Turk J Agric For
26 (2002) 211-216

Determination of Genetic Variation in Populations of *Bemisia tabaci* in Antalya

Hüseyin GÖÇMEN
Akdeniz University, Faculty of Agriculture, Department of Plant Protection, Antalya - TURKEY
Zübeyir DEVRAN
Citrus and Greenhouse Research Institute, Antalya - TURKEY

Received: 21.11.2001

Abstract: Although a number of investigations on the molecular genetics of *Bemisia tabaci* (Gennadius) have appeared in the literature, no such studies are known from Turkey. In order to fill this gap the amplified fragment length polymorphism (AFLP) technique was used to assay eight populations of *B. tabaci* collected from cotton, protected vegetables (tomato, cucumber and eggplant) and peppermint in various locations in the province of Antalya, Turkey. Eleven AFLP primer pairs detected a total of 547 AFLP fragments in these populations and 389 of these showed polymorphism. Cluster analyses of AFLP data clearly separated these populations into two groups with a genetic variability between 42% and 81%. The results indicated that *B. tabaci* may have biotypes based on adaptation to certain host plant species in Turkey.

Key Words: *Bemisia tabaci*, AFLP, genetic variability, Antalya

Antalya'da *Bemisia tabaci* Populasyonları Arasındaki Genetik Variyasyonun Saptanması

Özet: *Bemisia tabaci* (Genn.) üzerinde bir çok ülkede molekuler genetik çalışmalar yapılırken Türkiye'de bu tür bir çalışma bilinmemektedir. Bu boşlugu doldurmak amacıyla Antalya'nın degişik yerlerinden pamuk, örtü altında yetiştirilen sebzeler (domates, hıyar, patlıcan) ve yabani nane üzerinden toplanan sekiz *B. tabaci* populasyonu arasındaki genetik varyasyonun araştırılmasında AFLP (Amplified Fragment Length Polymorphism) teknigi kullanılmıştır. Onbir AFLP primeri kulanılarak yapılan çalışmada 547 AFLP fragmenti saptanmış ve bunlardan 389'u polymorfizm göstermiştir. AFLP verilerinin cluster analizi sonucu bu populasyonlar %42 ile %81 arasında genetik benzerlik gösteren iki gruba ayrılmışlardır. Bu sonuçlar *B. tabaci*'nin Türkiye'de konukçuya özelleşmiş biotiplerinin bulunabilecegini göstermektedir.

Anahtar Sözcükler: *Bemisia tabaci*, AFLP, genetik variyasyon, Antalya

Introduction

The sweet potato whitefly, *Bemisia tabaci* (Gennadius), is a major pest all over the world (including glasshouses in moderate to cold climatic zones) and causes serious losses due to direct damage (phloem feeding) or indirect damage (transmitting TYLCV, excreting honeydew). *B. tabaci* has been known as a major pest causing economic losses in cotton and protected vegetables in Turkey since 1966 (Kaygısız, 1976). Furthermore serious losses due to tomato yellow leaf curl virus (TYLCV), which is transmitted by *B. tabaci* in protected tomatoes, are being experienced (Yılmaz, 1999). The first serious outbreaks of *B. tabaci* were recorded on cotton in the Çukurova region of Turkey in 1974 (Şengonca and Yurdakul, 1975).

Whitefly identification is based on the morphological characters of the 4th instar (Martin, 1987), which is referred to as a pupa. The characteristics of the pupal case have been used to identify individuals at the species level. The taxonomic status of *B. tabaci*, especially at the biotpype level, is confused by the high degree of plasticity in the morphological characters of the pupal case. The morphology of the 4th instar vary in response to differences in leaf surface topology and environmental and physical factors such as temperature and humidity (Martin, 1987; Mound, 1966).

Ambiguity in the identification of *B. tabaci* biotypes can be eliminated using molecular markers. A major advantage of employing DNA markers is that they are not influenced by environmental effects or epistatic

interactions. They detect variation at the level of the DNA sequence and have proved to be an extremely effective tool for distinguishing between closely related genotypes. The analysis of genetic variation using DNA fingerprinting techniques has become an important approach in taxonomic, population genetic and evolutionary studies of a variety of insect species. The most frequently used DNA markers include restriction fragment length polymorphism (RFLP), PCR-based markers such as DNA fingerprinting of microsatellite or minisatellite sequences, and random amplified polymorphic DNA (RAPD) (Reineke et al., 1998). The recently developed amplified fragment length polymorphism (AFLP) technique offers great potential for the identification polymorphism (Vos et al., 1995). The AFLP technique was first applied in microbiology and botany (Vos et al., 1995) and adapted to insects in 1998 (Reineke et al., 1998). It has now been used to investigate relationships in many arthropods (McMichael and Prowell, 1999).

No attempts have been made since the first outbreaks in 1973 to detect the existence of biotypes or races of *B. tabaci* in Turkey. Our research focused on investigating the genetic variability of *B. tabaci* in Antalya province.

Materials and Methods

Bemisia tabaci cultures

The populations of *B. tabaci* used in this study were collected from host plants and locations in Antalya province in Turkey in 1998 (Figure 1 and Table 1). More than 100 adult insects were aspirated off each host plant. All specimens were maintained at –20 ºC until required.

Table 1. Codes, host plants and localities of populations of *B. tabaci* collected from Antalya.

Code	Host-plant	Location
B1	Tomato	Alanya
B2	Cotton	Aksu
B3	Cucumber	Alanya
B4	Eggplant	Kumluca
B5	Cucumber	Uncalı
B6	Cotton	Manavgat
B7	Peppermint	Göynük
B8	Cotton	Serik

Figure 1. Map showing the location of the sampling sites of *Bemisia tabaci*.

DNA isolation

DNA isolation procedures were performed as described by Ainsworth et al. (1996) with minor modifications. In brief, more than 100 adult insects were ground with a micropestle (pre-cooled with liquid N_2) in a 1.5 ml microcentrifuge tube containing 750 µl extraction buffer (100 mM TrisCl pH 8.0, 50 mM EDTA pH 8.0, 500 mM $NaCl_2$ and 10 mM mercaptoethanol) and 60 µl of 20% SDS were added to each sample. Samples were then inverted gently several times to mix and incubated for 15 min at 65 ºC. Subsequently, 250 µl of 5 M potassium acetate was added and the samples were mixed and incubated on ice for 20 min. Samples were then centrifuged at 12,000 rpm for 15 min at room temperature. Supernatants were transferred into clean 1.5 ml microcentrifuge tubes, 300 µl isopropanol was added and mixed and then DNA was centrifuged at 5,000 rpm for 15 min. Supernatant was gently poured off and pellet lightly dried by inverting the tubes on paper towels for 10 min. DNA pellet was solved in TE (300 µl) (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) and added to an equal volume of phenol/chloroform (500 µl), mixed and centrifuged at 5,000 rpm for 10 min and then the upper phase was transferred to a clean 1.5 ml microcentrifuge tube. A 0.6 vol of isopropanol and 0.2 vol 3 M Na acetate were added to precipitate nucleic acids. The pellets were washed with 70% ethanol, dried, and resuspended in TE buffer (100 ml). The DNA was quantified fluorometrically and verified against DNA concentration standards using agarose gel electrophoresis.

AFLP analyses

AFLP analyses were performed using a kit from Life Technologies (Paisley, UK) except for the γ-^{33}P (Amersham, UK) and additional primers were synthesized

by Bioline (London, UK). The sequence information on the primers are given in Table 2. Manufacturers' instructions were followed with minor modifications. PCR product was separated on 5% polyacrylamide gels (19: 1 acrylamide: N, N' methlyene-bis-acrylamide; 8.0 M urea; 1xTBE buffer) using a gel electrophoresis apparatus (Microcomputer Electrophoresis Power Supply PS 2000). Gels were pre-run for 20 min before 3-5 µl samples were loaded in each well. The gel was dried for 2 to 3 h at 70 ºC with gel dryer (Slab Gel Dryer SGD4050) and exposed to X-ray film (Kodak BioMax). The gels were analysed according to population specific loci (Nei and Li, 1979). Fragments of the same size in two different populations were considered to represent homologous DNA sequences. Each fragment on the gel was treated as a dominant allele of a unique AFLP locus. The matrix values were calculated. Polymorphisms within populations were evaluated according to Numerical Taxonomy and Multivariate Analysis System (NTSYS) (Rohlf, 1994). A dendrogram was generated using the Unweighted Pairgroup Method Average (UPGMA) clustering procedure.

Table 2. The adapter and primer sequences used for AFLP analysis in *B. tabaci*.

Primer name	Primer type	Sequences (5'-3')
EcoRI	Adapter	CTCGTAGACTGCGTACC CATCTGACGCATGGTTAA
E+T	+1	GACTGCGTACCAATTCT
E+A	+1	GACTGCGTACCAATTCA
E+TAT	+3	GACTGCGTACCAATTCTAT
E+AGG	+3	GACTGCGTACCAATTCAGG
E+TCA	+3	GACTGCGTACCAATTCTCA
E+TTC	+3	GACTGCGTACCAATTCTTC
E+TAG	+3	GACTGCGTACCAATTCTAG
E+ACA	+3	GACTGCGTACCAATTCACA
E+AGA	+3	GACTGCGTACCAATTCAGA
MseI	Adapter	GACGATGAGTCCTGAG TACTCAGGACTCAT
M+C	+1	GATGAGTCCTGAGTAAC
M+G	+1	GATGAGTCCTGAGTAAG
M+GCT	+3	GATGAGTCCTGAGTAAGCT
M+GCA	+3	GATGAGTCCTGAGTAAGCA
M+CAC	+3	GATGAGTCCTGAGTAACAC
M+CTG	+3	GATGAGTCCTGAGTAACTG
M+CTA	+3	GATGAGTCCTGAGTAACTA
M+GAT	+3	GATGAGTCCTGAGTAAGAT
M+CAA	+3	GATGAGTCCTGAGTAACAA
M+GAC	+3	GATGAGTCCTGAGTAAGAC
M+GGA	+3	GATGAGTCCTGAGTAAGGA

Results and Discussion

We investigated the extent and nature of intraspecific variability of different populations of *B. tabaci*. Using the AFLP technique, the genetic variability of eight populations of *B. tabaci* collected from Antalya province was shown. Analysis of the eight *B. tabaci* populations with 11 AFLP primer pairs identified a total of 547 fragments, of which 389 were polymorphic. An example of typical AFLP variation for a single AFLP primer pair in an arbitrary sample of eight sweet potato whitefly populations is shown in Figure 2.

Similarity values was constructed to estimate the level of DNA polymorphism among eight populations. The calculation of similarity values was based on the presence or absence of discrete characters (AFLP fragments) from paired samples. Thus, the matrix values estimated the number of AFLP fragments shared (or not shared) between two individuals. The similarity values ranged from 42% to 81% (Table 3). The highest genetic similarity (81%) was found between B3 (cucumber, Alanya) and B4 (eggplant, Kumluca) and between B3 (cucumber, Alanya) and B5 (cucumber, Uncalı); the lowest genetic similarity (42%) was found between B5 (cucumber, Uncalı) and B6 (cotton, Manavgat). The genetic similarity of other populations ranged between the values mentioned above. Our results suggest that the effect of host ranges on populations of *B. tabaci* is more important than that of geographical location, since *B. tabaci* showed high intraspecific variability supported by the results of cluster analyses.

The similarity value was then used to cluster the data using the unweighted pair-group method with arithmetic average (UPGMA). Populations of *B. tabaci* collected from various vegetable crops and cotton were established as two distinct groups (Figure 3). The results indicated that adaptation to certain host plant species may have led to genome variations in *B. tabaci* or individuals with varying genomes may differ in their host preference. Brown et al. (1995) reported that *B. tabaci* could exhibit host-plant

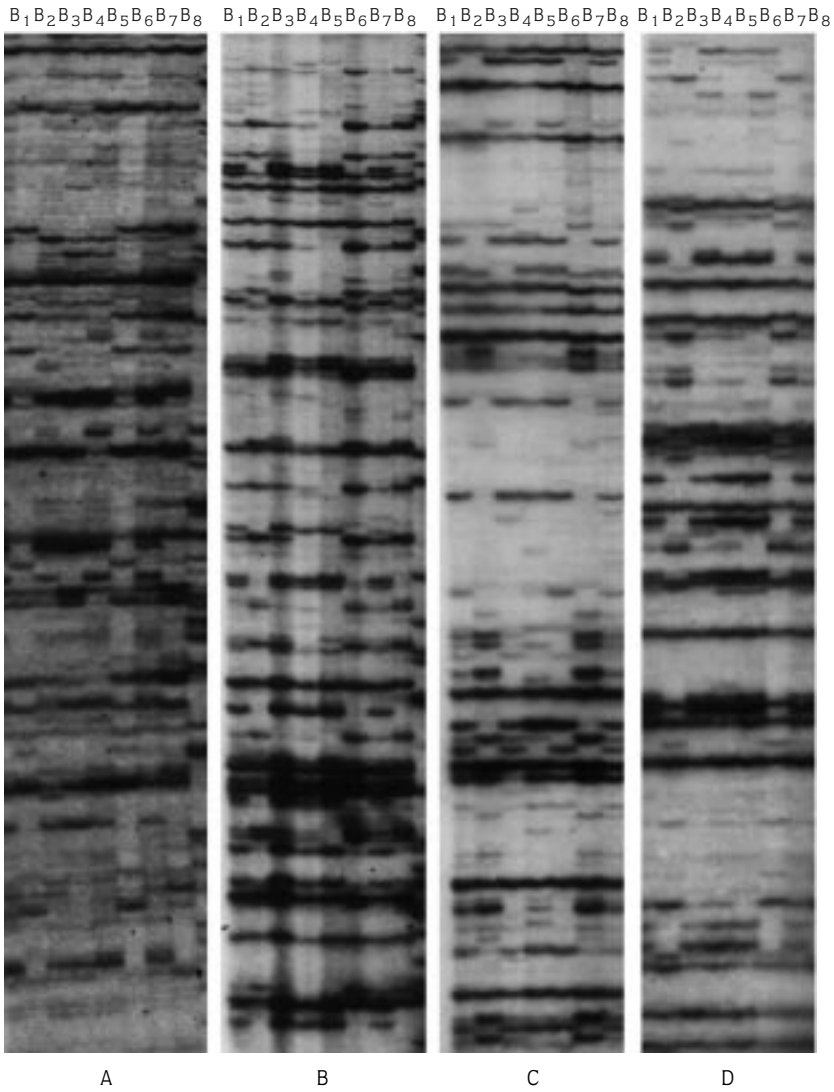

Figure 2. Autoradiogram obtained with four primer combination. AFLP fingerprints produced by primer pairs EcoRI-TAT/MseI-CTA (A), EcoRI-AGA/MseI-GCT (B), Eco-RI,AGA/MseI-GAC (C) and Eco-RI-AGA (D) for eight population of *Bemisia tabaci*.

Table 3. Genetic similarity matrix in populations of *B. tabaci* (%).

Populations	B1	B2	B3	B4	B5	B6	B7
B1	-						
B2	44	-					
B3	78	44	-				
B4	79	59	81	-			
B5	75	52	81	79	-		
B6	55	79	49	48	42	-	
B7	77	62	75	72	73	55	-
B8	60	73	56	58	52	76	60

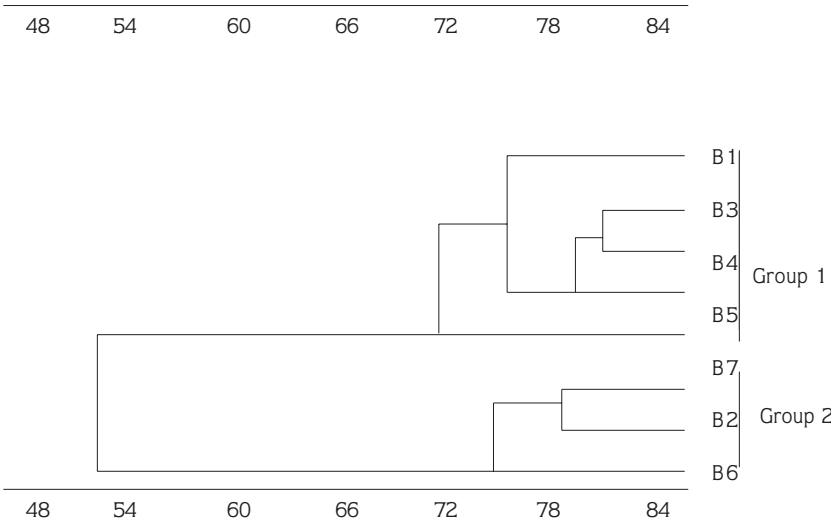

Fig. 3. Dendrogram of the eight populations of *B. tabaci* derived from the genetic similarity index.

adaptability. The dendrogram shows that there is a close relationship (80%) between B3 (cucumber, Alanya) and B4 (eggplant, Kumluca), and a wide genetic distance between (52%) B7 (peppermint, Göynük) and B8 (cotton, Serik) (Figure 3). Group 1 populations displayed a high similarity (> 72%) although they originated from diverse hosts and geographic locations. All of vegetable populations were clustered in Group 1 and cotton populations were in Group 2. Group 1 was more polymorphic than Group 2, because populations in Group 1 were obtained from various vegetables and locations.

The percentage of polymorphic AFLP fragments in our study was similar to the percentage of polymorphic RAPD markers found in similar studies (Guirao et al., 1997). RAPD-PCR has been used to delineate the taxa of fungi, plants (Williams et al., 1991), and insects (Black et al., 1992). In the organisms analysed so far by RAPD – PCR intraspecies similarity ranges from 80 to 100%, while interspecies similarities range from 0 to 30% (Perring et al., 1993 a). In this study, intraspecies similarity ranges (42-82%) were lower than the values mentioned above. The wide variability found in our study may have been due to the use of different methods since AFLP can detect more genetic loci.

In different countries, A and B biotypes of *B. tabaci* have been found. To distinguish the characteristics of the A and B biotypes, host range (Burban et al., 1992; Bedford et al., 1994; Brown et al., 1995), honeydew production (Byrne and Miller, 1990), egg production (Bethke et al., 1991), esterase patterns (Wool et al., 1989; Costa and Brown, 1991; Liu et al., 1992; Perring et al., 1992), plant cause (Yokomi et al., 1990; Schuster et al., 1990; Bharathan et al., 1990), crossing experiments (Perring et al., 1993b) and molecular work (Perring et al., 1993a; Gawel and Bartlett, 1993) have been used. In the United States, the B biotype referred to as *B. tabaci* strain B or *B. tabaci* poinsettia strain was described as a new species (Bemisia argentifolii Bellows & Perring) (Bellows et al., 1994). AFLP analysis of eight natural populations of *B. tabaci* from Antalya indicated genetic variations among the populations and these variations may account for new strains, biotypes or even species.

Since the technique employed here is robust and repeatable, to determine the populations of *B. tabaci* in Turkey, whether as strains, biotypes or new species, future studies will encompass the genotypic screening of a broader temporal and geographic sample of *B. tabaci* alongside the other known strains or biotypes.

Acknowledgments

We would like to thank Professor İrfan Tunç for his critical review of the manuscript. This study was supported by Akdeniz University Research Fund grant number 98.01.0121.19.

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