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Genetic diversity and identification of some Turkish cotton genotypes (*Gossypium hirsutum* L.) by RAPD-PCR analysis

Yonca SURGUN, Bekir ÇÖL, Betül BÜRÜN
Department of Biology, Faculty of Science, Muğla University, 48000 Muğla - TURKEY

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Abstract: Cotton (*Gossypium hirsutum* L.) is the leading fiber crop and the second most important oil seed crop in the world. This study aimed to analyze the genetic differences among 9 cotton genotypes of cultivars from Turkey using the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) method. A total of 42 RAPD primers were used to determine polymorphism among the genotypes and 34 of the primers produced scorable results, with 319 RAPD-PCR bands obtained. The rate of polymorphism was calculated to be 18.1%, and genetic similarity between any 2 genotypes ranged from 90.2% to 96.5%. By evaluating the presence and absence of the RAPD-PCR bands, a dendrogram indicating the relative genetic similarity of the Turkish cotton genotypes was constructed and presented. The results also showed that the genotypes can be separated from each other at the molecular level by taking advantage of some of the RAPD markers. Specific markers were proposed for the identification of genotypes. To our knowledge, this is the first study to use RAPD markers to investigate the genetic relationships among the *Gossypium* genotypes grown in Turkey.

Key words: Cotton (*Gossypium hirsutum* L.), RAPD, genetic relationship

Bazı Türk pamuk (*Gossypium hirsutum* L.) genotiplerinin RAPD-PZR analizi ile identifikasyonu ve genetik çeşitliliği

Özet: Pamuk (*Gossypium hirsutum* L.) dünyada önde gelen lif bitkilerinden birisidir ve dünyada ikinci en önemli yağlı tohum kaynağıdır. Bu çalışmanın amacı, Türkiye'de kültürü yapılan 9 pamuk genotipinin genetik farklılığının RAPD-PZR tekniği ile belirlenmesidir. Genotipler arasında, polimorfizmi saptamak amacıyla, toplam 42 adet RAPD primeri kullanılmış olup, bu primerlerden 34 tanesinden değerlendirilebilir sonuçlar alınmış ve toplam 319 RAPD-PZR bandı elde edilmiştir. Çalışılan genotipler arasında polimorfizm oranı % 18,1 olarak saptanırken, herhangi 2 genotip arasında genetik benzerlik % 90,2 ile % 96,5 arasında değişmiştir. RAPD-PZR bantlarının varlığı ve yokluğu değerlendirilerek, pamuk genotiplerinin genetik benzerliğini gösteren dendrogram oluşturulmuştur. Sonuçlar, genotiplerin moleküler seviyede RAPD markörleri yardımıyla birbirinden ayrılabildiğini ortaya koymuştur. Kültürü yapılan çeşitlere ait genotiplerin teşhisi için spesifik markörler önerilmiştir. Bilgimize göre, bu çalışma, Türkiye'de yetiştirilen *Gossypium* genotiplerinin RAPD markörleri ile genetik akrabalığının araştırılması ile ilgili yapılan ilk çalışmadır.

Anahtar sözcükler: Pamuk (*Gossypium hirsutum* L.), RAPD, genetik akrabalık

Introduction

Within the field of biodiversity, it is of great importance to determine the genetic diversity of plants in order to promote sustainable agriculture. The success of plant breeding projects greatly depends on our ability to understand the genetic parameters and genetic diversity of the species and cultivated varieties. The extent of genetic diversity in species is thought to be important in determining the selection to be performed, and for the ability to adapt to variable environmental conditions. At the onset of a breeding program, information concerning genetic relationships can be used to improve the breeding population by complementing phenotypic features. The ability to predict genetic similarities or differences among genotypes is vital for the accurate selection of parental combinations for populations exhibiting segregation, the production of expected hybrids, and the maintenance of sufficient diversity in breeding programs (1-3).

Genetic markers are important tools in the determination of genetic diversity, which is the first step for breeding projects and the protection of genetic resources (1). Researchers denote specific locations on chromosomes that constitute important clues for genome analysis. In general, morphological and molecular markers are used. Molecular markers can be divided into 2 groups: biochemical and DNA markers (4).

DNA markers indicate the differences in the sequences of nucleic acid at a particular location or locations in the genome. In addition, they are independent of external factors, and the rates of polymorphism in these variable regions are high (2). One of the DNA marker techniques, random amplified polymorphic DNA (RAPD), is based on the amplification of unknown DNA sequences and uses a single short and random oligonucleotide primer (5). This technique, which is both fast and inexpensive, can detect DNA variations even at the single-base level. Hence, it has become a commonly used technique for exploring intraspecies and interspecies diversity (6-9). Comparative studies of RAPD and simple sequence repeat (SSR) markers in a wide range of crop species, including corn, soybean, barley, sorghum, rice, bluegrass, and wheat, have generally revealed good congruence between the genetics patterns shown by the 2 genetic markers (9,10). Ali et al (2008) utilized RAPD analysis in order to investigate hybrid authentication in upland cotton in Pakistan. Comparing the RAPD band pattern of the parents with that of the respective hybrids resulted in the clear identification of genuine hybrids (11). Farzaneh et al. (2010) performed a cytogenetic and molecular study using 10 tetraploid cotton cultivars (*Gossypium hirsutum* L.), including parental genotypes and F_1 progenies. Based on cytogenetic and RAPD data, the results indicated that the parental genotypes and their hybrids were different from each other (12). In yet another RAPD study involving 13 African and American cultivars (*Gossypium hirsutum* L. race *latifolium* H.) and 8 inbred lines, approximately 90% of commercial cultivars originally identified as African were placed in 1 cluster while the remaining American cultivars were clustered into other groups (13).

The objectives of this study were to evaluate the level of genetic diversity among the 9 cotton genotypes in Turkey using RAPD markers and to identify the genotypes' specific RAPD markers in order to fingerprint the 9 genotypes.

Materials and methods

Plant materials

There are 9 different genotypes of the cultivars of *Gossypium hirsutum* L. (2n = 4x = 56), namely Nazilli 84, Nazilli 84-S, Şahin 2000, Carmen, Flora, $BA_{mig}119$, $72D4/F_1$, $608D4/F_1$, and $608D1/F_1$, which are the choice cotton crops cultured in the southwestern and southeastern regions of Turkey. Seeds of these genotypes were procured from cotton seed companies and the Nazilli Cotton Research Institute. The genotypes were maintained under the required cultural conditions in a greenhouse environment.

Genomic DNA isolation

Fresh young leaves from cotton plants, which were 5-6 weeks old, were collected from each genotype and stored at –80 °C until DNA isolation. The total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (14). The DNA concentration of the samples was measured by the ethidium bromide spot test (15). After quantitation, the DNA samples were diluted to 5 ng μL^{-1} with distilled H_2O. The DNA was then stored in a freezer (–20 °C) until it could be used for polymerase chain reaction (PCR) amplification.

RAPD analysis

In this study, 42 operon primers were used: OP-A11, OP- B 1-20, OP-C04, and OP-M 1-20. RAPD analysis was done using a modified protocol (5). The primers were applied and those that provided good and scorable amplification products were selected. Ultimately, 34 oligonucleotide primers were chosen for RAPD analysis. PCR amplification was carried out in a reaction volume of 15 µL containing the following: 1.5 µL of 10× Taq buffer with KCl [100 mM Tris-HCl (pH 8.8), 500 mM KCl, 0.8% Nonidet 40], 1 µL of primer (10 µM), 2.4 µL of magnesium chloride (25 mM), 1 µL of dNTPs (10 mM), 0.25 µL of Taq DNA polymerase, 3.85 µL of ddH_2O, and 25 ng of genomic DNA as a template. PCR amplification conditions were as follows: first denaturation at 94 °C for 1 min, followed by 45 cycles of 94 °C for 1 min, 36 °C for 1 min, and 72 °C for 2 min. After the completion of 45 cycles, a final extension was carried out at 72 °C for 10 min. Subsequently, PCR products were analyzed using agarose gel electrophoresis; the gel was stained with ethidium bromide and visualized under UV light. A 1.0-kb DNA ladder was used as a molecular size standard (Fermentas).

Data analysis

For all of the genotypes, the RAPD bands were scored as 1 for present or 0 for absent. The presence/absence data was used to produce a matrix and the genetic distance in the 9 cotton genotypes was calculated using the Nei (1972) genetic distance formula present in the PHYLIP 3.5 software program (16). The similarity coefficient was calculated using JMP 7.0 (SAS Institute, 1994) software.

Results and discussion

The RAPD-PCR products yielded by the primers used in this study ranged from 1 (OPM-08, OPM-09, and OPM-11) to 17 (OPC-04). A total of 319 fragments were generated by 34 primers. It was observed that 261 of the scored RAPD-PCR bands were monomorphic (81.81%) and 58 were polymorphic (18.1%). Figures 1a and 1b show 2 of the gel pictures. The rate of polymorphism among the 9 Turkish cotton genotypes is 18.1% (Table 1).

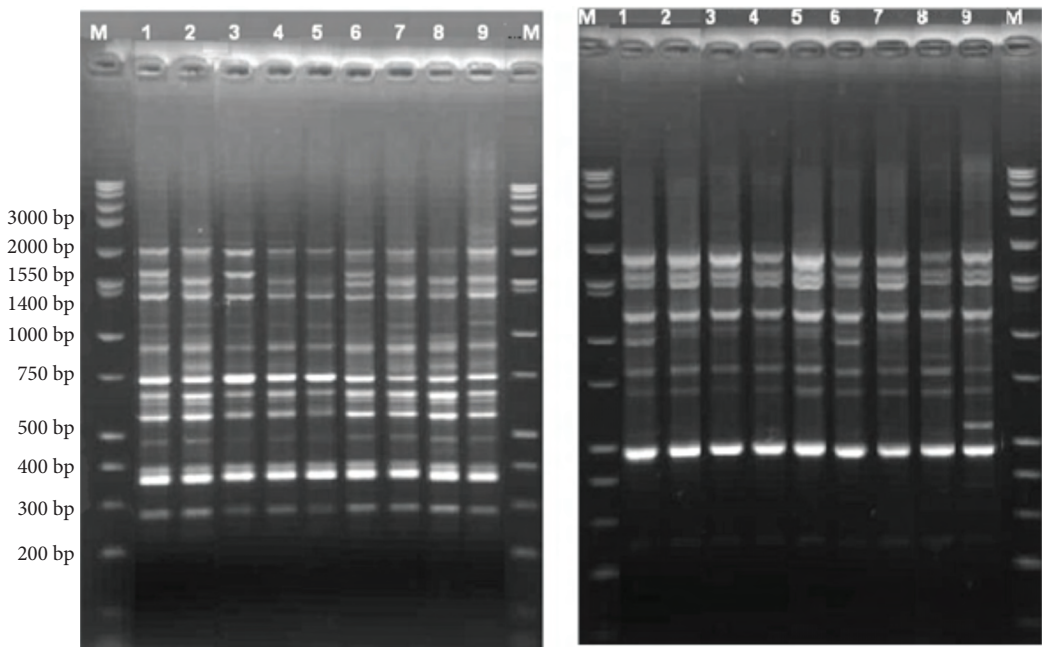

Figure 1. Examples of the RAPD profiles for the 9 genotypes using some primers. OPM-13 (a) and OPM-15 (b) were used as primers and PCR was carried out using the genomic DNA isolated from the genotypes as described. M: marker (1.0 kb), 1: genotype 72D4/F_1, 2: BA_{mig}119, 3: 608D1/F_1, 4: Şahin 2000, 5: Carmen, 6: 608D4/F_1, 7: Nazilli 84, 8: Nazilli 84-S, 9: Flora.

Table 1. The presence or absence of polymorphic RAPD bands for the cotton genotypes studied (+ and - indicate the presence and absence of polymorphic bands, respectively).

Primer name	Cotton Genotype Name									
	72D4/F_1	BA_{mig}119	608D1/ F_1	Şahin 2000	Carmen	608D4/ F_1	Nazilli 84	Nazilli 84-S	Flora	RAPD band size (bp)
OPA-11	+	+	+	+	+	-	+	+	+	2800
OPB-01	-	-	+	-	+	+	-	-	+	1600
	+	+	+	+	-	+	+	-	-	525
OPB-05	+	+	+	+	+	+	+	-	-	2200
	+	+	+	+	+	+	+	-	+	1200
OPB-07	-	-	-	-	-	+	-	-	-	875
	+	+	-	-	+	+	-	-	+	850
	+	+	+	-	+	+	+	+	+	525
	-	-	+	-	-	-	-	+	-	500
OPB-08	+	+	-	-	-	-	-	-	-	700
OPB-09	+	+	+	+	+	-	-	-	+	1850
	+	+	+	+	+	-	+	-	+	1600
OPB-10	-	+	+	+	+	+	+	+	+	2100
	-	-	-	-	-	-	-	+	-	1100
OPB-11	+	+	-	-	-	-	-	-	-	1000
	-	-	-	+	+	+	+	+	-	650
	-	-	-	-	-	-	-	-	+	500
OPB-12	+	+	+	+	-	+	+	-	+	1500
	+	+	+	+	-	+	+	+	+	900
	+	+	-	-	-	+	+	-	-	550
OPB-15	-	-	-	-	-	-	-	-	+	2000
	+	+	+	+	+	+	+	+	-	850
	+	+	+	-	+	+	+	+	+	375
OPB-16	-	-	-	+	-	+	-	-	-	650
	+	+	+	-	+	-	+	+	+	625
	-	-	-	-	-	-	+	+	-	600
	+	+	+	+	+	+	+	-	-	540
	-	+	+	+	-	+	+	+	-	420
	+	+	-	+	+	+	-	-	-	270
OPB-17	-	+	+	+	+	+	+	+	+	950
OPB-18	+	+	+	+	-	+	+	+	+	800
OPB-20	-	+	+	+	+	+	+	+	+	1200
	-	-	-	+	+	+	+	+	+	1150
	+	+	+	-	+	+	+	+	+	650
OPC-04	+	+	+	-	+	+	+	+	+	1150
	-	+	-	-	+	-	-	-	-	700
	-	-	+	+	-	+	-	-	-	625
	-	+	+	+	+	+	+	+	+	600
OPM-02	+	+	+	+	-	+	-	-	-	2700
	+	+	+	+	+	-	+	+	-	1100
	+	+	-	+	+	+	+	+	+	900
OPM-03	+	-	-	-	-	+	+	+	-	1600
OPM-04	+	+	+	-	-	+	-	+	+	1900
	+	+	+	+	+	+	+	+	-	1500
	+	+	+	+	+	+	+	-	-	850
OPM-05	+	+	-	-	-	+	+	+	+	1000
	+	+	+	+	+	+	+	+	-	800
	+	+	+	+	+	+	+	+	-	775
OPM-06	-	+	+	-	+	+	+	+	+	650
OPM-07	+	+	+	-	+	+	-	-	+	550
OPM-12	+	+	+	+	+	-	+	+	+	900
OPM-13	+	-	+	-	-	+	-	-	-	1600
	+	+	+	-	-	-	+	+	-	1000
	-	+	-	+	-	-	+	+	+	800
	+	+	+	+	-	+	+	+	+	675
OPM-15	+	-	-	-	-	+	-	-	-	950
	-	-	-	-	-	-	-	-	+	525
OPM-16	+	+	+	+	+	+	+	-	+	200

The results of the RAPD-PCR analysis indicated that some polymorphic RAPD bands are specific markers for a genotype or genotypes. While some of the polymorphic bands are only present in only one genotype, some were found to be shared among more than one genotype (Table 2). For example, a band of approximately 875 bp produced by primer OPB-07 is only specific to genotype 608D4/F_1, while primer OPB-07 produced a 500-bp band specific to genotypes 608D4/F_1 and Nazilli 84-S. Primers OPB-08 (700 bp) and OPB-11 (1000 bp) produced polymorphic bands that are specific to genotypes 72D4/F_1 and BAmig119. Primers OPB-11 (500 bp), OPB-15 (2000 bp), and OPM-15 (525 bp) produced polymorphic bands that are only specific to genotype Flora. Similar conclusions can be drawn to discriminate the genotype based on the RAPD results summarized in Table 2. The genetic similarities of the 9 genotypes were relatively high, ranging from 90.1% to 96.8% according to the Nei (1972) algorithm. Based on these values, genotypes Nazilli 84 and Nazilli 84-S were found to have the greatest similarity (96.8%), followed by the genetic similarity between genotypes 72D4/F_1 and BA_{mig}119 (96.4%). Using the JMP 7.0 (SAS Institute, 1994) statistical program package, a dendrogram was constructed for the 9 cotton genotypes based on the analysis of the polymorphic and monomorphic bands (Figure 2 and Table 3). The genotypes were classified into 2 main branches. The first branch was only for genotype Flora, whereas the second branch was divided into 2 subgroups that have the subbranches seen in Figure 2.

In the present study, only 18.1% of the DNA fragments generated by the 34 primers were polymorphic. These results indicate that the level of polymorphism was relatively low among the 9 genotypes. Wendel et al. (1992) applied allozyme analysis to 50 US upland cultivars to measure genetic diversity and detected a polymorphism rate of 28% (17). Brubaker and Wendel (1994) applied the method of restriction fragment length polymorphism to 23 cultivars and came up with only 7% polymorphism (18). Lu and Myers (2002) used 86 RAPD primers to analyze 10 upland cotton cultivars and observed a polymorphism rate of 13.5% and genetic similarity ranging from 92.7% to 97.6% (19). Sheidai et al. (2007) used 27 RAPD primers to determine the genetic relations of 10 tetraploid cotton variants cultured in Iran and found a polymorphism rate of 19% (20). These results bear similarities to the results obtained in the present study. In other studies conducted to determine the origins of and

Table 2. Specific RAPD markers for the identification of some genotypes (+ and - indicate the presence and absence of polymorphic bands, respectively).

Primer name	Cotton genotype name									
	72D4/F_1	BA_{mig}119	608D1/F_1	Şahin 2000	Carmen	608D4/F_1	Nazilli 84	Nazilli 84-S	Flora	RAPD band size (bp)
OPB-07	-	-	-	-	-	+	-	-	-	875
	-	-	-	-	-	+	-	+	-	500
OPB-08	+	+	-	-	-	-	-	-	-	700
OPB-10	-	-	-	-	-	-	-	+	-	1100
OPB-11	+	+	-	-	-	-	-	-	-	1000
	-	-	-	-	-	-	-	-	+	500
OPB-15	-	-	-	-	-	-	-	-	+	2000
OPB-16	-	-	-	+	-	+	-	-	-	650
	-	-	-	-	-	-	+	+	-	600
OPC-04	-	+	-	-	+	-	-	-	-	700
OPM-15	+	-	-	-	-	+	-	-	-	950
	-	-	-	-	-	-	-	-	+	525

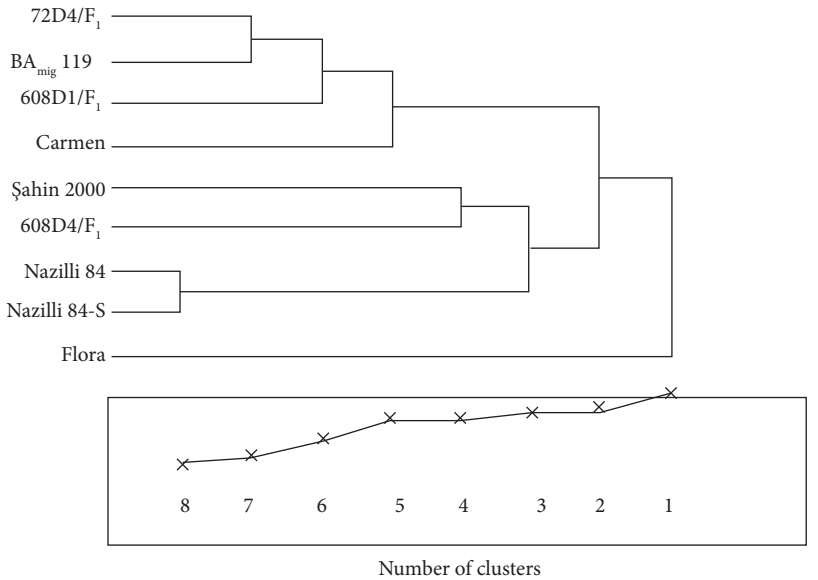

Figure 2. The RAPD-PCR results generated by 34 primers were applied to construct a dendrogram using the JMP 7.0 program (http://www.jmp.com).

Table 3. The numbers in the table represent similarity values among the genotypes in the dendrogram (http://www.jmp.com).

Cluster number	Distance	Leader	Joiner
8	5.259	Nazilli 84	Nazilli 84-S
7	5.533	72D4/F_1	BA_{mig}119
6	6.697	72D4/F_1	608D1/F_1
5	7.802	72D4/F_1	Şahin 2000
4	8.120	Carmen	608D4/F_1
3	8.344	Carmen	Nazilli 84
2	8.656	72D4/F_1	Carmen
1	9.459	72D4/F_1	Flora

the genetic relationships among cotton genotypes, different polymorphism rates were obtained. Punitha and Raveendran (2004) observed a polymorphism rate of 76.3% as a result of the RAPD analysis of 11 colored and 4 white cotton genotypes using 32 different 10-mer primers of arbitrary sequences (21). Vafaie-Tabar et al. (2004) found a rate of 87% polymorphism and a genetic similarity rate of 30% as a result of testing 50 random decamer primers on 22 cotton genotypes belonging to tetraploid and diploid cotton species (22). Their analysis revealed that tetraploids show a much narrower genetic base (with a similarity range of 65%-95%) than diploids (at a similarity range of 54%-88%). Saravanan et al. (2006) observed a polymorphism rate of 69.7% among 10 races and a genetic distance ranging from 71.2% to 88% (23). Khan et al. (2010) found 70.2% polymorphism in 11 colored cottons (10 genotypes belonging to *Gossypium hirsutum* and 1 to *Gossypium arboreum*) and 5 white-linted genotypes (4 of these belonging to *Gossypium hirsutum* and 1 to *Gossypium arboreum*) using 25 polymorphic decamer randomly sequenced primers for RAPD analysis (24). In another study, Mahmood et al. (2009) observed a high level of genomic variability within the 20 different species of *Gossypium* with 63 random 10-mer primers; in that study, maximum similarity was 64%, between *G. arboreum* and *G. herbaceum*, and minimum similarity was 3%, between *G. stocksii* and *G. longicalyx* (25). In our opinion, the higher polymorphism rates observed in the above studies may result from the fact that the cotton species and cultivars used by those researchers come from quite different parts of the world. Furthermore, different germplasms were compared to those reported in

this study. Genotypes were studied from individuals belonging to diploid and tetraploid species, and the polymorphism rates were calculated by considering both diploid and tetraploid individuals together. On the other hand, Erkılınç and Karaca (2005) used the SSR technique to detect the genetic variation among some cotton genotypes cultivated in Turkey. Of the genotypes they examined, 4 (Carmen, Nazilli 84, Nazilli 84-S, and Şahin 2000) were also utilized in the current study (26). The SSR technique revealed that the genome base of the cotton genotypes examined is very narrow; the dendrogram constructed in that study showed the genotypes divided into 3 main groups. In keeping with those findings, the present study revealed that the Nazilli 84 and Nazilli 84-S genotypes are placed in the same group and occur very close to each other in the dendrogram. In the present study, Nazilli 84 and Nazilli 84-S and $BA_{mig}119$ and $72D4/F_1$, which are genetically closer to each other than the other genotypes, are in the same group, consolidating the relationship between these genotypes. Furthermore, some of the 9 Turkish cotton genotypes could be individually characterized with genotype-specific RAPD marker(s) or a combination of 2 or more markers. These findings enable researchers to identify or distinguish between particular genotypes under investigation by using a specific RAPD marker or the combination of more than 1 RAPD marker.

The main goal of plant breeding is to develop new and more versatile genotypes by introducing and manipulating genetic variation (27). RAPD is the most widely used technique because it allows for a rapid and inexpensive assay with a large number of markers. Due to the technical simplicity and speed of the RAPD method, it has been successfully used for the generation of genetic similarities and phylogenetic analysis (25). Estimations of genetic diversity and the relationship between varieties are valuable resources for cotton breeding. This is the first study to use RAPD markers to investigate the genetic relationship of the *Gossypium* genotypes grown in Turkey. The results of the present investigation confirmed that these molecular markers are capable of detecting polymorphism among *Gossypium* genotypes, estimating relatedness, and identifying genotypes with genotype-specific RAPD marker(s). Finally, this study is thought to contribute vital information for breeding programs and the development of germplasm resources.

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Corresponding author:

Betül BÜRÜN

Department of Biology,

Faculty of Science,

Muğla University,

48000 Muğla - TURKEY

E-mail: bbetul@mu.edu.tr

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