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Population variability of *Bemisia tabaci* (Genn.) in different hosts

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ABSTRACT. The silverleaf whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), is a cryptic species complex that contains some of the most damaging pests in tropical and subtropical regions. Recent studies have indicated that this complex is composed of at least 24 distinct and morphologically indistinguishable species that mainly differ in their ability to transmit phytoviruses, adapt to hosts, and induce physiological changes in certain hosts. The importance of this species has been increasing worldwide, because it serves as a phytovirus vector, particularly for geminiviruses, in economically important crops. Here, we aimed to examine the population variability of *B. tabaci* populations inhabiting 6 agricultural crops grown in 5 regions of Brazil and 1 region of the USA; BRrep [Brasília (DF, Brazil) - cabbage], ILsoj [Urbana (IL/USA) - soybean], BJabo [Bom Jesus da Lapa (BA, Brazil) - pumpkin], CPsoj [Campinas (SP, Brazil)

- soybean], UBman [Ubatuba (SP, Brazil) - cassava], and PEmel [Petrolina (PE, Brazil) - melon]. Thirteen polymorphic loci with 50 alleles were observed, with an average of 2.37 (range: 2.00-2.91) alleles per population. The UBman and PEmel *B. tabaci* populations were the most differentiated, which was probably caused by insect adaptation to the host plant and the use of insecticides. A 33.87% inter-population variation was observed, indicating that microsatellites may be used to measure differentiation among these *B. tabaci* populations. Based on the comparison of microsatellites in the current study, only the Middle East-Asia Minor 1 population of *B. tabaci* was found in the six populations.

Key words: Genetic variability; Molecular marker; Population variability; Silverleaf whitefly

INTRODUCTION

More than 1500 species of whitefly (Hemiptera: Aleyrodidae) have been reported in the published literature (Martin and Mound, 2007); however, only a small number of these insects has been subject to detailed study (Byrne et al., 1990). One of the most important whitefly species is *Bemisia tabaci* (Genn.). The existence of different *B. tabaci* whitefly biotypes was proposed in the 1950s, to describe distinct populations of insects based on host plant adaptation and the ability to transmit viruses (Brown et al., 1995; Perring, 2001). This species is considered to be a species complex (Perring, 2001; Berry et al., 2004), with a broad host spectrum. *B. tabaci* is found in more than 900 plant species (Jones, 2003; Berry et al., 2004), and is able to carry more than 110 different phytoviruses. In addition, it is the only whitefly species that transmits geminiviruses (Mound, 1963).

The *B. tabaci* biotype B was probably first introduced to Brazil through the importation of plant material. The first massive infestation of economically important crops by this biotype was documented in 1991 (Lourenção and Nagai, 1994) and reached the country's main agricultural regions in just a few years (Villas Bôas et al., 1997; Sosa-Goméz et al., 1997; Arruda et al., 1998; Lourenção et al., 2001; Lima et al., 2002; Yuki et al., 2002; Tamai et al., 2006).

Recent studies (De Barro et al., 2011) have indicated that there is sufficient evidence to state that *B. tabaci* is not made up of biotypes, and that the use of biotype is misleading. *B. tabaci* is a complex of well-defined high-level groups containing at least 24 morphologically indistinguishable species. These species are clearly defined by comparison against consensus sequences, and may be delimited by 3.5% mtCOI1 sequence pairwise genetic distance divergence (De Barro et al., 2011). As a result, *B. tabaci* biotype B has now been placed in the Middle East-Asia Minor 1 (MEAM1) group (De Barro et al., 2011).

The main studied phenotypic differences among *B. tabaci* groups include host specialization, a wide range of polyphagous haplotypes, dispersal behavior, mating behavior, reproductive compatibility, differential resistance to many classes of insecticides, and variable efficiency in the transmission of phytoviruses (Perring, 2001; Brown, 2010).

Molecular markers have provided valuable information toward clarifying the behavior

and dynamics of natural *B. tabaci* populations. Hence, the determination of *B. tabaci* genetic differentiation in different populations might contribute toward improving pest control in Brazil and other areas of the world, because different host plants are found in different geographical regions, with intra-population genetic variation. Microsatellites are ideal for studying the population genetic structure of different *B. tabaci* biotypes (Simón et al., 2007). For instance, Tsagkarakou and Roditakis (2003), De Barro et al. (2003, 2005), and Delatte et al. (2006) isolated and characterized *B. tabaci* microsatellites that may be used to characterize biotypes derived from different regions and/or host plants.

This study aimed to determine the genetic variability of *B. tabaci* populations in certain hosts from five regions in Brazil and one region in the USA using microsatellite markers.

MATERIAL AND METHODS

The variability of 6 *B. tabaci* populations (Table 1) was studied using microsatellite markers. Five of the study populations originated from states in Brazil (Bahia, Distrito Federal, São Paulo, and Pernambuco), while the other originated from the USA (Urbana/Illinois). The Campinas population (CP) was previously described as belonging to biotype B (Fontes et al., 2010; do Valle et al., 2011) and had been classified as MEAM1 (De Barro et al., 2011); hence, it was used as a reference for the characterization of the other populations studied.

Table 1. *Bemisia tabaci* populations from different locations and their respective host plants.

Population	Host plant	Scientific name	Latitude - longitude	Location
BRrep	Cabbage	*Brassica oleracea*	15°46'47"S - 47°55'47"W	Brasília, DF, Brazil
ILsoj	Soybean	*Glycine max*	40°06'38"N - 88°12'26"W	Urbana, IL, USA
BJabo	Pumpkin	*Cucurbita moschata*	13°15'42"S - 43°24'28"W	Bom Jesus da Lapa, BA, Brazil
CPsoj	Soybean	*Glycine max*	22°54'20"S - 47°03'39"W	Campinas, SP, Brazil
UBman	Cassava	*Manihot esculenta*	23°26'13"S - 45°04'08"W	Ubatuba, SP, Brazil
PEmel	Melon	*Cucumis melo*	09°23'55"S - 40°30'03"W	Petrolina, PE, Brazil

Maintenance of *B. tabaci* populations

Adults from various whitefly *B. tabaci* populations were collected in the field during 2009 and 2010, and stored in 70% ethanol at -4°C for subsequent molecular analysis. Table 1 shows the locations where the whitefly populations analyzed were collected, in addition to providing information about their respective host plants.

DNA extraction and quantification

The procedure for the extraction of DNA from *B. tabaci* followed the protocol of Silva (2006). The DNA was extracted from female whiteflies that were macerated in a 1.5-mL tube, followed by maceration in 60 µL extraction buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA, 0.3% Triton X-100, 60 µg/mL proteinase K). The homogenate was incubated for 15 min at 65°C, and then boiled at 95°C for 10 min. Subsequently, the samples were stored at -20°C. After electrophoresis, the sample was quantified from the fluorescence intensity emitted by Syber Safe under UV on 1.4% (w/v) agarose gel, and compared to standards with known molecular weights and concentrations (λ phage DNA).

Population variability of *B. tabaci* using microsatellite markers

The microsatellite loci for the characterization of *B. tabaci* were obtained from 12 individuals from each population (Table 1). Thirteen pairs of primers that had been previously described in the literature were used (De Barro et al., 2003; Tsagkarakou and Roditakis, 2003; Tsagkarakou et al., 2007; Gauthier et al., 2008) (Table 2).

Table 2. Polymorphic primers evaluated in the study of *Bemisia tabaci* variability.

Primer name	Expected size (bp)	Forward/reverse	Annealing temperature (°C)	Reference
BT-e 49	304-368	F: GATCGAATCCAATCTCCCAC R: GAGCTCTGCAATACTGCCAA	64	Tsagkarakou et al., 2007
BT-b 103	140-142	F: GCTCAACCGAATACATCCAC R: AAGTCTAAAGGAAGCGTGGAA	56	Tsagkarakou and Roditakis, 2003
BEM 12	100-304	F: CTATCGTTGACTGATTTTTG R: AAATTACCTACACCTGCCT	56	De Barro et al., 2003
BEM 25	95-188	F: AAGTATCAACAAATTAATCGTG R: TGAAGAATAAGAATAAAGAAGG	60	De Barro et al., 2003
BEM 37	72-87	F: TGACGACCTGAGGCTGAGAG R: TGCAACGGCAACAGCAAGCAA	60	De Barro et al., 2003
1.2	299-355	F: CTTACCTTCCATTCACC R: ATCCCGAGTCTTATGTTG	53	Gauthier et al., 2008
1.4	162-232	F: GAAATCCTCACACTGGC R: CACTGATGTCAGACCTGG	53	Gauthier et al., 2008
1.9	262-282	F: GTGTTTGAGGAGGTGGG R: CTATTTATCTATTTGGGTCA	60	Gauthier et al., 2008
1.11	106-136	F: ATGTTATGACTATCGCAATC R: GCAGTGTGTAAGGGTGTC	58	Gauthier et al., 2008
1.12	158-220	F: AATCCTCACACTGGCTG R: CCTGATGTCAGACATGG	62	Gauthier et al., 2008
2.3	134-156	F: CAGAACGACAGGTCGAG R: CAAAATTAATGGTATTGACTC	58	Gauthier et al., 2008
1.13	113-121	F: CTAAGACCGATTCCTCC R: GAATACTACACCTTCAATTACC	62	Gauthier et al., 2008
1.17	111-133	F: GTGACCGAGTAAGGCCA R: CACGTCCGCTCTCTATG	58	Gauthier et al., 2008

All of the analyzed microsatellite loci were amplified by PCR in a final volume of 20 µL that contained 3 µL DNA (approximately 20 ng), 2 µL of the forward and reverse primers (0.2 µM), 0.25 µL dNTPs (0.15 mM), 2 µL 1X buffer (50 mM KCl, 100 mM Tris-HCl, pH 8.5), 1.60 µL $MgCl_2$ (4.0 mM), 0.5 U Taq DNA polymerase, and 10.65 µL autoclaved MilliQ water.

The amplifications were performed in a thermocycler (MyCycler, BioRad). The PCR protocol for the BT-e 49 primer was: an initial denaturation step of 15 min at 95°C; 8 cycles of 30 s at 94°C, 90 s at 67-53°C (-2°C per cycle), and 60 s at 72°C; 40 cycles of 30 s denaturation at 94°C, 90 s annealing at 64°C, and a 1-min extension at 72°C; and a final extension for 30 min at 72°C. Table 2 shows the annealing temperatures for the other primers used.

Single sequence repeat (SSR) genotyping of polymorphic primers

SSR genotyping was performed on 7% denaturing polyacrylamide gels stained with silver nitrate (Creste et al., 2001). The gels were run in a vertical chamber (BioRad Gene

Sequence) for 150 to 210 min, depending on the primer used, at 70 W using a 10-bp ladder (Invitrogen) as the molecular weight marker.

Genetic analysis - microsatellite locus statistics

From the acrylamide gels, we obtained the genic (allelic) and genotypic frequencies at each locus. Tests of Hardy-Weinberg equilibrium (HWE) were estimated for each locus by FSTAT v.2.9.3.2 (Goudet, 2001). The genetic diversity [number of alleles per locus (N_A), observed (H_O) and expected (H_E) heterozygosities, and F-statistics (F_{IS}, F_{ST}, and F_{IT})] were estimated by Genetix v.4.05 (Belkhir et al., 2002).

The variability structure was visualized through dendrograms constructed by Cavalli-Nei's genetic distance (Nei, 1978) matrix and by the neighbor-joining clustering criterion using the PHYLIP software (Felseinstein, 1989). Group stability was tested by employing the resampling procedure based on 10,000 bootstrap samples.

For the analysis of the diversity structuring with no *a priori* hierarchy, the Bayesian approach was performed with the Structure software. For each value of the *K*-group number (*K* 1-11), 5 independent simulations were performed. Each simulation contained 500,000 iterations with 50,000 burn-in iterations. The optimal clustering number (ΔK) was chosen from the prediction method based on the *K* values (Evanno et al., 2005).

RESULTS

For each primer, six populations [Brasília, DF (BRrep, cabbage); Urbana, IL, USA (ILsoj, soybean); Bom Jesus da Lapa, BA (BJabo, pumpkim); Campinas, SP (CPsoj, soybean); Ubatuba, SP (UBman, cassava); and Petrolina, PE (PEmel, melon); Table 1] were genotyped for a total of 72 *B. tabaci* individuals. All 13 primers that were used were polymorphic. These loci discriminated 50 different alleles in the studied populations.

Genetic variation

When tested with the primers BT-b 69, BEM 6, BEM 23, BEM 37, 1.1, 1.6, 2.4, and 1.14, the 6 *B. tabaci* populations did not exhibit any genetic variation. However, genetic variation was identified using the primers BT-e 49, BT-b 103, BEM 12, BEM 25, BEM 37, 1.2, 1.4, 1.9, 1.11, 1.12, 2.3, 1.13, and 1.17 (Table 3).

Table 3. Estimates of genetic diversity parameters in 6 *Bemisia tabaci* populations.

Population	N	N_A	H_E	H_O	*f*
BRrep	12	2.17	0.529	1.000	-0.973
ILsoj	12	2.25	0.510	0.917	-0.866
UBman	12	2.55	0.552	0.992	-0.864
BJabo	12	2.91	0.579	1.000	-0.817
CPsoj	12	2.00	0.473	0.800	-0.745
PEmel	12	2.38	0.476	0.875	-0.909
Mean	12	2.37	0.520	0.931	-0.862

N = number of individuals sampled; N_A = average number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity under Hardy-Weinberg equilibrium; *f* = fixation index. For other abbreviations, see Table 1.

A low level of genetic polymorphism was observed in the present study. However, 50 alleles were found in the 13 polymorphic loci. The average N_A per locus was 2.37, and the average H_E was 0.5199. The average N_A ranged from 2.00 alleles (CPsoj) to a maximum of 2.91 alleles (BJabo) (Table 3). The H_O varied among populations, ranging from 0.800 (CPsoj) to 1.000 (BRrep and BJabo), with a mean of 0.931. In contrast, the smallest H_E was obtained in the CPsoj (0.473) population, while the BJabo population had the highest value for this parameter (0.579).

After correcting the significance level with the Bonferroni correction ($P \leq 0.05$), it was observed that the majority of the populations were in HWE for most loci. For some studied loci, the following populations were not in HWE: locus 2.3, ILsoj population; locus BT-b 103, BJabo and PEmel populations; locus BT-e 49, ILsoj, BJabo, and UBman populations; and locus BEM 25, BRrep, CPsoj, BJabo, UBman, and PEmel populations. It is important to note that the deviations from the HWE might be related to the small sample sizes of some populations.

A small number of exclusive alleles were observed in certain populations, including 1 in the CPsoj population, 2 in the BJabo population, 3 in the ILsoj population, and 6 in the UBman population (Table 4). The frequencies of these alleles are shown in Table 4.

Table 4. Exclusive alleles obtained in microsatellite loci and their frequencies in six *Bemisia tabaci* populations.

Loci	Allele (bp)	Frequency (%)	Population
2.3	55	8.33	ILsoj
2.3	56	8.33	ILsoj
1.11	75	100.00	CPsoj
1.11	108	100.00	ILsoj
BEM 37	129	50.00	UBman
BEM 37	130	50.00	UBman
1.13	136	50.00	UBman
1.12	156	50.00	UBman
1.12	158	50.00	UBman
1.17	160	50.00	UBman
1.4	201	50.00	BJabo
1.4	204	50.00	BJabo

Genetic structure

The optimal clustering number (Evanno et al., 2005) was 7 (Figure 1) in the diversity structuring analysis, when using the Bayesian approach in the Structure software. From a total of 72 samples of adult whitefly, all 6 populations were found to be homogeneous, each of which may be visually identified by its specific color (Figure 1). The BJabo and CPsoj populations exhibited higher diversity among individuals within each population; these 2 populations also presented a slightly different pattern of diversity compared to the other populations.

The species rate of fixation ($\hat{F}_{IS}$) estimated from 13 SSR loci was negative and significant, indicating an excess of heterozygotes. The inter-population variation index was high ($F_{ST} = 0.34$), indicating that the populations differed to one another, as evidenced by the large number of clusters detected by the Bayesian analysis. These estimates were relatively consistent and significantly different from zero (Table 5). These differences might be primarily related to the adaptation of the insects to their specific host plants and geographic isolation. The two most distinct populations were BJabo, which might be the result of geographic distance, and CPsoj, which might be because of the greater adaptation of the pest to the host plant.

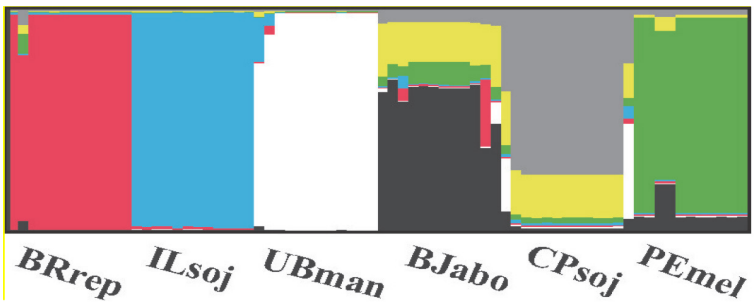

Figure 1. Probable genetic structures assigned to female *Bemisia tabaci* evaluated in 6 populations (K = 7) according to the Structure software. The females sampled in the populations are represented by colored vertical bars. The same color in different populations indicates that they belong to the same group. Different colors on the same bar indicate the percentage of the genome shared with each group.

Table 5. Wright's F-statistic estimates (Weir and Cockerham, 1984) in 6 natural *Bemisia tabaci* populations.

	$f(F_{IS})$	F (F_{IT})	θ (F_{ST})
Estimate	-0.91029	-0.26328	0.33870
Upper limit (95%CI)	-0.82419	-0.06981	0.44480
Lower limit (95%CI)	-0.98558	-0.49070	0.21509

95%CI = confidence interval at 95% probability.

The Nei genetic distances calculated among the populations ranged from 0.000 to 0.909 (Table 6). The genetic variability structure of these populations is illustrated with a dendrogram (Figure 2).

Table 6. Nei's genetic distances (1978) calculated among the *Bemisia tabaci* populations (below the diagonal) and the F_{ST} values calculated pairwise (above the diagonal).

	BRrep	ILsoj	UBman	BJabo	CPsoj	PEmel
BRrep	0.000	0.179	0.294	0.323	0.308	0.197
ILsoj	0.203	0.000	0.292	0.362	0.219	0.188
UBman	0.909	0.749	0.000	0.235	0.286	0.102
BJabo	0.324	0.598	0.484	0.000	0.254	0.244
CPsoj	0.421	0.373	0.306	0.395	0.000	0.161
PEmel	0.240	0.295	0.424	0.181	0.064	0.000

For abbreviations, see Table 1.

The dendrogram obtained from the similarity measures was verified by the neighbor-joining clustering method (Figure 2). One group contained just the Ubatuba population, while a second group contained all the other populations. The genetic distance values shown in the dendrogram indicated a pattern of genetic variability among the populations, with the Ubatuba population being the most different compared to the other study populations. This difference might be caused by the lower adaptation of insects to the host plant in the case of cassava in Ubatuba. It should be noted that the populations of Bom Jesus da Lapa and Petrolina were phylogenetically closer to one another; therefore, some differentiation might have occurred in

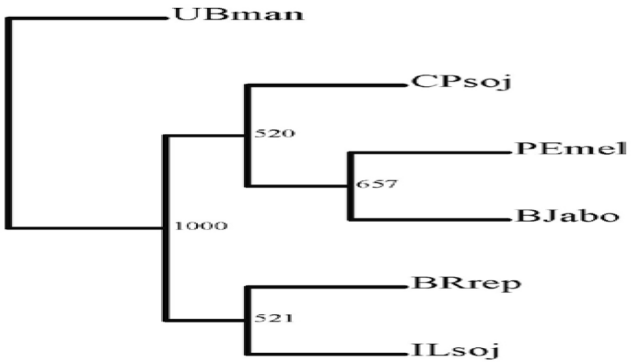

Figure 2. Genetic divergence pattern among 6 *Bemisia tabaci* populations as defined by neighbor joining clustering based on the genetic identity obtained from Nei's genetic distances (1978) for microsatellite loci: UBman = cassava, Ubatuba (SP); CPsoj = soybean, Campinas (SP); PEmel = melon, Petrolina (PE); BJabo = pumpkin, Bom Jesus da Lapa (BA); BRrep = cabbage, Brasília (DF); ILsoj = soybean - Urbana (IL).

these populations because of their geographical isolation in relation to other populations. The ILsoj (USA) population was observed to be very similar to the populations in Brazil.

DISCUSSION

Genetic variationw

When tested with the primers BT-b 69, BEM 6, BEM 23, BEM 37, 1.1, 1.6, 2.4, and 1.14, the 6 *B. tabaci* populations did not exhibit any genetic variation. These data differ to those previously obtained in the published literature (De Barro et al., 2003; Tsagkarakou and Roditakis, 2003; Tsagkarakou et al., 2007; Gauthier et al., 2008). However, these authors analyzed the polymorphisms in different biotypes collected from a larger geographic range compared to the present study. In contrast, the primers BT-e 49, BT-b 103, BEM 12, BEM 25, BEM 37, 1.2, 1.4, 1.9, 1.11, 1.12, 2.3, 1.13, and 1.17 (Table 3) revealed genetic variation among the 6 study populations, corroborating the results of previous publications (De Barro et al., 2003; Tsagkarakou and Roditakis, 2003; Tsagkarakou et al., 2007; Gauthier et al., 2008).

A low level of genetic polymorphism was observed in the present study, compared to previous study on biotype B (Simón et al., 2007; Dalmon et al., 2008; do Valle et al., 2011). However, 50 alleles were found in the 13 polymorphic loci. The average N_A per locus was 2.37, and the average H_E was 0.5199, which was higher compared to that found in a similar study (Delatte et al., 2006) of different whitefly populations using microsatellite markers. The population parameters obtained in the current study may be compared with those estimated by Delatte et al. (2006), because these authors studied biotype B, which is the same biotype evaluated in this study, but is now referred to as MEAM1.

Genetic structure

The optimal clustering number (Evanno et al., 2005) was 7 (Figure 1) in the diversity structuring analysis, when using the Bayesian approach in the Structure software. From a total

of 72 samples of adult whitefly, all 6 populations were found to be homogeneous. The BJabo and CPsoj populations exhibited greater diversity among individuals. In addition, these 2 populations also exhibited slightly different patterns of diversity compared to the other populations.

The $\hat{F}_{IS}$ estimated from 13 SSR loci was negative and significant, indicating an excess of heterozygotes. This result contradicted the results of a previous study (Evanno et al., 2005), which showed a positive value of 0.18, indicating the occurrence of intrapopulation endogamy. However, this previous study used different populations and different microsatellite loci, which might explain this discrepancy.

The F_{ST} was high ($F_{ST} = 0.34$), indicating that the populations differed to one another, as evidenced by the large number of clusters detected by the Bayesian analysis. These estimates were relatively consistent and significantly different from zero (Table 5). These differences might be primarily related to the adaptation of the insects to the host plants and geographic isolation. The two most distinct populations were BJabo, which might be because of geographic distance, and CPsoj, which might be because of the greater adaptation of the pest to the host plant and/or to the reproductive isolation. Previous studies have confirmed that specific populations reproductively isolated because of copulation and post-copulation barriers (Wang et al., 2010; Xu et al., 2010; Sun et al., 2011).

The high F_{ST} value (Table 5) observed in the current study was consistent with the F_{ST} values documented in the published literature using random amplified polymorphic DNA markers (Moya et al. 2001; $F_{ST} = 0.34$), and was consistent with those obtained between genetic group B and the Indian Ocean group Ms (Delatte et al., 2006; $F_{ST} = 0.32$) using microsatellite markers.

Based on the comparison of the microsatellites used in this study, only MEAM1 is present in the *B. tabaci* populations collected from the 5 regions in Brazil and the single population in Urbana, IL, USA.

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