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REGULAR ARTICLE

Genotyping of Albania olive (*Olea europaea*) germplasm by SSR molecular marker

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ABSTRACT

The increasing interest in olive varieties with high productivity and quality is the basis of modern olive growing. The molecular characterization of olive varieties is essential to maximize the genetic diversity in the *in situ* and *ex situ* collections of the olive germplasm. The importance of studies aimed at identification and selection of genotypes that meet the requirements for plant nursery certification, for oils traceability and to preserve genotypes that are not widespread from risks of extinction, is evident. In this context, DNA fingerprinting represents a valid tool because the productivity and quality of olive oil are intrinsic characteristics of the original varieties. To date, the Albanian olive-growing heritage has been little studied and the number of varieties has not yet been well defined. As a consequence, it doesn't a precise characterization of the Albanian genetic entities. The aim of this work was to contribute in clarifying the identity of the Albanian olive cultivars, using the SSR molecular markers. We have genotyped olive trees at the level of nine nuclear microsatellite loci or SSR, pre-selected among those present in the literature and we have identified 38 unique genotypes. It has not been possible to establish a clear correlation between clustering of plants, depending on molecular profiles, and the geographical distribution of origin or maturation period or the intended use of drupes. However, our results show a high genetic diversity and a high discrimination capacity of the tested SSR markers.

Keywords: Fingerprinting; Genotyping; Molecular mark*ers; Olea europaea;* SSR

INTRODUCTION

In recent years, the development of the olive sector and the way of olive tree cultivation has changed profoundly. Virgin olive oil is considered a real food, beneficial for human health, as widely documented in the literature (Cimato et al., 2008; Ghanbari et al., 2012; Franco et al., 2014; Hernáez et al., 2015; Georgakouli et al., 2016). In olive oil, some compounds such as tocopherols and polyphenols play a protective action in oxidative degenerative processes and act on Low Density Lipoprotein (LDL) preventing the development of arteriosclerotic lesions. The fatty acids, especially monounsaturated, present in the oil are a source of energy and play a preventive action for cardiovascular diseases (Ghanbari et al., 2012; Violi et al., 2015). Moreover, tocopherols and polyphenols ensure the preservability and therefore the nutritional value of this food (Franco et al., 2014; Ghanbari et al., 2012; Hernáez et al., 2015; Georgakouli et al., 2016). The consumer's awareness of the health properties of this food and the importance of the oil in the diet have increased its consumption and pushed the market to look for typical products for organoleptic and nutritional characteristics (Cimato et al., 2008).

These aspects have created a strong international competition in the field of olive growing, with increases in olive tree cultivation both in the Mediterranean basin, which remains the most important reservoir in terms of extension and economic impact of world olive production, and in non-Mediterranean countries such as Argentina, Chile, the USA, Mexico and South Africa and in non-traditional oil producers such as Australia (FAOSTAT, 2004; Cimato et al., 2008). In the Mediterranean area, Albania belongs to the climatic zones favourable to the growth of olive tree; it has a rich biological and landscape diversity of olive trees and olive cultivation is one of the most important sectors of the Albanian national economy.

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The olive genetic variability coupled with migration of clones has led to confusion in the identity and nomenclature of olive cultivars, therefore many varieties present in the Mediterranean are uncertain (Bartolini et al., 2005). In general, there is taxonomic confusion that brings different varieties to be called with the same name (homonymy), while on the other hand, the same variety could be called with different names (synonymy).

In this context, the importance of studies which aimed at identification and selection of genotypes that meet the requirements for plant nursery certification, for the traceability of oils and for the need to preserve genotypes that are not widespread from risks of extinction, is urgent and evident. The molecular characterization of olive germplasm is an essential prerequisite for a wide and correct use of plant material and represents the first step towards defining the roles that varieties can play in sustainable production. In the last years, olive DNA fingerprinting is considered very important, since both the productivity and the quality of olive products are intrinsic characteristics of the original varieties.

Olive cultivation is rooted in Albanian culture and tradition since ancient times. Olive growing is mainly present in the hilly areas and on the Adriatic and Ionian coast. The most important olive-growing districts are located at Valona, which is the district with the highest number of plants (550.000), and that of Tirana (280.000 plants), the most economically and organizationally important district, where the institutions and ministries responsible for development of agriculture, in particular olive growing, are located.

At present, Albania has sixty thousand hectares of olives and ten million olive trees. Among them, 1.3 million are centenarians, while others are aged up to eighty years. Genetic resources are rich and the Albanian varieties cultivated are 22 of which only 6 varieties are important: 'Kaninjot', 'Kryps Berati', 'Bardhi Tiranës', 'Kryps Elbasani', 'Mixani' and 'Himara' which occupy the 85% of the olive-growing area. The Kaninjot cultivar covers 50% of the olive production area (Ismaili et al., 2014).

However, in recent times it has been witnessing the replacement of old olive trees with new varieties and more rustic cultivars with other more productive ones. In addition, foreign varieties are becoming more widespread, such as 'Frantoio', 'Leccino', 'Carolea', 'Pendolino', 'Nocellara Messinese', typically Italian, and Greek varieties such as 'Kalkidhikia', 'Cunatis', 'Koroneqis', 'Amigdanolia' and 'Calamon', that are profoundly changing the Albanian olive growing (Gixhari et al., 2014).

The purpose of this work was to contribute to clarify the identity of the Albanian olive cultivars, using, as experimental approach, the molecular SSR markers that allowed us to genotype the recovered germplasm. Olive trees were genotyped at the level of nine nuclear microsatellites loci, selected among those available in literature and that in previous works were suitable for the characterization and the varietal identification of the olive trees (Muzzalupo et al., 2006; 2014).

MATERIALS AND METHODS

Vegetal materials

Thirty-eight trees of olive cultivars (*Olea europaea* subsp. *europaea* var. *europaea*) were chosen for this study (Table 1). The thirty-eight samples are archived as a part of the olive collections of the Quendra e trasferimit te teknologjive bujqesore collection of Vlore, Albania.

DNA extraction from leaves

Ten leaves were collected from any olive trees. They were immediately placed in a paper envelope with silica gel. Samples were kept for 72 hours in a box to dehydrate. 200 mg of leaves were mechanically disrupted by TissueLyser II (QIAGEN) through high-speed shaking in plastic tubes with tungsten carbide (QIAGEN). A volume of 100 µL of water was added to 1 mg of disrupted tissue and then mixed by pipetting. 1 µL of this flow fraction was used as DNA sample for PCR reaction using KAPA3G Plant DNA polymerase (KAPA Biosystems) in the Veriti™ thermal cycler (Applied Biosystems) according to Chiappetta et al., (2017). Samples were amplified in triplicate.

PCR amplification from leaves

PCR reactions were carried out in 50 µL final volumes containing: DNA (1 – 5 µL of the solutions described previously) 1X KAPA Plant PCR Buffer, 100X of KAPA Plant PCR Enhancer, 1.5 mM $MgCl_2$, 0.3 mM of each dNTPs, 0.2 µM forward and reverse primers, 2 U of DNA Polymerase (KAPA3G Plant DNA Polymerase) (Kapa Biosystems, Inc., Boston, MA, USA).

The amplification program was an initial denaturation step at 95 °C for 5 min, followed by 40 cycles at 95 °C for 30 s, 15 s at the appropriate annealing temperature (Table 2) and 30 s at 72 °C, with a final elongation at 72 °C for 30 s. Each sample was amplified in triplicate. PCR products were analysed using a Bioanalyzer 2100 (Agilent Tecnologies, Waldbronn, Germany) with a DNA 1000 LabChip following the manufacturer's instructions; the system provides the exact length of amplicons and offers a number of benefits. A typical analysis is completed in around 30 min without the use of gels, buffers, staining or

Table 1: List of Albanian olive tree genotypes taken from the "*Quendra e Transferim te Teknologjive Bujqesore*". The table also shows the geographical distribution of the samples, the maturation period and the intended use of drupes

Nr	Cultivar name	Geographic area	Maturation	Intended use
1	Boç	Tirane/Center	Early	Dual attitude
2	Frengu	Kruje/Center	Medium	Dual attitude
3	Kalinjot	Vlore/South	Late	Dual attitude
4	Kallmet 1	Lezhe/North	Medium	Dual attitude
5	Kallmet 2	Lezhe/North	Medium	Dual attitude
6	Karen	Tirane/Center	Early	Dual attitude
7	Kokërrmadh Berati	Berat/South	Early	Table olives
8	Kokërrmadh Elbasani 1	Elbasan/Center	Early	Table olives
9	Kokërrmadh Elbasani 2	Elbasan/Center	Early	Table olives
10	Kotruvs	Berat/South	Medium	Oil
11	Krypsi Kruje 1	Kruje/Center	Medium	Dual attitude
12	Krypsi Kruje 2	Kruje/Center	Medium	Dual attitude
13	Kushan 1	Preze-Tirane/Center	Early	Dual attitude
14	Kushan 2	Preze-Tirane/Center	Early	Dual attitude
15	Kushan KM v3	Preze-Tirane/Center	Early	Dual attitude
16	Managjel	Kruje/Center	Early	Dual attitude
17	Mixani a	Elbasan/Center	Late	Oil
18	Mixani b	Elbasan/Center	Late	Oil
19	Mixani v3	Elbasan/Center	Late	Oil
20	Nisjot	Mallakaster/South	Early	Oil
21	Nisjot v3	Mallakaster/South	Early	Oil
22	Nisjot 1 v3	Mallakaster/South	Early	Oil
23	Nisjot 2v3	Mallakaster/South	Early	Oil
24	Nisjot 3v3	Mallakaster/South	Early	Oil
25	Nisjot 4v3	Mallakaster/South	Early	Oil
26	Olivaster e kuqeTiranës	Tirane/Center	Early	Oil
27	Pulazeqin	Vlore/South	Early	Oil
28	Ulliri i Bardhe Kruje	Kruje/Center	Medium	Oil
29	Ulliri Bardhe Pobrat	Berat/South	Late	Dual attitude
30	Ulliri i BardheTirane 1	Tirane/Center	Late	Oil
31	Ulliri i BardheTirane 2	Tirane/Center	Late	Oil
32	Ulliri i Kuq	Tirane/Center	Medium	Oil
33	Ulliri i Kuq v3	Tirane/Center	Medium	Oil
34	Ulliri Zi v3 1	Tirane/Center	Early	Oil
35	Ulliri Zi v3 2	Tirane/Center	Early	Oil
36	Unafka	Berat/South	Early	Dual attitude
37	Vajsi Peqin 1	Peqin, Elbasan/Center	Medium	Oil
38	Vajsi Peqin 2	Peqin, Elbasan/Center	Medium	Oil

Table 2: Characteristics of microsatellites markers used for the genotyping of olive trees in the present study

Locus	Directed sequence (5' → 3')	Annealing temperature (Ta)
ssrOeUA-DCA9*	AATCAAAGTCTTCCTTCTCATTTCG	64°C
ssrOeUA-DCA18*	AAGAAAGAAAAAGGCAGAATTAAGC	63°C
GAPU59**	CCCTGCTTTGGTCTTGCTAA	55°C
GAPU71A**	GATCATTTAAAATATTAGAGAGAGAGA	57°C
GAPU71B**	GATCAAAGGAAGAAGGGGATAAA	56°C
GAPU103A**	TGAATTTAACTTTAAACCCACACA	55°C
UDO99-012***	TCACCATTCTTAACTTCACACCA	56°C
UDO99-028***	CTGCAGCTTCTGCCCATAC	54°C
UDO99-039***	AATTACCATGGGCAGAGGAG	55°C

**Sefc et al., 2000; **Carriero et al., 2002; ***Cipriani et al., 2002*

imaging systems with minimal sample consumption (1 µl PCR product). Furthermore, the ease of use and low cost benefits may provide an alternative method for determining genetic modifications in foodstuffs compared to other technologies (Muzzalupo et al., 2007).

SSR analysis

The samples (leaves) were genotyped using nine nuclear microsatellite markers, carefully chosen from those available in literature, and demonstrated to be proper for the characterization and identification of olive varieties in previous studies (Baldoni et al., 2009; Muzzalupo et al., 2014): GAPU59, GAPU71A, GAPU71B, GAPU103A (Carriero et al., 2002), UDO99-012, UDO99-028, UDO99-039 (Cipriani et al, 2002), ssrOeUA-DCA9 and ssrOeUA-DCA18 (Sefc et al., 2000). The characteristics of these loci SSRs are described in Table 2 and 3.

Genetic analysis

To estimate genetic parameters such as number of alleles (*Na*), effective number of alleles (N*e*), observed (*Ho*) and expected (*He*) homozygosity, and F-statistics (*Fst*) we used the PopGene32 version 1.32 software. The power of discrimination (*PD*) and Polymorphism Information Content (*PIC*) were calculated for each SSR locus according to Brenner and Morris (1990).

Moreover, the alleles detected for each microsatellite were recorded into a data matrix of presence (1) and absence (0) of bands by using FreeNA software (Chapuis and Estoup, 2007). The binary matrix representing the molecular dataset was transformed into a similarity matrix using the NTSYSpc program version 2.2 (Rohlf, 1998). The similarity coefficients used for the new matrices were based on Nei's coefficients. The new similarity matrices were inferred using the Unweighted Pair Group Method (UPGMA) algorithm based on the NTSYSpc program version 2.2.

RESULTS AND DISCUSSION

Genetic diversity of olive plants

We used the nine microsatellite markers to investigate the genetic variation level among thirty-eight Albania olive varieties examined in this study (Table 1). The data obtained from the analysis have shown that the number of alleles per locus was highest at the GAPU103A locus and lowest at the GAPU71B locus (Table 3). We detected a total of 68 alleles at 9 loci with a mean value of the *Ne* equal to 4.8. We calculated the *Ho* and *He* for all studied varieties. The *He* ranged from 0.108 to 0.415 whilst the *Ho* ranged from 0.000 to 0.605. Therefore, the *Ho* (mean = 0.187) was lower than expected (mean *He* = 0.224) in accordance with that reported in previous studies (Baldoni et al., 2009). The F_{ST} values, calculated for each analysed locus, ranged from 0.362 at the UDO99-039 locus to 0.750 at the ssrOeUA-DCA18 locus. The PIC ranged from 0.536 at the UDO99-039 locus to 0.869 at the GAPU103A locus. The PD ranged from 0.698 (UDO99-028) to 0.891 (GAPU103A) with an average value of 0.736. This is higher than that found by Cipriani et al., (2002) in 12 Italian olive cultivars, but comparable to Rekik et al., (2008) in 20 Tunisian cultivars.

Clustering analysis

We tested the dendrogram (Fig. 1) obtained utilizing the UPGMA method that elaborates the similarity matrix, obtained using the NTSYS PC software and the WinBoot software. It highlights the presence or absence of mislabeling, redundancies, homonymy, and synonymy in olive tree datasets analysed in this study. The clustering analysis based on Nei's coefficient showed two different clusters namely A and B (Fig. 1). In cluster **A** it was possible to distinguish three olive varieties ('Boc', 'Nisjot' e 'Kallmet 2'), while in cluster B 34 olive plants were present. The only

Table 3: Genetic diversity parameters for each locus are reported (Na=number of alleles detected, Ne=effective number of alleles, Ho=observed homozygosity, He=expected homozygosity, FST=F-statistics, PD=Power of Discrimination; PIC=Polymorphism Information Content)

	Na	Ne	Ho	He	Fst	PD	PIC
ssrOeUA-DCA9*	10	4.7	0.026	0.202	0.382	0.749	0.750
ssrOeUA-DCA18*	8	4.7	0.605	0.201	0.750	0.880	0.762
GAPU59**	7	3.0	0.237	0.327	0.426	0.792	0.630
GAPU71A**	7	6.3	0.000	0.149	0.405	0.866	0.819
GAPU71B**	6	4.6	0.105	0.209	0.427	0.841	0.746
GAPU103A**	16	8.3	0.158	0.108	0.522	0.891	0.869
UDO99-012***	7	4.5	0.184	0.214	0.474	0.837	0.732
UDO99-028***	10	5.0	0.105	0.190	0.441	0.698	0.776
UDO99-039***	7	2.4	0.263	0.415	0.362	0.740	0.536
Mean	8.7	4.8	0.187	0.224	0.470	0.810	0.736
St. Dev	3.1	1.7	0.180	0.093	0.117	0.068	0.100

**Sefc et al., 2000; **Carriero et al., 2002; ***Cipriani et al., 2002*

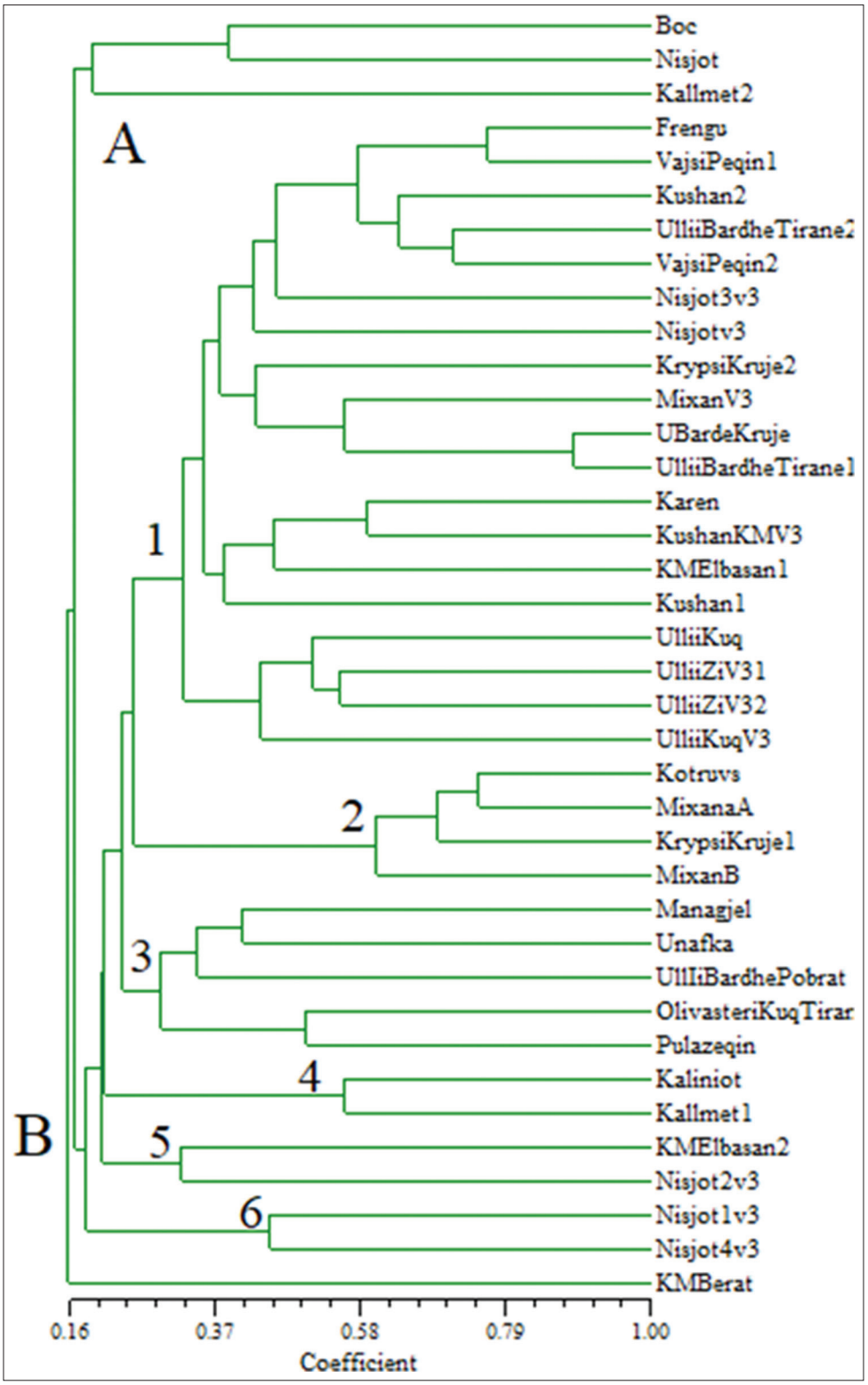

Fig 1. Dendrogram of thirty-eight olive genotypes from the Albanian region, generated by UPGMA cluster analysis based genetic identity (Nei, 1972).

accession 'Kokërrmadh Berati' not joins with the other two groups. Overall, the data in the present study clearly indicate that using the SSR loci for cultivar genotyping allowed for variety identification and distinguished all cultivars.

The clustering analysis of olive plants showed, in the cluster B, six different sub-clusters namely 1 - 6 (Figure 1). In sub-cluster 1 we distinguished 19 olive accessions ('Frengu', 'Vajsi Peqin 1', 'Kushan 2', 'Ulliri i BardheTirane 2', 'Vajsi

Table 4: Size (in base pairs) of allelic profiles found in the thirty-eight Albanian olive samples studied

	Gapu59		Gapu71A		Gapu71B		Gapu103A		UDO99-012		UDO99-028		UDO99-039		ssrOeUA-DCA 9		ssrOeUA-DCA 18	
Boc	208	208	210	212	121	138	136	170	160	164	154	166	125	213	184	210	173	173
Frengu	208	218	212	221	121	138	150	170	160	177	182	200	125	213	166	182	169	190
Kalinjot	208	208	214	224	121	138	197	197	160	177	182	200	213	213	162	172	175	175
Kallmet 1	208	226	214	224	121	138	150	184	166	182	182	200	125	213	162	172	177	177
Kallmet 2	208	214	212	221	121	138	190	211	166	166	154	154	170	185	182	198	173	173
Karen	208	218	212	221	124	126	203	211	160	166	182	200	125	220	198	210	169	175
Kokërrmadh Berati	212	232	210	214	121	126	203	203	164	182	150	161	125	125	166	188	169	183
Kokërrmadh Elbasani 1	208	208	214	221	126	130	170	203	164	177	182	200	125	125	198	210	169	175
Kokërrmadh Elbasani 2	212	218	214	221	124	130	150	184	177	200	161	175	125	125	166	194	175	175
Kotruvs	208	226	214	224	124	130	184	203	164	177	175	196	125	220	166	198	169	175
Krypsi Kruje 1	208	226	214	224	121	126	184	203	164	177	175	196	125	220	188	198	169	175
Krypsi Kruje 2	208	218	214	221	121	126	157	191	177	200	182	200	125	125	166	182	177	177
Kushan 1	208	208	210	212	121	126	150	176	177	200	182	200	125	220	182	198	169	169
Kushan 2	208	218	212	221	124	126	190	211	160	177	150	182	125	220	166	182	169	190
Kushan KM v3	208	226	212	221	124	126	150	184	160	177	182	200	125	220	198	210	169	169
Managjel	208	218	218	228	121	126	191	211	166	193	161	182	125	125	166	182	175	175
Mixani a	208	226	214	224	126	130	184	203	164	177	175	196	125	220	166	184	169	169
Mixani b	208	226	214	224	126	130	184	203	160	177	175	196	125	220	166	182	173	173
Mixani v3	208	208	214	224	126	130	184	203	164	177	182	200	125	125	166	184	173	173
Nisjot	208	218	210	212	121	144	157	157	160	177	154	166	125	125	194	210	169	175
Nisjot v3	208	218	210	221	124	124	150	150	160	177	210	210	125	213	166	182	163	163
Nisjot1 v3	220	220	212	218	124	138	170	170	160	182	143	143	125	220	166	182	163	190
Nisjot2 v3	212	220	214	221	124	138	150	184	160	166	166	182	125	213	198	198	163	175
Nisjot3 v3	208	232	210	218	124	126	150	184	166	166	182	200	125	213	166	182	169	169
Nisjot4 v3	220	220	210	218	124	138	170	203	177	177	143	143	125	213	166	176	163	163
Olivaster e kuqeTiranës	208	220	214	228	124	124	176	213	166	166	182	200	125	232	182	198	169	169
Pulazeqin	208	220	214	228	126	126	170	197	160	166	154	182	125	232	166	182	169	169
Ulliri i Bardhe Kruje	208	226	214	221	126	130	150	184	160	177	182	200	125	213	166	182	173	173
Ulliri Bardhe Pobrat	208	208	218	228	124	126	190	211	160	177	143	166	125	220	166	182	169	169
Ulliri i BardheTirane 1	208	226	214	221	126	130	150	184	160	177	182	200	125	220	166	182	173	173
Ulliri i BardheTirane 2	208	218	210	218	124	126	190	212	160	177	182	200	125	220	166	182	169	190
Ulliri i Kuq	208	218	210	212	121	138	188	209	164	166	182	200	125	125	166	182	163	169
Ulliri i Kuq v3	208	220	210	212	121	138	150	184	166	177	182	200	125	125	166	188	163	190
Ulliri Zi v3 1	208	218	210	218	121	138	150	150	160	160	182	200	125	220	166	182	163	163
Ulliri Zi v3 2	208	218	212	221	121	138	190	211	160	160	182	200	125	125	166	182	163	175
Unafka	208	208	218	228	124	124	188	209	166	193	161	182	125	209	182	184	179	179
Vajsi Peqin 1	208	208	212	221	124	126	150	184	160	177	182	200	125	213	166	182	169	169
Vajsi Peqin 2	208	218	212	218	124	126	190	213	160	177	182	200	125	213	166	182	169	190

Peqin 2', 'Nisjot 3 v3', 'Nisjont v3', 'Krypsi Kruje 2', 'Mixani v3', 'Ulliri i Bardhe Kruje', 'Ulliri i BardheTirane 1', 'Karen', 'Kushan KM v3', 'Kokërrmadh Elbasani 1', 'Kushan 1', 'Ulliri i Kuq', 'Ulliri Zi v3 1', 'Ulliri Zi v3 2', 'Ulliri i Kuq v3'), in sub-cluster 2 we distinguished 4 olive accessions ('Kotruvs', 'Mixani a', 'Krypsi Kruje 1', 'Mixani b'), in sub-cluster 3 we distinguished 5 olive accessions ('Managjel', 'Unafka', 'Ulliri Bardhe Pobrat', 'Olivaster e kuqeTiranës', 'Pulazeqin'), while sub-clusters 4, 5 and 6 grouped only two olive accessions each: 'Kalinjot' and 'Kallmet 1'; 'Kokërrmadh Elbasani 2', 'Nisjot 2 v3', 'Nisjot 1 v3' and 'Nisjot 4 v3', respectively.

Within sub-category 1, which includes most of the cultivars, we established that all come from the Center of Albania and are distributed between the regions of Tirana, Kruje and Elbasan that are adjacent to each other. The only variety found in the Mallakaster region was the 'Nisjot v3'. Furthermore, we distinguished a sub-cluster composed of 4 plants that all come from the Tirana region: 'Ulliri i Kuqi', 'Ulliri Zi v3 1', 'Ulliri Zi v3 2' and 'Ulliri i Kuq v3'. These 4 cultivars are all intended for oil production. In sub-cluster 1 there was also the pair of genotypes with greater similarity value (vs = 0.889, data not shown) composed of 'Ulliri i Bardhe Kruje' and 'Ulliri i BardheTirane 1'. In sub-cluster 2 we found 4 cultivars ('Kotruvs', 'Mixani a', 'Krypsi Kruje 1', 'Mixani b') all intended for oil production, with the exception of 'Krypsi Kruje 1' which is also used for the production of table olives. In sub-cluster 3 we found two cultivars for oil production ('Olivaster e kuqeTiranës', 'Pulazeqin') and three dual-purpose cultivars ('Managjel', 'Unafka', 'Ulliri Bardhe Pobrat'). In sub-cluster 4 we found two cultivars ('Kalinjot', 'Kallmet 1') both with dual attitude. In sub-cluster 5 we had 2 cultivars ('Kokërrmadh Elbasani 2', 'Nisjot 2 v3') both characterized by early maturation. Finally, in the sub-cluster 6 we found the only individuals named 'Nisjot' that group together ('Nisjot 1 v3', 'Nisjot 4 v3').

The high levels of inter- and intra-varietal variability of the Albanian olive germplasm was already observed in previous work as evidenced in a study conducted on the clones of the Kalinjot cultivar (Ismaili et al., 2013) and on 19 varieties of native Albanian olive trees by using the RAPD as molecular markers (Belaj et al., 2002).

CONCLUSIONS

The analysis of the products of the nine SSR loci taken into consideration, allowed us to determine the genetic profiles of the Albanian olive varieties, examined in this study, in a quick and precise way, by using small sample quantities, simplifying the study of genotyping compared to classical analysis conducted by sequencing.

The molecular characterization of olive varieties is essential to avoid the redundancy of genotypes and to maximize the genetic diversity in the *in situ* and *ex situ* collections of the olive germplasm. The ability to distinguish the cultivars unequivocally and to clarify the synonymies and homonymies present, is of great importance for solving the problems related to the management of the olive germplasm of a nation.

The analysis by SSR molecular markers of the Albanian olive germplasm examined, led to the identification of 38 unique genotypes. It has not been possible to establish a clear correlation between clustering of plants, according to molecular profiles, and the geographical distribution of origin or the period of maturation or the intended use of drupes. However, our results show a high genetic diversity and a high discrimination capacity of the tested SSR markers.

The great diversity of varieties found in our study, considering the small area of cultivation, is probably due to their different indigenous origin and the reduced pressure of selection carried out by Albanian farmers throughout history.

The grouping of the varieties in the same region or in neighbouring regions suggests that these cultivars have a common genetic base and moreover a native origin. This result agrees with the hypothesis of native origin of most varieties as well as their limited diffusion from their centres of origin.

In conclusion, the use of SSR molecular markers is essential to build a database for the analysis of olive cultivars, for traceability of processed foods and for the appropriate management of olive tree germplasm collections. This work represents one of the first studies in which such a large number of Albanian olive varieties were analysed using the SSR molecular markers.

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