Biochem Genet (2011) 49:555–561
DOI 10.1007/s10528-011-9430-z

SSR Marker-Based DNA Fingerprinting and Cultivar Identification of Olives (*Olea europaea*)

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Received: 9 March 2010 / Accepted: 3 January 2011 / Published online: 8 April 2011

Abstract Four well-known commercial olive cultivars (Domat, Edremit, Gemlik, and Memecik) and six local cultivars (Ziraat, Isrange, Tuz, Patos, Yag, and Marantelli) from northeastern Turkey were analyzed for genetic diversity and relationships using seven SSR primers (DCA-4, DCA-09, DCA-11, DCA-16, DCA-17, GAPU-89, UDO-14). The number of markers ranged from 3 (DCA-04 and DCA-17) to 6 (DCA-11, DCA-16, GAPU-89), with an average of 4.57 alleles per primer. UPGMA cluster analysis based on a simple matching similarity matrix grouped cultivars into two main clusters. Three pairs of cultivars (Ziraat and Gemlik, Isrange and Tuz, and Patos and Yag) were thought to be different cultivars although they produced identical SSR profiles. The results indicate the efficiency of SSR markers for evaluation of genetic diversity in olives and identification of misnamed individuals of the same genotype.

Keywords Olive · Molecular markers · SSR · Genetic diversity

Introduction

The olive tree is believed to be the first tree cultivated on Earth, and its production is one of the oldest agricultural activities, going back to the first civilization. The olive originated in southeastern Anatolia and eventually spread to other Mediterranean countries, to Asia, and to America. It has been an important part of all the civilizations established on the shores of the Mediterranean Sea (Mendilcioglu 2001).

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Olives have great commercial importance in Turkey. They are consumed as table olives and olive oil and used for soap production. Turkey is also a major exporter of olive oil. Olive growing in Turkey is well established mainly around the Aegean and Mediterranean regions, but also in the Marmara, southeastern Anatolia, and Black Sea regions (Ercisli 2004).

According to Food and Agriculture data from the United Nations, Mediterranean countries produce 90% of world olives, and the biggest olive producers are Spain, Italy, Greece, Turkey, Tunisia, Morocco, Syria, and Portugal (FAO 2008). Although Turkey ranks fourth in the production of fresh olives, it is the second greatest producer of table olives, after Spain (FAO 2008). The top three table olives in Turkey, in order of production, are the black, green, and kalamata types (Unal and Nergiz 2003). It has been estimated that Turkey has approximately 100 local olive cultivars (Aktan and Kalkan 1999).

Previously, olive cultivars were characterized by morphological traits, including tree, fruit, and leaf characteristics (Aydin and Yunculer 1983; Canozer 1991; Kaya and Tekintas 2006). Most morphological traits are influenced by environmental factors, plant age, and phenology. Since objectivity is crucial to accurate morphological typing, it is difficult to use such traits in plant identification and characterization of genetic relationships (Ercisli et al. 2008). Moreover, the simultaneous presence of local cultivars and those with a patchy distribution and ambiguous naming, continuous interchange of plant materials among the olive production regions and countries, long juvenile period, the presence of varietal clones, and problems of varietal certification in nurseries have complicated identification of olive cultivars (Sarri et al. 2006).

The availability of molecular tools able to detect genetic differences even at the clonal level permits a more rapid and reliable approach to these problems. DNA marker technologies based on polymerase chain reaction (PCR), such as simple sequence repeat (SSR), are now available for many crop species, including the olive (Sefc et al. 2000; Cipriani et al. 2002; Khadari et al. 2003; Belaj et al. 2003; Sarri et al. 2006), grape (Benjak et al. 2005), apricot (Pedryc et al. 2009), apple (Galli et al. 2005), quince (Dumanoglu et al. 2009), and others.

SSRs are PCR-based molecular markers valued for their abundant and uniform genome coverage, high levels of polymorphism, codominance, reproducibility, and ease of genotyping. SSRs are largely used for the characterization and differentiation of fruit species, in particular closely related accessions. SSRs require little DNA for the amplification. Every SSR locus is defined by a unique pair of primers; therefore, information exchange between laboratories is easy and allows individuals to be uniquely genotyped (Powell et al. 1996).

SSR markers have been developed for the olive by several groups (Sefc et al. 2000; Carriero et al. 2002; Cipriani et al. 2002), and this marker system was found to be the most reliable, effective, and easy to use for identification of olive cultivars (Sarri et al. 2006). The use of this method, however, to determine genetic relationships between old olive cultivars grown in Turkey is limited. Therefore, the objectives of this study were to use seven SSR primers to characterize a total of 10 olive cultivars grown in Turkey.

Materials and Methods

A total of 10 olive (*Olea europea* L.) cultivars were used for SSR analysis. Four (Domat, Edremit, Gemlik, and Memecik) are well-known commercial cultivars in Turkey. The remaining six cultivars (Ziraat, Isrange, Tuz, Patos, Yag, and Marantelli) are local cultivars grown in a microclimate in Trabzon province in the Black Sea region of Turkey; they were found together in Mersin village in Trabzon province. The commercial cultivars were found together in the Atatürk Central Horticultural Research Institute, Yalova, in Turkey.

Young leaves of olive trees were sampled for DNA extraction. Lyophilized leaf samples were ground to a fine powder using a mortar and pestle. DNA samples were extracted from 150 mg powdered leaf samples using a modified CTAB method described by Futterer et al. (1995). The concentrations of each DNA sample were measured using a Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA) and adjusted to 50 ng/ml for analysis.

Seven previously developed SSR primers were used for amplification (Table 1). Each 20 µl PCR mixture for amplification consisted of 0.75 U DNA polymerase (Fermentas, Hanover, MD, USA) with the reaction buffer supplied at 1× concentration, 0.4 uM each primer, dNTPs at 0.25 mM each, and 50 ng template DNA. Thermal cycling conditions were 2 min at 94°C; 10 cycles of 45 s at 94°C, 1 min at 65°C (annealing temperature was reduced 1°C after every cycle), and 1 min 30 s at 72°C; 35 cycles of 45 s at 94°C, 1 min at 55°C, and 1 min 30 s at 72°C; and a final extension step of 5 min at 72°C; using an Applied Biosystems Thermal Cycler. PCR products were separated on a 4% agarose SFR gel (Amresco Inc., Solon, OH, USA) in 0.5 M Tris–borate–EDTA. Gels were stained with ethidium bromide (0.5 mg/ml; Sigma, St. Louis, MO, USA) and photographed.

SSR markers were scored as present (1) or absent (0), since allelic constitution of these SSR markers was not known in the plant materials studied. Simple matching similarity coefficients (Sneath and Sokal 1973) were calculated for all pairwise comparisons among 10 olive cultivars (Table 2). A dendrogram demonstrating the relative genetic relationships was generated using NTSYSpc version 2.11V (Exeter Software, Setauket, NY; Rohlf 2004), based on the unweighted pair-group method of arithmetic mean (UPGMA) cluster analysis (Fig. 1).

Table 1 SSR primers and markers used in the DNA fingerprinting of Turkish olive accessions

SSR primer pair	SSR markers
DCA-04[a]	3
DCA-09	4
DCA-11	6
DCA-16	6
DCA-17	3
GAPU-89[b]	6
UDO-14[c]	4
Total	32

[a] Sefc et al. (2000)

[b] Carriero et al. (2002)

[c] Cipriani et al. (2002)

Table 2 Simple matching similarity matrix of 10 olive cultivars calculated from 32 SSR markers

Cultivars	Domat	Edremit	Ziraat	Patos	Isrange	Marantelli	Tuz	Memecik	Yag	Gemlik
Domat	1.00									
Edremit	0.66	1.00								
Ziraat	0.50	0.53	1.00							
Patos	0.55	0.62	0.79	1.00						
Isrange	0.56	0.47	0.81	0.72	1.00					
Marantelli	0.59	0.50	0.47	0.62	0.66	1.00				
Tuz	0.56	0.47	0.81	0.72	1.00	0.66	1.00			
Memecik	0.50	0.47	0.63	0.66	0.56	0.66	0.56	1.00		
Yag	0.56	0.59	0.81	1.00	0.75	0.59	0.75	0.63	1.00	
Gemlik	0.50	0.53	1.00	0.79	0.81	0.47	0.81	0.63	0.81	1.00

Results and Discussion

The seven SSR primers identified 32 polymorphic alleles in the 10 olive cultivars (Table 1). The number of polymorphic alleles ranged from 3 (DCA-04 and DCA-17) to 6 (DCA-11, DCA-16, GAPU-89), with an average of 4.6 fragments per primer, indicating that the primers DCA-11, DCA-16, and GAPU-89 were the most effective for discriminating among the cultivars analyzed here. The high level of polymorphism in olive cultivars revealed by SSR markers was evident (Carriero et al. 2002; Sarri et al. 2006; Muzzalupo et al. 2006; Gomes et al. 2009; Noormohammadi et al. 2009). We obtained a higher average number of polymorphic alleles (4.6) than Noormohammadi et al. (2009) and Cipriani et al. (2002). Our results are also comparable to Carriero et al. (2002), Belaj et al. (2003), Sarri et al. (2006), and Muzzalupo et al. (2006). This high level of polymorphism in olives could be important for future breeding efforts in Turkey. Our results also highlighted the genetic diversity of olive cultivars grown in Turkey. It seems that the seven SSR primer pairs used in this study had high discriminating capacity for the 10 olive cultivars. SSR markers have been previously used in genetic diversity and relationship studies in olive cultivars, and most scientists conclude that SSR markers are a powerful tool for cultivar identification and analysis of genetic structure (Khadari et al. 2003; Belaj et al. 2003; Gomes et al. 2009). Variations reported in the number of alleles in olive cultivars by different scientists may be related to variation in the loci studied as well as the number of genotypes and their localities (Lopes et al. 2004)

The dendrogram derived from UPGMA cluster analysis of the 32 SSR markers showed two main, distinct groups (Fig. 1). Group I consisted of the important commercial cultivars Domat and Edremit. Group II was divided into two subgroups. In group II, the local cultivar Marantelli and the well-known standard cultivar Memecik clustered together in subgroup 1, suggesting that grouping genotypes by geographic origin is not useful in the olive. Besnard et al. (2001) found that olive genotypes from different countries clustered together within a group, and they did not find any grouping based on geographic origin to be useful in the case of olives.

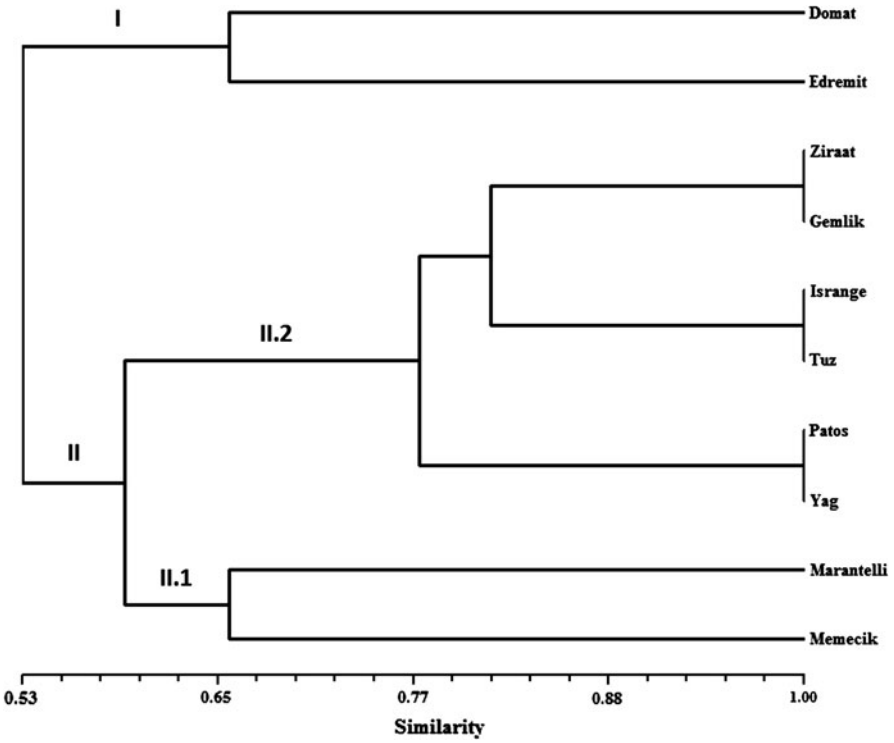

Fig. 1 The relative similarity of 10 olive cultivars from Turkey. UPGMA dendrogram based on simple matching similarity matrix (Table 2) obtained using 32 SSR markers

This result indicates that olive genotypes have been freely exchanged among collectors in different regions. Subgroup 2 within group II included cultivars Ziraat, Gemlik, Isrange, Tuz, Patos, and Yag. Three pairs of these cultivars (Ziraat and Gemlik, Isrange and Tuz, and Patos and Yag) shared identical SSR banding profiles. Gemlik is a well-known table olive not only in Turkey but also worldwide; it has been misnamed "Ziraat" in Trabzon. Olive cultivars have been recently introduced to Trabzon province (a new locality) mainly from western Turkey, where the cultivar Gemlik is largely grown. In Trabzon province, the Isrange and Patos cultivars are also misnamed or renamed "Tuz" and "Yag," respectively. In Turkish, "tuz" means salt and "yag" means oil; therefore, "Tuz" and "Yag" may not be the correct or original names for the Isrange and Patos cultivars.

The use of synonymous names or mislabeling is one of the most important problems in Turkish olive germplasm. It has been reported that synonymous naming is also common in the olive germplasm in other olive-growing Mediterranean countries. For example, identification of synonymous cases in Italian and Portuguese olive germplasm has been reported using SSR and other molecular markers (Muzzalupo et al. 2006; Gomes et al. 2009).

In conclusion, SSR analysis was found to be useful for detection of genetic differences among olive accessions from Turkey. The outcome of this study could

be useful for a varietal survey and the construction of a database of olive cultivars in Turkey.

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