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Molecular Characterization of Some Turkish Olive Cultivars Using Random Amplified Polymorphic DNA (RAPD) Markers

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Abstract: Olive (*Olea europea* L.) is one of the oldest cultivated plants characteristic in the Mediterranean area, where it is the most important oil-producing crop. The cultivated olive (*O. europaea* L. var. *europaea*) is propagated by cutting or grafting, whereas wild olive (*O. europaea* L. var. *sylvestris*) is reproduced from seeds. These two olive types are interfertile and have led to a large number of varieties. Morphological descriptions are not entirely reliable, due to numerous synonyms and homonyms in designations, labelling mistakes, the presence of varietal clones, and the uncertain identification methods thus far applied. Molecular markers, as random amplified polymorphic DNA (RAPD) markers, are environment-independent and efficient to identify olive varieties and to detect synonymous and homonymous. In this study, fifteen selected RAPD markers are used for determination of relationships among twenty individuals belonging to four important Turkish olive cultivars. Our results showed that RAPD markers can be used to differentiate olive cultivars.

Rastgele Çoğaltılmış Polimorfik DNA (RAPD) Markörler Kullanılarak Bazı Türk Zeytin Çeşitlerinin Moleküler Karakterizasyonu

Keywords
DNA
Polimorfizm
RAPD Markörler
Zeytin (*Olea europea* L.).

Özet: Zeytin (*Olea europea* L.), Akdeniz Bölgesi'ne özgü en eski kültür bitkilerinden biridir ve bölgede yağ verimi sağlayan en önemli üründür. Kültür zeytini (*O. europaea* L. var. *europaea*), tohumla çoğaltılan yabani zeytin (*O. europaea* L. var. *sylvestris*)'in çeliklenmesi ya da aşılanması ile yetiştirilir. Her iki zeytin çeşidi de kendi içlerinde tozlaşabilmekte ve pek çok sayıda varyete oluşumuna yola açabilmektedir. Zeytinde morfolojik tanılar, çok sayıda sinonim ve homonim içermesi, etiketleme hataları, varyasyonel klonlar ve günümüze kadar yapılmış net olmayan tanılar nedeniyle tam anlamıyla güvenilir değildir. Rastgele çoğaltılmış polimorfik DNA (RAPD) gibi moleküler markörler, çevreden bağımsız ve zeytin çeşitlerinin tanısında, sinonim ve homonimlerin belirlenmesinde oldukça etkilidir. Bu çalışmada seçilen onbeş adet RAPD markör primeri, dört önemli Türk kültür çeşidine ait yirmi bireyin aralarındaki akrabalık düzeylerini belirlemek amacıyla kullanılmıştır. Elde ettiğimiz sonuçlar RAPD markörlerin zeytin kültür çeşitlerindeki farklılıkları ortaya çıkarmak için kullanılabileceğini göstermiştir.

1. Introduction

Olive (*Olea europaea* L.), which has nearly more than 1200 cultivars, is one of the oldest cultivated plants of the Mediterranean area, where it is the most important oil-producing crop (Rugini and Lavee, 1992; Zohary and Hopf, 1994; Bartolini et al., 2005). It is determined in two forms, former is wild (*O. europaea* subsp. *europaea* var. *sylvestris*) and latter is cultivated form (*O. europaea* subsp. *europaea* var. *europaea*). The cultivated olive (*O. europaea* L. var. *europaea*) is propagated by cutting or grafting, whereas wild olive (*O. europaea* L. var. *sylvestris*) reproduced from seeds (Green, 2002).

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Olive trees have high levels of heterozygosity and genetic diversity among cultivars so that they are predominantly allogamus. This variability, coupled with the confusion in olive cultivar identification, make necessary the evaluation and characterization of olive genetic resources that have been recognized as very important, since both olive productivity and oil quality are traits inherited to a variety (Angiolillo et al., 1999; Rallo et al., 2000; Diaz et al., 2006a,b). Therefore, molecular markers, such as random amplified polymorphic DNA (RAPD) markers are environment-independent and efficient to identify olive varieties and to detect synonymous and homonymous (Besnard et al., 2001; Bronzini de Caraffa et al., 2002; Fabbri et al., 1995; Hess et al., 2000).

Thus, fifteen selected RAPD markers are used for determination of relationships among twenty individuals belonging to important Turkish olive cultivars ("Gemlik", "Hatay", "Mardin" and "Mugla"). Our results showed that retrotransposon-based IRAP markers can be used to differentiate olive cultivars.

2. Material and Methods

2.1. Plant Materials and DNA Extraction

Twenty individuals belonging to important Turkish olive cultivars Bursa-Gemlik cv. "Gemlik"; Hatay cv. "Halhali" (wild type); Mardin cv. "Halhali"; Mugla cv. "Domat") were collected from different places of Turkey. The total genomic DNA was extracted by using CTAB method (Doyle and Doyle, 1987) from young leaf tissue ground to a fine powder. DNA sample concentration was determined using a nanodrop spectrophotometer (BioSpec-nano; Shimadzu-Biotech) and the DNA samples were diluted to 50 ng/ml prior to IRAP (PCR) amplification.

2.2. RAPD (Random Amplified DNA Polymorphisms) PCR

PCR DNA amplification was performed using fifteen RAPD primers (Table 1). Amplifications were performed according to Willams et al. (1990) and Martins-Lopes et al. (2007) in a 25 ml reaction volume containing PCR Buffer (1x final concentration, invitrogen), 2,5 mM MgCl2, 0.4 mM each dNTP, 0,4 mM RAPD primer, 50 ng genomic DNA, and 2 unit Taq DNA polymerase. Amplification conditions (thermocycler Model-9700, Perkin-Elmer, Boston, MA, USA) were initial denaturation at 94 ^{0}C for 3 min and 35 cycles at 94 ^{0}C for 1 min, and then 38 ^{0}C for 1 min, a ramp to 72 ^{0}C for 1 min, followed by 6 min at 72 ^{0}C and indefinite soak at 4 ^{0}C. Amplicons were separated on 1.5% agarose gel at 80 V. Gels were then stained with 0.5 ml/ml ethidium bromide solution, visualized by illumination under UV light, and then documented using a gel documentation and image analysis system (BIORAD, Molecular Imager®, ChemiDocTM XRS+ with Image LabTM Software).

Table 1. RAPD Primers and primer base sequence.

Primers	Sequence
A - 01	5'- CAG GCC CTT C - 3'
A - 02	5' - TGC CGA GCT G - 3'
A - 10	5' - GTG ATC GCA G - 3'
A - 19	5' - CAA ACG TCG G - 3'
A - 20	5' - GTT GCG ATC C - 3'
C - 09	5'- CTC ACC GTC C - 3'
C - 15	5'- GAC GGA TCA G - 3'
D - 03	5' - GTC GCC GTC A - 3'
D - 08	5' - GTG TGC CCC A - 3'
D - 15	5'- CAT CCG TGC T - 3'
OPAH -02	5'- CAC TTC CGC T - 3'
OPJ1	5'- CCC GGC ATA A - 3'
OPJ6	5'- TCG TTC CGC A - 3'
OPJ - 18	5'- TGG TCG CAG A - 3'
OPX-6	5'- ACG CCA GAG G - 3'

3. Result and Discussion

Molecular characterization of twenty individuals belonging to important four Turkish olive cultivars ("Gemlik", "Hatay", "Mardin" and "Mugla") was assayed using fifteen RAPD markers. We obtained a total of 351 band profile which varied in size from 200 bp to 4500 bp. from fifteen RAPD primers, 237 of them were polymorphic. The best polymorphism was obtained from primer A01(23 bands) and OPAH-02 (19 bands) (Figure 1). Four cultivars of Turkish olive examined were genetically distinct, and these differences provided for the development of strategies for genetic analyses and crop improvement in these cultivars.

The high level of polymorphism observed in this study (Figure 2) agrees with results of previous studies carried out in olive cultivars with RAPDs (Fabbri et al., 1995; Weismann et al., 1998; Belaj et al., 2001; Besnard et al. 2001; Sanz- Cortés et al. 2001). However, the polymorphism level yielded by RAPD markers in this study was higher than in other cases, possibly due to the better representativeness of olive cultivar diversity in the Mediterranean basin and a higher resolution provided by polyacrylamide gels.

The genetic variability detected among Turkish cultivars could be of major importance for solving problems concerning the management of the Turkish olive germplasm. The overall findings were that enough genetic diversity could be detected, from the comparative study of molecular and morphological approaches, using RAPD markers combined with morphological criteria, to differentiate registered olive varieties. This may help to the registration of new varieties which could be of a great interest for

breeders. However, to construct a molecular data base that can be used to make a reference collection, more primers and preferably other markers such as microsatellites may be necessary and should be compared with the present assay.

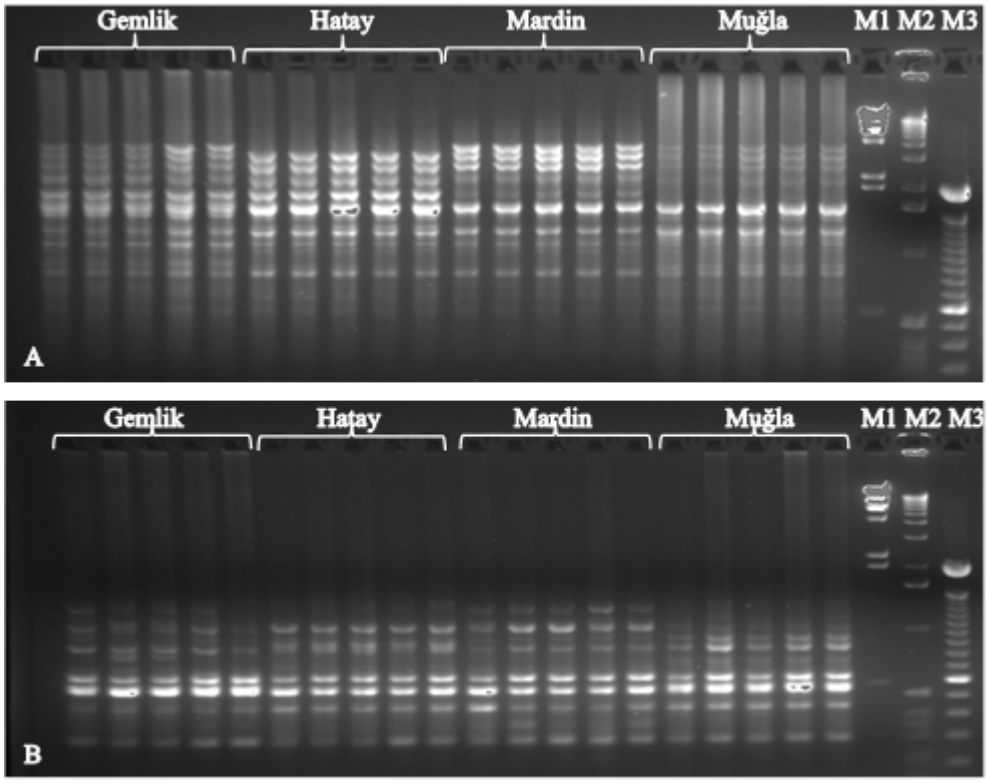

Figure 1. RAPD amplification products obtained in 1.5% agarose gel from eight Turkish olive cultivars (each cultivars have five different samples) with RAPD primer A. A01, B. OPAH-02 [M1, Lambda DNA/Hind III marker (vivantis); M2, 1kb ladder (vivantis), M3, 100bp ladder (vivantis)].

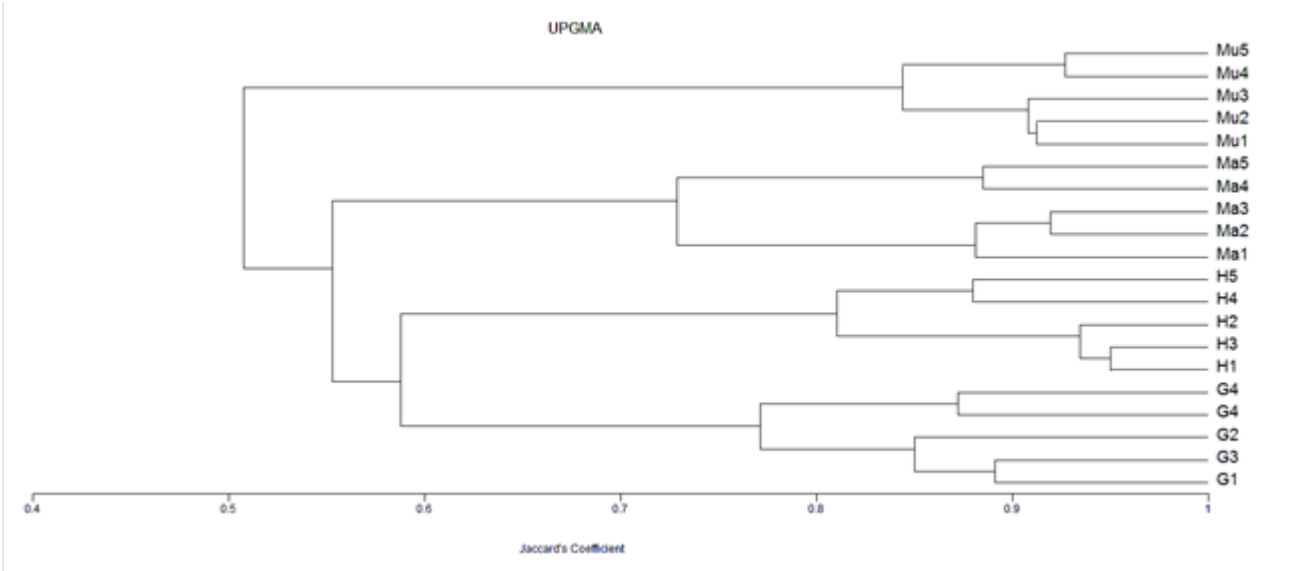

Figure 2. Dendogram of the Turkish four turkish olive cultivars (each cultivars have five different samples) (G 1-5, Bursa-Gemlik cv. "Gemlik"; H 1-5, Hatay cv. "Halhali" (wild type); Ma1-5, Mardin cv. "Halhali"; Mu1-5, Mugla cv. "Domat"), derived from UPGMA analysis.

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Abbreviations

CTAB: Cetyl Trimethyl Ammonium Bromide
PCR: Polymerase Chain Reaction
RAPD: Random Amplified Polymorphic DNA