

VARIABILITY OF SWEET CHESTNUT (*Castanea sativa* Mill.) IN MONTENEGRO ACCORDING TO MORPHOLOGICAL TRAITS OF FRUITS AND UPOV DESCRIPTORS

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In this preliminary research phenotypic diversity of 40 chestnut trees in four natural sweet chestnut populations in Montenegro (Koštanjica, Ostros, Stoliv and Kostanjica) was investigated. The variations within and among the populations were analyzed based on fruit morphology traits and descriptors established by The International Union for the Protection of New Varieties of Plants (UPOV) in order to assess sweet chestnut genetic diversity. The research conducted in 2018 examined six measured and six derived fruit morphology traits as well as seven UPOV descriptors of fruit morphology and leaf bud burst phenology. The following morphometric traits were analyzed: Fruit weight, Fruit height, Fruit width, Fruit thickness, Scar length and Scar width. The following UPOV descriptors were examined: Fruit embryony, Degree of penetration, Fruit shape, Fruit size, Fruit color, Kernel color, Time of leaf bud burst. The results showed that the Mediterranean populations (Stoliv and Kostanjica) have smaller fruits than the sub-Mediterranean populations (Koštanjica and Ostros); their fruits have stronger pellicle intrusion and they exhibit polyembryony more frequently. The average value of Shannon's diversity index for six UPOV descriptors was 0.66. Cluster analysis with the UPGMA method was used for classification of the trees into groups. Cluster grouping is in concordance with populations' geographical distribution. Sub-Mediterranean populations Ostros and Koštanjica formed 2 clusters with trees from both populations, and another two clusters were formed by trees from Mediterranean populations Kostanjica and Stoliv. Our results indicate a high variability within populations which is important for the conservation of genetic pool of the species. To fully comprehend the variability of sweet chestnut natural populations in Montenegro

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the research should be expanded on data provided by molecular markers like SSRs as well as further and more detailed assessment of morphological characters.

Keywords: sweet chestnut, variability, morphological traits, Shannon index, cluster analysis

INTRODUCTION

The sweet chestnut (*Castanea sativa* Mill.) is a species of the *Fagaceae* family, common to Europe and Asia. It is mostly distributed in the Mediterranean area, from Caspian Sea to the Atlantic Ocean, with areal of 2.530.000 ha (FERNANDEZ-LOPEZ and ALIA, 2003; POLJAK *et al.*, 2012).

The species is highly appreciated in Balkans where it represents an important economic value for its fruit and timber, its landscaping and environmental characteristics. Its fruits have been a part of Mediterranean diet since ancient times which have an increased value due to not only nutritional properties but also health benefits related to their consumption (DE VASCONCELOS *et al.*, 2010; BARREIRA *et al.*, 2008).

Montenegro is one of the rare countries in Europe without commercial chestnut production which is the reason why the consumption of this fruit relies completely on production from natural forests. In Montenegro, the sweet chestnut grows mainly in the Mediterranean area in the altitudinal belt of 200 to 600 m where it mostly forms mixed forests with oak (*Castaneo-Quercetum submediterraneum* Wraber) or pure acidophilic chestnut stands growing on dry southern and eastern slopes of mountains Rumija and Orjen (MAYRHOFER *et al.*, 2013). There are several sites of natural chestnut populations in Montenegro with four largest being located in the Mediterranean and sub-Mediterranean area. Two of the natural populations are located in the Mediterranean region of Boka Bay (Stoliv and Kostanjica) and other two in sub-Mediterranean region in the vicinity of Skadar Lake (Ostros and Koštanjica). The two populations in the vicinity of Skadar lake form the largest natural chestnut forest in Montenegro which covers the area of 300 ha (ODALOVIĆ *et al.*, 2013). Four sweet chestnut natural populations are included in this study: Stoliv, Kostanjica, Ostros and Koštanjica.

Sweet chestnut has been on the list of priority species for the conservation of genetic resources since the colonization of its habitat by chestnut blight (*Cryphonectria parasitica*). The disease first appeared in Italy in 1938 (BIRAGHI, 1946) and has since moved eastwards in Slovenia (JURC, 2002), Croatia (HALAMBEEK, 1988) and Bosnia and Herzegovina (TREŠTIĆ *et al.*, 2001). Chestnut blight is present in all four mentioned populations in Montenegro but there still hasn't been an official report on this important pathogen. Chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu) is an important pest of sweet chestnut which is mostly present in Mediterranean populations (Kostanjica and Stoliv) but there hasn't been any official report yet. The area and density of sweet chestnut are gradually decreasing due to mentioned pathogen and pest attacks, changes in soci-economic structure of Montenegro's costal area and deforestation in favor of urbanization. The preservation of sweet chestnut germplasm from genetic erosion is an important task in which the investigation of existing variability of this species plays a crucial role.

Within mentioned populations great variability is observed, with types differing in size, color and quality. In order to assess genetic diversity of sweet chestnut populations various molecular markers have been used: isozymes (VILLANI *et al.*, 1991), ISSR markers (MATTIONI *et*

et al., 2008), RAPD markers (SOLAR *et al.*, 2005) and microsatellite markers or SSRs (SKENDER *et al.*, 2017). Alongside molecular markers, researchers in various countries have shown that studies of morphological traits play an important role in determining the difference between trees in various habitats of sweet chestnut: Turkey (SERDAR *et al.*, 2011), Bosnia and Herzegovina (DANIČIĆ *et al.*, 2018; MUJIĆ *et al.*, 2010), Croatia (POLJAK *et al.*, 2012), Italy (BORGHETTI *et al.*, 1986) and Slovenia (SOLAR *et al.*, 2005). Although the expression of morphological characteristics depends largely on developmental phase, environmental factors and applied cultivation techniques etc., morphological traits are a useful tool for preliminary evaluation of genetic diversity of sweet chestnut among different populations.

The aim of this study was to characterize and quantify the variability of morphological traits in sweet chestnut populations in Montenegro and to identify associations within studied populations. The level of phenotypic diversity of the autochthonous types of sweet chestnut in four natural sweet chestnut populations in Montenegro was assessed by combined investigation of The International Union for the Protection of New Varieties of Plants (UPOV) descriptors and morphological traits.

MATERIAL AND METHODS

Plant material

In the areas with natural populations of sweet chestnut in Montenegro, four main populations were included in the study; two populations from Mediterranean region: Kostanjica (KZ) and Stoliv (SZ) and two populations from sub-Mediterranean region: Ostros (OT) and Koštanjica (KSJ) (Figure 1). The study was carried out on 40 sweet chestnut trees in total, growing in four mentioned populations (ten trees per each population). The morphological analyses encompassed about 15 fruits per tree which were collected in November 2018.

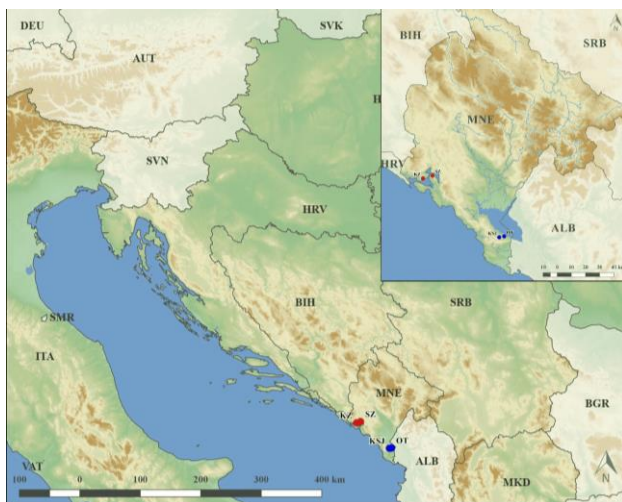


Figure 1. Sampled populations: Stoliv (SZ), Kostanjica (KZ), Ostros (OT), Koštanjica (KSJ).

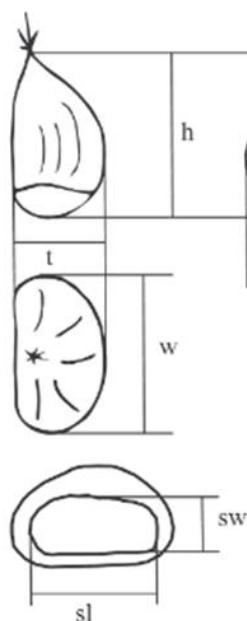


Figure 2. Measured fruit traits: height (h), thickness (t), width (w), scar width (sw), scar length (sl).

The following measured fruit traits were taken: Fruit weight (M), Fruit height (H), Fruit width (W), Fruit thickness (T), Length of hilum (SL), Width of hilum (SW) (Figure 2). According to POLJAK *et al.*, (2012), following derived variables were calculated from the latter: Fruit height/Fruit width (H/W), Fruit thickness/Fruit height (T/H), Fruit thickness/Fruit width (T/W), Hilum length/Fruit width (SL/W), Hilum width/Fruit thickness (SW/T), Hilum width/Hilum length (SW/SL).

Employing the method of UPOV (UPOV, 1989) for all observed genotypes the following nominal descriptors were used: Time of leaf bud burst (BB), Fruit embryony (FE), Degree of penetration of seed coat into embryo (DP), Fruit shape (FSH), Fruit color (FC), Fruit size (FS) and Kernel color (KC). Time of leaf bud burst is classified with 1 as very early (before or on 1 April), 3 early (Between 2-5 April), 5 medium (Between 6-9 April), 7 late (Between 10-13 April), 9 very late (Later than 14 April). The monoembryonic fruits are estimated at point 1, polyembryonic fruits are assessed at point 2. The level of pellicle intrusion is estimated at 3 (weak), 5 (medium), or 7 (strong). The fruit shape can be ovoid (point 1), broad ovoid (point 2), globose (point 3), transversally elliptical (point 4), and transversally broad elliptical (point 5). The color of fruits' pericarp may be light brown (point 1), brown (point 2), dark brown (point 3), reddish brown (point 4), and blackish brown (point 5). The fruit size is estimated at 3 (small), 5 (medium), 7 (large). Kernel color may be white (point 1) and cream (point 2).

Data analysis

Statistical analyses were performed on the mean tree values for each trait, calculated on the basis of one-year study. Descriptive analysis was performed in order to characterize the variation existing on the four locations and between all 40 trees. For studied traits following statistical parameters were calculated: mean, minimum and maximum value as well as coefficient of variation (CV %). The analyzed trees were grouped in clusters regarding their six measured and six derived fruit morphology traits using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical method and Euclidian distance as a measure of dissimilarity. The Shannon and Weaver diversity index (H') was determined as a measure of morphological diversity of the genotypes for studied UPOV descriptors. The estimated H' value was determined using following formula:

$$H' = -\sum_{i=1}^n P_i \log_2 P_i$$

where n is the number of nominal classes of traits and P_i is the proportion of accessions in the i th class of a trait. Normalization of H' value was performed by dividing each H' value with its maximum value, which ensured that H' values were in the range of 0-1. The Shannon diversity index was determined by the number of UPOV categories and evenness in the distribution of genotypes in each category (SHANNON, 1949). In case of a higher value of H' index, the distribution of genotypes within phenotypic class is even while in case of a low H' index the distribution of genotypes is unbalanced and reveals a lack of diversity (MORENO, 2013). Statistical analysis of morphological traits for 40 sweet chestnut genotypes was performed using the program R (R CORE TEAM, 2015).

RESULTS

Variation of quantitative traits

The greatest variation among the populations was observed regarding the average fruit weight. Mean fruit weight varied from 8.2 g (SZ) to 10.7 g (OT). The highest variability in fruit weight was determined for trees from population SZ (CV=18.0 %) and the lowest variability was determined in OT (CV=7.4 %). Mean fruit thickness varied from 17.7 mm (SZ) to 19.3 mm (OT). The highest coefficient of variation was defined in the fruits of trees from SZ (CV=7.8%) while the lowest was found in the fruits of trees from KSJ (CV=5.0 %) and KZ (CV=5.0 %) (Table 1, Figure 3).

Mean fruit height varied from 26.5 mm (SZ) to 28.8 mm (OT). The highest variability among trees in fruit height appears in population KZ (CV=4.0 %) while the lowest variability was determined among trees from OT (CV=2.0 %). Mean fruit width varied from 28.5 mm (SZ) to 31.6 mm (OT). The highest variability among trees in fruit width was observed in populations KZ (CV=5.0 %) and SZ (CV=5.0 %) while the lowest was observed in trees from OT (CV=3.0 %). The mean scar length for all populations was 21.4 mm. The highest variability among trees in scar length was observed in population SZ (CV=9.0 %) while the lowest was observed in trees from population KSJ (CV=6.0 %). The mean scar width in all populations was 11.5 mm. The highest variability among trees for scar width was observed in population SZ (CV=13.0 %) while the lowest was noted in population KSJ (CV=7.0 %) (Table 1).

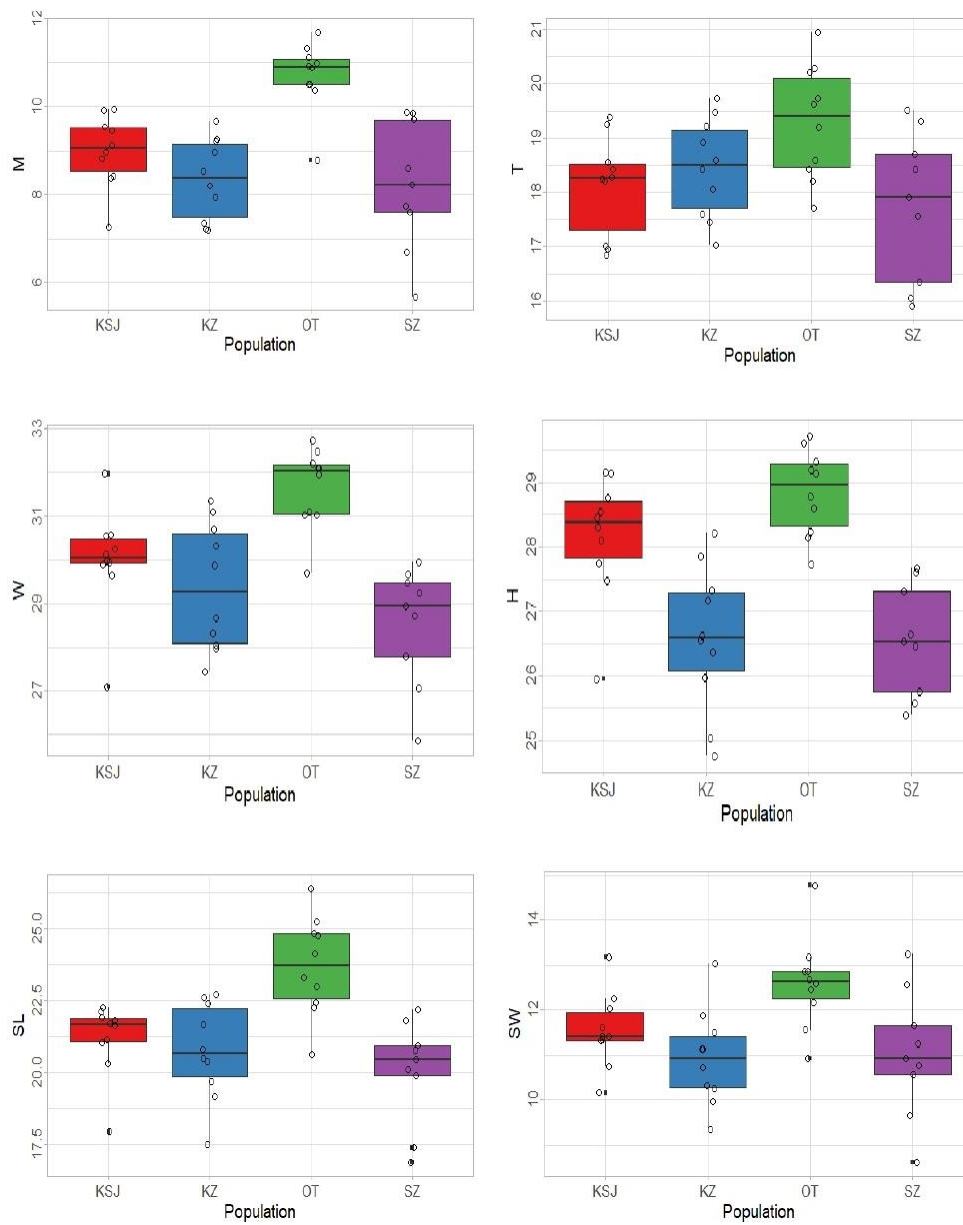


Figure 3. Classification of chestnut trees from the populations SZ, KZ, OT and KSJ with regard to weight (M), thickness (T), width (W), height (H), scar length (SL), scar width (SW) of fruits.

Table 1. Descriptive statistical parameters for measured morphological traits

| Populations | Traits | | | | | | | | | | | | |
|-----------------|--------|--------|-------|-------|-------|-------|--------|-------|-------|-------|-------|--------|--------|
| | M | T | H | W | SL | SW | H/W | T/H | T/W | SL/W | SW/T | SW/SL | |
| KSJ | Mean | 8,98 | 18,12 | 28,16 | 30,00 | 21,18 | 11,54 | 0,94 | 0,65 | 0,61 | 0,71 | 0,64 | 0,56 |
| | Min | 7,26 | 16,85 | 25,96 | 27,09 | 17,94 | 10,15 | 0,89 | 0,58 | 0,56 | 0,66 | 0,60 | 0,51 |
| | Max | 9,29 | 19,38 | 29,15 | 31,97 | 22,25 | 13,17 | 0,98 | 0,69 | 0,63 | 0,73 | 0,69 | 0,61 |
| | CV | 9,07% | 5,00% | 3,00% | 4,00% | 6,00% | 7,00% | 3,00% | 6,00% | 4,08% | 3,60% | 4,34% | 5,63% |
| KZ | Mean | 8,35 | 18,45 | 26,59 | 29,38 | 20,75 | 10,92 | 0,91 | 0,70 | 0,63 | 0,71 | 0,60 | 0,54 |
| | Min | 7,20 | 17,02 | 24,75 | 27,44 | 17,49 | 9,33 | 0,86 | 0,67 | 0,60 | 0,64 | 0,54 | 0,48 |
| | Max | 9,66 | 19,73 | 28,21 | 31,35 | 22,72 | 13,04 | 0,98 | 0,72 | 0,69 | 0,79 | 0,70 | 0,58 |
| | CV | 10,93% | 5,00% | 4,00% | 5,00% | 8,09% | 9,00% | 5,00% | 2,00% | 4,18% | 6,24% | 9,58% | 5,98% |
| OT | Mean | 10,71 | 19,30 | 28,85 | 31,65 | 23,70 | 12,61 | 0,92 | 0,67 | 0,61 | 0,75 | 0,67 | 0,55 |
| | Min | 8,78 | 17,72 | 27,73 | 29,69 | 20,64 | 10,93 | 0,87 | 0,61 | 0,55 | 0,70 | 0,63 | 0,49 |
| | Max | 11,70 | 20,96 | 29,73 | 32,74 | 26,41 | 14,78 | 0,96 | 0,74 | 0,66 | 0,82 | 0,71 | 0,62 |
| | CV | 7,37% | 5,42% | 2,00% | 3,00% | 7,00% | 8,00% | 3,00% | 6,00% | 5,01% | 4,95% | 4,26% | 7,97% |
| SZ | Mean | 8,22 | 17,75 | 26,55 | 28,52 | 20,04 | 11,03 | 0,94 | 0,67 | 0,62 | 0,70 | 0,63 | 0,56 |
| | Min | 5,67 | 15,90 | 25,40 | 25,85 | 16,87 | 8,60 | 0,87 | 0,60 | 0,55 | 0,65 | 0,55 | 0,50 |
| | Max | 9,87 | 19,50 | 27,67 | 29,96 | 22,18 | 13,24 | 1,03 | 0,72 | 0,65 | 0,79 | 0,72 | 0,67 |
| | CV | 18,00% | 7,77% | 3,22% | 5,00% | 9,00% | 13,00% | 5,00% | 7,00% | 5,24% | 6,56% | 10,60% | 10,04% |
| All populations | Mean | 9,07 | 18,41 | 27,54 | 29,89 | 21,42 | 11,53 | 0,93 | 0,67 | 0,62 | 0,72 | 0,64 | 0,55 |
| | Min | 5,67 | 15,90 | 24,75 | 25,85 | 16,87 | 8,60 | 0,86 | 0,58 | 0,55 | 0,64 | 0,54 | 0,48 |
| | Max | 11,70 | 20,96 | 29,73 | 32,74 | 26,41 | 14,78 | 1,03 | 0,74 | 0,69 | 0,82 | 0,72 | 0,67 |
| | CV | 11,34% | 5,80% | 3,06% | 4,25% | 7,52% | 9,25% | 4,00% | 5,25% | 4,63% | 5,34% | 7,20% | 7,40% |

In order to quantify the shape of fruit, derived variables were obtained from the studied morphological traits, on the basis of which fruit shape could be defined. From these variables it can be concluded that the populations KSJ and SZ have approximately equally high and wide fruits ($H/W=0.94$) while other two populations had on average wider fruits which is the reason why their derived ratios were lower in comparison to the other two ($OT=0.92$ and $KZ=0.91$). The average ratio of fruit thickness and height (T/H) for all populations was 0.67 while the average ratio for fruit thickness and width (T/W) was 0.62 which is similar to the results reported by POLJAK *et al.*, (2012). The derived variable SW/SL was obtained from the variables scar length and scar width and had the average value of 0.55 (Table 1). From this result it can be concluded that the length of fruits' hilum of all studied populations is on average two times bigger than its width which is similar to the results reported by POLJAK *et al.*, (2012).

Diversity for qualitative traits

The descriptors, the genotype distribution, as well as Shannon's diversity index values (H') for each of the descriptors are summarized in Table 2. The total mean diversity index of all qualitative traits was 0.66 and varied from 0.36 to 0.98. The index value was the lowest for descriptor Kernel color, moderate to high for Time of leaf bud burst, Fruit embryony and Fruit size while the highest values were observed for Degree of penetration, Fruit color and Fruit shape. Higher values of Shannon index revealed even distribution of genotypes within the category while the low values indicated a lack of diversity.

Table 2. Descriptors, descriptors designations and categories, categories' frequency and Shannon's diversity indices

| Descriptor | Descriptor designation | Categories | Genotype distribution (%) | H index |
|------------------------|------------------------|------------------------------|---------------------------|-------------|
| Fruit embryony | FE | 1-Monoembryonic | 89,26 | 0,49 |
| | | 2-Polyembryonic | 10,74 | |
| Degree of penetration | DP | 3-Weak | 23,09 | 0,98 |
| | | 5-Medium | 40,74 | |
| | | 7-Strong | 36,18 | |
| Fruit shape | FSH | 1-Ovoid | 5,88 | 0,83 |
| | | 2-Broad ovoid | 19,26 | |
| | | 3-Globose | 45,00 | |
| | | 4-Transverse ellipsoid | 24,56 | |
| | | 5-Transverse broad ellipsoid | 5,29 | |
| Fruit size | FS | 3-Small | 13,53 | 0,68 |
| | | 5-Medium | 60,29 | |
| | | 7-Large | 26,18 | |
| Fruit color | FC | 1-Light brown | 1,62 | 0,87 |
| | | 2-Brown | 32,65 | |
| | | 3-Dark brown | 21,47 | |
| | | 4-Reddish brown | 13,82 | |
| | | 5-Blackish brown | 30,44 | |
| Kernel color | KC | 1-White | 6,91 | 0,36 |
| | | 2-Cream | 93,09 | |
| Time of leaf bud burst | BB | 1-Very early | 0,00 | 0,42 |
| | | 3-Early | 61,18 | |
| | | 5-Medium | 38,82 | |
| | | 7-Late | 0,00 | |
| | | 9-Very late | 0,00 | |

The highest ratio of polyembryonic fruits is found in population SZ (16.0 %). In population KSJ the ratio is only 8.0 %. Monoembryonic fruits were represented by 89.3 % in all studied trees. The population SZ has the highest ratio of the trees with very strong pellicle intrusion (68.1%) while KSJ has the highest ratio of trees with weak intrusion (52.4 %). Globose fruits were found in 45.0 % of the samples while 24.6 % of fruits were transversally elliptical. Only 5.9 % of fruits are ovoid, 19.3 % are broad ovoid, 5.3 % are transversally broad elliptical. The highest ratio of fruit samples is of medium size (60.3 %) and 32.6 % of all fruit samples have brown pericarp. The highest ratio of fruit samples (93.1 %) have cream kernel color while only 6.9% have white kernel color. All fruit samples had present glossiness immediately after opening involucre.

Cluster analysis

Matrix presenting Euclidian distances between examined trees is displayed in the Figure 4. The smallest observed distance was 0.050 while the maximum observed distance amounted 0.581. The average distance for all pairs of genotypes was 0.303.

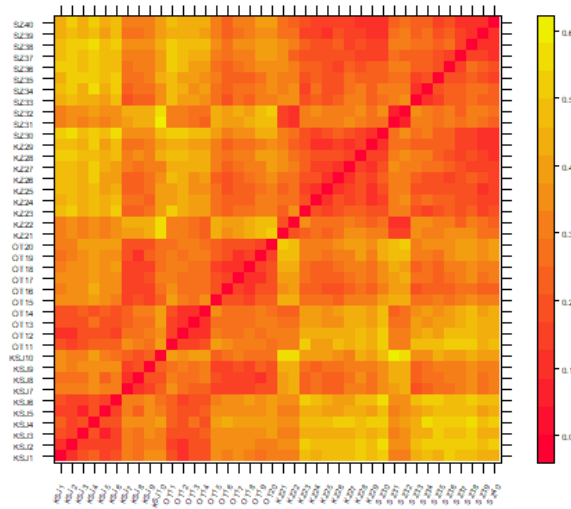


Figure 4. Distance matrix for the 40 genotypes of sweet chestnut calculated using the simple matching coefficient of similarity of morphological markers (genotypes of sweet chestnut are numbered from 1 to 40)

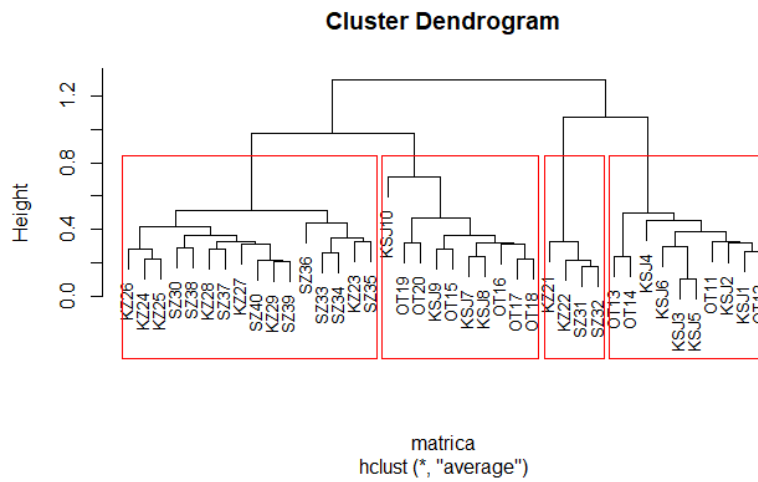


Figure 5. Dendrogram of sweet chestnut genotypes obtained by UPGMA cluster analysis of morphological traits

According to UPGMA cluster analysis studied trees are grouped into four main clusters (Figure 5). The first cluster comprises of 16 trees, all from Mediterranean populations: KZ and SZ. The cluster is split into two sub-clusters. The first sub-cluster comprises of seven trees from KZ and four from SZ; second sub-cluster comprises of four trees from SZ and one from KZ. The second cluster includes six trees from OT and four trees from KSJ. The third cluster comprises only four trees, namely, two trees from KZ and two trees from SZ. The fourth cluster includes six trees from KSJ and four trees from OT.

DISCUSSION

The group of chestnut trees from the Mediterranean region populations (KZ and SZ) differs from the group of trees from sub-Mediterranean populations (OT and KSJ) in most of the pomological characteristics. The Mediterranean population SZ demonstrated the highest variability and the lowest values for most of the observed pomological traits. The sub-Mediterranean population OT showed the highest values for most of the observed pomological traits and together with population KSJ, the lowest variability for most of the observed pomological traits.

The weight of fruits (M) is the most variable pomological trait in all studied populations. It has been observed that trees from sub-Mediterranean populations (KSJ and OT) have on average fruits heavier than 8.5 g which is similar to the reports presented by researchers from Croatia (POLJAK *et al.*, 2012), Slovenia (SOLAR *et al.*, 2005), Italy (BORGHETTI *et al.*, 1986) and Turkey (SERDAR, 1999).

It can be concluded that among four studied sweet chestnut populations in Montenegro, the heaviest, the thickest, the highest and the widest fruits with the lowest level of pellicle intrusion grow in the vicinity of Skadar lake.

The fruits from this area are on average higher than the fruits of the trees grown in northwest Croatia where they reach 23.6-26.6 mm in height (POLJAK *et al.*, 2012) and longer than the fruits of genotypes from natural populations in Bosnia and Herzegovina where they reach 16.8-19.8 mm in average height (DANIČIĆ *et al.*, 2018). The height of fruits from Mediterranean populations (KZ and SZ) is similar to height of fruits growing in northwest Croatia (POLJAK *et al.*, 2012) but shorter than fruits of traditional Croatian cultivar 'Lovran Marron' and trees grown from marron seed on Mount Učka in sub-Mediterranean area of Croatia (POLJAK *et al.*, 2016).

With regard to the fruits' width, the Montenegrin chestnut trees have on average more narrow fruits than fruits of most natural populations in Slovenia (2.8-3.1 cm) (SOLAR *et al.*, 2005), narrower than the fruits of trees grown from marron seed on Mount Učka (28.4- 34.7 mm) in sub-Mediterranean area of Croatia (POLJAK *et al.*, 2016) but wider than fruits of natural populations in Bosnia and Herzegovina (14.9 -16.9 mm) (DANIČIĆ, 2018).

The fruits of traditional Croatian cultivar 'Lovran Marron' (21.4 mm \pm 3.0) and trees grown from marron seed on Mount Učka (19.82 mm \pm 2.88) in sub-Mediterranean area of Croatia were on average thicker than the fruits of studied trees.

With regard to the mean length and width of hilum, the mean values for the two parameters are similar to those reported by other researchers (SOLAR *et al.*, 2005; POLJAK *et al.*, 2012).

Pellicle intrusion is an important feature which affects peeling and fruits' appeal. In most of the studied trees the epidermis protrudes into the embryo medium strongly to strongly which is similar to reports of POLJAK *et al.*, (2012). The study showed that fruits of trees from sub-Mediterranean region (KSJ and OT) had weaker average pellicle intrusion than the trees from Mediterranean region (KZ and SZ). Mediterranean populations (KZ and SZ) had higher portion of polyembryonic fruits (12.5 and 16.0 %, respectively) than sub-Mediterranean populations (KSJ and OT, 7.8 and 9.2% respectively). These results correspond to those reported by POLJAK *et al.*, (2012) for sweet chestnut natural populations in Croatia.

Important trait for consumer appeal is fruit shape. In our study, the most prevalent fruit shape was globose. The most represented pericarp color in our study is brown which is similar to the reports for sweet chestnut populations in Bosnia and Herzegovina (DANIČIĆ *et al.*, 2018). Sub-Mediterranean populations (KSJ and OT) are mostly represented with blackish brown fruits while Mediterranean populations (KZ and SZ) are mostly represented with brown fruits. In Slovenian and Romanian types, the pericarp is most frequently dark brown (SOLAR *et al.*, 2005).

The UPGMA clustering produced a dendrogram with four separate clusters for which grouping was in accordance with their geographical origin. Clustering analysis made a clear distinction between Mediterranean and sub-Mediterranean populations. However, it appears that the differences between studied populations within Mediterranean and sub-Mediterranean region were not strong enough to clearly separate the populations within the regions.

The total mean diversity index for qualitative traits was 0.66 and varied from 0.36 to 0.98 between traits. One of the aims in sweet chestnut breeding is to select genotypes with no or weak pellicle penetration which influences peeling and in that sense is an important trait for marketing. Descriptor Degree of penetration had high diversity index (0.98) which confirmed high variability of studied material. Splitting of the pericarp and polyembryony are important problems with monoembryony being a very desirable trait of sweet chestnut fruits. Fruit embryony showed low diversity index (0.49) with 89.3 % ratio of monoembryonic genotypes. Another important trait is the shape of fruits. The most commercialized chestnut cultivars are generally of transverse ellipsoid or transverse broad ellipsoid fruit shape (SERDAR *et al.*, 2011; DINIS *et al.*, 2011). Fruit shape and Fruit colour showed relatively high diversity index (0.83 and 0.87, respectively) implying that these descriptors could be useful for genotype discrimination in breeding programs. Kernel colour and Time of leaf bud burst showed low to moderate diversity index (0.36 and 0.42, respectively).

Evaluation of sweet chestnut germplasm, revealed phenotypic diversity among studied populations in spite of small geographical distances between them. The results of the preliminary study conducted on 40 chestnut trees grown at four populations in two regions of Montenegro demonstrate phenotypic diversity and provide the basis for future research aiming for conservation of sweet chestnut genetic resources. Due to the lack of suitable chestnut varieties and good quality, chestnut types from autochthonous populations can be interesting for market production of high-quality chestnut in Montenegro. With this research we aim to explore chestnut types in autochthonous populations and select ones with big nuts which can be interesting for various purposes such as: processing, fresh consumption, candied fruit. In order to gain broader knowledge on the variability of sweet chestnut in Montenegro the research should be expanded to the use of molecular markers such as SSRs markers.

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VARIJABILNOST PITOMOG KESTENA (*Castanea sativa* Mill.) NA PODRUČJU CRNE GORE PREMA MORFOLOŠKIM OBELEŽJIMA PLODA I UPOV DESKRIPTORIMA

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Izvod

U radu je prikazana varijabilnost četiri populacije pitomog kestena (*Castanea sativa* Mill.) na području Crne Gore (Koštanjica, Ostros, Stoliv i Kostanjica). Unutarpopulacijska i međupopulacijska varijabilnost utvrđena je na osnovu šest morfoloških karakteristika plodova, šest izvedenih odnosa kao i sedam deskriptora za morfologiju ploda i fenofazu otvaranja lisnih pupoljaka razvijenih od strane Međunarodne unije za zaštitu novih biljnih sorti (UPOV). Analizirane su sledeće osobine ploda: masa, dužina, širina i debljina ploda; dužina i širina hiluma. Ispitani su sledeći UPOV deskriptori: embrionija ploda, stepen ureza endokarpa u plod, oblik, veličina i boja ploda, boja mezokarpa i vrijeme otvaranja lisnih pupoljaka. Rezultati su pokazali da se mediteranske populacije (Stoliv i Kostanjica) odlikuju manjim plodovima od submediteranskih populacija (Ostros i Koštanjica); njihovi plodovi se odlikuju većim stepenom ureza endokarpa u plod kao i većim udelom poliembrionalnih plodova. Prosečna vrednost Šenon indeksa raznovrsnosti za sedam ispitivanih UPOV deskriptora iznosila je 0.66. UPGMA klaster analiza morfoloških karakteristika i njihovih odnosa omogućila je deskripciju četiri grupe pri čemu je grupisanje stabala bilo u skladu sa geografskim porieklom; mediteranske populacije formirale su dva klastera koja su sadržala stabla iz mediteranskih populacija dok isti slučaj važi i za submediteranske populacije. Rezultati ove preliminarne studije ukazuju na veliku varijabilnost ispitivanih populacija što je jedan od preduslova za očuvanje genetskog fonda ove vrste. Za kompletniju studiju varijabilnosti pitomog kestena u prirodnim populacijama Crne Gore, istraživanje je potrebno proširiti na primenu genetičkih markera. U toku je dalja studija morfoloških svojstava ove vrste.

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