasion of the B biotype of *B. tabaci* in different regions of Brazil occurring in habitats previously occupied by the native Brazilian biotype. In addition, there apparently does not exist a host crop preference, indicating the extreme adaptability of this pest to different nutritional conditions.

The RAPD molecular profiles obtained in this study confirmed the descriptions previously set forth by Lima et al. (2002) for the native biotype, as well as permitting the monitoring of dispersion and occurrence of whitefly populations belonging to other species or biotypes.

The molecular strategy used can address the need to detect the presence of the B biotype and other biotypes in Brazilian agriculture from samples stored in alcohol relatively quickly and accurately in order to prepare plant protection reports.

Analysis of the genetic variability of whitefly population in Brazil

From the information generated by the RAPD marker profiles, a study of the genetic variability of populations from different crops in the Northeast, Midwest and Southeastern regions of Brazil, as well as from the United States, was carried out for comparison purposes. By submitting the DNA of individuals from ten whitefly populations to RAPD analysis with in decameric oligonucleotides, differences were observed RAPD fragment profiles for the various samples (Table 2).

The ten RAPD oligonucleotides produced 103 *loci* and each sample produced 76.8±5.6 polymorphic fragments, on average. Regarding the number of polymorphic fragments, it was observed that sample 88 produced the highest number (82.7%) and the population represented by sample 86 produced the smallest number (63.5%). Each initiator generated 13±3.2 fragments per sample (Table 3).

The OPA-02 oligonucleotide generated an average of 15.4±1.8 polymorphic RAPD fragments for the study of genetic variability of whitefly populations. The OPA-05 primer produced the lowest average of polymorphic fragments. From the RAPD marker analysis, it was observed that the selected oligonucleotides showed potential for use in the generation of molecular profiles of various samples of *B. tabaci* (Table 4).

Once more, the molecular analysis confirmed the potential use of the OPA-10 and OPA-13 oligonucleotides to identify biotypes of *B. tabaci*, confirming the information by Lima et al. (2000) on the application of these oligonucleotides to discriminate the B biotype of *B. tabaci*. Noteworthy is also the potential of the oligonucleotides OPA-05, OPA-11 and OPA-15 in the identification of *B. tabaci* biotype B. Then, the binary data generated by RAPD oligonucleotides using *B. tabaci* samples 74-93 were used to generate a dendrogram and for analyses of molecular variance (AMOVA). The analyses showed differences among the analyzed samples. In the generation of groupings of whitefly samples by UPGMA the formation of specific groups for each location and crop was observed (Figure 3).

The dendrogram analysis showed that biotype A of *B. tabaci* from the United States differed from the other biotypes as it showed around 8% of genetic similarity.

For the remaining populations previously identified by the molecular method as biotype B, separation was observed among them based on location and crop of collection, so that it is not possible to establish a pattern among these parameters. Regarding *B. tabaci*

Table 4. DNA fragments obtained by the use of different RAPD oligonucleotides which can be applied to the identification of the B and native biotypes of *B. tabaci*.

Oligonucleotide	Biotype	RAPD fragment (pb)
OPA-05	B	600 and 900
OPA-10	B	400 and 900
	BR	1000
OPA-11	B	550 and 1100
	BR	500
OPA-13	B	600, 1000 and 1600
	BR	800
OPA-15	B	700
	BR	700 and 1100

native Brazilian biotype, it was observed that this did not form a separate grouping from the B biotype. However, the population supposedly defined as a native biotype, showed 25% similarity with the B biotype. This biotype was collected mainly on cotton, suggesting that the biotype presents greater preference for that crop. In addition, the initial molecular identification using the OPA-10 oligonucleotide showed that the native Brazil biotype had a similar molecular profile to biotype A from the United States. However, subjecting these samples to an analysis with more oligonucleotides showed a different grouping compared to biotype A.

From the information obtained in the dendrogram, an analysis of molecular variance (AMOVA) was carried out in order to better understand the dynamics of these whitefly populations. Analyzing the differences among biotypes A, B and the native Brazilian biotype, it was observed that 22.99% of the variation originated between the biotypes, 76.78% between samples within the biotypes, and 0.22% within samples from the same biotype. Following AMOVA analysis with all the biotypes grouped, it was observed that 99.68% of the variation was between the samples and 0.32% within the samples.

The analysis of only samples initially identified as biotype B of *B. tabaci* indicated a value of 99.75% between the samples and 0.25% variation within the samples. Comparing the A biotype and the native Brazilian biotype, it was found that 25.67% of the variation was between the biotypes, 74.33% between samples within the group, and 0% within the biotypes samples. The comparison between the B biotypes and the native biotype found in Brazil indicates a variation of 16.79% between groups, 83.04% between samples within the group, and 0.17% within the samples.

Regarding the B-biotype samples, the AMOVA values indicate differences between the samples of this whitefly biotype. In other words, despite the molecular identification revealing molecular markers for this specific biotype, it was observed that the samples belonging to this biotype have distinct gene pools. This was confirmed due to the high variation found among the samples.

This phenomenon has also been described by Silva et al. (2009) using RAPD markers. The authors collected whiteflies on okra, bean and pepper crops in São Luís (MA), and from the 96 RAPD molecular markers generated, a dendrogram was constructed which showed that populations on okra, beans and peppers were grouped according to the host crops. These data corroborate the findings of this study. However, in this work it was possible to analyze a larger number of locations and describe the phenomenon of grouping of whitefly populations as a function of crop that may be a phenomenon independent of location, suggesting a pattern of dispersion and allocation of this biotype in the field. This phenomenon can be explained partly by the reproductive strategy of the species, which has parthenogenetic arrhenotokous cycles, generating haploid males. Thus, the data obtained in the dendrogram indicate that each population established on a given crop is composed of clones due to the asexual reproduction of *B. tabaci*. This observation is supported by the low variability within the samples, observed to be less than 1%.

Helmi (2011) used random primers to assess the genetic variability of *B. tabaci* occurring in six hosts, that is, eggplant, tomato, cotton, watermelon, squash and okra. The results obtained showed that the whitefly may have different genotypes on adaptations to certain host plant species in Egypt, suggesting host specificity amongst under study *B. tabaci* populations based on RAPD molecular markers. The clustering pattern observed in the dendrogram showed that distinct genotypes exist among populations collected within the Egyptian region. After these information the author concludes these differences may be influencing some biological features

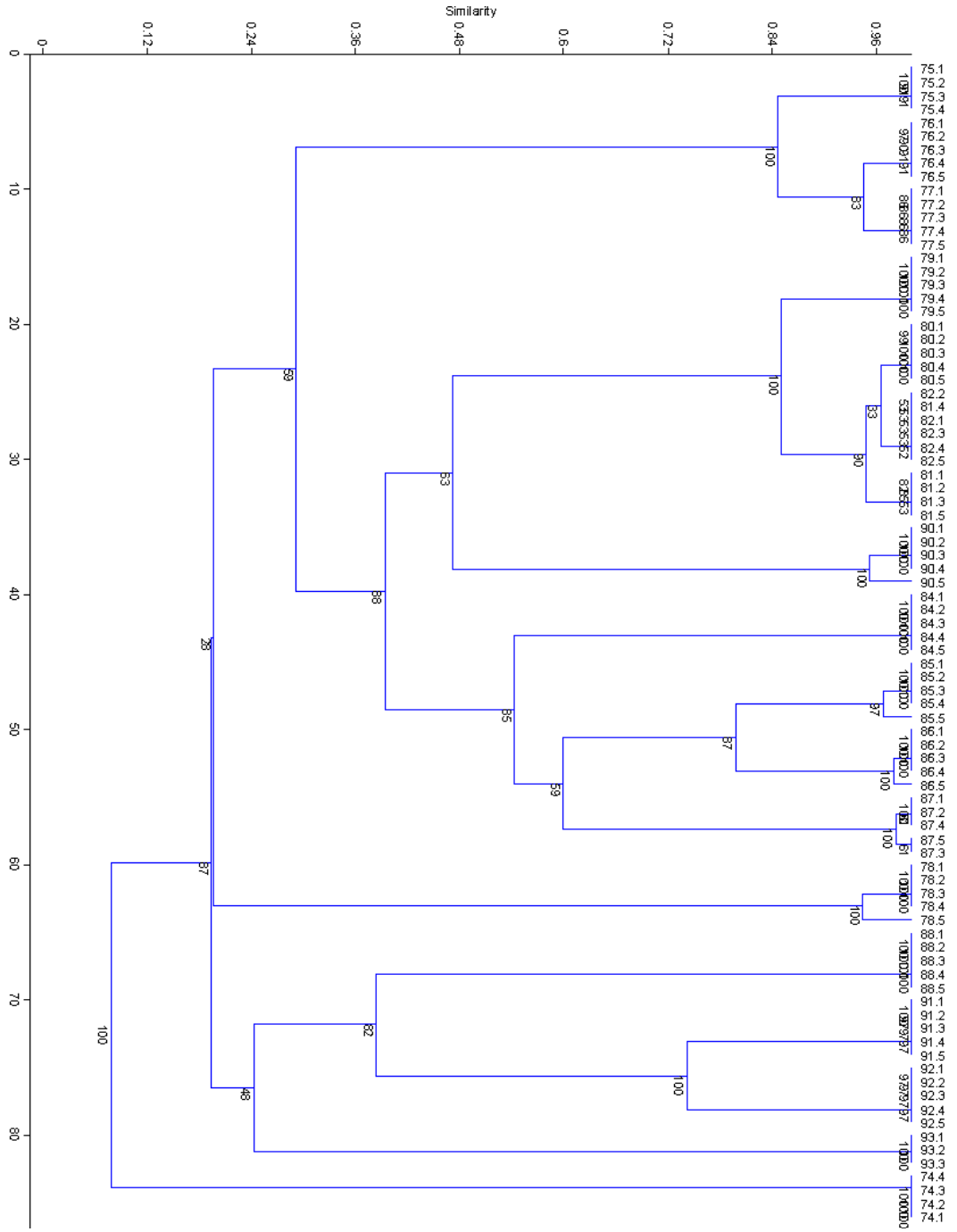

Figure 3. Dendrogram of genetic similarity classified by unweighted pair group method with arithmetic mean (UPGMA) determined by DICE coefficient (Cophenetic correlation coefficient = 0.9852) of *B. tabaci* populations present in different crops and regions in Brazil. The numbers are in accordance with Table 1.

like as virus vectoring capabilities and insecticide susceptibilities for example.

Furthermore, the availability of diet and the location of establishment of the sample may exert some influence on the ecological dynamics of these individuals. Bernays (1999) presents *B. tabaci* behavior parameters for the selection of the host plant. Experiments with *B. tabaci* adult females showed that when a mixture of host plants is found in an area, the insect performance is decreased when compared to the same experiment

using only a plant known as the best host. In mixtures of host plants, whiteflies spend less time on the leaf surface, moving most of the time and more often to new feeding places, spending less time at a given nutrition point. The author suggests that *B. tabaci* eventually perceives alternative feeding locations and that the observed performance must be related to the insect's information processing mechanisms and decision-making. The data pointed by the author suggest that the observed behavior is the result of a characteristic loss of attention when the insect is facing a complex mixture of sensory signals. This can result in distraction and consequent reduction in decision making by the insect.

Finally, the phenomenon observed is due probably to the fact that the clonal populations established in a given area when at a low migration frequency and under selection pressure caused by chemical agents can contribute to the selection of a profile of molecular markers that are found specifically in a given *B. tabaci* population.

Using molecular analysis, it was observed that gene flow between two biotypes of the same population is smaller than between populations of identical biotypes (Moya et al., 2001). The present work confirmed this finding, since it was possible to identify specific molecular markers for tested biotypes independently of the sample origin. Thus, molecular techniques based on DNA proved useful to confirm the existence of biotypes and the degree of separation between them. For epidemiological and agro-economic purposes, it is essential to understand the intimate relationship developed between *B. tabaci* local populations and especially any differentiation among populations that is related to the host (Burban et al., 1992).

This information may serve as a basis for studies aiming to identify which factors influence the population distribution in a given area, but also, it may be useful in determining whitefly chemical control methods in relation to a specific host crop. It becomes very important to obtain information with respect to the sources of variation occurring within the B biotype of *B. tabaci*, since resistant populations of whitefly can be quickly selected after chemical insecticide applications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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