

Morphological, Chemical and Molecular Characterization of 'Old olive' (*Olea europaea* L.) from Montenegro

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ABSTRACT

Thousands of years of tradition of olive cultivation in Montenegro is witnessed with the 'Old olive' estimated to be more than 2000 years old. 'Old olive' draws attention to its genetic potential and the relation to the closest grown olive cultivars. Therefore, the aim of this paper was to give the basic description of 'Old olive' (through 20 morphological and 37 chemical properties) as well as to determine its DNA profile and to examine the closeness with major olive cultivar grown, cv. 'Žutica'. The obtained data showed the differences between examined samples. 'Old olive' started flowering (27. May) more than 10 days later than cv. 'Žutica' (14. May), and began ripening 15 days later (20th October and 5th October, respectively). From 21 morphological parameters analysed all traits of fruit (weight 2.11g in 'Old olive' and 3.55g in cv. 'Žutica'), and inflorescence density (6.39 versus 5.55) showed very significant and significant differences, respectively. The chemical properties also showed very significant differences in olive oil content in the fresh (11.28 versus 19.68%) and dry matter (27.97 versus 47.78%), and significant differences in the content of linolenic acid (0.76 versus 0.54g/100 of FA and of campesterol (3.4 versus 3.1%). Three out of eleven SSR markers (DCA05, DCA09, DCA16) distinguished obtained DNA fragments, and showed as important tool in differentiation of two samples. Further study of the 'Old olive' as a living memory of olive cultivation in Montenegro, should be to examine its relation with other ancient olive trees and local varieties.

Key words: 'Old olive' tree, morphology, oil, SSR, cv. 'Žutica', Montenegro



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INTRODUCTION

The survival of very ancient olive trees has been reported through the entire Mediterranean area. The ancient trees are the living proof of the very long tradition of olive cultivation. These trees have main genetic, agronomical, naturalistic, landscape and historical importance [1]. Thousands of years of tradition of olive cultivation in Montenegro is linked to the 'Old olive' (Fig. 1), the oldest exemplar in the region and beyond. 'Old olive' (in Montenegrin 'Stara maslina') is the name by which this tree is known among the people and registered as the 'monument' of nature since 1963. Its estimated age is of more than 2000 years [2], recently confirmed [3].

The 'Old olive' tree is a vital part of the visual and spiritual identity of the Montenegrin Coastal area, valuable evidence of ancient civilization in this area. The importance to take care of 'Old olive' passed down from generation to generation. Among people it is said that the tree is older than Christ, while the older people still remember the strict prohibition of removing leaves and cuttings from the tree, which contributed to its preservation until today. The place, where the 'Old olive' is situated, has always had a great socio-cultural significance for the area of Bar. It is believed that the place Mirovica was named after the meetings and counselling kept under the crown of the tree, with aim to make peace among people at loggerheads, and where 'giving faith' for omission of evil [4]. The last private owner of 'Old olive', bought it together with a large estate, in the thirties of XXth century, and in sharing the estate with his brothers gave 20 other 'roots' (trees) of olive trees in exchange. During the Second World War the Italians wanted to buy the 'Old olive' tree for 2.5 million golden liras, but the owner did not accept the offer. In the mid-sixties 'Old olive' was recognized as 'general interest' and the Municipality of Bar brought it from the owner. Until the nineties of the XXth century the olive oil processing company 'Primorka' was responsible for maintenance of 'Old olive' when it was overtaken by the City of Bar and stayed nowadays. The 'Old olive' tree had hard periods, one of which happened in the 60s of last century when, due to the people's negligence the part of the trunk was burnt down (Fig. 1A). Today, the 'Old olive' is an attractive tourist destination (Fig. 1D) and remains a lasting inspiration for native artists. Under the 'Old olive' tree, a trademark of Bar, various cultural events are held and most recognized are International Film Festival and the Days of poetry, awarded with the prize which symbolize the 'Old olive'.

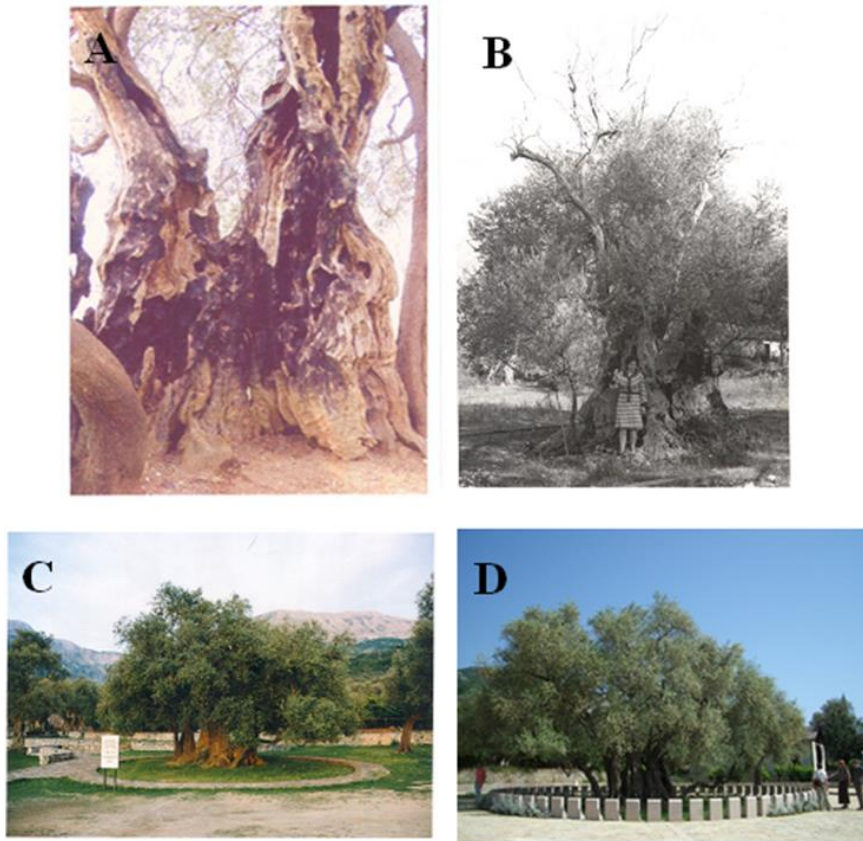


Figure 1. Recent history of 'Old olive' tree: A – Consequences of the fire occurred in the sixties are still obvious (photo from 2004); B – 'Old olive' tree recovering from the fire (photo from 1972, [5]); C – The 'Old olive' tree on Mirovica in the end of XXth century; D – Actual view of the 'Old olive' tree.

Beside historical and cultural significance, 'Old olive' draws attention to examine its genetic potential, interest indicated through the works on the old olive trees in other countries [6,7,8,9]. The age of this tree and the fact that it is located in a monovarietal region, cause attention from morphological to molecular aspects. Classical approaches for identifying olive cultivars based on morphology [10] provide a very useful tool for identification, but it has limitations because of environmental influences, and the need for extensive observations of mature plants. Therefore, DNA-based SSR markers as independent of the environment exhibit a higher degree of polymorphism and provide an opportunity for genetic characterization and biodiversity studies in olive [11,12].

The presumption that the 'Old olive' is actually a rootstock on which it was grafted cv. 'Žutica', because the long-thought was that 'Old olive' is of 'Žutica' variety [5], which developed from the root neck, after a fire burnt the plant mass. Such presumption opens many issues for more reasons as: on the same location around the 'Old olive' there are relatively old trees of 'Žutica' variety (500-800 of years) on their own roots; in the southern area of the Montenegro coast for a long time olives were propagated by separation of the shoots from the trunk base (root neck) with a part of the mother tree so called tubers (spheroblasts), which was abundant in 'Žutica' variety [13]. That seems contrary to the findings of Díez *et al.* [8] and Barazani *et al.* [14], which suggest that grafting pre-existing wild olives with olive cultivars was linked to the beginnings of olive growing. Another speculation on the origin of 'Old olive' lies in the traditional practice of planting a wild olive (oleaster), for better pollination. These pollinators are characterized with small fruit and poor quality oil.

Along the way on answering if the 'Old olive' is a mother tree (or what is the relation) to other old trees grown in the area, this paper is to give the basic description of the 'Old olive' specimen and in comparison with major characteristics of the 'Žutica' cultivar.

MATERIALS AND METHODS

Plant material

'Old olive' tree is located on Mirovica near Bar, on 42°04'48.53" North, 19°07'45.96" East, 47 m above sea level, in the Southern region, named Bar-subregion. In this area in the olive cultivation where predominate the indigenous variety 'Žutica' (around 98%). Therefore, both samples were treated to belong to the prevalent cv. 'Žutica'. In order to evaluate potential relationship between 'Old olive' and cv. 'Žutica', morphological data of plant organs, chemical parameters of fruit and olive oil quality and their SSR profiles were compared. The trees were situated at the same location, to avoid possible environmental influence on the parameter measurements and observations.

Morphological characteristics

To provide more precise average values the data were collected during five years period (2012-2016). The measures were performed according to the olive descriptor [10,15] used as standard in evaluation of the morphological and agronomic properties of olive varieties. Observation was performed on flowering (beginning and end of flowering, intensity and duration) and ripening (beginning and full) stages, and the number of days from end of flowering to the full ripening. For yield data, the fruits were harvested and measured in full ripening stage. From the plant organs the following properties of leaf (length, width and shape), internode length, inflorescences (length, number of flowers, number and percentage of imperfect flowers, inflorescence density), fruit (length, width, form, weight, pulp weight and percentage, pulp/endocarp ratio, and number of fruits in 1kg), and endocarp (length, width, form, weight) were measured.

For the fruit analysis a sample of 100 fruits was taken from each accession from all sides of the crown and from the mid treetop section. The fruits were at the beginning of ripening phase, reaching the full fruit size characteristic of 'Old olive' tree and of variety 'Žutica' trees. The harvested sample was divided into two sub-samples: one of 40 fruits was used for characterising the fruit and endocarp, while 100 g from the second subsample was used for determining moisture and dry matter.

Olive oil analysis

Olive oils were extracted from fruit samples (3-4 kg each sample) during three years period (2012-2014) from 'Old olive' tree (1) and cv. 'Žutica' (2), picked at full maturation (maturation index = 6-7). Olive oil extracted on the laboratory olive mill (Abencor system) was used for further analyses.

Olive oils quality parameters were determined (free acidity, peroxide value, and specific coefficients of extinction) according to the Regulation EEC 2568/91 of the European Union Commission (Annex II, III and IX [16]). Fatty acids composition (Annex XA and Annex XB [16]), sterolic fraction (Annex V [16]), wax (Annex IV [16]) and total phenols content (determined colorimetrically using the Folin-Ciocalteu reagent [17]) were analysed. The oils were also tested by an organoleptic panel

(according to the Panel test method (Annex XII [16]) to characterize the sensorial composition as well as nature and defects of the olive oils from 'Old olive' tree.

Molecular analyses

DNA was extracted from young leaves of 'Old olive' tree and cv. 'Žutica' with the Plant Mini Kit according to the manufacturer's instructions. The DNA analyses were performed using 11 SSRs developed for high levels of polymorphism: *ssrOeUA-DCA03*, *ssrOeUA-DCA05*, *ssrOeUA-DCA09*, *ssrOeUA-DCA14*, *ssrOeUA-DCA16*, *ssrOeUA-DCA18* [18]; *EMO90* [19]; *GAPU71B*, *GAPU101*, *GAPU103A* [20] and *UDO99-43* [21]. All these loci are included in the list of best SSR markers for cultivar fingerprinting [22]. Capillary electrophoresis was performed on an automated sequencer (ABI 3130 Genetic Analyzer Applied Biosystems, Foster City, CA, USA). Molecular data, fragment sizes were automatically calculated to two decimal places using the Gene Mapper 3.7 (Applied Biosystems).

Data analysis

To determine the significance between morphological parameters (leaf, inflorescence fruit and endocarp) and chemical composition of fruits of 'Old olive' tree and cv. 'Žutica', ANOVA, LSD test for materiality threshold of $P < 0.05$ was used. The data were analysed in the software STATISTIX 7.0 (General AOV; Statistix 7, Analytical Software, Tallahassee, Florida, USA).

RESULTS AND DISCUSSION

Morphological and biological characteristics

Morphological characteristics are presented in the Figure 2 and Tables 1 and 2. Beginning of flowering of 'Old olive' differs from the variety 'Žutica' and occurs on May 27, which is 13 days later than that of cv. 'Žutica' (May 14). Flowering lasts for 12 days and ends at the end of the first week of June (June 7). Flowering intensity varies from low to abundant, in average medium extensive (grade 3). Fruit ripening begins in the second half of October, 15 days later than in 'Žutica', and full ripening when most of the fruits were fully coloured, stands in mid-November. From the end of flowering to full ripening of the 'Old olive' was 161 days, similar to 'Žutica' (166 days). The yield of 'Old olive' is regular, medium to high (around 120 kg), and thanks to the size of the tree [23], it is much more productive than the average tree of 'Žutica' variety (40 kg).

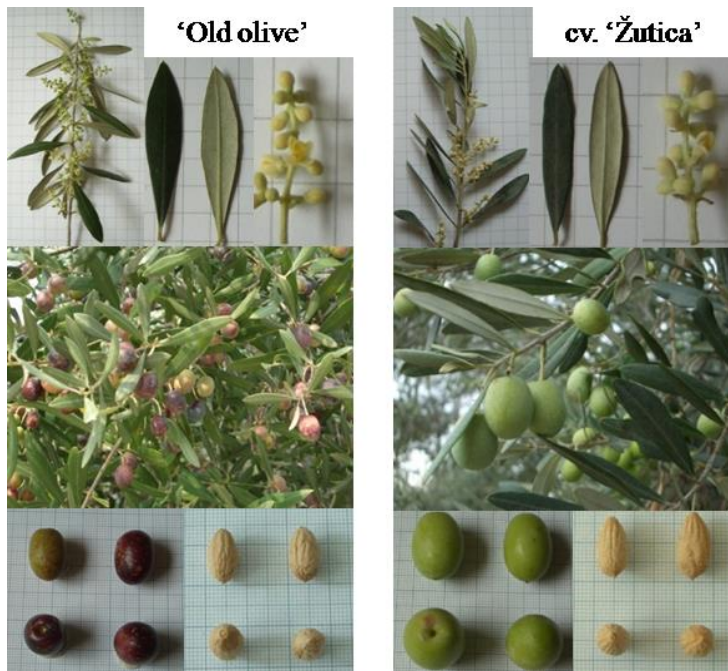


Figure 2. Morphological description of 'Old olive' tree and cv. 'Žutica' trees organs.

Table 1. Flowering and ripening period of 'Old olive' and cv. 'Žutica' trees.

Biological characteristics	'Old olive'	cv. 'Žutica'
Beginning of flowering	May 27	May 14
End of flowering	June 7	May 25
Flowering intensity	3	4
Duration of flowering	12 days	11 days
Beginning of ripening	October 20	October 5
Full ripening	November 15	November 7
Days from end of flowering to full ripening	161	166

The biometric data of olive organs is very important to discriminate olive cultivars [10,24,25]. The measurements of 21 parameters of olive leaf, inflorescence, fruit and endocarp are presented in Table 2. Very significant differences between two samples were shown in the fruit trait and significant in inflorescence density.

The leaf of 'Old olive' is elliptic-lancelet, medium long and narrow, with a maximum diameter in the middle. Colour of the leaf surface is green and grey-green-silvery on the reverse side. The internodes of 'Old olive' are medium-long (1.66 cm); inflorescences are compact, medium in length with a medium number of flowers (18.35), while cv. 'Žutica' has a low number of flowers (15.13) and lower density of flowers in bloom. There was no significant difference in number of perfect flowers although their proportion varies with inflorescence, accessions and the prevailing environmental conditions [26]. Number of imperfect flowers was similar and low (3.96 and 3.34, respectively) [10], and with no influence to the yield, since if 1% to 2% of flowers set the fruit is enough for a good yield [27].

The fruit of both samples belong to the medium category according to the olive descriptor [10]. However the fruit 'Old olive' is smaller (2.11g), oval, symmetric, with a rounded base, peak without bulge and with lots of big lenticels on the green fruit, unlike the cv. 'Žutica' (3,55g) that has few tiny lenticels (Lazovic et al., 2016). Pulp/endocarp ratio was much higher in cv. 'Žutica' (7.57) than in 'Old olive' (4.09) and belong to the different categories [28], very high and high, respectively. Fruit

parameters of two accessions are statistically significantly different at $P < 0.05$, confirming that differences in the fruit size are mainly of genetic origin [29]. The endocarp traits, although have been recognised as the most affective among morphological data, due to their high discrimination capacity and low sensitivity on environmental influence [1], did not show significant differences between the two analysed samples. This might also be the reason of the lack in distinguish these two samples in the past. 'Old olive' endocarp is elliptical, medium (0.41 g), symmetric, rounded top and base, smooth surfaces with a high number of grooves evenly divided. The difference, however is observed visually (Fig. 2), especially in the prominence of the base and the largest diameter in cv. 'Žutica' which is closer to the top of the endocarp.

Table 2. Morphological characteristics of leaves, inflorescences, fruits and endocarps from 'Old olive' tree and cv. 'Žutica' trees.

Plant organ	'Old olive' tree	cv. 'Žutica'	LSD F-value ^a	P-value
Leaves				
Leaf length (cm)	5.84±0.27 ^b	5.80±0.31	0.04 ns	0.8467
Leaf width (cm)	1.23±0.10	1.25±0.06	0.21 ns	0.6578
Shape (L/W)	4.76±0.39	4.65±0.26	0.47 ns	0.5066
Internode length	1.66±0.14	1.54±0.20	1.11 ns	0.3171
Inflorescences				
Length (cm)	2.87±0.39	2.72±0.40	0.40 ns	0.5418
Number of flowers	18.35±1.86	15.13±3.36	3.71 ns	0.0831
Number of imperfect flowers	3.96±0.79	3.34±2.20	0.36 ns	0.5592
Imperfect flowers (%)	21.60±4.83	22.04±8.80	0.02 ns	0.8792
Inflorescence density (flowers/length)	6.39±0.41	5.55±0.68	6.43 *	0.0295
Fruits				
Length (cm)	1.94±0.07	2.19±0.11	20.50 **	0.0011
Width (cm)	1.38±0.04	1.68±0.04	126.33 **	0.0000
Form (L/W)	1.40±0.03	1.31±0.04	18.25 **	0.0016
Weight (g)	2.11±0.18	3.55±0.28	98.85 **	0.0000
Pulp weight (g)	1.70±0.14	3.13±0.24	136.31 **	0.0000
Pulp percentage in fruit	80.35±0.96	88.33±0.72	272.64 **	0.0000
Pulp/endocarp ratio	4.09±0.25	7.57±0.51	193.52 **	0.0000
Fruits in 1kg (n)	474±41.11	282±22.82	109.69 **	0.0000
Endocarp				
Length (cm)	1.38±0.04	1.39±0.06	0.05 ns	0.8339
Width (cm)	0.72±0.03	0.74±0.02	2.68 ns	0.1329
Form (L/W)	1.94±0.09	1.87±0.07	1.65 ns	0.2278
Weight (g)	0.41±0.04	0.41±0.04	0.00 ns	0.9854

^aLSD Least Significant Difference test F-values. **Significant at $P > 0.01$; *Significant at $P > 0.05$; ns—not significant ($P < 0.05$).

^b Mean±S.D.

Chemical characteristics of fruit

Basic parameters of 'Old olive' fruit composition (Table 3) showed that the most important parameter, oil content in the fruit, is according to the descriptor very low in fresh matter (11.28%) and dry matter (27.97%), and significantly differ ($P < 0.05$) from the cv. 'Žutica', with an average of around 20% oil in the fruit [30,31]. According to Malheiro *et al.* [32] fat content in olives is mainly influenced by the olive variety and the maturation degree, since olives were picked at full maturation (6-7 index), fat was completely formed in the fruits. Furthermore, the differences found in fat content are important to differentiate olive samples since according to Di Bella *et al.* [33] fat content in olive fruits is regulated by genetic factors, highlighting differences between olive cultivars.

Table 3. Chemical composition of fruits (g/100 g of fruits) from 'Old olive' and cv. 'Žutica' trees.

Fruit composition	'Old olive' tree	cv. 'Žutica'	LSD F-value ^a	P-value
Dry matter	40.33±2.71 ^b	41.18±2.41	0.28 ns	0.6134
Humidity (%)	59.67±2.71	58.82±2.41	0.28 ns	0.6134
Oil in fresh matter	11.28±2.22	19.68±0.82	62.83 **	0.0000
Oil in dry matter	27.97±3.82	47.78±3.86	68.18 **	0.0000

^aLSD Least Significant Difference test F-values. **Significant at P>0.01; *Significant at P>0.05; ns—not significant (P<0.05).

^bMean±S.D.

Chemical characteristics of oil

The prevalent cv. 'Žutica' enables production of mono-varietal oil. However, different cultivars or biodiversity of olive trees is the source of value which has to be recognised and preserved [34]. The research on biodiversity can help us to get oils with different taste which can satisfy demanding consumers [35]. In spite of the increasing appreciation of typical extra virgin olive oils, based on use of local traditional varieties, very few studies have focused on the genetic characterization of olive cultivars of regional or local interest [36]. The olive oils extracted from fruits of 'Old olive' and cv. 'Žutica' were analyzed regarding their quality parameters (Table 4), fatty acids and sterolic profiles (Tables 5 and 6 respectively). Regarding quality the oils from both 'Old olive' and cv. 'Žutica' are of good quality, with a low content of free fatty acids (0.31 and 0.45% respectively) and a low peroxide number (4.9 and 7.03 meq. O₂/kg, respectively). Even more, the results of specific extinction coefficients K₂₃₂ and K₂₇₀, as well as ΔK, presented good values. According to the data obtained for the quality parameters of olive oils from the studied samples complied with the Regulation limits for the extra virgin olive [37].

Table 4. Quality parameters of olive oils from 'Old olive' and cv. 'Žutica' trees.

Quality parameter	'Old olive' tree	cv. 'Žutica'	LSD F-value ^a	P-value
Free acidity (%)	0.31±0.09 ^b	0.45±0.25	0.84 ns	0.4111
Peroxide value (meq. O ₂ /kg of oil)	4.90±1.60	7.03±0.32	5.13 ns	0.0863
K ₂₃₂	1.69±0.27	1.60±0.12	0.25 ns	0.6417
K ₂₇₀	0.11±0.01	0.06±0.00	27.84 **	0.0062
ΔK	-0.004±0.0002	-0.002±0.0005	3.77 ns	0.1242

^aLSD Least Significant Difference test F-values. **Significant at P>0.01; *Significant at P>0.05; ns—not significant (P<0.05).

^bMean±S.D.

Fatty acid composition of the oil (Table 5) depends on genotype and indicates a high content of oleic acid (average 73.03%), similar to previously obtained in Leccino (73,8%) by Lazovic *et al.* [34]. The contents of miristic, behenic, eueic and lignoceric acids were not reported, and the *trans* forms were absent in the oils of both specimen examined. Differences in oil of 'Old olive', especially the higher content of palmitic, margaric, margaroleic and linolenic acids, and lower content of palmitoleic stearic, oleic, linoleic, arachidic and gadoleic acids, were observed in comparison to the oil of cv. 'Žutica'. The differences found in fatty acids profile are important tools for varietal authentication. Fatty acids profiles could be used as fingerprints of olive varieties [38], processed table olives [32] and in olive oils as well [39, 40]. Therefore, the obtained data in the fatty acids profile of 'Old olive' and cv. 'Žutica' could be indicative that cv. 'Žutica' is not provenience from 'Old olive'. Results obtained of fatty acids analysis are in conformity to those defined by the more recent European Regulation [41].

Table 5. Fatty acids composition (g/100g of fatty acids) in olive oils from 'Old olive' and cv. 'Žutica' trees.

Fatty acids	'Old olive' tree	cv. 'Žutica'	LSD F-value ^a	P-value
Palmitic (C _{16:0})	13.62±1.75 ^b	12.61±0.77	1.70 ns	0.2191
Palmitoleic (C _{16:1})	1.07±0.11	1.19±0.30	1.01 ns	0.3371
Margaric (C _{17:0})	0.23±0.13	0.20±0.17	0.12 ns	0.7342
Margaroleic (C _{17:1})	0.26±0.19	0.21±0.15	0.28 ns	0.6079
Stearic (C _{18:0})	2.76±0.70	3.29±0.39	2.62 ns	0.1339
Oleic (C _{18:1})	72.85±1.39	73.21±0.51	0.35 ns	0.5655
Linoleic (C _{18:2})	7.96±0.66	8.10±1.32	0.06 ns	0.8178
Linolenic (C _{18:3})	0.76±0.17	0.54±0.09	7.42 *	0.0198
Arachidic (C _{20:0})	0.30±0.05	0.38±0.04	7.78 *	0.0176
Gadoleic (C _{20:1})	0.20±0.11	0.29±0.14	1.82 ns	0.2042
MUFA ^c	74.38±1.25	74.90±0.63	0.84 ns	0.3784
PUFA ^d	8.72±0.72	8.63±1.34	0.02 ns	0.8828
SFA ^e	16.91±1.28	16.47±0.82	0.52 ns	0.4876
<i>trans</i> fatty acids	n.d. ^f	n.d.		

^aLSD Least Significant Difference test F-values. **Significant at P>0.01; *Significant at P>0.05; ns—not significant (P<0.05).

^b Mean±S.D.

^c monounsaturated fatty acids

^d polyunsaturated fatty acids

^e saturated fatty acids

^f n.d. – not detected

Sterol fraction of olive oils from 'Old olive' and cv. 'Žutica' is presented in Table 6. β -sitosterol was the main sterol found (around 94%) in both olive oils studied. There was no evidence of brassicasterol content, 24-methyl cholesterol and Δ -7-campesterol. The content of campesterol was significantly higher than in the oil of 'Žutica'. Again, similarly to fatty acids profile, sterolic fraction can also be used as a chemical marker of olive varieties as observed by several studies [42,43], confirming that sterolic composition of oils varies on the variability of gene expression [44] and of good adaptation of the autochthonous accessions to the local environment [45].

Table 6. Composition of the sterolic fraction of olive oils from 'Old olive' and cv. 'Žutica' trees.

Sterols (%)	'Old olive' tree	cv. 'Žutica'	LSD F-value ^a	P-value
Cholesterol	0.3±0.09 ^b	0.3±0.05 ^a	0.01 ns	0.9315
Campesterol	3.4±0.17	3.1±0.10	8.42 *	0.0337
Campestanol	0.2±0.09	0.2±0.05	0.41 ns	0.5517
Stigmasterol	1.0±0.38	1.4±0.37	2.46 ns	0.1773
Δ -5,23-stigmastadienol	0.4±0.25	0.4±0.20	0.09 ns	0.7796
Clerosterol	1.0±0.08	1.0±0.05	0.73 ns	0.4319
β -sitosterol	81.8±1.58	81.5±0.45	0.09 ns	0.7737
Sitostanol	0.6±0.34	0.5±0.05	0.45 ns	0.5334
Δ -5-avenasterol	10.0±1.36	10.3±0.72	0.15 ns	0.7134
Δ -5,24-stigmastadienol	0.5±0.17	0.4±0.05	0.62 ns	0.4665
Δ -7-stigmastenol	0.2±0.05	0.2±0.05	0.10 ns	0.7663
Δ -7-avenasterol	0.4±0.08	0.5±0.05	3.08 ns	0.1395
β -sitosterol total (sum: 1-6)	94.2±0.55	94.1±0.68	0.14 ns	0.7275
Eritrodiol+Uvaol	1.9±0.25	1.8±0.44	0.16 ns	0.7125

^aLSD Least Significant Difference test F-values. **Significant at P>0.01; *Significant at P>0.05; ns—not significant (P<0.05).

^b Mean±S.D.

No difference in wax content (data not shown) among examined oils of 'Old olive' and cv. 'Žutica' was found (111 and 112 mg/kg, respectively). Higher wax content was reported by Piscopo *et al.* [44], with possible explanation of stronger response of examined samples to environmental factors as heat, humidity and irradiance, also

reported in hotter climates [46]. Total polyphenols (data not shown) were slightly higher in 'Old olive' (140 mg/kg) than in cv. 'Žutica' (120 mg/kg). Both the oils of 'Old olive' and cv. 'Žutica' have a relative low polyphenol content under the Bar climatic conditions, and much lower than in Leccino (195 mg/kg) [34]. Furthermore, since olives were collected at full maturation, low values of polyphenols are expected, since polyphenols decrease during olive maturation.

According to the sensory panel evaluation the flavour of 'Old olive' oils is characterized to have a distinct bitterness, which is also different from the oil of cv. 'Žutica', which is only slightly bitter. However, both oils are of good sensory properties.

Molecular analysis

The application of molecular tools in this research was to overcome the limitations of morphological analyses. Molecular analysis has also been able to identify cases of errors among accessions due to the mislabelling, propagation mistakes etc. [1]. Molecular identification of some ancient olive trees has demonstrated that they are remains of ancient olive cultivation and often represent unknown genotypes [47,8]. However, these trees should undergo an overall survey and a molecular together with agronomic evaluation. In our study, the molecular analysis with eleven SSR primers showed differences between 'Old olive' and cv. 'Žutica' (Table 7). The obtained DNA fragments were distinguished by primers DCA05, DCA09, DCA16. Also a single fragment difference was obtained in cv. 'Žutica' variety with DCA03, DCA18, EMO90, GAPU71B, and GAPU103A primers. Other primers gave the same fragments for both of the tested samples. After all, majority of primers applied distinguished samples analysed as two distant genotypes, confirming and emphasizing the differences showed with morphological and chemical analyses.

Table 7. Genotype profiles from 'Old olive' and cv. 'Žutica' trees obtained with 11 SSRs primers.

SSRs primer	'Old olive' tree	cv. 'Žutica'
DCA03	232-239	232-243
DCA05	220-220	202-202
DCA09	192-206	204-204
DCA14	177-189	177-189
DCA16	148-152	152-156
DCA18	179-179	171-179
EMO90	186-188	184-188
GAPU71B	124-124	124-127
GAPU 101	190-198	190-198
GAPU103A	178-178	178-182
UDO99-043	180-180	180-180

Taking into account the analysis presented in this paper, and additional parameters such as adaptation to environmental conditions, productivity, estimated age [3], and olive oil quality, 'Old olive' represent very interesting genotype that should be safeguarded as original mother plant, which can be propagated and conserve and evaluated in the collections and field plots.

CONCLUSION

The 'Old olive' is the oldest olive tree of numerous ancient olive trees in Montenegro. It represents valuable specimen in olive genetic resources and makes this small country comparable to the much larger countries. Ancient trees, as the 'Old olive',

represent the result of the centuries of experience in their productive behaviour or a high degree of environmental compatibility [47]. They are very useful for creating database/catalogue and to locate the hotspot areas of ancient olives in order to avoid losing forever this priceless olive germplasm [1]. Outstanding performance of the 'Old olive' makes its agronomic evaluation very useful with possibility to involve it in the breeding program in the future.

The molecular/SSR markers application was useful in proving the difference between 'Old olive' and cv. 'Žutica', showed to some extent with morphological and chemical analyses. Results clearly showed that treating 'Old olive' as of the same as cv. 'Žutica' was an example of homonymy. Thus, further analyses of the origin of the 'Old olive' and its relation to the local germplasm should be a future task. Important would be to continue research on uncharacterised olive samples from ancient trees which are still grown by the local farmers. This study also gives valuable data about the morphological and chemical characterization of 'Old olive' and cv. 'Žutica'. This is important for 'Old olive' which is a living memory of olive cultivation in Montenegro and the heritage that needs to be preserved, maintained and valorised at all levels.

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