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# Low polymorphism revealed in new microsatellite markers for *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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**ABSTRACT.** The silverleaf whitefly (*Bemisia tabaci*) is actually a complex of morphologically indistinct species that vary in their capacity to transmit plant viruses and to induce physiological disorders in plants of economic importance. The worldwide impact of this whitefly has increased greatly, as it is a vector of various types of phytovirus, especially geminiviruses, in plants of economic importance. The adaptability of *B. tabaci* to many regions of the world has fostered the appearance of various biotypes that attack a broad spectrum of host plants. We developed microsatellite markers to study genetic variability and population structure of this whitefly in Brazil. Thirteen polymorphic microsatellite markers were isolated and characterized in 20 individuals from a natural population that were collected in soybean in Campinas (SP). The number of alleles per locus ranged from one to two, and the expected heterozygosity ranged from 0.000 to 0.505. These microsatellite

 

markers will be useful for studies and management of *B. tabaci*. The low polymorphism found in these molecular markers is probably associated with homology of genes expressed in these markers.

**Key words:** Silverleaf whitefly; Homology; Molecular markers; Monomorphic microsatellite; Whitefly

## INTRODUCTION

More than 1500 whitefly species (Hemiptera: Aleyrodidae) are known in the whole world (Martin and Mound, 2007); however, very few of these insects have been studied more specifically (Byrne et al., 1990). *Bemisia tabaci* (Genn.) is one of the most important species, considered as a complex (Brown et al., 1995; Perring, 2001) and has a wide spectrum of hosts. *B. tabaci* develops in more than 900 host plants (Perring, 2001; Berry et al., 2004), where it is responsible for more than 110 different viruses and transmitting geminivirus (Jones, 2003). Therefore, the objective of the present research was to develop microsatellite molecular markers through genomic-enriched library protocols for future studies of genetic variability, population structure and gene flow of *B. tabaci*.

## MATERIAL AND METHODS

Genomic DNA was extracted from a whitefly female using an extraction protocol described by Lima et al. (2000). Only one whitefly female was macerated in 60 µL extraction buffer (1 mM Tris-HCl, pH 8.0, 5 mM NaCl, 1 mM EDTA, pH 8.0, 3% Triton X-100, 60 µg/mL proteinase K). The macerate was incubated for 15 min at 65°C, followed by heating for 10 min at 95°C. A microsatellite-enriched library was obtained using adapted protocols from Billotte et al. (1999). Genomic DNA from one genotype of *B. tabaci* was digested with *Afa*I (Invitrogen) and enriched in microsatellite fragments using $(CT)_8$ and $(GT)_8$ motifs. The enriched fragments were cloned into pGEM77 T (Promega) and ligation products were used to transform Epicurian Coli XL1-Blue *Escherichia coli* competent cells. The positive clones were selected using the β-galactosidase gene and then grown overnight in the presence of ampicillin. A total of 117 clones were sequenced in an ABI 3770 automated 79 sequencer (PE Applied Biosystems) using a Big-Dye terminator cycle sequencing kit (Applied Biosystems). About 10 primer pairs were designed for SSR flanking regions using Primer 3 (Rozen and Skaletsky, 2000) and tested in 20 specimens of *B. tabaci* collected from different regions in Brazil. PCRs were performed in a 20-µL reaction volume containing 1X reaction buffer (100 mM Tris-HCl, pH 8.5, 50 mM KCl) containing 20 ng genomic DNA, 0.2 µM of each primer, 0.25 mM dNTPs, 0.5 U *Taq* DNA polymerase, and 4 mM $MgCl_2$. The PCR program consisted of an initial denaturing step at 95°C for 2 min followed by 45 cycles of amplification [95°C (1 min), 1 min at the specific annealing temperature of each primer pair (Table 1), and 72°C (1 min)], and a final elongation step at 72°C for 5 min. Amplification products were resolved by electrophoresis on 7% denaturing polyacrylamide gels and visualized by silver staining (Creste et al., 2001). The allele scoring was done using the 10-bp DNA Ladder (Invitrogen) for size standards. The population's parameters of the 13 loci obtained were estimated by using the Genetix 4.05.2 program (Belkhir et al., 2004) and by genotyping 20 individuals of *B. tabaci* from a natural population collected in Campinas, SP, Brazil.

 

**Table 1.** Characteristics of 13 microsatellite loci from *Bemisia tabaci*.

| Locus | Repeat motif | Primer sequence (5'-3') | Ta (°C) | Size range (bp) | N | $H_O$ | $H_E$ | PIC | GenBank accession No. |
|---|---|---|---|---|---|---|---|---|---|
| Bta1 | $(CTT)_4$ | F: TGAAGGAGACTTGGCAACG<br>R: ATCTCTCTCACCGCCATCAT | 62.8 | 224 | 2 | 0.000 | 0.337 | 0.269 | GF111984 |
| Bta2 | $(TACC)_3$ | F: AAAAGTCGTCCGCTGTTACC<br>R: CGGGGAAATAAAAGCAACTG | 63.8 | 177 | 1 | 0.000 | 0.000 | 0.000 | GF111977 |
| Bta3 | $(GAAA)_3$ | F: TATACTTGGGCATCGTCAG<br>R: GCGTAGGAGAGTTGGAATG | 57.7 | 228 | 1 | 0.000 | 0.000 | 0.000 | GF111978 |
| Bta4 | $(TTTA)_3$ | F: CGCTCCTCAAGTTTTCTGT<br>R: CGGCAGTCAGGGTTATTA | 58.9 | 175 | 2 | 0.000 | 0.442 | 0.332 | GF111985 |
| Bta5 | $(AAAG)_3$ | F: TATACTTGGGCATCGTCAGC<br>R: GCGTAGGAGAGTTGGAATGC | 62.7 | 228 | 1 | 0.000 | 0.000 | 0.000 | GF111979 |
| Bta6 | $(AATA)_3$ | F: TTGTTGGTTCACCTGTAATTGG<br>R: GCGTGGACTAGCTGAAAAAGA | 64.7 | 150 | 2 | 0.000 | 0.505 | 0.365 | GF111980 |
| Bta7 | $(TTCT)_4$ | F: CTGTAACGGACACACACACA<br>R: GGTCATTTTGGTGTGGAA | 58.1 | 244 | 1 | 0.000 | 0.000 | 0.000 | GF111986 |
| Bta11 | $(TCAT)_3$ | F: TTTTTCGGTTGGTTGGTG<br>R: GCTAATTGCTTCTTTCCTTG | 60.8 | 155 | 1 | 0.000 | 0.000 | 0.000 | GF111987 |
| Bta12 | $(AAAT)_6$ | F: TCCCATCTCCGCCTTATCTA<br>R: GACCTCCGCAAGTTTTTCC | 64.1 | 166 | 2 | 0.000 | 0.505 | 0.365 | GF111981 |
| Bta13 | $(CT)_{12}(CT)_8$<br>$(CACT)_3(CT)_7$ | F: AAACGTGGTCCCTTGGAATA<br>R: AACGCCCTTTCACAATTCA | 63.8 | 248 | 1 | 0.000 | 0.000 | 0.000 | GF111982 |
| Bta16 | $(TCAT)_3$ | F: GGCTAATTGCTTCTTTCCTTG<br>R: GTTTTTCGGTTGGTTGGTG | 63.8 | 155 | 1 | 0.000 | 0.000 | 0.000 | GF111988 |
| Bta17 | $(TACG)_3$ | F: CACCCTCACCTACACACACAC<br>R: TCACAACAAATGCGACCTT | 61.6 | 206 | 1 | 0.000 | 0.000 | 0.000 | GF111983 |
| Bta18 | $(TCAT)_3$ | F: GGTGGTTTGTTTAGAAGAGTGG<br>R: GGCTAATTGCTTCTTTCCTTG | 63.0 | 193 | 1 | 0.000 | 0.000 | 0.000 | GF111989 |

F = forward primer sequence; R = reverse primer sequence; Ta = annealing temperature; N = number of alleles; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; PIC = polymorphic information content.

## RESULTS AND DISCUSSION

From the library, 344 clones were sequenced and 103 SSRs were found: 17.5% mononucleotides, 33.0% dinucleotides, 0.9% trinucleotides, 15.5% tetranucleotides, 8.8% pentanucleotides, and 24.3% compounds. Of these sequences, 13 had a flanking region of adequate size for the design of forward and reverse primers. The number of alleles observed for each locus ranged from one to two. We found a low level of polymorphism among 20 individuals analyzed with the new locus developed. Thirteen loci were developed but only four were polymorphic with two alleles per locus. We showed a low level of polymorphism in the genotypes evaluated. The expected heterozygosity ranged from 0.000 to 0.505. For all loci, the observed heterozygosity was lower than or equal to the expected heterozygosity. The low polymorphism found in these molecular markers is probably associated with homology to the genes expressed in all these markers (Table 2). The studies of genetic variability of insect pests, in the context of insect resistance management, can provide useful information about distribution patterns of genetic variability and gene flow that allow the determination of focus areas for monitoring and the development of strategies to mitigate the development of resistance. Obtaining specific primers that access microsatellite loci in *B. tabaci* enables the study of this genetic variability and generates information that could be used in future management programs of this pest. A previous study with other molecular markers (SSRs) for *B. tabaci* also showed a low level of polymorphism (Valle et al., 2011). A recent tool for genetic analysis was developed by Nazereno and Reis (2011), which consisted in evaluating monomorphic

microsatellites; therefore, in new and future studies, these analyses will be used to determine the reason for the low polymorphism found in this study.

**Table 2.** Homology of 13 microsatellite loci from *Bemisia tabaci*.

| Primer | Homology | GenBank accession No. | E value | Max ident (%) |
|---|---|---|---|---|
| Bta1 | Rat alpha-amylase (Amy-1) gene, 5'-end, and parotid-specific promoter region | M14151.1 | 0.002 | 90 |
| | *Mus musculus* mRNA for mKIAA0164 protein | AK129071.1 | 0.020 | 90 |
| Bta2 | *Fragaria* x *ananassa* gene encoding methionine sulfoxide reductase | AJ297967.1 | 5e-10 | 83 |
| | *Gallus gallus* mRNA for hypothetical protein, clone 22o14 | AJ720655.1 | 5e-10 | 83 |
| Bta3 | Uncultured archeon partial 16S rRNA gene, isolate AEXO15n.4 | FM242736.1 | 2e-18 | 84 |
| | Uncultured bacterium partial 16S rRNA gene, isolate EXO15432 | FM242723.1 | 2e-18 | 84 |
| | *Bacterium* AK-MB19 partial yfkC gene for putative reverse transcriptase maturase protein, isolate MB 19 | AM997282.1 | 3e-15 | 81 |
| Bta4 | *Fragaria* x *ananassa* gene encoding methionine sulfoxide reductase | AJ297967.1 | 5e-10 | 83 |
| | *Gallus gallus* mRNA for hypothetical protein, clone 22o14 | AJ720655.1 | 5e-10 | 83 |
| | *Arabidopsis lyrata* clone MITE7 transposon-insertion display band genomic sequence | EU558532.1 | 6e-09 | 82 |
| Bta5 | *Macrococcus caseolyticus* JCSC5402 DNA, complete genome | AP009484.1 | 0.056 | 83 |
| | *Paramecium tetraurelia* hypothetical protein (GSPATT00012894001) partial mRNA | XM001444769.1 | 0.20 | 83 |
| | *Oryzias marmoratus* PSMB10, PSMB8 genes for proteasome subunit, beta type 10, proteasome subunit, beta type 8, partial and complete cds, isolate: *Oryzias marmoratus* 15 | AB551024.1 | 0.68 | 93 |
| | *Oryzias matanensis* PSMB10, PSMB8 genes for proteasome subunit, beta type 10, proteasome subunit, beta type 8, partial and complete cds, isolate: *Oryzias matanensis* 6, haplotype: N | AB551021.1 | 0.68 | 93 |
| Bta6 | *Bemisia tabaci* isolate Mogi Guaçu haplotype 4 cytochrome oxidase subunit | JN689359.1 | 7e-94 | 100 |
| | *Bemisia tabaci* isolate Sumare haplotype 3 cytochrome oxidase subunit | JN689358.1 | 7e-94 | 100 |
| | *Bemisia tabaci* isolate Ilha Solteira haplotype 2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | JN689357.1 | 7e-94 | 100 |
| Bta7 | *Sorghum bicolor* hypothetical protein, mRNA | XM002441933.1 | 4e-07 | 85 |
| | *Solanum lycopersicum* goblet gene, complete cds | HM210879.1 | 3e-08 | 85 |
| | *Picea glauca* clone GQ03118_G07 mRNA sequence | BT108209.1 | 1e-08 | 86 |
| | *Petunia hybrida* NAM gene | X92204.1 | 4e-07 | 82 |
| | *Zea mays* clone 345601 CUC2 mRNA, complete cds | EU970442.1 | 1e-06 | 84 |
| | *Amborella trichopoda* mRNA for the NAM protein | FR668080.1 | 5e-06 | 83 |
| Bta11 | *Solanum tuberosum* no apical meristem (NAM) mRNA, complete cds | FJ435166.1 | 3e-09 | 89 |
| | *Picea glauca* clone GQ03118_G07 mRNA sequence | BT108209.1 | 1e-08 | 86 |
| | *Sorghum bicolor* hypothetical protein, mRNA | XM002462559.1 | 3e-08 | 86 |
| | *Zea mays* partial mRNA for hypothetical protein (nam1 gene) | AJ833966.1 | 4e-07 | 86 |
| | *Amborella trichopoda* mRNA for the NAM protein | FR668080.1 | 5e-06 | 86 |
| | *Brassica oleracea* cup-shaped cotyledon 2 (CUC2) gene, partial cds | HQ703968.1 | 2e-04 | 87 |
| | *Ginkgo biloba* partial mRNA for the NACa protein | FR668082.1 | 2e-04 | 87 |
| Bta12 | *Mus musculus* chromosome 5, clone RP24-465G18, complete sequence | AC133456.11 | 7e-16 | 87 |
| | *Mus musculus* chromosome 5, clone RP23-37G13, complete sequence | AC161225.14 | 7e-16 | 87 |
| | *Homo sapiens* X BAC RP11-126O22 (Roswell Park Cancer Institute Human BAC Library) complete sequence | AC073488.23 | 1e-11 | 93 |
| | Human T-cell receptor alpha delta locus, complete sequence | AE000521.1 | 1e-05 | 88 |
| Bta13 | *Bacteriovorax marinus* SJ genome | FQ312005.1 | 4.3 | 89 |
| | *Gossypium hirsutum* TM-1 D-subgenome 14-3-3-like protein (14-3-3L | HQ142993.1 | 4.3 | 88 |
| | *Karlodinium micrum* clone 17645 cytochrome oxidase subunit III (COX3) mRNA, partial cds; mitochondrial | EF443024.1 | 0.35 | 81 |
| Bta16 | Zebrafish DNA sequence from clone DKEY-246M18 in linkage group 15 | BX571794.22 | 1e-05 | 73 |
| | PREDICTED: *Acyrthosiphon pisum* tyrosine-protein phosphatase non-receptor type 21-like (LOC100161359), mRNA | XM001951853.2 | 2e-20 | 84 |
| Bta17 | *Homo sapiens* N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits (GNPTAB), RefSeqGene on chromosome 12 | NG021243.1 | 0.38 | 85 |
| | *Karlodinium micrum* clone 1939 cytochrome oxidase subunit III (COX3) mRNA, partial cds; mitochondrial | EF443026.1 | 0.38 | 81 |
| | *Homo sapiens* KIAA0438 mRNA, partial cds | AB007898.1 | 1.3 | 96 |
| | *Pinellia cordata* voucher T.S. Yi 08015 rps16 gene, intron; chloroplast | JQ237171.1 | 4.6 | 90 |
| | *Arcobacter* sp L DNA, complete genome | AP012048.1 | 4.6 | 81 |
| | *Puccinia graminis* f. sp *tritici* CRL 75-36-700-3 hypothetical protein, mRNA | XM003319919.1 | 4.6 | 84 |
| | *Gossypium hirsutum* TM-1 D-subgenome 14-3-3-like protein (14-3-3L) gene, complete CDs | HQ142993.1 | 4.6 | 88 |
| Bta18 | *Flavobacterium columnare* ATCC 49512, complete genome | CP003222.2 | 0.11 | 79 |
| | *Solanum lycopersicum* strain Heinz 1706 chromosome 1 clone slm-15p5 map 1, complete sequence | AC246100.4 | 0.38 | 85 |
| | *Gossypium hirsutum* TM-1 D-subgenome 14-3-3-like protein (14-3-3L) gene, complete cds | HQ142993.1 | 4.6 | 88 |

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