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Genetic diversity of some Palestinian and Turkish olive (Olea europaea L.) germplasm determined with SSR markers

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Abstract

Olive (Olea europaea L.) is one of the most important crops in the Mediterranean region including Palestine and Turkey. In this study, the genetic diversity and population structure of 19 olive cultivars (15 from Palestine and 4 from Turkey) was assessed using simple sequence repeat (SSR) markers. The 14 SSR markers yielded 110 alleles with 91% polymorphism and 7.8 alleles per marker. Genetic dissimilarity ranged from 0.00 to 0.51 with an average of 0.35. Cluster analysis using the Dice coefficient and the neighbor joining algorithm showed that the 19 cultivars grouped into three clusters, with all of the Turkish cultivars in one subcluster. Nabali Baldi from the West Bank and Souri from the Gaza Strip were identical and therefore, synonyms. The highest dissimilarity was between the Turkish Mut and Spanish Arbequina cultivars. In contrast to our expectation, two of the cultivars of supposed Palestinian origin clustered with an Italian cultivar suggesting that it may have originated there. Population structure analysis that assigned the 19 cultivars to two subpopulations.

Keywords:

Olea europaea L.,
Genetic diversity,
Population structure,
Microsatellite,
Molecular

1. Introduction:

Olive (*Olea europaea* L.) is perhaps the best known Mediterranean crop and has significant economic value in this region (Hagidimitriou et al., 2005). The history of olive cultivation goes back to the third millennium B.C. when it was first cultivated in the eastern Mediterranean region (Gomes et al., 2012). Olive was initially grown in Palestine, Lebanon, Syria and southern Turkey and then spread to Greece, Egypt and Western Turkey as evidenced by various archeological sites. Olive cultivation then expanded to include Sicily, Sardinia, Italy, France, Spain, Portugal, Algeria, Tunisia, and Morocco (Vossen, 2007). As many as 1250 different cultivars have been used globally to produce olives in 54 countries and this material has been conserved in over 100 collections (Bartolini, 2008). There are 805 million olive trees worldwide; however, more than 90% of olive cultivation and production still take place in Mediterranean countries (Gomes et al., 2012). Olives are grown on 10.3 million hectares worldwide with yearly production of about 15.4 million tons of olives and 3.05 million tons of virgin olive oil (FAOSTAT, 2014).

Cultivation of olive as a crop plant in Palestine started 5000 years ago (Zohary & Spiegel-Roy, 1975). Historically, there is not much information about olive cultivation in Turkey; however, archeological evidence shows that the first olive cultivars were brought to Anatolia before 1,000 B.C (Owen et al., 2005). Olive has considerable value to the Turkish national economy as it is considered a main product of the agricultural sector (Kaya et al., 2010). Eighty seven local olive cultivars are documented in Turkey (Ipek et al., 2012). Turkey

produces 1.77 million tons of olives and 73,915 tons of olive oil yearly from trees grown on 826.092 hectares. Thus, Turkey ranks fourth in global olive production after Spain, Italy and Greece (FAOSTAT, 2014). Olives are also one of the most powerful and major components of the Palestinian economy which is, to a great extent, based on agriculture. Olives represent up to 19% of the country's agricultural output and about 25% of its Gross Domestic Product (GDP) (World Bank, 2013). Olive trees comprise about 67% of the 13.3 million horticultural trees in the Palestinian Territories with 88% in the West Bank and 12% in the Gaza Strip (PCBS, 2011). Olive oil is the main end product of about 95% of the olive harvest in the Palestinian Territories and the rest of the crop is used for pickling and table olives (World Bank, 2013). In 2014, the Palestinian territories produced a total of 93,146 tons of olives and 24,759 tons of olive oil (FAOSTAT, 2014) .

The study of genetic resources is important for determining their diversity, relatedness and geographical distribution. In addition, such work allows cultivar discrimination which is important for identifying synonyms and ensuring the quality of both olives and their oil (Hagidimitriou et al., 2005). Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR) and Sequence Characterized Amplified Region (SCAR) are among the PCR-based methods used for olive diversity evaluation and germplasm characterization. However, SSR markers are the most popular marker to study genetic diversity in plants as they are codominant, transferable markers that are very reproducible especially when used with high resolution PCR fragment analysis as reviewed by Gomes et al. (2012).

The objective of this study was to use SSR markers to analyze the genetic diversity of 19 olive cultivars, of which 15 were collected in Palestine. The Palestinian material included five cultivars that originated in Palestine, three each from Italy and Tunisia, two from Greece and one each from Spain and Syria. Four Turkish cultivars were included for comparison because we supposed that the Turkish and the Palestinian cultivars had a common origin in the Mediterranean region, these two regions are a part of olive origin that extend from Palestine to southern Turkey as reported by vossen (2007).

1. Material and Methods:

Olive leaf collection

The first group (cultivars 1 to 15) of olive leaf samples was supplied by the Palestine Ministry of Agriculture (Table 1). Cultivars 1 to 12 were collected from the National Agricultural Research Centre, Qabatya, Jenin, West Bank, Palestine. Samples 13 to 15 were collected from Gaza Strip, Palestine. The Turkish samples (cultivars 16 to 19) were supplied by the Aegean Agricultural Research Institute (AARI, Izmir, Turkey).

DNA Extraction

Leaves were ground in liquid nitrogen and 25 mg olive leaf powder was used for DNA extraction according to Doyle & Doyle (1990). DNA integrity was assessed by electrophesis using 1% agarose gel stained with ethidium bromide. DNA was quantified by spectrophotometer (Thermo Scientific, Multiskan GO).

SSR marker genotyping

The genotyping for olive cultivars was performed using 14 nuclear SSR markers which were selected from previously published work (Table 2). Polymerase chain reactions (PCRs) were conducted in a final volume of 20 µl containing 30 ng DNA, 1× PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1 pmol forward and reverse primers, 1 U Taq Polymerase. PCR conditions were 94˚ C for 3 min, 36 cycles of 94˚C for 30 sec, 45 sec at annealing temperature (Table 2) and 72˚C for 45 sec, the final extension was

at 72˚C for 5 min. PCR reactions were performed in a Bio-Rad C1000 Thermal Cycler. PCR products were separated using capillary electrophoresis instrument (Fragment Analyzer Automated CE System, Advanced Analytical) according to manufacturer's instructions. SSR fragments were visualized using the PROSize 2.0 software version 1.2.1.1 (Advanced Analytical).

Data analysis

Genotypes was scored for presence (1) or absence (0) of each amplified SSR allel. Gene diversity was calculated depending on the frequency of the allele for each SSR marker and the calculations were performed by GDdom online (http://plantmolgen.iyte.edu.tr/GDdom/) computer program (Abuzayed et al., 2016) using the formula of Roldan et al. (2000):

$GD_i = 2f_i (1 - f_i)$.

Where GD_i is the gene diversity of marker 'i', f_i is the frequency of the amplified allele (band presence), and $1 - f_i$ is the frequency of the null allele. The Dice coefficient was used to produce a dissimilarity matrix (Dice, 1945) as implemented by Darwin 5 computer software (http://darwin.cirad.fr/product.php). The distance data were used to construct a dendrogram using unweighted neighbor-joining method. A Mantel statistic test was used to determine the fit of the dissimilarity matrix and dendrogram. Principal coordinate analysis (PCoA) was conducted using genetic distance pairwise matrix with Darwin 5 computer software. Marker data were used to infer population structure of the 19 olive cultivars with the Structure computer program (Pritchard et al., 2000), models with 1 to 10 subpopulations (K) were tested for 20 iterations. Burn-in period was 10,000 and number of Monte Carlo Markov Chain repeats was 100,000. Structure Harvester computer program (Earl & vonHoldt, 2012) was used to calculate for each model based on posterior probabilities. The model with the highest ΔK was selected as the best. Inferred ancestry threshold was set as ≥ 0.70 to assign the accessions to subpopulations.

Table 1 *Olive cultivars included in this study and their countries of origin*

No.	Cultivar name	Origin	Collection location
1	Nabali Baladi	Palestine	West Bank
2	Nabali Muhasan	Palestine	West Bank
3	Nasouhi Jabaa 1	Palestine	West Bank
4	Nasouhi Jabaa 2	Palestine	West Bank
5	Sant Augustine	Italy	West Bank
6	Chemlali	Tunisia	West Bank
7	Telmesani	Tunisia	West Bank
8	Yonani	Greece	West Bank
9	Barouni	Tunisia	West Bank
10	Cucca	Italy	West Bank
11	Frantoio	Italy	West Bank
12	Arbequina	Spain	West Bank
13	Kalamata	Greece	Gaza
14	Zaity	Syria	Gaza
15	Souri	Palestine	Gaza
16	Hamza Celebi	Turkey	Turkey
17	Mut	Turkey	Turkey
18	Mars	Turkey	Turkey
19	Karayaglik	Turkey	Turkey

Table 2 *SSR primers and repeat motifs used to characterize olive cultivars*

Locus	Repeat motif	Annealing temp. (°C)	Primer reference
DCA4	$(GA)_{16}$	60	(Sefc et al., 2000)
DCA07	$(AG)_{19}$	60	(Sefc et al., 2000)
DCA15	$(CA)_3G(AC)_{14}$	56	(Sefc et al., 2000)
DCA18	$(CA)_4CT(CA)_3(GA)_{19}$	55	(Sefc et al., 2000)
EMO13	$(CT)_4(CA)_8$	60	(De La Rosa et al., 2002)
EMO90	$(CA)_{10}$	60	(De La Rosa et al., 2002)
GAPU59	$(CT)_9$	55	(Carriero et al., 2002)
GAPU71B	$GA(AG)_6(AAG)_8$	60	(Carriero et al., 2002)

GAPU101	$(GA)_8(G)_3(AG)_3$	60	(Carriero et al., 200
UDO43	$(GT)_{12}$	60	(Cipriani et al., 200
UDO39	$(AT)_5(GT)_{11}$	56	(Cipriani et al., 200
UDO12	$(GT)_{10}$	56	(Cipriani et al., 200
UDO09	$(AG)_{16}$	56	(Cipriani et al., 200
UDO24	$(CA)_{11}(TA)_2(CA)_4$	56	(Cipriani et al., 200

2. Result and Discussion:

Study of the genetic diversity of olive is important for genotype characterization, breeding and germplasm conservation. There is very little information about the genetic diversity of Palestine olives. Basheer-Salimia et al. (2009), studied the genetic diversity among five Palestinian olive cultivars using SNPs. Obaida et al. (2014) studied three Palestinian cultivars using five SSR markers (17 alleles). Jaber (2013) used six SSR markers (23 alleles) to study genetic diversity within 92 individuals of the ancient olive cultivar Roumi and three common cultivars in Palestine. In contrast, there are many studies about the genetic diversity of Turkish olives (Ipek et al., 2009; Kaya et al., 2010; Seker et al., 2010; Isik et al., 2011; Ercisli et al., 2012; Ipek et al., 2012; Kaya et al., 2013a, b; Akcay et al., 2014; Yoruk & Taskin, 2014; Kaya, 2015).

In this study, 14 SSR published primer pairs were employed to investigate the genetic variation among 15 (five originally Palestinian cultivars and 10 from other countries but commonly grown in Palestine) and four Turkish olive cultivars (Table 1). A total of 110 alleles with 91% polymorphism were detected with individual markers providing from 5 (DCA15, EMO13 and EMO90) to 16 alleles (UDO43). Thus, an average of 7.8 alleles per primer were obtained with an average GD value of 0.36 (Table 3). GD values for individual SSR markers ranged from a minimum of 0.25 to a maximum of 0.46, indicating moderate discrimination power.

Table 3 *Gene diversity (GD) values for the simple sequence repeat (SSR) loci*

SSR marker	Gene diversity* (GD)	Polymorphic/total alleles	Polymor-phism %
UDCA4	0.26 ± 0.08	4/6	66.7
DCA07	0.46 ± 0.01	8/8	100
DCA15	0.34 ± 0.05	5/5	100
DCA18	0.37 ± 0.06	7/7	100
EMO13	0.25 ± 0.09	3/5	60
EMO90	0.32 ± 0.04	5/5	100
GAPU59	0.39 ± 0.06	6/7	85.7
GAPU71B	0.39 ± 0.03	9/9	100
GAPU101	0.42 ± 0.03	8/8	100
UDO43	0.35 ± 0.04	15/16	93.8
UDO39	0.46 ± 0.01	7/7	100
UDO12	0.35 ± 0.06	7/8	87.5
UDO09	0.30 ± 0.05	9/11	81.8
UDO24	0.40 ± 0.03	8/8	100
Average	0.36		91.1

* For each marker, average gene diversity ± standard error is presented

A dendrogram was constructed using the neighbor joining method. The 19 cultivars grouped in three clusters (A, B and C) (Figure 1). Based on a Mantel test, the correlation between the dendrogram and distance matrix was r = 0.97 indicating a very good fit between the tree and distance data. Genetic dissimilarity ranged from 0.00 to 0.51 with an average of 0.35. The highest dissimilarity was between Arbequina from Spain and Mut from Turkey.

Cluster A had two subclusters, one of these subclusters had all the Turkish olive cultivars as one group. The dissimilarity between the Turkish cultivars ranged between 0.21 and 0.34 with an average of 0.27. The lowest dissimilarity was between Mut and Hamza Celebi and the highest was between Mars and Mut. Two-dimensional PCoA clearly showed that Turkish olives cultivars were distinct from the other clusters and located in one group (Figure 2). The Greek cultivars that are

grown in Palestine, Kalamata and Yonani, grouped together in cluster A with a dissimilarity value of 0.02. Also in cluster A, Souri from Gaza and Nabali Baldi from the West Bank clustered with a dissimilarity of 0 indicating that these two cultivars are synonyms. This finding agrees with the fact that sometimes Souri is called Nabali Baladi by farmers (Assaf, 1994; Ayoub et al., 2009). Vossen (2007) reported that it's common in olives to see same cultivars have different names or in some cases one name for different cultivars and this confusion can be sorted out using DNA identification.

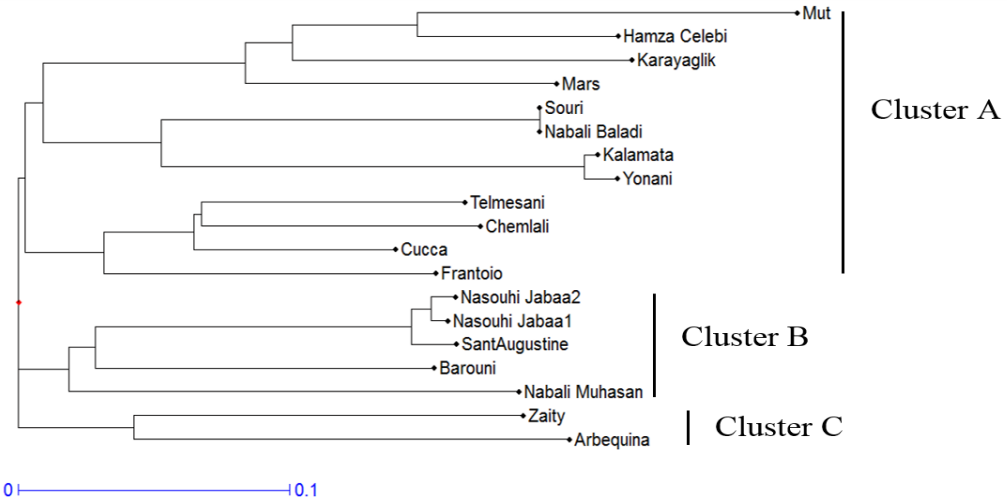

Figure 1 *Unweighted neighbor-joining dendrogram of 19 olive cultivars based on 110 simple sequence repeat (SSR) alleles.*

In cluster B, Palestinian cultivars Nasouhi Jabaa 1 and Nasouhi Jabaa 2 were grouped together but not with the other Palestinian cultivars (Figure 1). Instead, they were most closely related to the Italian cultivar Sant Augustine. Al-Taher (2002) reported that the Nasouhi Jabaa cultivars were not Palestinian and hypothesized that they came to the holy land with monks from Italy hundreds of years ago. Two-dimensional PCoA also supported this hypothesis as Nasouhi Jabaa 1, Nasouhi Jabaa 2 and Sant Augustine fell into one group (Figure 2). Nabali Muhasan ("Muhasan" means "improved" in English) is planted in Palestine and Jordan and was also located in cluster B. This cultivar takes its name from Nabali Baladi and showed high dissimilarity (0.45) with Nabali Baladi which clustered in A, indicating that they are not genetically similar. Thus, the name is misleading. According to Jaber (2013), Nabali Baladi and Nabali Muhasan clustered in two separated group with the same amount of dissimilarity found in our work (0.45). Assaf (1994) also reported that there is no scientific reason to justify its name as it is reported to have worse performance than Nabali Baladi. According to Hassawi & Hdeib (2004), Nabali Muhasan had 0.76 similarity with the Nabali Baladi and did not fall in the same cluster when six RAPD primers were used in diversity analysis of 44 olive cultivars. Cluster C had only two cultivars and grouped Zaity from Syria and Arbequina from Spain with 0.30 dissimilarity.

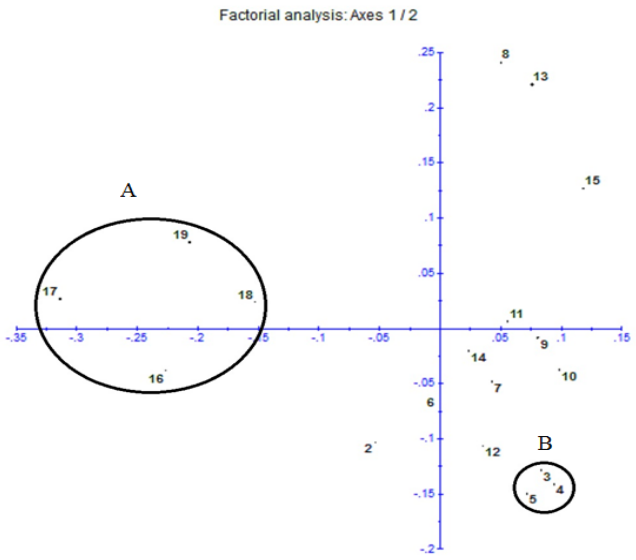

Figure 2 *Two-dimensional principal coordinate analysis (PCoA) generated from genetic distance of olive cultivars. The numbers represent the 19 olive cultivars listed in Table 1. The Turkish cultivars are in circle A. Nasouhi Jabaa 1, Nasouhi Jabaa 2 and Sant Augustine are in circle B. Genotypes 1 and 15 overlapped.*

The SSR data were used to study the population structure of 19 olive cultivars. This analysis assigned the cultivars to two subpopulations (Table 4 and Figure 3), the first subpopulation included 16 cultivars, the second included 3 cultivars. All cultivars from cluster A were assigned to subpopulation 1 while 3 (Nasouhi Jaba1, Nasouhi Jaba2 and Sant Augustine) cultivars from cluster B were assigned to subpopulation 2, these results coincide with the reporting of Al-Taher (2002) that Nasouhi Jabaa 1 and Nasouhi Jabaa 2 were not Palestinian and that they may have come from Italy. The two cultivars of Cluster C were assigned to subpopulation 1.

Table 4 *Cluster assignments of 19 Olive cultivars according to Structure and DARwin analyses*

Sample No.	Sample Name	Inferred ancestry subpopulation		Subpopulation assignment*	Cluster**
		1	2		
1	Nabali Baladi	0.97	0.03	1	A
2	Nabali Muhasan	0.87	0.13	1	B
3	Nasouhi Jabaa 1	0.00	1.00	2	B
4	NasouhiJabaa 2	0.01	0.99	2	B
5	Sant Augustine	0.01	0.99	2	B
6	Chemlali	0.98	0.02	1	A
7	Telmesani	0.97	0.03	1	A
8	Yonani	1.00	0.01	1	A
9	Barouni	0.78	0.22	1	B
10	Cucca	0.91	0.09	1	A
11	Frantoio	0.99	0.01	1	A
12	Arbequina	0.96	0.05	1	C
13	Kalamata	0.99	0.01	1	A
14	Zaity	0.99	0.01	1	C
15	Souri	0.97	0.03	1	A
16	Hamza Celebi	0.99	0.01	1	A
17	Mut	1.00	0.01	1	A
18	Mars	0.99	0.01	1	A
19	Karayaglik	0.99	0.01	1	A

*Accessions were assigned to subpopulations based on the proportion of inferred ancestry with a threshold of ≥0.7

**Cluster assignments based on the neighbor-joining dendrogram

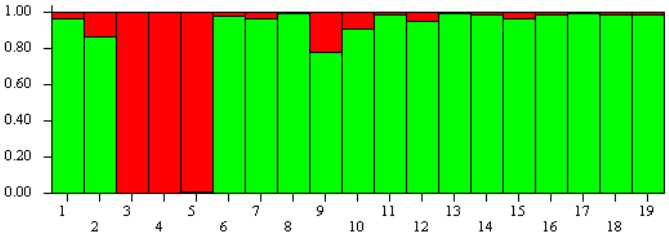

Figure 3 *Structure bar plot representing assignments of olive cultivars to two supopulations at K = 2.*

This study demonstrated the usefulness of SSR markers as a powerful tool for olive characterization. The markers revealed the genetic diversity of olive cultivars grown in Palestine and detected synonyms as well as misleading naming practices. The work also showed that such analysis can enlighten us about the genetic history of olives in Palestine. This study may also help olive breeders in their selection of suitable genotypes and for future improvement in growth and production.

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