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## Mirjana ADAKALIĆ, Biljana LAZOVIĆ, Tatjana PEROVIĆ, Miroslav ČIZMOVIĆ<sup>1</sup>

### VARIABILITY OF BIO-POMOLOGICAL PROPERTIES OF OLIVE VARIETY CRNICA IN MONTENEGRO

#### SUMMARY

Crnica is the second important olive variety on Montenegrin Coast, after variety Žutica. It is the most present in the area of Boka-kotorska sub region, and in lesser extent in the area of Budva - sub region of Bar. Crnica variety is for dual purposes of the fruit, for table use and for oil production. Long period of cultivation and potential mixing with other genotypes and unsystematic selection resulted in differences that are observed in the phenotypic properties of individuals within this variety. The intensity of differences was investigated in eleven Crnica individuals analyzing 22 biological and pomological parameters. Significant variability between individuals was shown. Analyzed accessions were distinguished based on fruit size, oil content in the fruit, etc. Results obtained in this study showed the presence of the clones with bigger fruit and therefore more suitable for table fruit processing and clones with higher oil content that are the potential for olive oil production improvement respectively. Results also suggest the need for further research within this variety.

Keywords: variability, clone, olive, bio-pomological characteristics

### **INTRODUCTION**

Crnica (synonym Crnjaka) is a domestic olive variety that is grown in the area of Budva and in sub region of Boka-kotorska with presence of around 30% (Miranović, 1978). Its total presence in Montenegrin olive assortment is around 15%. Crnica was described as variety of medium vigor, adapted to the poor soils, medium productive and alternate. The name comes from the color of the fruit which turn black during ripening (Miranović, 2006; Lazović and Adakalić 2012a).

Crnica (Crnjaka) is old variety cultivated long period together with other autochthonous and domesticated olive varieties in the area of Montenegrin Coast. During this period the variety was spread mainly by vegetative parts of the mother plants. Potentially it was mixed with a rich olive assortment of Bokakotorska sub area.

In order to examine the autochthonous olive assortment larger number of trees of different local varieties were marked. The idea was to examine the level of variability of morphological and pomological level already recognized in old

<sup>&</sup>lt;sup>1</sup> Mirjana Adakalić (corresponding author: adakalic@yahoo.com), Biljana Lazović, Tatjana Perović, Miroslav Čizmović, University of Montenegro, Biotechnical faculty-Podgorica, Centre for Subtropical Cultures-Bar, Montenegro

varieties (Lavee *et al.*, 1999; Lazović *et al.*, 2002; Strikić *et al.*, 2007). Additionally, Crnica is the cultivar for dual fruit purposes, oil and table use, presenting the potential to solve the lack of table varieties in the Montenegrin olive assortment.

The aim of this study was to test biological and pomological characteristics of chosen individuals/clones of Crnica variety. With use of statistical programs the existence and intensity of the variability of traits will be recognized, and be a guideline for selection and further research within this variety.

#### MATERIAL AND METHODS

During three-year period (2009 – 2011) eleven accessions/clones of Crnica variety from: Budva (2BD and 11BD), Bar (9BR), Grbalj (1GR, 3GR and 10GR), Herceg Novi (4HN, 5HN and 7HN) and Luštica (6LU and 8LU) were studied.

The morphological properties of leaf (length-LL, width-LW and index of shape-LI), internodes (length-INT), inflorescence (length-IL, number of flowers in inflorescence-IF, compactness of inflorescence-CI and percentage of aborted flowers-AF), fruit (length-FL, width-FW, index of shape-FI, weight-FWe, flesh ratio-FR and fruit ratio-F/E) and endocarp (length-EL, width-EW, index of shape-EI and weight-EWe) were analyzed according to the descriptor of Barranco *et al.* (2000). In addition, parameters on moisture content (FM), dry matter (DM), oil content in fresh (OFM) and in dry matter (ODM), were determined.

To determine the significance of differences observed in studied parameters among the accessions LSD0.05 test was applied using Statistix 7.0 program. Hierarchical cluster analysis was performed, data standardized and dendrogram constructed using the unwieghted pair-group average method (UPGMA) with the squared Euclidean distance in Statistika 5.0 program.

### **RESULTS AND DISCUSSION**

Morphological characteristics of leaves, internodes and inflorescence are presented in Table 1. Morphological characterization is a classic method which continues to be the first step for the description and classification of olive germplasm. Statistical analyzes of variance and cluster analysis can be very helpful in assessing the variability within varieties (Cantini *et al.*, 1999). In this analysis, 22 morphological characteristics of leaves, internodes, flowers, fruit and endocarp showed different significance in distinguishing the tested accessions of olive variety Crnica. High significant variation in the average values for the length and width of the leaf was recorded, while the other differences observed on characteristics of leaves, internodes and inflorescences were no statistically significant according to analysis of variance.

All of 11 studied accessions have a leaf of medium length (5-7 cm) and width (1-1.5 cm), elliptical - lancelet shape (4-6) and internodes of medium length (1-3 cm). There were two types of inflorescence, short (< 2.5 cm) in six

accessions (2BD, 4HN, 7HN, 8LU, 10GR and11BD) to medium length (2.5-3.5 cm) in five accessions (1GR, 3GR, 5HN, 6LU and 9BR) and with a small to medium number of flowers in six accessions (2BD, 5HN, 8LU, 9BR, 10GR and 11BD) and five accessions (1GR, 3GR, 4HN, 6LU and 7HN), respectively. Only clones 4HN and 7HN have short inflorescences with medium number of flowers. Average percentage of imperfect flowers in bloom was from 30.31% in accession 6LU to 75.91% in 11BD, showing different productive potential. According to the descriptor (Baranco *et al.*, 2000), percentage of imperfect flowers in bloom had medium value (20-60%) in most accessions, while in three accessions (2BD, 9BR and 11BD) this parameter was high (> 60%). The variability of this parameter indicated by high coefficient of variation may be related to the age of the plants or climatic conditions. It can also be related to the degree of flowering as well as the position of the flowers on twig (Lombardo *et al.*, 2006).

Table 1: Characteristics of leaf, internodes and inflorescence of Crnica accessions

| Accession              | LL                     | LW                 | LI                 | INT                | IL                  | IF                  | CI                  | AF                  |
|------------------------|------------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|
|                        | (cm)                   | (cm)               | (LL/LW)            | (cm)               | (cm)                |                     | (IF/IL)             | (%)                 |
| 1GR                    | 5,95 <sup>bc (1)</sup> | 1,29 <sup>a</sup>  | 4,60 <sup>c</sup>  | 1,82 <sup>ab</sup> | 2,63 <sup>abc</sup> | 18,86 <sup>ab</sup> | 7,17 <sup>bc</sup>  | 54,98 <sup>ab</sup> |
| 2BD                    | 5,83 <sup>bc</sup>     | 1,13 <sup>bc</sup> | 5,14 <sup>bc</sup> | 1,41 <sup>c</sup>  | 2,28 <sup>cd</sup>  | 16,58 <sup>b</sup>  | 7,27 <sup>bc</sup>  | 74,82 <sup>a</sup>  |
| 3GR                    | 5,90 <sup>bc</sup>     | 1,15 <sup>bc</sup> | 5,14 <sup>bc</sup> | 1,59 <sup>bc</sup> | 2,91 <sup>a</sup>   | 19,68 <sup>ab</sup> | 6,77 <sup>bc</sup>  | 44,84 <sup>bc</sup> |
| 4HN                    | 5,79 <sup>bcd</sup>    | 1,15 <sup>bc</sup> | 5,05 <sup>bc</sup> | 1,46 <sup>c</sup>  | 2,47 <sup>bcd</sup> | 20,96 <sup>a</sup>  | 8,48 <sup>a</sup>   | 57,87 <sup>ab</sup> |
| 5HN                    | 6,01 <sup>bc</sup>     | 1,14 <sup>bc</sup> | 5,26 <sup>ab</sup> | 1,62 <sup>bc</sup> | 2,66 <sup>abc</sup> | 16,72 <sup>b</sup>  | 6,29 <sup>c</sup>   | 45,26 <sup>bc</sup> |
| 6LU                    | 6,27 <sup>b</sup>      | $1,09^{bc}$        | $5,77^{a}$         | 1,36 <sup>c</sup>  | 2,61 <sup>abc</sup> | 19,19 <sup>ab</sup> | 7,35 <sup>abc</sup> | 30,31 <sup>c</sup>  |
| 7HN                    | 6,89 <sup>a</sup>      | 1,29 <sup>a</sup>  | 5,33 <sup>ab</sup> | 1,67 <sup>bc</sup> | 2,46 <sup>bcd</sup> | 18,86 <sup>ab</sup> | 7,67 <sup>ab</sup>  | $40,05^{bc}$        |
| 8LU                    | 5,99 <sup>bc</sup>     | 1,14 <sup>bc</sup> | 5,23 <sup>ab</sup> | 1,56 <sup>bc</sup> | 2,36 <sup>bcd</sup> | 16,10 <sup>b</sup>  | 6,82 <sup>bc</sup>  | 44,41 <sup>bc</sup> |
| 9BR                    | 5,76 <sup>cd</sup>     | 1,15 <sup>b</sup>  | 4,99 <sup>bc</sup> | 1,52 <sup>bc</sup> | 2,75 <sup>ab</sup>  | 17,36 <sup>ab</sup> | 6,31°               | 62,18 <sup>ab</sup> |
| 10GR                   | 6,12 <sup>bc</sup>     | 1,32 <sup>a</sup>  | 4,63 <sup>c</sup>  | 2,10 <sup>a</sup>  | $2,08^{d}$          | 16,01 <sup>b</sup>  | $7,70^{ab}$         | 42,79 <sup>bc</sup> |
| 11BD                   | 5,34 <sup>d</sup>      | 1,04 <sup>c</sup>  | 5,14 <sup>bc</sup> | 1,46 <sup>c</sup>  | 2,39 <sup>bcd</sup> | 16,40 <sup>b</sup>  | 6,88 <sup>bc</sup>  | 75,91 <sup>a</sup>  |
| Average                | 5,99                   | 1,17               | 5,10               | 1,60               | 2,51                | 17,88               | 7,13                | 51,80               |
| P-value <sup>(2)</sup> | 0,0032**               | 0,0071**           | 0,1160ns           | 0,0828ns           | 0,1316ns            | 0,5066ns            | 0,0880ns            | 0,1026ns            |
| LSD <sub>0,05</sub>    | 0,4916                 | 0,1163             | 0,5813             | 0,3258             | 0,3995              | 4,0086              | 1,1518              | 23,565              |
| CV (%)                 | 8,3049                 | 9,7135             | 9,5465             | 17,837             | 13,305              | 16,765              | 13,853              | 39,056              |

<sup>(1)</sup> Values of traits marked with different letter are statistically significant on the level P < 0.05 (LSD test) <sup>(2)</sup> P-values are highly significant (\*\*), significant (\*) or not significant (ns).

Morphological characteristics of fruit and endocarp (Table 2) showed a statistically high significant variation among accessions of Crnica for five traits: fruit weight (FWe), flesh percentage (FR), the ratio of the flesh of the fruit/endocarp (F/E), the oil content in fresh (OFM) and oil content in dry matter (ODM). Most of the accessions had oval fruit (PI 1.25-1.45), while in accessions 4HN and 11BD the fruit shape was spherical (FI < 1.25). Shape of endocarp was oval (EI 1.4-1.8) in four accessions (4HN, 6LU, 8LU and 11BD) and elliptical (EI 1.8-2.2) in other accessions. Large fruits of over 4 g (4-6 g) had accessions 2BD, 3GR, 9BR and 11BD. Other accessions/clones had fruits just under 4 g and belong to the category of medium- large (2-4 g).

Since one of the purpose of the fruit of Crnica variety is for table use, it is very important to recognize variability among accessions/clones regarding fruit

weight. Bigger fruited clones are more suitable for table use and therefore potential for improving table production in Montenegro. The endocarp in all tested accessions was very large > 0.45 g. Obtained results are in accordance with the average data for Crnica variety presented in previous studies (Miranović, 1978; Lazović and Adakalić 2012 b).

| Accession                        | FL<br>(cm)   | FW<br>(cm)   | FI<br>(FL/FW)   | FWe<br>(g)   | FR<br>(%)   | EL<br>(cm)  | EW<br>(cm)  | EI<br>(EL/EW)   | EWe<br>(g)  | F/E  |
|----------------------------------|--|--|---|--|---|---|---|---|---|--|
| 1GR                              | 2,22 <sup>ab (1)</sup>   | 1,77 <sup>a</sup>  | 1,26 <sup>cd</sup>  | 3,90 <sup>ab</sup>   | 83,42 <sup>b</sup>  | 1,61 <sup>abc</sup>   | 0,87 <sup>ab</sup>  | 1,84 <sup>ab</sup>  | 0,65 <sup>abc</sup>   | 5,03 <sup>c</sup>  |
| 2BD<br>3GR                       | 2,34 <sup>a</sup><br>2,38 <sup>a</sup>   | 1,81 <sup>a</sup><br>1,84 <sup>a</sup>   | 1,29 <sup>abc</sup><br>1,29 <sup>abc</sup>                            | 4,55 <sup>a</sup><br>4,50 <sup>ab</sup>                                | 85,59 <sup>ab</sup><br>84,79 <sup>ab</sup>  | 1,65 <sup>ab</sup><br>1,69 <sup>a</sup>   | $0,87^{ab}$<br>$0,91^{a}$   | 1,89 <sup>a</sup><br>1,86 <sup>ab</sup>                                 | $0,66^{ab}$<br>$0,68^{a}$   | 5,94 <sup>ab</sup><br>5,58 <sup>bc</sup>                             |
| 4HN<br>5HN                       | 2,23 <sup>ab</sup><br>2,23 <sup>ab</sup>   | 1,80 <sup>a</sup><br>1,76 <sup>ab</sup>  | 1,24 <sup>cd</sup><br>1,27 <sup>bc</sup>                              | 3,90 <sup>ab</sup><br>3,92 <sup>ab</sup>                               | 84,19 <sup>b</sup><br>84,62 <sup>ab</sup>   | 1,55 <sup>abcd</sup><br>1,58 <sup>abc</sup>   | $0,88^{ab} \\ 0,86^{ab}$  | 1,77 <sup>ab</sup><br>1,83 <sup>ab</sup>                                | $0,62^{abc}$<br>$0,60^{abc}$  | 5,32 <sup>bc</sup><br>5,50 <sup>bc</sup>                             |
| 6LU<br>7HN<br>8LU<br>9BR<br>10GR | 2,22 <sup>ab</sup><br>2,35 <sup>a</sup><br>2,03 <sup>c</sup><br>2,21 <sup>ab</sup><br>2,16 <sup>bc</sup> | 1,76 <sup>ab</sup><br>1,74 <sup>ab</sup><br>1,51 <sup>c</sup><br>1,75 <sup>a</sup><br>1,61 <sup>bc</sup> | $1,26^{cd}$<br>$1,35^{a}$<br>$1,35^{a}$<br>$1,26^{cd}$<br>$1,34^{ab}$ | $3,96^{ab}$<br>$3,86^{bc}$<br>$2,89^{d}$<br>$4,00^{ab}$<br>$3,18^{cd}$ | 83,70 <sup>b</sup><br>84,86 <sup>ab</sup><br>79,38 <sup>c</sup><br>86,88 <sup>a</sup><br>80,23 <sup>c</sup> | 1,53 <sup>bcd</sup><br>1,57 <sup>abc</sup><br>1,51 <sup>bcd</sup><br>1,45 <sup>cd</sup><br>1,55 <sup>abcd</sup> | $0,88^{ab}$<br>$0,84^{b}$<br>$0,86^{ab}$<br>$0,81^{b}$<br>$0,86^{ab}$ | $1,74^{ab}$<br>$1,87^{ab}$<br>$1,75^{ab}$<br>$1,80^{ab}$<br>$1,80^{ab}$ | $0,65^{abc}$<br>$0,58^{bc}$<br>$0,60^{abc}$<br>$0,53^{c}$<br>$0,63^{abc}$ | $5,14^{bc}$<br>$5,61^{bc}$<br>$3,85^{d}$<br>$6,62^{a}$<br>$4,06^{d}$ |
| 11BD                             | 2,17 <sup>bc</sup>   | 1,82 <sup>a</sup>  | 1,19 <sup>d</sup>   | 4,15 <sup>ab</sup>   | 85,40 <sup>ab</sup>   | 1,41 <sup>d</sup>   | 0,84 <sup>b</sup>   | 1,69 <sup>b</sup>   | 0,61 <sup>abc</sup>   | 5,85 <sup>abc</sup>  |
| Average                          | 2,23   | 1,74   | 1,28  | 3,89   | 84,14   | 1,55  | 0,86  | 1,80  | 0,62  | 5,30   |
| P-value <sup>(2)</sup>           | 0,1298ns   | 0,1305ns   | 0,1113ns  | 0.0398**   | 0,0016**  | 0,2252ns  | 0,5005n<br>s  | 0,8576n<br>s  | 0,4541ns  | 0,0021**   |
| $LSD_{0,05}$                     | 0,1622   | 0,1506   | 0,0757  | 0,6806   | 2,3298  | 0,1514  | 0,0630  | 0,1972  | 0,0951  | 0,8648   |
| CV (%)                           | 6,0729   | 7,1928   | 5,0306  | 15,342   | 2,9219  | 7,8666  | 5,5723  | 7,7898  | 11,809  | 16,466   |

Table 2: Characteristics of fruit and endocarp of Crnica accessions

<sup>(1)</sup> Values of traits marked with different letter are statistically significant on the level P < 0.05 (LSD test)

<sup>(2)</sup> *P*-values are highly significant (\*\*), significant (\*) or not significant (ns).

According to the data on fruit chemical traits presented in Table 3, the oil content in fresh matter was lower in the majority of studied Crnica accessions (8-10%) than previously published average data for this variety of 14.43% (Miranović, 1978). However, among the studied material in particular stands out accessions 7HN and 9BR who had significantly higher oil content in fresh matter of 18.74 and 16.70%, and 54.26 and 42.20% in dry matter, respectively. This result suggests the presence of more oily clones within Crnica variety that can also improve oil production.

There is no published data on the oil quality of Crnica variety. However, some results obtained recently, showed much higher content of total biophenols in comparison to the oil of Žutica, major variety in Montenegro named for oil production (Lazovic *et al.*, 2012), suggesting that oil of Crnica variety has potentially better sensorial and storage capacities.

Based on the results of 22 bio-pomological characteristics of examined Crnica clones and the distance matrix calculated, the dendrogram was created (Figure 1). The clones are grouped together in one main cluster consisted of six accessions of larger fruit (1GR, 5HN, 3GR, 4HN, 6LU and 2BD), two pairs and one independent accession (11BD).

| Accession              | FM                      | DM                   | OFM                | ODM                  |
|------------------------|-------------------------|----------------------|--------------------|----------------------|
|                        | (%)                     | (%)                  | (%)                | (%)                  |
| 1GR                    | 61,93 <sup>bc (1)</sup> | 38,08 <sup>ab</sup>  | 8,08°              | 21,21 <sup>ef</sup>  |
| 2BD                    | 67,38 <sup>ab</sup>     | $32,62^{bc}$         | $10,00^{bc}$       | 30,64 <sup>cd</sup>  |
| 3GR                    | 58,75 <sup>c</sup>      | $41,26^{a}$          | 9,10 <sup>bc</sup> | 22,06 <sup>ef</sup>  |
| 4HN                    | 64,22 <sup>bc</sup>     | 35,78 <sup>ab</sup>  | 8,46 <sup>bc</sup> | 23,63 <sup>def</sup> |
| 5HN                    | 61,75 <sup>bc</sup>     | 38,26 <sup>ab</sup>  | 9,87 <sup>bc</sup> | $25.80^{de}$         |
| 6LU                    | 64,60 <sup>bc</sup>     | 35,40 <sup>ab</sup>  | 9,15 <sup>bc</sup> | 25,85 <sup>de</sup>  |
| 7HN                    | 65,47 <sup>abc</sup>    | 34,53 <sup>abc</sup> | 18,74 <sup>a</sup> | 54,26 <sup>a</sup>   |
| 8LU                    | 58,39°                  | 41,61 <sup>a</sup>   | 7,94°              | $19,08^{\rm f}$      |
| 9BR                    | 60,43 <sup>bc</sup>     | 39,57 <sup>ab</sup>  | $16,70^{a}$        | 42,20 <sup>b</sup>   |
| 10GR                   | 62,35 <sup>bc</sup>     | 37,65 <sup>ab</sup>  | 10,85 <sup>b</sup> | 28,82 <sup>cd</sup>  |
| 11BD                   | 73,64 <sup>a</sup>      | 26,36 <sup>c</sup>   | 9,23 <sup>bc</sup> | 35,02°               |
| Average                | 63,54                   | 36,46                | 10,74              | 29,44                |
| P-value <sup>(2)</sup> | 0,4188ns                | 0,4188ns             | 0,0006**           | 0,0002**             |
| LSD <sub>0,05</sub>    | 8,3979                  | 8,3979               | 2,5461             | 6,7031               |
| CV (%)                 | 7,4370                  | 12,988               | 33,593             | 35,860               |

Table 3: Chemical properties of olive fruit samples

<sup>(1)</sup> Values of traits marked with different letter are statistically significant on the level P < 0.05 (LSD test) <sup>(2)</sup> *P*-values are highly significant (\*\*), significant (\*) or not significant (ns).



Figure 1: UPGMA dendrogram of Crnica accessions obtained by comparison of 22 bio-pomological characteristics

The clones with highest oil content in fresh and dry matter (7HN and 9BR) were linked together. Clones 8LU and 10 GR were different from the others by a lower weight of the fruit, flesh ratio and fruit/endocarp ratio. Separation of accession 11BD was due to the lower values of majority of parameters

#### CONCLUSIONS

Analysis of 22 bio pomological characteristics of eleven clones of Crnica olive variety showed that the properties of fruit and endocarp, and also flowers, leaves and internodes are of great importance in assessing the variability in olive. Significant variability between analyzed accessions/clones was recorded. The accessions were mainly distinguished according to the fruit weight, fruit and flesh ratio and oil content in the fruit. Important result is the knowledge on statistically significant differences regarding two main fruit purposes of Crnica variety, fruit weight and olive oil content. Results suggest the existence of extent variability within Crnica variety and thus the presence of individuals with larger fruits and with higher oil content in the fruit. Results also suggest the need to work on further selection of new individuals within this variety. Furthermore, in order to check the stability of the traits it is recommendable to include analyses on molecular level that are not influenced by environmental factors.

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## Mirjana ADAKALIĆ, Biljana LAZOVIĆ, Tatjana PEROVIĆ, Miroslav ČIZMOVIĆ

## VARIJABILNOST BIO-POMOLOŠKIH OSOBINA MASLINE SORTE CRNICA U CRNOJ GORI

# SAŽETAK

Crnica je druga po važnosti i zastupljenosti sorta masline na Crnogorskom primorju, posle sorte Žutica. Najviše je zastupljena u Boko-kotorskom podrejonu i u manjem obimu na području Budve u Barskom podrejonu. Sorta Crnica je kombinovanih svojstava ploda, za jelo i za proizvodnju ulja. Dugi period gajenja te potencijalno miješanje i nesistematska selekcija, rezultirali su razlikama koje se zapažaju na fenotipu individua u okviru ove sorte. Intenzitet različitosti istraživan je na jedanaest aksešena sorte Crnica analizom 22 biološka i pomološka parametara. Utvrđena je značajna varijabilnost između analiziranih individua. Izdvajaju se aksešeni prema krupnoći ploda, sadržaju ulja u plodu i dr. Dobijeni rezultati ukazuju na postojanje klonova koji su krupnijeg ploda te pogodniji za konzerviranje odnosno sa većim sadržajem ulja u plodu koji su potencijal za poboljšanje proizvodnje maslinovog ulja. Rezultati takođe upućuju na potrebu daljih istraživanja u okviru ove sorte.

Ključne riječi: varijabilnost, klon, maslina, biopomološke karakteristike