NOTAS CIENTÍFICAS

MOLECULAR CHARACTERIZATION OF WHITEFLY (*BEMISIA* SPP.) IN BRAZIL[1]

MARIA REGINA VILARINHO DE OLIVEIRA[2], MYRIAN SILVANA TIGANO[3] and SALAH ALJANABI[4]

ABSTRACT - RAPD analysis was used to evaluate the genetic variability of 12 Brazilian populations of *Bemisia* spp. (Hemiptera: Aleyrodidae). Ten primers were analysed in order to detect the polymorphism between the samples tested. The results showed that individuals from a colony maintained since 1983 had similar patterns of *Bemisia tabaci* originated from California, USA. The other samples analysed showed similar patterns to *B. tabaci* biotype B (=*B. argentifolii*), also from California, USA, indicating a great dissemination of this biotype in Brazil. Phenetic analysis of populations revealed high homogeneity between individuals of the B biotype of *B. tabaci*.

CARACTERIZAÇÃO MOLECULAR DA MOSCA-BRANCA (*BEMISIA* SPP.) NO BRASIL

RESUMO - A análise de RAPD foi utilizada para avaliar a variabilidade genética de 12 populações brasileiras de *Bemisia* spp. (Hemiptera: Aleyrodidae). Foram analisados dez *primers* que permitiram a detecção de polimorfismo entre as amostras testadas. Os resultados obtidos mostraram que os indivíduos analisados provenientes de uma colônia mantida desde 1983 apresentaram perfis de RAPD próximos do padrão de *B. tabaci* oriunda da Califórnia, EUA. As outras populações analisadas apresentaram padrões semelhantes ao de *B. tabaci* raça B (=*B. argentifolii*), também oriunda da Califórnia, EUA, indicando a grande disseminação deste último biótipo no Brasil. A análise fenética dos dados dessas populações revelou uma alta homogeneidade entre os indivíduos do biótipo B de *B. tabaci.*

The pest status of whiteflies of the genus *Bemisia* increased greatly in Brazil in the last few years. The type species of the genus, *Bemisia tabaci* (Gennadius), used to be considered important as a vector of virus disease, rather than as an insect pest.

---

[1] Accepted for publication on June 11, 1999.

[2] Biologist, Dr., Embrapa-Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia (Cenargen), Caixa Postal 02372, CEP 70849-970 Brasília, DF. E-mail: vilarin@cenargen.embrapa.br

[3] Agronomist, Dr., Embrapa-Cenargen. E-mail: myrian@cenargen.embrapa.br

[4] Agronomist, Ph.D., Biology Dept., MSIRI, Reduit-Mauritius. E-mail: sjanabi@hotmail.com

A population of *B. tabaci* that was initially associated with unprecedent whitefly infestations in greenhouse-grown ornamentals in the USA, Caribbean Basin, and Europe, in late 1985 was designated as the B-biotype of *B. tabaci* (Costa & Brown, 1991; Brown et al., 1995) or *B. argentifolii* Bellows & Perring (Perring et al., 1993; Bellows et al., 1994). The existence of biotypes or host races of the whitefly, *B. tabaci* was proposed in the 1950s after the discovery that morphologically indistinguishable populations of *B. tabaci* exhibited measurably different biological traits with respect to host range, host-plant adaptability, and plant virus-transmission capabilities (Costa & Russell, 1975; Bird & Maramarosch, 1978; Brown et al., 1995). Recently recognized differences among populations of *B. tabaci*, the sweetpotato whitefly, and *B. argentifolii*, the silverleaf whitefly, represent either different biotypes of *B. tabaci* or are members of a species complex (Brown et al., 1995). The B type of *B. tabaci* (=*B. argentifolii*) has spread to many areas world wide due, probably, to the international trade of plants.

The introduction of *B. tabaci* biotype B in Brazil was first reported in 1991/92, in the State of São Paulo (Melo, 1992; Lourenção & Nagai, 1994). Investigations made after the first detection suggested that the insect was introduced into the country on ornamental plants commonly sold in the international market; a very heavy infestation of *B. tabaci* was observed in a greenhouse planted with *Crysanthemum* sp. (Lima et al., 1992; Oliveira & Lima, 1997). Since then, Villas Bôas et al. (1997) and Pedrosa et al. (1997), reported individuals of this species associated to symptoms of geminivirus in tomatoes and cabbage crops of central area of Brazil, in 1993. The favourable climate conditions and a great number of host plants may have played an important role on dissemination of this biotype.

The identification of a new species has been mainly based on crossing experiments, mating behavior, and isozymes analysis (Perring et al., 1993). The morphological characters used for whiteflies identification are not clearly and easily able to differentiate *B. tabaci* from *B. tabaci* biotype B (Bellows et al., 1994).

The analysis of RAPD markers has been showed to be useful to recognize inter and intraspecific differences among *Bemisia* spp. (Gawel & Bartlett, 1993; Perring et al., 1993; Barro & Driver, 1997). Compared to isozymes, this analysis has the advantages to allow the use of alcohol preserved material, and to extensively cover the genome.

The purpose of this study was to evaluate the genetic variability of the complex species of *Bemisia* occurring in Brazil, using RAPD analysis.

Adults of *Bemisia* spp. were collected in different crops and regions in the years 1996/1997 (Table 1). They were kept in 100% ethanol until use. Adults from colonies in California, USA (Perring et al., 1993) were used as standards for *B. tabaci* and *B. tabaci* biotype B. Species identification was based on morphological characteristic. Four female specimens from each sample were selected for molecular analysis.

A modified DNA extraction protocol of Kazmer et al. (1995) was used to extract DNA. Individual insect was ground in a 1.5 mL microcentrifuge tube with 59 μL of well-stirred, sterile 5% Chelex-100 using a disposable micropestle, after which 1 μL of proteinase K (20 mg/mL) was added and mixed. The samples

were incubated at 65°C for one hour and then for ten minutes at 95ºC. The tubes containing the DNA solution were spun for five seconds and then stored at -20°C until needed.

The PCR reactions were performed in a 50 μL volume containing 2 μL of previously prepared template DNA solution, two units of Taq polymerase (Cenbiotec, Porto Alegre, RS, Brazil), 5 μL of 10x polymerase's recommended buffer, 200 mm of each dNTP (Pharmacia), and 0.4 mm primer. Amplification was carried out in 96-well PCR plates using the PTC-100 programmable thermal controller (MJ Research). The temperature profile used was one step at 94°C for three minutes, followed by 45 cycles of 93°C for one minute, 35°C for one minute and 72°C for two minutes, with final extension step at 72°C for five minutes. The reactions were maintained at 4°C until electrophoresis. PCR products were electrophoresed in 2% LE agarose gel prepared in 0.5x Tris-borato-EDTA (TBE) buffer, stained with ethidium bromide (Sambrook et al., 1989), and photographed under UV light. Primers were selected according to Gawel & Bartlett (1993). Ten 10-mer primers (Operon Technologies, Alameda, CA) were used: OPA-02, OPA-03, OPA-04, OPA-05, OPA-10, OPA-11, OPA-13, OPA-15, OPA-17 and OPA-20.

DNA fingerprints were scored directly from the photographs. Only well resolved products were scored. The presence or absence of each fragment was considered as an independent character. RAPD markers were analyzed using NTSYS-pc V1.8 (Rohlf, 1993). A similarity matrix was calculated using Jaccard similarity coefficient. Clustering was done using the unweighted mean pair group arithmetic mean method (UPGMA) (Sneath & Sokal, 1973).

The DNA extraction protocol adopted proved to be simple and produced consistent yield within the time frame, which is conducive to performing large-scale population genetics analysis on these insects. The amount of crude DNA extracted of each specimens in this study was more than sufficient to prepare all the PCR reactions.

The analysis of RAPD markers proved to be an efficient method to distinguish species on *Bemisia* samples collected in Brazil. Examples of fingerprinting data obtained is shown in Figs. 1 and 2. Specimens of *B. tabaci* and

TABLE 1. Origin of Brazilian *Bemisia* spp. populations.

Population	Crop	Geographical origin	Collection date
1	Bean	Goiânia, GO	May 1997
2	Bean	Jaboticabal, SP	April 1997
3	Soybean	Campinas, SP	March 1997
4	Squash	Petrolina, PE	December 1996
5	Tomato	Petrolina, PE	December 1996
6	Watermelon	Itaberaba, BA	December 1996
7	Squash	Juazeiro, BA	December 1996
8	Melon	Aracati, CE	December 1996
9	Melon	Aracati, CE	January 1997
10	Melon	Aracati, CE	January 1997
11	Melon	Aracati, CE	January 1997
12	Cabbage	Itaiçaba, CE	January 1997
13	Cotton	Almeida, PB	May 1997

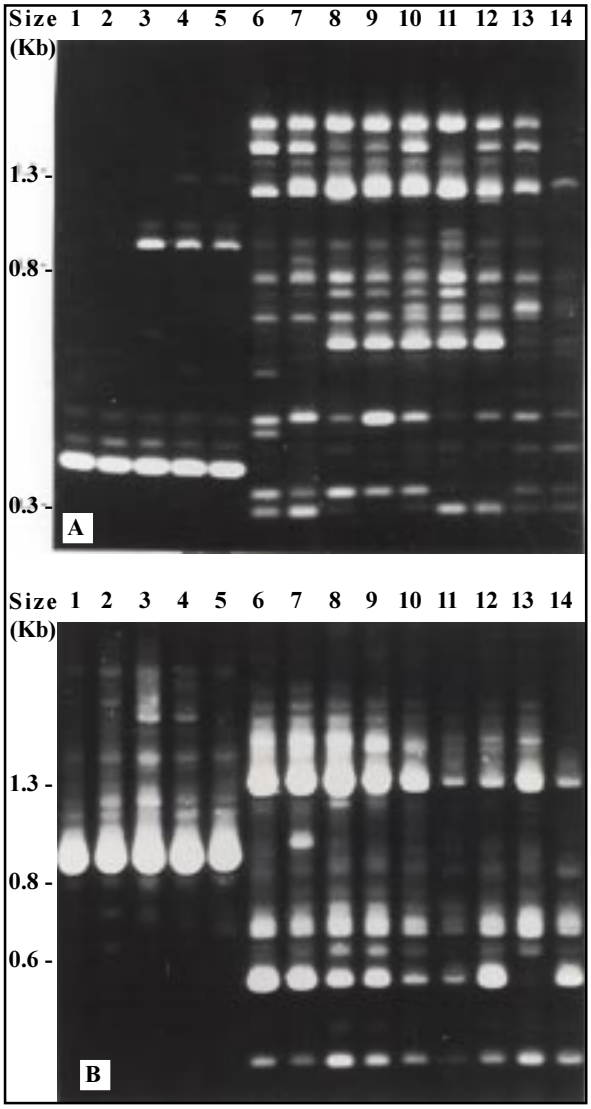

FIG. 1. Agarose gel showing results from RAPD fingerprinting of *Bemisia* spp. Primers used: (A) OPA-02 and (B) OPA-04. Lane 1 and 2: specimens from *B. tabaci*, California, USA; lane 3, 4 and 5: specimens from population 1, Goiânia, GO; lanes 6 and 7: specimens from *B. argentifolii*, California, USA; lane 8: specimen from population 2, Jaboticabal, SP; lane 9 and 10: specimens from population 3, Campinas, SP; lane 11: specimens from population 8, Aracati, CE; lane 12: specimen from population 12, Itaiçaba, CE; lane 13 and 14: specimens from population 13 Almeida, PB.

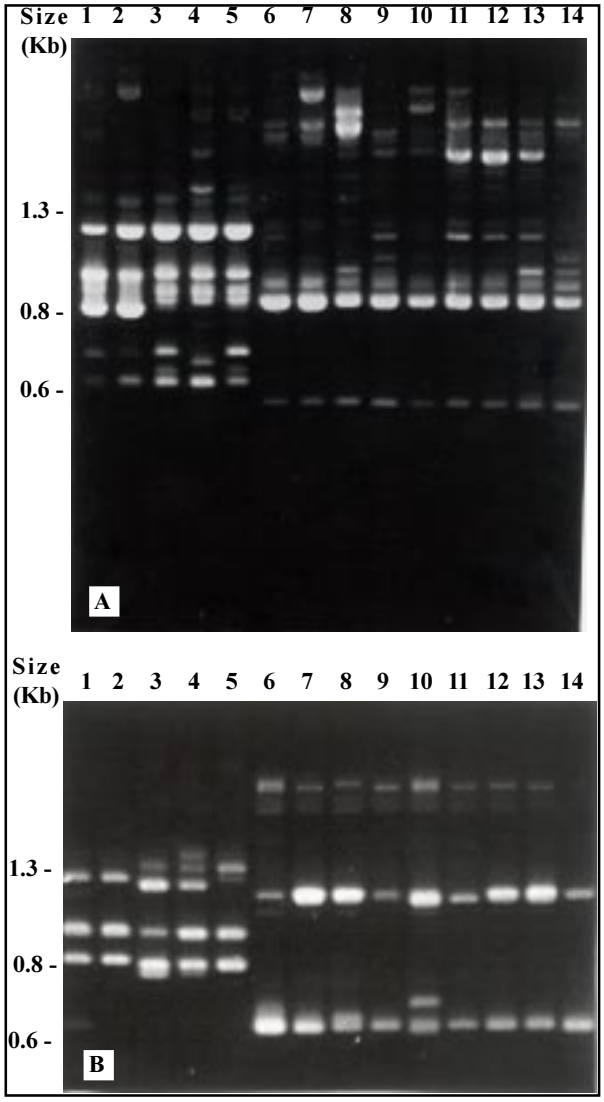

FIG. 2. Agarose gel showing results from RAPD fingerprinting of *Bemisia* spp. Primers used: (A) OPA-05 and (B) OPA-13. Lane 1 and 2: specimens from *B. tabaci*, California, USA; lane 3, 4 and 5: specimens from population 1, Goiânia, GO; lanes 6 and 7: specimens from *B. argentifolii*, California, USA; lane 8: specimen from population 2, Jaboticabal, SP; lane 9 and 10: specimens from population 3, Campinas, SP; lane 11: specimens from population 8, Aracati, CE; lane 12: specimen from population 12, Itaiçaba, CE; lane 13 and 14: specimens from population 13 Almeida, PB.

biotype B from California, USA, were used as reference for these species. Only individuals from sample 1 (Table 1) presented similar RAPD patterns to individuals of *B. tabaci* from the USA. All the other specimens from different populations analyzed showed to be related to *B. tabaci* biotype B (Figs. 1 and 2). Therefore, the spread of biotype B associated with phytotoxic symptoms was confirmed by the RAPD analysis. Individuals presented no common bands with *B. tabaci*.

RAPD patterns showed to be also efficient markers to detect genetic variability within biotypes. A total of 25 bands were scored with the five primers selected, OPA-02, OPA-03, OPA-04, OPA-15 and OPA-20, presenting ten polymorphic bands among *B. tabaci* biotype B samples. An example of fingerprinting among these samples is shown in Fig. 3. Although, different genotypes were detected within and among samples, cluster analysis of the RAPD characters did not show clear phenetic groups related to host plants or geographical region.

This study is the first of a series that will be analysing the complex population of *B. tabaci* in Brazil, in order to develop integrated pest management programs. The identification of the biotype B of *B. tabaci* in our agricultural systems may have severe implications as most of the crops produced are for subsistence, cultivated in small areas such as 1 ha to 10 ha.

These results showed that RAPD markers could be a useful tool to resolve identification questions and to analyze genetic variability of *Bemisia* spp. in Brazil. Recognition of species and population variation is important to develop strategies for pest management.

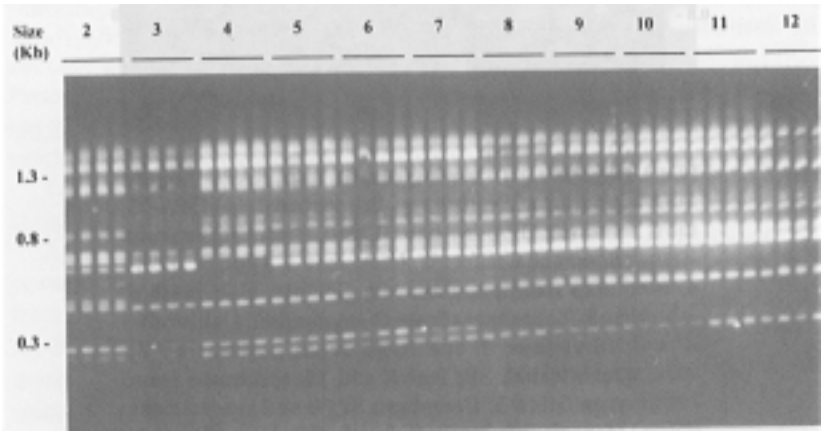

FIG. 3. Agarose gel showing results from RAPD fingerprinting of *B. argentifolii* populations. Numbers (2 to 12) represent results from four specimens of respective population (Table 1). The primer used was OPA-02.

ACKNOWLEDGEMENTS

To G. G. Soares (AgroGenesis, San Diego, CA, USA) and T. Perring (University of California, Riverside, CA, USA) for the kind supply of material and information; to Brazilian Ministry of Agriculture and Food Supply for collecting part of the whiteflies samples; to CNPq and Embrapa for supporting this work.

REFERENCES

BARRO, P.J.; DRIVER, F. Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). **Australian Journal of Entomology**, Canbera, v.36, p.149-152, 1997.

BELLOWS, T.S.J.; PERRING, T.M.; GILL, R.J.; HEADRICK, D.H. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). **Entomological Society of America Annals**, Lanham, v.87, p.195-206, 1994.

BIRD, J.; MARAMAROSCH, K. Viruses and virus diseases associated with whiteflies. **Advanced Virus Research**, San Diego, v.22, p.55-110, 1978.

BROWN, J.K.; FROHLICH, D.R.; ROSELL, R.C. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? **Annual Review of Entomology**, Palo Alto, v.40, p.511-534, 1995.

COSTA, A.S.; RUSSELL, L.M. Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera, Aleyrodidae). **Ciência e Cultura**, São Paulo, v.27, p.388-390, 1975.

COSTA, H.S.; BROWN, J.K. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction. **Entomologia Experimentalis et Applicata**, Dordrecht, v.61, p.211-219, 1991.

GAWEL, N.J.; BARTLETT, A.C. Characterization of differences between whiteflies using RAPD-PCR. **Insect Molecular Biology**, Oxford, v.2, p.33-38, 1993.

KAZMER, D.J.; HOPPER, K.R.; COUTINOT, D.M.; HECKEL, D.G. Suitability of random amplified polymorphic DNA for genetic markers in the aphid parasitoid, *Aphelinus asychis* Walker. **Biological Control**, San Diego, v.5, p.503-512, 1995.

LIMA, L.H.C.; OLIVEIRA, M.R.V. de; GOMES, A.C.M.M.; FERREIRA, D.N.M. **Análise eletroforética em populações da mosca-branca, *Trialeurodes vaporariorum* e *Bemisia* sp. (Homoptera, Aleyrodidae)**. Brasília : Embrapa-Cenargen, 1992. 5p. (Embrapa-Cenargen. Pesquisa em Andamento, 5).

LOURENÇÃO, A.L.; NAGAI, H. Surtos populacionais de *Bemisia tabaci*, no Estado de São Paulo. **Bragantia**, Campinas, v.53, p.53-59, 1994.

MELO, P.C.T. **Mosca-branca ameaça produção de hortaliças**. Campinas : Asgrow do Brasil Sementes, 1992. 2p. (Asgrow Sementes. Informe Técnico).

OLIVEIRA, M.R.V.; LIMA, L.H.C. Padrões isoenzimáticos de *Trialeurodes vaporariorum* e *Bemisia tabaci* (Homoptera, Aleyrodidae) e de *Encarsia formosa*

e *E. lycopersici* (Hymenoptera, Aphelinidae). **Pesquisa Agropecuária Brasileira**, Brasília, v.32, n.7, p.683-687, jul. 1997.

PEDROSA, F.N.H.; ALENCAR, J.A.; LIMA, M.F.; MATTOS, M.A.A.; HONDA, O.T.; HAJI, A.T. **Avaliação de produtos para o controle da mosca-branca (*Bemisia* spp.) na cultura do tomate (*Lycopersicum esculentum* Mill.)**. Petrolina : Embrapa-CPATSA, 1997. 6p. (Embrapa-CPATSA. Pesquisa em Andamento, 84).

PERRING, T.M.; COOPER, A.D.; RODRIGUEZ, R.J.; FARRAR, C.A.; BELLOWS JUNIOR, T.S. Identification of a whitefly species by genomic and behavioral studies. **Science**, Washington, v.259, p.74-77, 1993.

ROHLF, F.J. **Numerical taxonomy and multivariate analysis system**. New York : Setauket, 1993. 191p. (Applied Biostatistics, 18).

SAMBROOK, J.; FRITSCH, E.F.; MANIATIS, T. **Molecular cloning**: a laboratory manual. 2.ed. Nova York : Cold Spring Harbor Laboratory, 1989. 120p.

SNEATH, P.H.A.; SOKAL, R.R. **Numerical taxonomy**. San Francisco : Freeeman, 1973. 573p.

VILLAS BÔAS, G.L.; FRANÇA, F.H.; ÁVILA, A.C.; BEZERRA, I.C. **Manejo integrado da mosca-branca, *Bemisia argentifolii***. Brasília : Embrapa-CNPH, 1997. 11p. (Embrapa-CNPH. Circular Técnica, 9).