Advanced Journal of Plant Biology
Volume 1(1), pages 1-6, April 2020
Article Number: B79E855E1
ISSN: 2992-4928
https://doi.org/10.31248/AJPB2020.005
https://integrityresjournals.org/journal/AJPB

Full Length Research

Genetic characterization of olive (*Olea europaea* L.) genotypes from Adana and Osmaniye provinces by microsatellite markers

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Received 17th February, 2020; Accepted 30th March, 2020

ABSTRACT: Studies on the determination and domestication of the motherland of olive have shown that there are important gene centres (Adana and Osmaniye) in the Eastern Mediterranean Basin. In the study, 41 olive genotypes collected from Adana and Osmaniye provinces. Twenty-three domestic, eight local and five foreign reference olive cultivars were obtained from the olive germplasm within the Çukurova and Harran Universities. A total of 77 fresh olive leaves were included in the SSR analysis. At the genetic level allele profiles and genetic affinity, levels were determined by nine SSR markers. The total allele number was 101, while the lowest allele number per locus was three (UDO9) and the highest allele number was 31 allele/locus (DCA9). The average allele is 11.22 per locus. The mean expected (he) and observed heterozygosity (Ho) ratios were 0.512 and 0.675 respectively. The high genetic similarity was evident between Eşek zeytini and Ayvalık yağlik (0.78) genotypes, while the most genetically divergent genotypes were Melkabazi and Mavi (0.06) and Melkabazi and Eğriburun (0.06).

Keywords: Allele, genetic, genotypes, Mesopotamia, olive, SSR markers, Turkey.

INTRODUCTION

Olive is economically growing in 29 provinces of Turkey, accounting for 2.4% of total agricultural areas and about 23.5% of vineyards and horticultural areas. While 75.0% of olive growing on a hillside and inclined lands, 45.0% of the remaining production areas on irrigated lands. Olive production regions are; Marmara, Gediz, Körfez, South Aegean, coastal and Eastern Mediterranean, and eastern Black sea. In the production areas, 66.9% is the traditional, 31.4% is conventional and 1.7% is the dense planting area. 30.0% of the production areas can be irrigated and 70.0% of the dry conditions in the plots. There are 1,187 olive oil mills, 14 pomaces and 15 refinery plants in Turkey. 68.0% of the olive oil produced is natural olive oil and 32.0% is refining olive oil (Özaltaş et al., 2016). Gemlik, Ayvalık Yağlik, Memecik, Domat, Kilis Yaglik, Nizip Yaglik, Sarı Ulak and Halhalı are common olive cultivars mainly grown in Turkey for oil and table olive. Adana and Osmaniye provinces are important areas on the olive gene origin zone, although they are not at the forefront in terms of olive production in Turkey. Besides local cultivars, it is valuable in terms of hosting wild olive populations. The common problem encountered in all of the studies aimed at defining olive varieties and species is the variation of the characters, which has examined depending on the ecological conditions, and agricultural practices to define the varieties/species of the data obtained. DNA-based analyses are using to solve this problem. Among the current molecular marker techniques, the SSR technique, called simple sequence repeats or microsatellites, is a marker system that produces reliable results. SSR technique is a codominant marker system and is widely used in plant molecular breeding studies because of its high information content and reproducibility (Noormohammadi et al., 2014; Abdessemed et al., 2015;

Table 1. List of 36 olive cultivars and 41 selected genotypes analyzed in this study.

No.	Genotype code / Cultivars	Origin/Location	Utilization
1	ADA-100 Arbequina *FRC	Reus-SP	Oil
2	ADA-101 Ayvalık Yağlık *DRC	Ayvalık	Oil-Table
3	ADA-102	Adana	Table
4	ADA-103	Adana	Table
5	ADA-104	Adana	Table
6	ADA-105	Adana	Table
7	ADA-106 Sarı ULAK *DRC	Tarsus	Table
8	ADA-107	Adana	Table
9	ADA-108	İmamoğlu-Adana	Table-Oil
10	ADA-109	Ceyhan-Adana	Table
11	ADA-110	Ceyhan-Adana	Table-Oil
12	ADA-111	İmamoğlu-Adana	Oil-Table
13	ADA-112	Kozan-Adana	Table-Oil
14	ADA-113	Çukurören-Kozan	Oil-Table
15	ADA-114	Çukurören-Kozan	Oil-Table
16	ADA-115	Çukurören-Kozan	Oil-Table
17	ADA-116	Çukurören-Kozan	Oil-Table
18	ADA-117	Kozan	Oil-Table
19	ADA-118	Kozan	Oil-Table
20	ADA-119	Kozan	Oil-Table
21	ADA-120	Kozan	Oil-Table
22	ADA-121	Akkaya-Feke	Oil-Table
23	ADA-122	İslam-Adana	Oil-Table
24	ADA-123	İslam-Adana	Oil-Table
25	ADA-124	İslam-Adana	Oil-Table
26	ADA-125	Varsaklar-Kozan	Oil-Table
27	ADA-126	Adana	Oil-Table
28	ADA-127	KüçükdikiliAdana	Oil-Table
29	ADA-128	Küçükdikili-Adana	Oil-Table
30	ADA-129	Şambayat-Adana	Oil-Table
31	ADA-130	Şambayat-Adana	Table
32	ADA-131 Leccino *FRC	Toscana-IT	Oil
33	ADA-132	Sarıhuğlar-Adana	Table
34	ADA-133	Adana	Table
35	ADA-134	Küçük Dikili-Adana	Table
36	ADA-135	Küçük Dikili-Adana	Table
37	ADA-136	Zeytinli-Adana	Table
38	ADA-137 Mavi *DRC	Derik	Table
39	ADA-138 Belluti *DRC	Derik	Oil
40	ADA-139 Küçük Topak Ulak *DRC	Adana	Oil-Table
41	ADA-140 Kiraz *DRC	Kiraz	Table
42	ADA-141 Girit zeytini *DRC	Muğla	Oil
43	ADA-142 Dilmit *DRC	Muğla	Table-Oil
44	ADA- 143 Edremit Yağlık *DRC	Edremit	Oil
45	ADA-144 Çilli *DRC	İzmir	Table
46	ADA-145 Erkence *DRC	Karaburun	Table
47	ADA-146 Eşek zeytin *DRC	Ödemiş	Table
48	ADA-147 İri Yuvarlak *DRC	Adana	Table
49	ADA-148 Sayfi *DRC	Hatay	Table
50	ADA-149 Sarı Haşebi *DRC	Hatay	Table-Oil
51	ADA-150 Melkabazi *DRC	Mardin	Table-Oil

Table 1. Contd.

No.	Genotype code / Cultivars	Origin/Location	Utilization
52	ADA-151 Tavşan Yüreği *DRC	Antalya	Table
53	ADA-152 Elmacık *DRC	Hatay	Table
54	ADA-153 Adana Topağı *DRC	Adana	Table
55	ADA154	Adana	Table
56	ADA- 155 Memecik *DRC	Muğla	Oil
57	ADA-156	Kılbaş-Karaisalı	Table-Oil
58	ADA-157	Aflak-Karaisalı	Table-Oil
59	ADA-158	Sadıkali-Adana	Table-Oil
60	OSM-200	Bahçe-Osmaniye	Table-Oil
61	OSM-201	Bahçe-Osmaniye	Table-Oil
62	OSM-202	Bahçe-Osmaniye	Table-Oil
63	OSM-203	Kırmacalı-Osmaniye	Table-Oil
64	OSM-204	Kırmacalı-Osmaniye	Table-Oil
65	OSM-205	Kırmcalı-Osmaniye	Table-Oil
66	OSM-206	Sumbas-Osmaniye	Table-Oil
67	OSM-207	Kadirli-Osmaniye	Table-Oil
68	OSM-208	Kesmeburun-Osmaniye	Table-Oil
69	OSM-209	Kastabala-Osmaniye	Table-Oil
70	Domat *DRC	Akhisar	Table
71	Gemlik *DRC	Gemlik	Table-Oil
72	Gordal *FRC	Seville-SP	Table
73	Hojiblanca*FRC	Cordoba/Malaga-SP	Oil
74	Manzanilla *FRC	Seville-SP	Table
75	Saurani *DRC	Hatay	Table-Oil
76	Silifke yağlık *DRC	Silifke	Oil
77	Eğriburun*DRC	Gaziantep	Table-Oil

TR – Turkey, SP – Spain, IT – Italy, GR – Greece, *DRC – Domestic reference cultivar, *FRC – Foreign reference cultivar.

Ipek et al., 2012; Hatzopoulos et al., 2002; Ercişli et al., 2011; Sakar et al., 2016; Rallo et al., 2000, de la Rosa et al., 2003; Belaj et al., 2003; Mantia et al., 2005; Montemurro et al., 2005). In this study, the genetic affinitylevels of important olive genotypes found in Adana and Osmaniye provinces with reference olive varieties were determined by 9 SSR markers.

MATERIAL AND METHODS

Plant material

In the study, 41 olive genotypes were collected from Adana and Osmaniye provinces. Thirty-six samples were obtained from the Olive Germplasm Bank of the Çukurova and Harran Universities in Turkey. A total of 77 fresh leaves material used in the SSR analysis (Table 1).

DNA extraction

Fresh leaves were used for the Genomic DNA extraction as described by Saghai-Maroof et al. (1984) using the Wizard ® Genomic DNA purification kit (Promega, Madison, WI). Quantity and purity of the DNA checked on 1% (w/v) agarose gel and by NanoDrop®ND-1000 spectrophotometer.

Microsatellite Markers

Ten microsatellite markers were used in the study. Four markers (UDO-9, UDO-12, UDO-24, and UDO-26) were from the primer set design (Cipriani et al., 2002). Six (DCA-15, UDO-4, DCA-18, DCA-9, UDO-11, and DCA13) were from Mantia et al. (2005). On the other hand, SSR markers (DCA13), which cannot be obtained from all types of PCR products have been excluded from the evaluation.

PCR applications

PCR replications were performed at 10 µl volume, 15 ng DNA, 5 pmol each primary, 0.5 mm dNTP, 0.5 unit Go Taq

Table 2. Simple sequence repeats (SSRs), number of alleles, observed heterozygosity (Ho) and expected heterozygosity (He), information content of the polymorphism, allele size range(bp) of 9 SSR markers on 77 olive genotypes investigated.

SSR primers	Number of alleles (*n*)	Expected heterozygosity (*He*)	Observed heterozygosity (*Ho*)	PIC	Allele size range (*bp*)
UDO-9	3	0.298	0.443	0.395	96-114
UDO-12	4	0.376	0.647	0.586	153-165
UDO-24	7	0.506	0.706	0.655	166-196
UDO-26	8	0.337	0.693	0.652	96-112
DCA-15	12	0.597	0.592	0.535	240-284
UDO-11	7	0.571	0.719	0.672	112-124
UDO-4	7	0.350	0.469	0.416	137-153
DCA-18	22	0.740	0.876	0.866	140-204
DCA-9	31	0.831	0.932	0.929	162-214
Mean	11.22	0.512	0.675	0.634	96-284
Total	101	4.606	6.077	5.706	

DNA polymerase (1.5 mM MgCl2 containing) (Promega). Conditions for the primers were 35 cycles of 94°C for 3 minutes, 94°C for 1 minute; 55-60°C for 1 minute then 72°C for 2 minutes; the last cycle performed in 10 minutes at 72°C.

Fragment analysis

Fragment analysis of studied lockers was performed on Fragment Analyser (Qiagen). After the analysis, the allele sizes of the system were determined by using a fragment analysis program. It has viewed as heterozygote and homozygote considering the types of pics belonging to each locus. The reactions have been repeated at least twice to ensure the accuracy of the data.

Genetic analysis

The genetic analysis program “IDENTITY” was used for the determination of allele number (n), the allele frequency, the expected heterozygosity (he), observed heterozygosity (ho), the frequency (R) and the probability of identification (PI), similar genotypes and parents for each locus. Pic values were calculated according to Liu and Muse (2005) by using Power Marker v3.025. The forward (forward) primers have labelled fluorescently for fragment analysis of the primers. The data was converted to similarity values in Microsoft Excel. A dendrogram was created by MEGA6 (Tamura et al., 2006) and Power Marker software program V3.025.

RESULT AND DISCUSSION

The total alleles were determined as 101 alleles/primers in the 9 loci analyzed in the selected genotypes, the allele numbers changed between 3 allele/primers (UDO9) and 31 allele/primers (DCA9) and gave an average value of 11.22. The highest allele was DCA9 (31) and the lowest allele was UDO9 (Besnard et al., 2013). Considering the information content of the polymorphism (PIC); the most informative locus in olives was DCA9 with 31 alleles (PIC: 0.929) (Table 2). The dendrogram was generated based on the similarity index (genetic distance) with the obtained data of SSR analysis (Figure 1). The high genetic similarity was evident between Eşek Zeytini and Ayvalık yağlik (0.78) genotypes, while the most genetically divergent genotypes were Melkabazi and Mavi (0.06), Melkabazi and Eğriburun (0.06). In the second main group, the genotypes of ADA-113 (Çukurören-Kozan), ADA-119 (Kozan Castle-Kozan) and OSM-202 (Kırmacılı – Osmaniye) in the lower groups were found to be in the same cluster. The fact that OSM-202 genotype cultivars varieties in Osmaniye conditions are in the same cluster as the ADA-113 and ADA-119 holes in Kozan district suggests that OSM-202 genotype is in feral form considering its pomological and morphological characteristics. High genetic affinities have been identified between reference cultivars of wild olive trees and ancient olive cultivars. It shows that feral olive genotypes and old olive cultivars have a high genetic affinity with current olive cultivars (Barazani et al., 2016).

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

The author's thanks to Assoc. Prof. Özhan Şimşek at University of Erciyes and Dr. Dicle Dönmez at the University of Cukurova for their help in analyzing parts of the data.

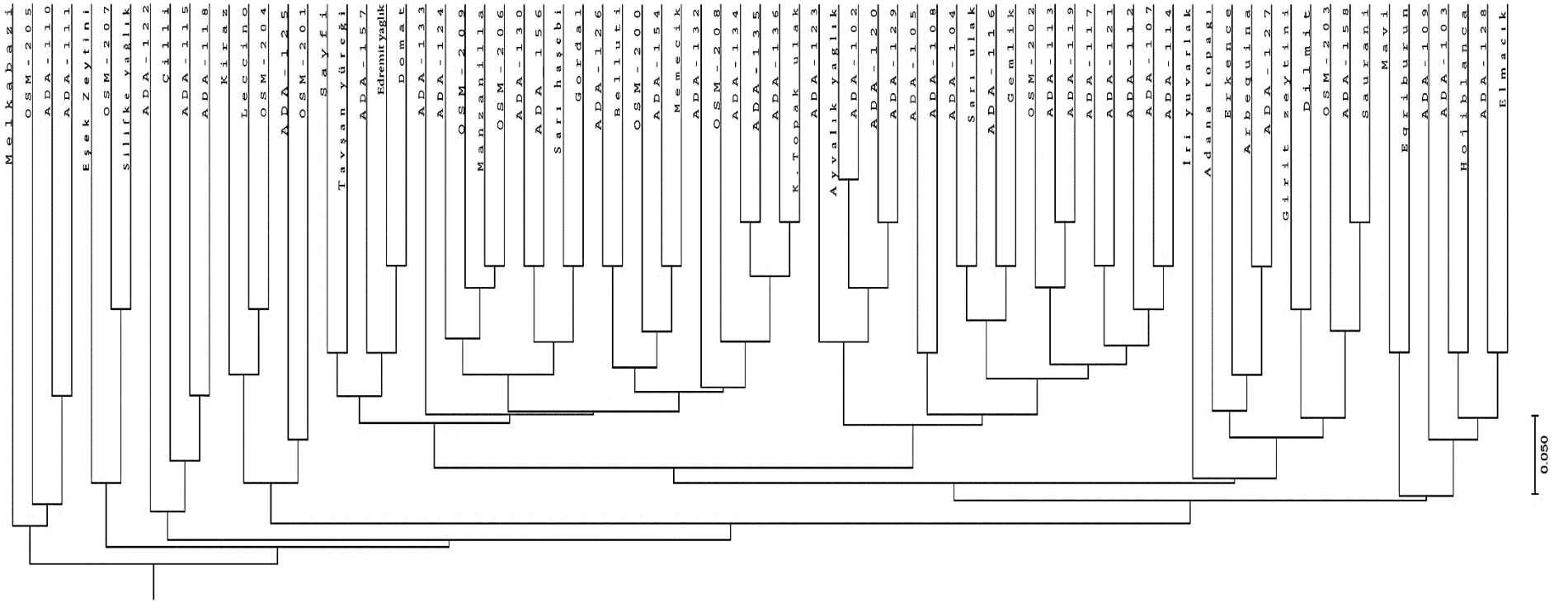

Figure 1. The PGMA dendrogram based on simple matching uniqueness matrix obtained using 9 SSR markers, illustrating the relative similarity among 77 olive genotypes.

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