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Orijinal araştırma (Original article)

Study on species composition of *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) on cotton in Çukurova plain, Turkey[1]

Çukurova'da pamukta bulunan *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae)'nin tür kompleksi üzerine çalışmalar

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Summary

The whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) is one of the most important pest species on cotton in the Çukurova Plain as well as in all cotton growing areas of Turkey. Although numerous studies on population dynamics, population development and parasitism were conducted on *B. tabaci* in the Çukurova Plain, there has been a few studies on the species complex of *B. tabaci* for this region. The aims of this study were to present genetic polymorphism and species composition of *B. tabaci* collected from cotton fields of Çukurova Plain of Turkey between 2006 and 2011 (except 2010). Polymerase chain reaction (PCR) products were generated from a mitochondrial gene, cytochrome oxidase I (COI), and a nuclear gene region (ITS1). PCR products were purified and sequenced. Maximum likelihood (ML) and Bayesian analyses were used to estimate trees. Restriction fragment length polymorphism (RFLP) method was also applied to support results of sequence analysis and differentiating the MED and MEAM1 species of *B. tabaci* populations. From 51 sequences 38 of them were determined as *B. tabaci* MED species, and 13 of them were *B. tabaci* MEAM1. All samples collected in 2006 were placed in the *B. tabaci* MEAM1 clade with some samples collected in 2011. All PCR products were studied by RFLP method and we were able to differentiate MED and MEAM1 species of *B. tabaci*. MED species was determined as dominant on cotton in Çukurova Plain of Turkey.

Key words: *Bemisia tabaci*, biotypes, cotton, sequence, PCR-RFLP, whitefly

Özet

Beyazsinek *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) Çukurova'da ve Türkiye'de pamuk üretimi yapılan diğer alanlarda, pamuğun en önemli zararlıları arasında yer almaktadır. Çukurova'da *B. tabaci*'nin populasyon dinamiği, populasyon gelişimi ve parazitlenme durumu ile ilgili birçok çalışma yapılmasına rağmen, *B. tabaci*'nin tür kompleksi hakkında çok az çalışma bulunmaktadır. Bu çalışmanın amacı 2006-2011 (2010 hariç) yılları arasında Çukurova'dan pamuk tarlalarından toplanan *B. tabaci*'nin genetik polimorfizmi ile tür kompleksinin ortaya çıkarılmasıdır. Polimeraz zincir reaksiyonu (PZR) ürünleri bir mitokondriyal gen olan sitokrom oksidaz I (COI) ve bir nüklear gen bölgesi olan ITS1'den elde edilmiştir. Elde edilen PZR ürünleri saflaştırılmış ve sekanslanmıştır. Filogenetik ağaç, Maximum likelihood (ML) ve Bayesian analiz yöntemleri kullanılarak oluşturulmuştur. Sekans analizi sonuçlarının desteklenmesi ve *B. tabaci*'nin MED (Q biyotip) ve MEAM1 (B biyotip) türlerinin belirlenmesinde Restriction Fragment Length Polymorphism (RFLP) metodu da kullanılmıştır. Toplam 51 sekans örneğinin 38 tanesi *B. tabaci*'nin MED türü olarak belirlenirken, 13 adedi MEAM1 olarak belirlenmiştir. 2011 yılında toplanan birkaç örnek ile beraber 2006 yılında toplanan örneklerin tamamı MEAM1 içerisinde yer almıştır. Ayrıca PZR sonucunda elde edilen ürünlerin tamamı RFLP metodu ile çalışılmış ve *B. tabaci*'nin MED ve MEAM1 türleri bu yöntemle de ayrılmıştır. Çukurova'da pamuk bitkisinde MED baskın tür olarak belirlenmiştir.

Anahtar sözcükler: *Bemisia tabaci*, biyotipler, pamuk, sekans PCR-RFLP, beyazsinek

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Introduction

Cotton is one of the main cash crops in Turkey, providing raw material for textile and many labor opportunities in agriculture and industry (Şekeroğlu et al., 1998). As the second largest cotton growing area of Turkey, the Çukurova Plain comprises the provinces Hatay, İçel and Adana with approximately 130.000 ha land devoted to cotton cultivation (Anonymous, 2012). The whitefly *Bemisia tabaci* (Gennadius) is widely regarded to be the most important pest species on cotton in the Çukurova Plain as well as in all cotton growing areas of Turkey (Karut & Naranjo 2009). It is a key pest in cotton due to significant economic losses each year caused by feeding damage to the plant and honeydew contamination of cotton lint. The management of *B. tabaci* is generally carried out by insecticide application (mostly neonicotinoids) in the region. Although numerous studies on population dynamics (Özgür & Şekeroğlu, 1986), population development (Özgür et al., 1989; Karut & Kazak, 2009) and parasitism (Karut & Naranjo, 2009) were conducted on *B. tabaci* in the Çukurova Plain, there has been no detailed record for *B. tabaci* species complex in this region.

The recognition of *B. tabaci* as a worldwide pest over the past 20 years has been driven by the spread of the highly invasive B and Q biotypes (Stansly & Naranjo, 2010). Establishment of either or both biotypes in new regions often resulted in intensified whitefly infestations that would lead to increased awareness of the pest status of *B. tabaci*. Studies revealed that indigenous populations of *B. tabaci* were different genetically from the invaded B or Q biotypes, thus initiating a keen interest in the identification and dynamics of biotypes within particular regions. Because of this phenomenon, studies of the phylogenetic structure and present geographical distribution of the *B. tabaci* complex have increased in recent years. At least 24 biotypes have been designated with still others remaining unassigned (Perring 2001), although a recent review proposed that many of these so-called biotypes are really cryptic species (De Barro & Ahmed, 2011). The geographical origins of many of the biotypes have been proposed including for the Q biotype with a native range extending from the western Mediterranean Basin through to Egypt (MED) , while B extends through the Middle East into Asia Minor (MEAM1) (Frohlich et al.1999; De Barro et al., 2000; Boykin et al., 2007). Today, *B. tabaci* is describe as a species complex consisting of at least 28 sibling species that can be identified only by molecular methods (Dinsdale et al., 2010; Hu et al., 2011). In this species complex, MEAM1 and MED (also referred to as biotypes B and Q respectively) are the two most invasive ones worldwide and cause the most significant economic losses.

Some recent studies have examined the species composition of *B. tabaci* in Turkey, but none of them provides a complete picture (Cervera et al., 2000; Ulusoy et al., 2002; De la Rua et al., 2006; Bayhan et al., 2006; Erdogan et al., 2008; Karut et al., 2012). Cervera et al. (2000) was one of the first to group *B. tabaci* species into geographical regions including; (i) Near East and Indian subcontinent species; (ii) B and Q species plus a Nigerian population from cowpea, *Vigna unquiculata* L.; (iii) New World A; and (iv) S and a Nigerian population from cassava, *Manihot esculanta* Crantz. They considered that the samples originating from Turkey, known as the M, occurred in the same group of populations collected from Pakistan and India Asian countries. This Oriental biotype disappeared from Turkey after 1999 according to Bayhan et al. (2006). The occurrence of *B. tabaci* MEAM1 in Turkey was reported for the first time in 1999 by Ulusoy et al. (2002) from the Çukurova Plain. De Barro et al. (2005) reported that populations from Turkey fell into the Mediterranean/Asia Minor/Africa and Asia Group I (using sequences from GeneBank). Subsequently, De la Rua et al. (2006) analyzed four *B. tabaci* populations collected from Turkey in their phylogenetic review that they identified as Turkey-biotype M, Turkey- tomato1, Turkey-tomato 2 and Turkey-tomato3. They observed that Turkey-biotype M belongs to the same genetic group as specimens from Thailand and Pakistan. In contrast, the other three Turkey strains were identified as MED and placed in the group with Spain and Morocco (Mediterranean). On the other hand Bayhan et al. (2006) reported that all of the samples collected from the Eastern Mediterranean region of Turkey were MEAM1 that grouped with North Africa and Mediterranean strains. Similarly, Erdogan et al. (2008) reported that all samples collected from different part of Turkey were MEAM1. Boykin et al. (2007) demonstrated that populations originating from Turkey fell into the Mediterranean/Asia Minor/Africa and Asia1 group. More recently, Karut et al. (2012) reported that although there are mixed MEAM1 and MED

species in Balcalı (Adana) on cucumber, eggplant, cotton and soybean crops, MED was dominant. The inconsistent data from these studies indicate that there is not a clear picture of the *B. tabaci* species complex in Turkey and that further investigation is warranted, especially in regard to the positive impact it could have on management of this insect.

The aim of this study was to present genetic polymorphism and species composition of *B. tabaci* collected from cotton fields of Çukurova Plain of Turkey over the past five years. It is thought that results obtained from this study will serve important knowledge for successful management of *B. tabaci* in the region.

Material and Method

Bemisia tabaci samples

Bemisa tabaci samples used in the analysis were collected from cotton (*Gossypium hirsutum*, Malvaceae) in Çukurova Plain in the years between 2006 and 2011 (except 2010). The collecting locality, date, and acronym of the samples are shown Table 1. For additional comparison, *B. tabaci* samples were collected from Aydıncık (Mersin), and Diyarbakır. Samples from Diyarbakır were collected from cotton, and samples from Aydıncık (Mersin) were collected from eggplant. Greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) was used as an out-group in the phylogenetic analyses. All samples of the adult female were kept in 95–100% ethanol prior to DNA extraction. DNA was extracted from single adult females (two individuals from each different population) with the PureLink genomic DNA kit (Invitrogen, Carlsbad, CA) according to instructions provided with the kit.

Primers, PCR and protocols

Polymerase chain reaction (PCR) products were generated from a mitochondrial gene, cytochrome oxidase I (COI), and a nuclear gene region (ITS1). Primers used for both amplification and sequencing were given in Table 2. The primer pair, C1-J-2195 and R-BQ-2819, were used because they are more effective for amplifying *B. tabaci* mtCOI gene fragment (Chu et al. 2011). PCR reaction components and final concentrations were 1.5 to 2.5 mM MgCl2, 0.2 mM dNTPs, and 1 unit Taq polymerase in a proprietary buffer (PCR Master Mix, Promega Biotechnology), 0.2 µM each primer, and 5 µl DNA template in a final volume of 25 µl. The PCR cycling protocol for COI was 95 ºC for 7 mins, followed by 40 cycles of 95 ºC for 1 min, 52 ºC for 1 min, and 72 ºC for 1 min 30 sec with a final extension at 72 ºC for 5 mins (Chu et al. 2011). Protocol for ITS1 was 94 ºC for 1 min followed by 35 cycles of 57 ºC for 1 min 15 sec, with a final extension at 72 ºC for 1 min 30 sec (De Barro et al. 2000).

Phylogenetic analyses

PCR products were purified and sequenced by IONTEK (İstanbul, Turkey). Contigs were assembled using CodonCode Aligner v. 3.7.1 (CodonCode Corp.) and multiple alignments were performed by using ClustalW in BioEdit.

Maximum likelihood (ML) and Bayesian analyses were used to estimate trees. The data were analysed for each fragments separately and with data for the two fragment combined. ML trees were constructed using Mega5.2.1 packed program, with two data partitions: codon positions for COI, and one partition for ITS1. A separate GTR, nucleotide substitution model was applied to each partition. 10.000 non-parametric bootstrap replicates were performed, with every 100 bootstrap tree used as the starting tree for ML optimisation. Phylogenies were also reconstructed with Bayesian inference methods using MRBAYES V. 3.1.2 (Ronquist & Huelsenbeck 2003) under the packed program Geneious 5.6. We applied a separate GTR model with gamma-distributed rates and a proportion of invariant sites (GTR) to each partition, using default priors. Four heated chains with 0.2 heated chain temperature, were run simultaneously for 1.100.000 generations, with trees sampled every 1.000 generations.

Table1. Collecting locality, host plant, and acronym of *Bemisia tabaci* samples

Date	Host plant	Location	Acronym
2006	Cotton	Ceyhan	TR-C-06-01-B
	Cotton	Yumurtalık	TR-Yu-06-02-A
	Cotton	Yumurtalık	TR-Yu-06-02-B
	Cotton	Yumurtalık	TR-Yu-06-03-A
	Cotton	Yumurtalık	TR-Yu-06-03-B
2007	Cotton	Yüreğir	TR-Yr-07-01-A
	Cotton	Yüreğir	TR-Yr-07-01-B
	Cotton	Yüreğir	TR-Yr-07-02-A
	Cotton	Yüreğir	TR-Yr-07-02-B
	Cotton	Yüreğir	TR-Yr-07-03-A
	Cotton	Yüreğir	TR-Yr-07-03-B
	Cotton	Karataş	TR-K-07-04-A
	Cotton	Karataş	TR-K-07-04-B
	Cotton	Karataş	TR-K-07-05-A
	Cotton	Karataş	TR-K-07-05-B
	Cotton	Karataş	TR-K-07-06-B
	Cotton	Yumurtalık	TR-Yu-07-07-A
	Cotton	Yumurtalık	TR-Yu-07-07-B
	Cotton	Yumurtalık	TR-Yu-07-08-A
	Cotton	Yumurtalık	TR-Yu-07-08-B
	Cotton	Yumurtalık	TR-Yu-07-09-A
	Cotton	Yumurtalık	TR-Yu-07-09-B
2008	Cotton	Sarıçam	TR-S-08-01-A
	Cotton	Sarıçam	TR-S-08-01-B
	Cotton	Yüreğir	TR-Yr-08-02-A
	Cotton	Yüreğir	TR-Yr-08-02-B
	Cotton	Karataş	TR-K-08-04-A
	Cotton	Karataş	TR-K-08-04-B
	Cotton	Yumurtalık	TR-Yu-08-05-A
	Cotton	Yumurtalık	TR-Yu-08-05-B
	Cotton	Yumurtalık	TR-Yu-08-06-A
	Cotton	Yumurtalık	TR-Yu-08-06-B
2009	Cotton	Sarıçam	TR-S-09-01-A
	Cotton	Sarıçam	TR-S-09-01-B
	Cotton	Yüreğir	TR-Yr-09-02-B
	Cotton	Karataş	TR-K-09-03-A
	Cotton	Karataş	TR-K-09-03-B
2011	Cotton	Ceyhan	TR-C-11-01-A
	Cotton	Ceyhan	TR-C-11-01-B
	Cotton	Ceyhan	TR-C-11-02-A
	Cotton	Ceyhan	TR-C-11-02-B
	Cotton	Yumurtalık	TR-Yu-11-03-A
	Cotton	Yumurtalık	TR-Yu-11-05-A
	Cotton	Yumurtalık	TR-Yu-11-05-B
	Cotton	Karataş	TR-K-11-06-A
	Cotton	Karataş	TR-K-11-06-B
	Cotton	Karataş	TR-K-11-08-A
	Cotton	Karataş	TR-K-11-08-B
	Eggplant	Aydıncık (Mersin)	TR-A-1
	Eggplant	Aydıncık (Mersin)	TR-A-2
	Cotton	Diyarbakır	TR-D-1

VspI-based mtCOI polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP)

PCR-RFLP was reported as a simple, quick, cost-effective and reliable alternative method to distinguish *B. tabaci* species (Khasdan et al. 2005; Ma et al. 2009; Chu et al. 2012). This method was applied to support results of sequence analysis and differentiating the MED and MEAM1 species of *B. tabaci* populations. Therefore all PCR products of COI region were digested by *VspI* enzyme. PCR products were digested according to procedure given by the producer (Fermentas), at 37 ^{o}C for 2 h with 2 U of *VspI,* a restriction endonuclease that cleaves DNA at “ATTAAT” sites. The *VspI* digested PCR

products were electrophoresed on a 1% agarose and visualized by ethidium bromide staining. Only three samples acronymed as TR-C-11-01-A, TR-C-11-01-B (representative of MED) and TR-K-11-06-A (representative of MEAM1) were gel electrophoresis. Based on the sizes of bands produced by *VspI* digestion, the MED was determined.

Table 2. Primer pairs used for amplifications

Region	Code	Primers 5'-3'	Reference
ITS1	TW81	GTTTCCGTAGGTGAACCTGC	De Barro et al. (2000)
	5.8 R	ATCCGCGAGCCGAGTGATCC	
COI	C1J2195	TTGATTTTTTGGTCATCCAGAAGT	Chu et al. (2011)
	R-BQ-2819	CTGAATATCGRCGAGGCATTCC	

Results and Discussion

A total of 51 sequences were obtained from the 28 different populations. The ITS1 gene region of B tabaci was successfully amplified from all samples collected in all different years. The COI region was also amplified successfully in all the samples except samples from 2006. The alignment contained 806 characters, of which 439 characters were from the COI dataset and 367 were from ITS1. The ML tree, with bootstrap proportions, (BS, each node) recovered from analysis of the combined data, is presented in Fig. 1 (the same structure of tree was received from the Bayesian analysis, here we are repsenting only ML tree). Two putative species were recovered with strong support (100 BS). From 51 sequences 38 of them were determined as *B. tabaci* MED species, and 13 of them were recovered as *B. tabaci* MEAM1 (confirmed by blasting and PCR-RFLP studies, please see the section about PCR-RFLP result and Fig. 2). Interestingly, all samples collected in 2006 were placed in the *B. tabaci* MEAM1 clade with some samples collected in 2011 and Aydıncık and Diyarbakır. On the other hand the majority (38 sequences from 51) of the samples were placed in the clade that contained the *B. tabaci* MED species. These results show that in the Çukurova Plain *B. tabaci* MED species is more common than *B. tabaci* MEAM1 species on cotton. These results are different from the results reported by Bayhan et al. (2006) and Erdoğan et al. (2008). Because neither Bayhan et al. (2006) or Erdoğan et al. (2008) were not able to detected MED from all examined *B. tabaci* samples collected from Turkey, all were MEAM1 species.

All PCR products were able studied by RFLP method and we could differentiate both MED and MEAM1 species of *B. tabaci*. PCR products of TR-C-11-01-A and TR-C-11-01-B-coded individuals (reported as MED species in the phylogenetic tree) were cut out to form two bands of 500 and 130 bp after digestion by *VspI* enzyme. PCR products of TR-K-11-06-A-coded individual (reported as MEAM1 species in the phylogenetic tree) was not cut out by *VspI* enzyme (Figure 2).

Bemisia tabaci population collected from cotton in southern Turkey in 1985 and designated as TC (M biotype) (Bedford et al., 1994; Brown et al., 1995) was not detected in this study. This agrees with the results of Bayhan et al. (2006) who were not able to identify M from *B. tabaci* populations collected from southern Turkey. They suggest that the MEAM1 species has displaced the indigenous M haplotype in southern Turkey. The authors also mention that the M haplotype was probably the predominant in Turkey previously, and until 1999 (Ulusoy et al., 2002), when the MEAM1 was introduced there.

We found that MED was dominant in the cotton growing areas of Çukurova Plain. This may be due to intensive use of neonicotionoid insecticides in management of *B. tabaci* in the cotton production in the region. Khasdan et al. (2005) reported similar results in cotton growing areas of Israel that they attributed to higher resistance of the MED to neonicotionid insecticides. This may be a contributing reason why the MEAM1 is still found in Aydıncık and Diyarbakır where neonicotinoid insecticides are not being commonly used.

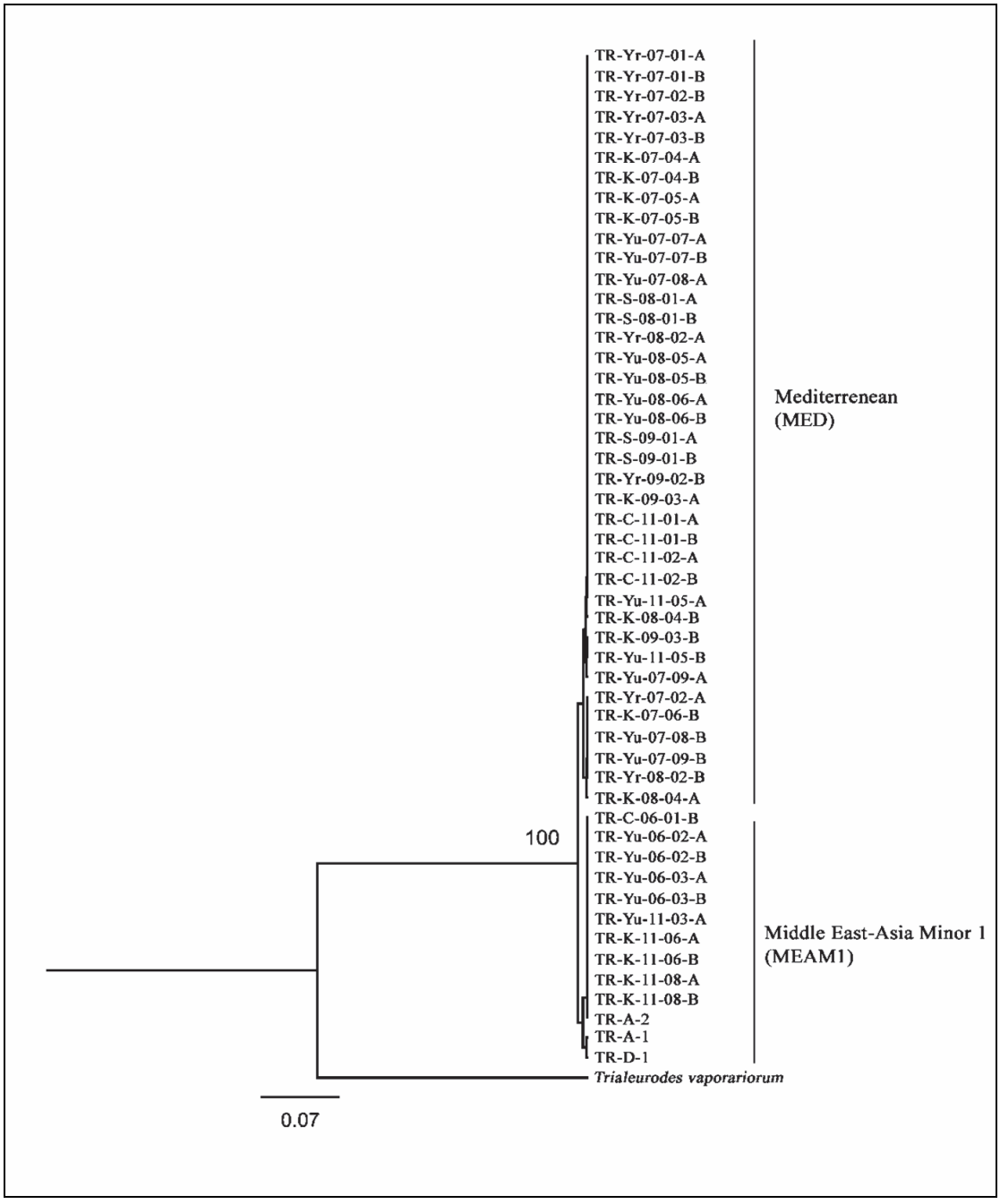

Figure 1. The ML tree, with bootstrap proportions, (BS, each node) recovered from analysis of the combined data (COI and ITS1 regions of *Bemisia tabaci*).

The greater occurrence of the MED over the MEAM1 in cotton growing areas of Çukurova Plain suggests that complete displacement of the MEAM1 could occur in this region. Chu et al. (2010) reported that MED was recorded for the first time in 2005 in Shandong region of China and until this date MEAM1 were dominant in the region. The authors reported that after three years, in 2008, due to neonicotionid insecticide used for management of *B. tabaci*, MEAM1 replaced by MED and MED became dominant in the region.

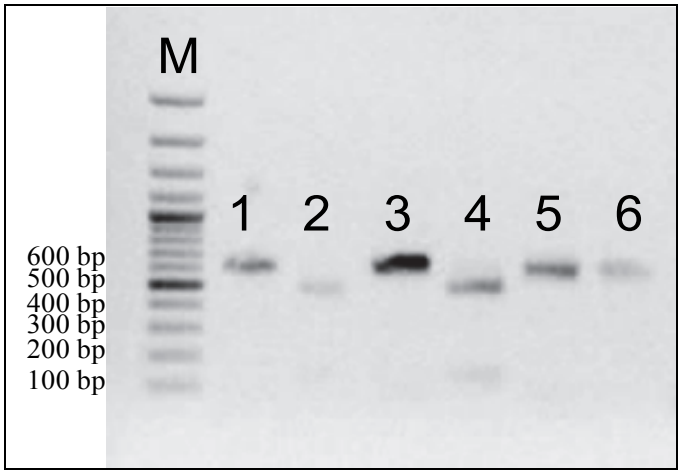

Figure 2. Species classification of the *Bemisia tabaci* collected from cotton determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques. 1, 3 and 5 shows VspI undigested PCR product of TR-C-11-01-A, TR-C-11-01-B, and TR-K-11-06-A, 2, 4 and 6 VspI digested PCR product of TR-C-11-01-A (MED), TR-C-11-01-B (MED), and TR-K-11-06-A (MEAM1) respectively.

In this study, species of *B. tabaci* were found to be in continuous change in cotton fields in Çukurova Plain. Thus, *B. tabaci* species should be monitored periodically by molecular diagnostic techniques such as sequencing or PCR-RFLP. In addition, these studies should be done to cover larger areas and other host plants of *B. tabaci* in Çukurova Plain. We thought that the findings of this study will contribute to the management of *B. tabaci* depending on the species structure in cotton areas of Çukurova Plain and other cotton growing areas of Turkey.

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