

## FRUIT AND ENDOCARP PROPERTIES IN RELATION TO INTRA-VARIETAL MORPHOLOGICAL DIVERSITY OF MONTENEGRIN OLIVE VARIETY ‘ŽUTICA’

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### ABSTRACT

‘Žutica’ is the most widespread olive variety of Montenegro, accounting for as much as 98% of olive trees in the southern part of Montenegrin coastal area – Bar subarea. The primary purpose of variety ‘Žutica’ is olive oil production due to its small fruits and high oil content, although it is also much appreciated as a table olive, prepared in local ways as green and black. As ‘Žutica’ is an old olive variety there are some phenotypic differences recorded in the fruit properties. Here we evaluated 22 accessions from the area of Bar and Ulcinj, where this variety is very widespread (almost mono-varietal), for 34 parameters of the fruit and endocarp. Significant differences in fruit properties were observed in the accessions. Average fruit size in nine accessions was above 3.5 g and in two accessions more than 4.0 g. Number of fruits per kg ranged from 224 to 330. Cluster analysis and principal component analysis (PCA) were used to group the accessions and evaluate the morphological variability. The accessions clustered into two groups, with two off group accessions of the highest fruit weight. The results showed differences among individuals, especially for large fruit accessions that should be investigated further.

**Key words:** olive, ‘Žutica’, accessions, fruit characteristics, table olives

### INTRODUCTION

Olive (*Olea europaea* L.) is an important fruit species in Montenegro. It occupies a quarter of the total area under fruit plantations [Statistical Year Book of Montenegro 2012; Lazović et al. 2014]. It is not known when the olive was introduced to the Montenegrin coast and the Adriatic coast in general. However, by the age of olive plantations [Lazović et al. 2016] and traditional knowledge it is believed that Greeks, who had colonies along the Adriatic, planted the first olives. Through the millennia, olive trees were distributed further, mainly by using root suckers for propagation [Drecun 1956]. The oldest examples of ancient olive trees are ‘Stara Maslina’ (meaning Old Olive) near Bar

and ‘Velja maslina’ (Great Olive) in Budva, which are believed to be more than 2000 years old [Miranović 1994, 2006].

Favourable environmental conditions of the coastal area of Montenegro helped olives to spread and adapt, and this is reflected in the relatively large number of varieties that have evolved over their time in cultivation. According to the environmental conditions and existing olive assortment, the Montenegrin coast is divided into two sub-regions: Bar, which covers the municipalities in the south – Budva, Bar, and Ulcinj, and in the north the Boka Bay subarea including the Bay of Kotor area and the municipalities Tivat, Kotor and Herceg Novi.

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The olive variety 'Žutica' dominates, representing 65% of all varieties, compared with other cultivated varieties such as 'Crnica', 'Sitnica', 'Lumbardeska', etc. 'Žutica' has special importance in the sub-region of Bar where it is almost exclusively present (around 98%). 'Žutica' variety is described as having small [Miranović 1994, 2006] to medium-large fruits [Lazović 2001, Lazović et al. 2002a] and with olive oil contents higher than 20%, intended predominantly for olive oil production. Previous studies showed it to have consistent morphological and physical properties, without notable diversity suggesting the existence of different forms [Miranović 1994]. However, later studies with isoenzyme markers showed the possible existence of diversity within this variety [Lazović et al. 2000, 2002b]. Those results implied the need for additional monitoring of morphological traits in a larger number of 'Žutica' accessions.

Morphological and agronomic characteristics have been used in many countries [Cantini et al. 1999; Barranco et al. 2000, Rotondi et al. 2003, Trentacoste and Puertas 2011] to identify and characterize cultivated olives, as essential components in evaluating genetic material. A number of clones with different morphological and agronomic characteristics can be found within a single olive variety [Bellini et al. 2008], revealing intra-varietal polymorphism [Lopes et al. 2004]. Clonal vegetative propagation has been practiced in olives for several thousand years [García-Díaz et al. 2003], and it was almost the only way

for olive propagation in Montenegro [Drecun 1956]. Therefore, analyses of morphological and agronomic characteristics can contribute to assessing diversity in an old olive variety such as 'Žutica'.

This study assessed the degree of variability of the variety 'Žutica' in the southern part of Montenegro based on fruit and endocarp characteristics.

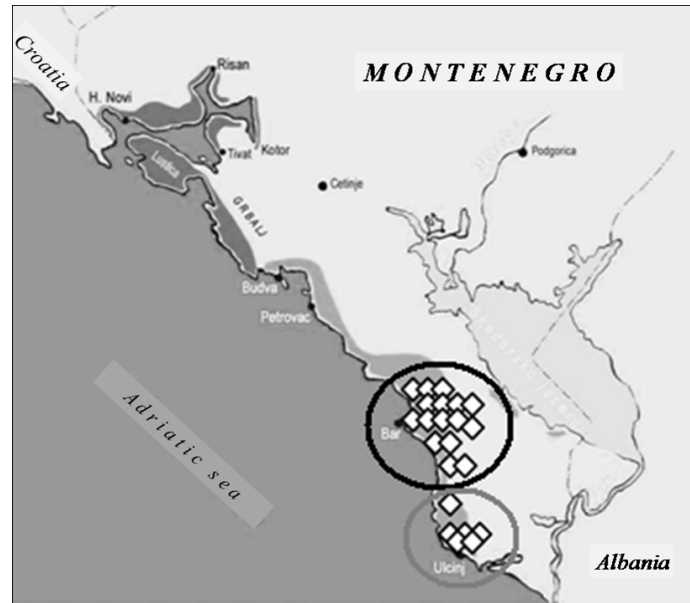
## MATERIALS AND METHODS

**Plant material.** During three consecutive years (2009–2011), 22 accessions inventoried in pure plantations of variety 'Žutica' in the southern part of Montenegro (Bar subarea) were studied. Accessions were labelled according to the locations. Plant material studied is listed in Table 1 and their locations identified in Figure 1, showing the accession name and the locality of distribution.

**Morphological characterization.** Morphological properties were measured during the period 2009–2011, according to the olive descriptor Barranco et al. [2000]. 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 variety 'Žutica'. 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 content in the fruit.

**Table 1.** Locations and codes of the variety 'Žutica' accessions from Bar and Ulcinj (grey) area

Location	Accession code	Location	Accession code
Kurilo	KUR	Dabanovo	DAB4
Bar	ZAVG	Sustaš	SUS1
Stari Bar	DM5	Sustaš	SUS2
Stari Bar	CSV1	Brinja	BRI1
Stari Bar	CSV2	Brinja	BRI2
Stari Bar	KAP1	Valdanos	VAL1
Stari Bar	KAP2	Valdanos	VAL2
Mirovica	STM1	Valdanos	VALL
Mirovica	STM2	Valdanos	VALD
Dabanovo	DAB2	Valdanos	VALVO
Dabanovo	DAB3	Kruče	STUL



**Fig. 1.** Olive growing areas in Montenegro, Bar (black oval) and Ulcinj (grey oval) showing the location of variety 'Žutica' accessions studied

A total of 34 characteristics of the fruit and endocarp were analysed, of which 14 were quantitative: 'length' (FL, EL), 'width' (FW, EW), 'shape index' of fruit (FI) and endocarp (EI), fruit (FWe) and endocarp 'weight' (EWe), 'fruit pulp weight' (FPW), 'fruit pulp percentage' (FPP), 'pulp/endocarp ratio' (P/E), 'fruits per kg' (FNo), 'water content' (FWC) and 'dry matter' (FDM), and 20 were qualitative, made up of 10 fruit characteristics: 'form' (FF-FL/FW), 'symmetry' (FSI), 'maximum diameter' (FMD), 'apex' (FA), 'base' (FB), 'nipple' (FN), 'presence of lenticels' (FPL), 'size of lenticels' (FSL), 'start of colour change' (FSC), 'colour at full maturity' (FCM), and 10 endocarp characteristics: 'form' (EF-EL/EW), 'symmetry A' (ESA), 'symmetry B' (ESB), 'maximum transversal diameter' (EMD), 'apex' (EA), 'base' (EB), 'surface' (ES), 'number of grooves' (ENG), 'groove distribution' (EGD) and 'apex termination' (EAT).

**Data analysis.** Means of each quantitative trait and accession was obtained based on three-year results, and subjected to one-way analysis of variance (ANOVA), testing for significance at the  $P < 0.05$

level. A pairwise comparison of means by least significant difference (LSD) was applied. The data were standardized and principal component analysis (PCA) was performed. Hierarchical cluster analysis was carried out using the unweighted pair group method using arithmetic average (UPGMA). A dendrogram was created using the squared Euclidean distance as the dissimilarity coefficient. All analyses were done using the software Statistica 5.0 (StatSoft Inc. Tulsa, USA).

## RESULTS AND DISCUSSION

**Bio-morphological evaluation of 'Žutica' accessions.** The use of morphological characteristics is the initial step in the description and classification of olive germplasm and biometric data should always be accompanied by detailed morphological descriptions [Barranco et al. 2000, Rotondi et al. 2003]. Fruit and stone traits are considered very efficient morphological parameters in distinguishing among cultivated olives [Cantini et al. 1999, Barranco et al. 2000, Belaj et al. 2011, Peres et al. 2011, Rotondi et al. 2011].

**Table 2.** Morphology of fruit and endocarp of 22 'Žutica' accessions (2009–2011)

Accession	FL <sup>(1)</sup> (cm)	FW (cm)	FI	EL (cm)	EW (cm)	EI
DAB2	2.03 <sup>fg (2)</sup>	1.65 <sup>ab</sup>	1.23 <sup>g</sup>	1.36 <sup>efgh</sup>	0.72 <sup>bc</sup>	1.88 <sup>cdef</sup>
DAB3	1.98 <sup>f</sup>	1.59 <sup>ab</sup>	1.25 <sup>fg</sup>	1.26 <sup>h</sup>	0.70 <sup>c</sup>	1.80 <sup>defg</sup>
DAB4	2.09 <sup>defg</sup>	1.60 <sup>ab</sup>	1.31 <sup>bcdefg</sup>	1.37 <sup>defgh</sup>	0.74 <sup>bc</sup>	1.86 <sup>cdef</sup>
SUS1	2.14 <sup>bcdefg</sup>	1.65 <sup>ab</sup>	1.30 <sup>defg</sup>	1.38 <sup>cdefgh</sup>	0.72 <sup>bc</sup>	1.92 <sup>abcd</sup>
SUS2	2.07 <sup>efg</sup>	1.61 <sup>ab</sup>	1.29 <sup>efg</sup>	1.34 <sup>gh</sup>	0.73 <sup>bc</sup>	1.83 <sup>cdefg</sup>
CSV1	2.03 <sup>fg</sup>	1.65 <sup>ab</sup>	1.23 <sup>g</sup>	1.27 <sup>gh</sup>	0.72 <sup>bc</sup>	1.76 <sup>fg</sup>
CSV2	2.07 <sup>efg</sup>	1.61 <sup>ab</sup>	1.29 <sup>efg</sup>	1.30 <sup>gh</sup>	0.72 <sup>bc</sup>	1.80 <sup>efg</sup>
DM5	2.34 <sup>ab</sup>	1.67 <sup>ab</sup>	1.40 <sup>ab</sup>	1.53 <sup>ab</sup>	0.75 <sup>bc</sup>	2.04 <sup>a</sup>
VAL1	2.33 <sup>abc</sup>	1.69 <sup>ab</sup>	1.38 <sup>abcd</sup>	1.51 <sup>abc</sup>	0.75 <sup>bc</sup>	2.01 <sup>ab</sup>
VAL2	2.41 <sup>a</sup>	1.67 <sup>ab</sup>	1.44 <sup>a</sup>	1.61 <sup>a</sup>	0.81 <sup>a</sup>	2.00 <sup>ab</sup>
VALD	2.22 <sup>abcdef</sup>	1.66 <sup>ab</sup>	1.34 <sup>bcde</sup>	1.38 <sup>defgh</sup>	0.75 <sup>bc</sup>	1.83 <sup>defg</sup>
VALL	2.20 <sup>abcdefg</sup>	1.65 <sup>ab</sup>	1.33 <sup>bcdef</sup>	1.35 <sup>gh</sup>	0.75 <sup>bc</sup>	1.79 <sup>efg</sup>
STUL	2.30 <sup>abcd</sup>	1.69 <sup>ab</sup>	1.36 <sup>abcde</sup>	1.50 <sup>abcd</sup>	0.77 <sup>ab</sup>	1.95 <sup>abc</sup>
VALVO	2.28 <sup>abcde</sup>	1.65 <sup>ab</sup>	1.38 <sup>abcd</sup>	1.49 <sup>abcd</sup>	0.79 <sup>ab</sup>	1.90 <sup>bcde</sup>
STM1	2.13 <sup>bcdefg</sup>	1.65 <sup>ab</sup>	1.29 <sup>defg</sup>	1.31 <sup>gh</sup>	0.76 <sup>b</sup>	1.74 <sup>g</sup>
STM2	2.34 <sup>ab</sup>	1.75 <sup>a</sup>	1.34 <sup>bcdef</sup>	1.48 <sup>abcde</sup>	0.81 <sup>a</sup>	1.83 <sup>cdefg</sup>
KAP1	2.04 <sup>fg</sup>	1.56 <sup>b</sup>	1.30 <sup>cdefg</sup>	1.36 <sup>efgh</sup>	0.75 <sup>bc</sup>	1.83 <sup>cdefg</sup>
KAP2	1.99 <sup>f</sup>	1.53 <sup>b</sup>	1.30 <sup>cdefg</sup>	1.31 <sup>gh</sup>	0.73 <sup>bc</sup>	1.80 <sup>efg</sup>
BRI1	2.11 <sup>cdefg</sup>	1.65 <sup>ab</sup>	1.28 <sup>efg</sup>	1.35 <sup>efgh</sup>	0.73 <sup>bc</sup>	1.84 <sup>cdefg</sup>
BRI2	2.18 <sup>bcdefg</sup>	1.66 <sup>ab</sup>	1.32 <sup>bcdefg</sup>	1.40 <sup>cdefg</sup>	0.75 <sup>bc</sup>	1.87 <sup>cdef</sup>
KUR	2.18 <sup>bcdefg</sup>	1.58 <sup>ab</sup>	1.38 <sup>abc</sup>	1.48 <sup>bcdef</sup>	0.79 <sup>ab</sup>	1.87 <sup>cdef</sup>
ZAVG	2.18 <sup>bcdefg</sup>	1.61 <sup>ab</sup>	1.36 <sup>bcde</sup>	1.34 <sup>gh</sup>	0.74 <sup>bc</sup>	1.82 <sup>cdefg</sup>
Average	2.17	1.64	1.32	1.39	0.75	1.86
F-value <sup>(3)</sup>	2.59**	0.62ns	3.11**	4.27**	1.63*	3.53**
p – value	0.0039	0.8791	0.0007	0.0000	0.0215	0.0002
LSD <sub>0.05</sub>	0.2229	0.1804	0.0901	0.1303	0.0710	0.1206

<sup>(1)</sup> Explanations of character symbols are given in 'Materials and methods'

<sup>(2)</sup> Values of traits marked with different letters are significant at the level  $P < 0.05$  (LSD test)

<sup>(3)</sup> F-values are highly significant (\*\*), significant (\*) or not significant (ns) ( $p < 0.05$ )

**Table 3.** Physical characteristics of fruit in 22 'Žutica' accessions (2009–2011)

Accession	FWe <sup>(1)</sup> (g)	FPW (g)	EWe (g)	FPP	P/E	FNo	FWC (%)	FDM (%)
DAB2	3.47 <sup>bc(2)</sup>	3.10 <sup>abc</sup>	0.37 <sup>def</sup>	89.33 <sup>a</sup>	8.37 <sup>a</sup>	288 <sup>abc</sup>	55.06 <sup>cd</sup>	44.94 <sup>ab</sup>
DAB3	3.08 <sup>c</sup>	2.74 <sup>c</sup>	0.34 <sup>f</sup>	89.07 <sup>ab</sup>	8.15 <sup>ab</sup>	325 <sup>a</sup>	55.93 <sup>abc</sup>	44.07 <sup>bcd</sup>
DAB4	3.16 <sup>c</sup>	2.77 <sup>c</sup>	0.39 <sup>bcdef</sup>	87.57 <sup>bc</sup>	7.05 <sup>bcd</sup>	316 <sup>a</sup>	59.58 <sup>abc</sup>	40.43 <sup>bcd</sup>
SUS1	3.47 <sup>bc</sup>	3.09 <sup>abc</sup>	0.38 <sup>bcdef</sup>	88.94 <sup>abc</sup>	8.04 <sup>abc</sup>	288 <sup>abc</sup>	57.69 <sup>abc</sup>	42.31 <sup>bcd</sup>
SUS2	3.11 <sup>c</sup>	2.73 <sup>c</sup>	0.38 <sup>bcdef</sup>	87.65 <sup>abc</sup>	7.10 <sup>bcd</sup>	322 <sup>a</sup>	55.69 <sup>abc</sup>	44.32 <sup>bcd</sup>
CSV1	3.18 <sup>c</sup>	2.82 <sup>c</sup>	0.36 <sup>ef</sup>	88.77 <sup>abc</sup>	7.91 <sup>abc</sup>	315 <sup>a</sup>	55.41 <sup>abcd</sup>	44.59 <sup>abcd</sup>
CSV2	3.16 <sup>c</sup>	2.76 <sup>c</sup>	0.41 <sup>bcdef</sup>	87.15 <sup>bc</sup>	6.78 <sup>cd</sup>	316 <sup>a</sup>	57.62 <sup>abc</sup>	42.39 <sup>bcd</sup>
DM5	3.64 <sup>abc</sup>	3.16 <sup>abc</sup>	0.48 <sup>abcd</sup>	86.85 <sup>c</sup>	6.60 <sup>cd</sup>	274 <sup>abc</sup>	58.44 <sup>abc</sup>	41.56 <sup>bcd</sup>
VAL1	3.68 <sup>abc</sup>	3.19 <sup>abc</sup>	0.48 <sup>abc</sup>	86.82 <sup>c</sup>	6.59 <sup>cd</sup>	272 <sup>abc</sup>	56.66 <sup>abc</sup>	43.34 <sup>bcd</sup>
VAL2	4.46 <sup>a</sup>	3.88 <sup>a</sup>	0.59 <sup>a</sup>	86.85 <sup>c</sup>	6.61 <sup>cd</sup>	224 <sup>c</sup>	57.08 <sup>abc</sup>	42.92 <sup>bcd</sup>
VALD	3.56 <sup>bc</sup>	3.11 <sup>abc</sup>	0.45 <sup>bcde</sup>	87.41 <sup>bc</sup>	6.94 <sup>cd</sup>	281 <sup>abc</sup>	58.59 <sup>abc</sup>	41.41 <sup>bcd</sup>
VALL	3.46 <sup>bc</sup>	3.04 <sup>bc</sup>	0.42 <sup>bcdef</sup>	87.83 <sup>abc</sup>	7.22 <sup>bc</sup>	289 <sup>abc</sup>	55.23 <sup>bcd</sup>	44.77 <sup>abc</sup>
STUL	3.91 <sup>abc</sup>	3.44 <sup>abc</sup>	0.47 <sup>bcd</sup>	88.00 <sup>abc</sup>	7.34 <sup>abc</sup>	256 <sup>abc</sup>	56.78 <sup>abc</sup>	43.22 <sup>bcd</sup>
VALVO	3.57 <sup>bc</sup>	3.11 <sup>abc</sup>	0.46 <sup>bcde</sup>	87.18 <sup>c</sup>	6.80 <sup>cd</sup>	280 <sup>abc</sup>	59.33 <sup>abc</sup>	40.67 <sup>bcd</sup>
STM1	3.56 <sup>bc</sup>	3.14 <sup>abc</sup>	0.42 <sup>bcdef</sup>	88.19 <sup>abc</sup>	7.47 <sup>abc</sup>	281 <sup>abc</sup>	59.12 <sup>abc</sup>	40.88 <sup>bcd</sup>
STM2	4.23 <sup>ab</sup>	3.74 <sup>ab</sup>	0.49 <sup>ab</sup>	88.34 <sup>abc</sup>	7.57 <sup>abc</sup>	236 <sup>bc</sup>	61.31 <sup>a</sup>	38.69 <sup>d</sup>
KAP1	3.38 <sup>bc</sup>	2.96 <sup>bc</sup>	0.42 <sup>bcdef</sup>	87.70 <sup>abc</sup>	7.13 <sup>bcd</sup>	296 <sup>abc</sup>	58.95 <sup>abc</sup>	41.05 <sup>bcd</sup>
KAP2	3.03 <sup>c</sup>	2.66 <sup>c</sup>	0.38 <sup>cdef</sup>	87.62 <sup>abc</sup>	7.08 <sup>bcd</sup>	330 <sup>a</sup>	55.50 <sup>abcd</sup>	44.50 <sup>abcd</sup>
BRI1	3.23 <sup>c</sup>	2.85 <sup>c</sup>	0.38 <sup>cdef</sup>	88.24 <sup>abc</sup>	7.50 <sup>abc</sup>	310 <sup>ab</sup>	49.72 <sup>d</sup>	50.29 <sup>a</sup>
BRI2	3.40 <sup>bc</sup>	2.98 <sup>bc</sup>	0.43 <sup>bcdef</sup>	87.50 <sup>bc</sup>	7.00 <sup>bcd</sup>	294 <sup>abc</sup>	53.77 <sup>cd</sup>	46.23 <sup>ab</sup>
KUR	3.61 <sup>abc</sup>	3.13 <sup>abc</sup>	0.49 <sup>abc</sup>	86.57 <sup>d</sup>	6.44 <sup>d</sup>	277 <sup>abc</sup>	61.11 <sup>ab</sup>	38.89 <sup>cd</sup>
ZAVG	3.17 <sup>c</sup>	2.78 <sup>c</sup>	0.40 <sup>bcdef</sup>	87.54 <sup>bc</sup>	7.03 <sup>bcd</sup>	315 <sup>ab</sup>	56.09 <sup>abc</sup>	43.91 <sup>bcd</sup>
Average	3.48	3.05	0.43	87.76	7.17	288	57.03	42.97
F-value <sup>(3)</sup>	1.38*	1.19*	2.21**	1.23*	1.19*	1.06*	1.10*	1.10*
p - value	0.0390	0.0305	0.0135	0.0412	0.0485	0.0425	0.0396	0.0396
LSD <sub>0,05</sub>	0.8825	0.8193	0.1109	2.5940	1.7351	81.866	5.9489	5.9489

<sup>(1)</sup> Explanations of character symbols are given in 'Materials and methods'

<sup>(2)</sup> Values of traits marked with different letters are significant at the level  $P < 0.05$  (LSD test)

<sup>(3)</sup> F-values are highly significant (\*\*), significant (\*) or not significant (ns) ( $p < 0.05$ )

Analyses of all fruit and endocarp traits of the 22 'Žutica' accessions (tab. 2) showed significant differences for all traits except FW. Accession VAL2 had the highest FL, FI, EL and EW. Means for FI and EI ranged from 1.23 (DAB2 and CSV1) to 1.44 (VAL2), and from 1.74 (STM1) to 2.04 (DM5) respectively, dividing the accessions into two groups for both fruit and endocarp shape.

Based on FI variation among accessions, two categories of fruit, round and oval, were distinguished. The corresponding endocarp trait EI is less influenced by the environment and is used in pomologic classification [Roselli et al. 2002; Cantini et al. 2008; Trentacoste and Puertas 2011, Trujillo et al. 2013]. Oval and elliptic shapes were recognised.

The physical traits (tab. 3) showed significant differences amongst accessions, especially for parameters FWe, FPW, FNo, FDM, FWC, FPP and P/E, and differences for EWe were highly significant. The fruit characteristics, especially the fruit pulp, indicated not only the high diversity amongst 'Žutica' accessions, but also the impact of the year of sampling within each accession, which was found in earlier research on the two-year cycle of olive bearing [Lavee and Wonder 2004, Trentacoste et al. 2010, Trentacoste and Puertas 2011].

Average FWe and FPW for the accessions were 3.48 g and 3.05 g, with VAL2 having the highest and KAP2 the lowest values. The EWe was on average 0.43 g, being smallest in accession DAB3, which together with DAB2 had the highest FPP. The FDM was on average 43%, being highest in accession BRI1 and lowest in STM2.

Amongst the 20 qualitative parameters studied (listed in 'Materials and Methods', data not shown due to limited space), nine traits (FSI, FMD, FA, FN, FSC, EMD, EA, EGD and EAT) were monomorphic, showing no differences amongst accessions. However, eleven traits, five fruit-related (FF, FB, FPL, FS, FCM) and six endocarp-related (EF, ESA, ESB, EB, ES, ENG) showed significant differences among accessions. Only one accession (BRI2) differed in FCM from the others, while for EF and ESA the accessions were differentiated as follows: ovoid (5) :

elliptic (17), and symmetric (9) : slightly asymmetric (13), respectively.

**Principal component analysis.** The most significant variables in the study were identified with Analysis of Principal component (PCA) (tab. 4). Characterization of the material using multivariate analyses (PCA and cluster analysis) enabled us to gain an overall view of variability amongst the accessions based on the complete range of traits. PCA has been used in the evaluation of olive germplasm [Cantini et al. 1999, Trentacoste and Puertas 2011] and in analysis of olive genetic diversity [Bandelj et al. 2002, Hosseini-Mazinani et al. 2004, Hannachi et al. 2008, Uylaşer et al. 2008, Strikic et al. 2009, Zaher et al. 2011]. The PCA used in our work showed that more than 91% of observed variability was explained with the first three components, similar to the results reported by Cantini et al. [1999] for olive accessions in the germplasm collection in Italy.

Eigenvalues of the first three axes of the principal components accounted for 62.28, 16.56 and 12.34 i.e. 91.17% of the total variability observed. The largest portion in variability corresponded to nine traits of the first principal component PC1 (FL, FI, EL, EW, EI, FWe, FPW, EWe, FNo). The highest contribution to PC2 corresponded to fruit FW, FPP and P/E, and on PC3 the highest contribution was of FWC and FDM. Results obtained agreed with previous PCA of morphological characters in olive accessions grown in different olive areas [Cantini et al. 1999, Lavee and Wonder 2004, Taamalli et al. 2006, Ozkaya et al. 2006,; Trentacoste et al. 2010, Zaher et al. 2011]. Fruit size and endocarp morphology are the products of complex genetic and environmental parameters [Strikic et al. 2009].

The correlation matrix of variables obtained from PCA showed a negative correlation of dry matter content with FWC. Also, FPW was negatively correlated with variables related with the endocarp (EL, EW, EI, EWe) and fruit dimensions (FL, FW, FI, FWe), but positively with fruit pulp percentage (FPP) and fruit ratio and P/E.

Table 4 also shows the first three principal factor loadings in 22 'Žutica' accessions. Accessions VAL2

**Table 4.** The first three principal components (PC), and contributions to the total variation (%) in 22 'Žutica' accessions

Trait	Component loadings			Accession	Component loadings		
	PC1	PC2	PC3		PC1	PC2	PC3
FL	-0.94	—	—	DAB2	0.35	-1.91	-0.66
FW	-0.58	0.65	—	DAB3	-0.99	-1.39	—
FI	-0.89	-0.33	—	DAB4	-1.01	0.33	0.96
EL	-0.95	—	—	SUS1	0.34	-1.25	—
EW	-0.89	—	—	SUS2	-0.93	—	-0.42
EI	-0.68	—	-0.44	CSV1	-0.53	-1.45	—
FWe	-0.91	0.36	—	CSV2	-1.18	0.47	0.37
FPW	-0.88	0.43	—	DM5	0.65	1.38	—
EWe	-0.98	—	—	VAL1	0.79	1.25	-0.84
FPP	0.58	0.75	—	VAL2	2.22	1.22	-0.33
P/E	0.57	0.76	—	VALD	—	—	0.63
FNo	0.91	-0.36	—	VALL	—	—	-0.46
FWC	-0.50	-0.30	0.79	STUL	1.42	—	-0.39
FDM	0.50	0.30	-0.79	VALVO	0.33	0.80	0.68
Eigenvalue	8.72	2.32	1.73	STM1	—	-0.99	1.25
% Var.	62.28	16.56	12.34	STM2	2.03	-1.35	1.71
% Cum.	62.28	78.84	91.17	KAP1	-0.82	—	1.03
				KAP2	-1.63	0.53	—
				BRI1	—	—	-2.65
				BRI2	—	0.40	-1.30
				KUR	-0.31	1.42	1.65
				ZAVG	-0.79	0.60	-0.32

and STM2 had the highest positive values on PC1, related to the highest fruit and endocarp values and the highest negative scores for number of fruits/kg.

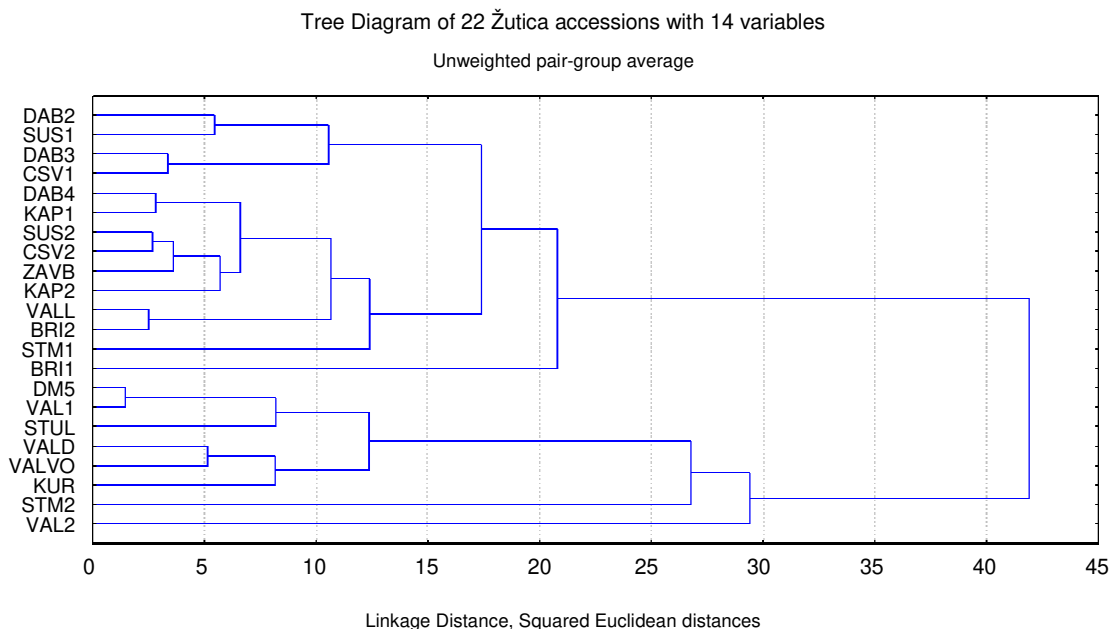
The data confirmed the suitability of multivariate analysis as a basis for further selection on a wider range of individuals. There was no grouping of 'Žutica' individuals according to the location, as was observed in studies of the varieties 'Zard' and 'Rowghani' [Hosseini-Mazinani et al. 2004] and 'Oblica' [Strikic et al. 2009].

In general, PCA may help the selection of accessions with better fruit quality traits. Those traits are also important in breeding, so more attention should be paid to them during further evaluations. Fruit traits are especially important as they have high heritability [Arias-Calderon et al. 2014], and endocarp properties are the least dependent on environmental conditions [Barranco et al. 2000]. This should be taken into account in further characterization of olive genetic resources in Montenegro.

**Cluster analysis.** To determine the hierarchical similarity among clones, a dendrogram was created

from UPGMA cluster analysis using 14 biomorphological traits selected in the PCA (tab. 4), presented in Figure 2. Using the value 21 as the standardized maximum distance for separation of groups, two main clusters were distinguished with smaller grouping within the clusters. The two groups showed dissimilarity between 26 and 42. Two 'Žutica' accessions (VAL2 and STM2) showed morphological differences which separated them from the rest of the individuals. These accessions were not included in clusters with others, as they were distant in terms of the highest fruit and endocarp traits and number of fruits per kg.

The first cluster consisted of thirteen accessions with one, BRI1, separated but connected to the group and characterised by the highest dry matter content (50.3%). The second cluster was composed of six accessions, of which four were from Ulcinj, suggesting a geographic connection to some extent. This cluster was connected with accession DM5 from Stari Bar, suggesting a clonal relation, also closely related to VAL1, while VALL was linked with BRI2 in the first cluster.



**Fig. 2.** UPGMA dendrogram obtained by comparison of 14 biopomological characteristics



The second cluster linked the accessions with higher values of FWe, EWe and FPW, while the first cluster contained individuals with higher pulp ratio, P/E and FNo.

Morphological variability among 'Žutica' accessions is not surprising having in mind the long period of cultivation, which supports previous studies of clones within predominant olive cultivars such as 'Picholine marocaine' [Zaher et al. 2011], and 'Oblica' [Strikic et al. 2009].

**Agronomic properties of 'Žutica' accessions.** Here we have demonstrated the presence of morphological variability within accessions of the variety 'Žutica' (tabs 2, 3 and 4). The most important variability occurred in fruit properties, indicating that characteristics of the fruit (FWe) for some 'Žutica' accessions are comparable to those obtained for foreign varieties grown under the conditions of the Montenegrin coast [Lazović 2001, Lazović et al. 2002a]. Accessions distinguished with high FWe, FPW, FPP and P/E ratio are important for further selection and good potential for producing table olives.

Table olives play an important role in the local diet, although Montenegro imports pickled olives from Greece, Italy and Spain. The variety 'Žutica' is appreciated for consumption prepared in local traditional ways. The quality classification of table olives is according to their size, represented as the number of fruits per kilogram, as well as pulp/endocarp ratio and total dry matter [Uyulaşer et al. 2008]. Therefore, the use of certain varieties for consumption depends on their agronomic and technological properties such as the fruit form, fruit ratio, texture, etc. [Rejano and Garrido, 2006].

The quality parameters presented in Table 4 showed significant variation, especially in the FWe, EWe and FDM. In this study, no accession had a fruit weight below 3 g, and in two accessions it was above 4 g (VAL2 and STM2). The fruit weight of these accessions is comparable with the average obtained for varieties Itrana in Ulcinjsko polje [Lazović 2001] and Manzanilla and Leccino in the conditions of Bar [Lazović et al. 2002a].

Significant differences were determined amongst the 22 accession for the parameters important for

table olives, such as fruit ratio (P/E – pulp/endocarp), the average number of fruits per kilogram (from 224 to 330), and moisture and dry matter content. No variation was found in fruit number per kg based on location that was also reported for the Turkish variety 'Gemlik' [Uyulaşer et al. 2008].

## CONCLUSION

Our analyses of fruit properties of 22 accessions of 'Žutica' olive variety clearly showed high variability in morphological characteristics. The accessions with fruit weight above 4.0 g (VAL2 and STM2) and even those over 3.5 g could be the subject of further studies on suitable agronomic traits. The differences expressed, especially in two off-group clustered accessions could be an example of homonymy. Olive variety 'Žutica' represents 98% of the olive material in the Bar sub-region, making the varietal characterization complex, but which is also important for producers to provide a unique product. The distance between variety 'Žutica' accessions suggests the existence of a certain level of intra-cultivar variation that should be further investigated with genetic markers. The intra-varietal classification allows access to unexplored sources of variability, which could be a reservoir of useful characters not yet found in other local cultivars.

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## REFERENCES

- Arias-Calderon, R., Rouiss, H., Rodríguez-Jurado, D., De la Rosa, R., León, L. (2014). Variability and heritability of fruit characters in olive progenies from open-pollination. *Sci. Hortic.*, 169, 94–98.
- Bandelj, D., Jakše, J., Javornik, B. (2002). DNA fingerprinting of olive varieties by microsatellite markers. *Food Technol. Biotech.*, 40(3), 185–190.

- Barranco, D., Cimato, A., Fiorino, P., Rallo, L., Touzani, A., Castañeda, C., Serafini, F., Trujillo, I. (2000). World catalogue of olive varieties. Consejo Oleícola Internacional, Madrid, pp. 360.
- Belaj, A., León, L., Satovic, Z., De La Rosa, R. (2011). Variability of wild olive (*Olea europaea* subsp. *europaea* var. *sylvestris*) analyzed by agro-morphological traits and SSR markers. *Sci. Hortic.*, 129, 561–569.
- Bellini, E., Giordani, E., Rosati, A. (2008). Genetic improvement of olive from clonal selection to cross-breeding programs. *Adv. Hortic. Sci.*, 22(2), 73–86.
- Cantini, C., Cimato, A., Graziano, S. (1999). Morphological evaluation of olive germplasm present in Tuscany region. *Euphytica*, 109, 173–181.
- Cantini, C., Cimato, A., Autino, A., Redi, A., Cresti, M. (2008). Assessment of the Tuscan olive germplasm by microsatellite markers reveals genetic identities and different discrimination capacity among and within cultivars. *J. Amer. Soc. Hortic. Sci.*, 133(4), 598–604.
- Drecun, V. (1956). Stanje i problematika maslinarstva na Crnogorskom primorju. *Poljoprivreda i šumarstvo/ Agricult. Forest.*, 2(2), 91–100.
- Garcia-Diaz, A., Oya, R., Sanchez, A., Luque, F. (2003). Effect of prolonged vegetative reproduction of olive tree cultivars (*Olea europaea* L.) in mitochondrial homoplasm and heteroplasm. *Genome*, 46, 377–381.
- Hannachi, H., Breton, C., Msallem, M., El Hadj, S.B., El Gazzah, M., Bervillé, A. (2008). Differences between native and introduced olive cultivars as revealed by morphology of drupes, oil composition and SSR polymorphisms: a case study in Tunisia. *Sci. Hortic.*, 116, 280–290.
- Hosseini-Mazinani, S.M., Mohammadreza Samaee, S., Sadeghi, H., Caballero, J.M. (2004). Evaluation of olive germplasm in Iran on the basis of morphological traits: Assessment of 'Zard' and 'Rowghani' cultivars. *Acta Hortic.*, 634, 145–151.
- Lavee, S., Wonder, M. (2004). The effect of yield, harvest time and fruit size on the oil content in fruits of irrigated olive trees (*Olea europaea*), cvs. Barnea and Manzanillo. *Sci. Hortic.*, 99, 267–277.
- Lazović, B. (2001). Osobine ploda nekih sorti masline (*Olea europaea* L.). *Poljoprivreda i šumarstvo/Agricult. Forest.*, 47(3–4), 15–25.
- Lazović, B., Adakalić, M., Perović, T. (2014). Olive growing in Montenegro – current state and perspectives. 6<sup>th</sup> Meeting of the IOBC/wprs WG Integrated Protection of Olive Crops, Bečići Montenegro, 12–15. May 2013. *IOBC-WPRS Bull.*, 108, 3–11.
- Lazović, B., Adakalić, M., Pucci, C., Perović, T., Bandelj, D., Belaj, A., Mariotti, R., Baldoni, L. (2016). Characterizing ancient and local olive germplasm from Montenegro. *Sci. Hortic.*, 209, 117–123.
- Lazović, B., Boskovic, R., James, C., Tobutt, K.R., Gasic, K. (2000). Izoenzimski polimorfizam u masline (*Olea europaea* L.). *Jugosl. Vocar.*, 34, 129–130.
- Lazović, B., Boskovic, R., James, C., Tobutt, K.R., Gasic, K. (2002b). Genetic diversity of olives grown along the Coast of Montenegro. *Acta Hortic.*, 586, 167–170.
- Lazović, B., Perović, T., Vuletić, V., Masoničić-Šotunova, R. (2002a). Karakteristike ploda introdukovanih sorti masline u uslovima Bara. *Poljoprivreda i šumarstvo/Agricult. Forest.*, 48(3–4), 43–51.
- Lopes, M.S., Mendonca, D., Sefc, K.M., Gil, F.S., Camara Machado, A. (2004). Genetic evidence of intra-cultivar variability within Iberian olive cultivars. *HortScience*, 39(7), 1562–1565.
- Miranović, K. (1994). Investigation of elayographic properties of the olive cultivar Žutica (*Olea europaea* L.). *Acta Hortic.*, 356, 74–77.
- Miranović, K. (2006). *Maslina (Olea europaea L.)*. Pobjeda, Podgorica, 498 pp.
- Ozkaya, M.T., Cakir, E., Gokbayrak, Z., Ercan, H., Taskin, N. (2006). Morphological and molecular characterization of Derik Halhali olive (*Olea europaea* L.) accessions grown in Derik–Mardin province of Turkey. *Sci. Hortic.*, 108, 205–209.
- Peres, A.M., Baptista, P., Malheiro, R., Dias, L.G., Bento, A., Pereira, J.A. (2011). Chemometric classification of several olive cultivars from Trás-os-Montes region (northeast of Portugal) using artificial neural networks. *Chemometr. Intell. Lab.*, 105, 65–73.
- Rejano, L., Garrido, A. (2006). El aderezo de las aceitunas. In: *El cultivo del olivo*. 4<sup>th</sup> ed. Barranco, D, Fernandez-Escobar, R, Rallo, L. (eds). Junta de Andalucía, Madrid, www.mundiprensa.com.
- Roselli, G., Petruccelli, R., Polsinelli, L., Cavalieri, D. (2002). Variability in the five Tuscan olive cultivars (*Olea europaea* L.). *J. Genet. Breed.*, 56, 61–60.
- Rotondi, A., Cultrera, N.G.M., Mariotti, R., Baldoni, L. (2011). Genotyping and evaluation of local olive varieties of a climatically disfavoured region through molecular, morphological and oil quality parameters. *Sci. Hortic.*, 130, 562–569.
- Rotondi, A., Magli, M., Ricciolini, C., Baldoni, L. (2003). Morphological and molecular analyses for the charac-

- terization of a group of Italian olive cultivars. *Euphytica*, 132, 129–137.
- Statistical Year Book of Montenegro (2012). Monstat.
- Strikić, F., Bandelj Mavsar, D., Perica, S., Cmelik, Z., Satović, Z., Javornik, B. (2009). The main Croatian olive cultivar, 'Oblica', shows high morphological but low molecular diversity. *J. Hortic. Sci. Biotech.*, 84(3), 345–349.
- Taamalli, W., Geuna, F., Banfi, R., Bassi, D., Daoud, D., Zarrouk, M. (2006). Agronomic and molecular analyses for the characterisation of accessions in Tunisian olive germplasm collections. *Electron. J. Biotechnol.*, 9(5), 467–481.
- Trentacoste, E.R., Puertas, C.M. (2011). Preliminary characterization and morpho-agronomic evaluation of the olive germplasm collection of the Mendoza province (Argentina). *Euphytica*, 177, 99–109.
- Trentacoste, E.R., Puertas, C.M., Sandras, V.O. (2010). Effect of fruit load on oil yield components and dynamics of fruit growth and oil accumulation in olive (*Olea europaea* L.). *Eur. J. Agron.*, 32, 249–254.
- Trujillo, I., Ojeda, M.A., Urdiroz, N.M., Potter, D., Barranco, D., Rallo, L., Diez, C.M. (2013). Identification of the Worldwide Olive Germplasm Bank of Córdoba (Spain) using SSR and morphological markers. *Tree Genet. Genomes.*, 10(1), 141–155.
- Uyulaşer, V., Tamer, C.E., Incedayi, B., Vural, H., Çopur, Ö.U. (2008). The quantitative analysis of some quality criteria of Gemlik variety olives. *J. Food Agric. Environ.*, 6 (3–4), 26–30.
- Zaher, H., Boulouha, B., Baaziz, M., Sikaoui, L., Gaboun, F., Udupa, S.M. (2011). Morphological and genetic diversity in olive (*Olea europaea* subsp. *europaea* L.) clones and varieties. *Plant Omics J.*, 4(7), 370–376.