Sauteria 18, 2009 323-333

Variation of Sweet Chestnut (*Castanea sativa* MILL.) Populations in Croatia According to the Morphology of Fruits

Populationen der Edelkastanie (*Castanea sativa* Mill.) in Kroatien in Beziehung zur Fruchtmorphologie

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Key words: sweet chestnut, fruits, morphology, Croatia.

Schlagwörter: Edelkastanie, Früchte, Morphologie, Kroatien.

Summary: In Croatia sweet chestnut grows in very diverse ecological conditions, in various forest communities, on an area of about 35000 ha. It provides direct and indirect benefits (high-quality wood, edible fruits, honey, firewood, preventing soil erosion, maintaining the watershed etc.) and it also contributes to the distinctive character of landscapes. Sweet chestnut is among the priority species for the conservation of genetic resources in Croatia. In the last 50 years it has been seriously threatened due to chestnut blight.

The aim of this study was to assess the interpopulation and intrapopulation variability of sweet chestnut populations in Croatia according to the morphology of fruits. Nuts were sampled in 10 natural populations, from the whole area of sweet chestnut distribution range in Croatia. Each population was represented by 10 mature trees and each tree by 30 nuts. Nine morphological traits and seven indices were analysed. Univariate and multivariate statistical techniques were used to evaluate the differences among and within populations.

Zusammenfassung: In Kroatien wächst die Edelkastanie unter sehr verschiedenen ökologischen Bedingungen in verschiedenen Wäldern, auf einer Fläche von 35000 ha. Ihr direkter und indirekter Nutzen ist groß (qualitativ hochwertiges Holz und Brennholz, essbare Früchte und Honig, Schutz vor Erosion und Wirkung auf das Grundwasser, etc.) und sie prägt das Landschaftsbild. So ist sie in Kroatien auch unter den Arten, de-

ren genetische Ressourcen geschützt werden. In den letzten 50 Jahren wurden ernsthafte Maßnahmen gegen den Kastanienrindenkrebs gesetzt.

Das Ziel dieser Studie ist die Variabilität von Inter- und Intrapopulationen auf Grund fruchtmorphologischer Unterschiede zu bewerten. Früchte aus 10 natürlichen Populationen aus dem gesamten Edelkastanienareal in Kroatien wurden gesammelt. Aus jeder Population wurden je 30 Nüsse von 10 fruchtenden Bäumen entnommen. Neun morphologische Merkmale und 7 Indices wurden analysiert. Uni- und multivariate statistische Verfahren wurden angewandt um die Unterschiede zwischen und innerhalb der Popolationen zu bewerten.

Introduction

Sweet chestnut is distributed across the Mediterranean region, from the Caspian Sea to the Atlantic Ocean. It is thought that the species survived in several refuges during the last ice age in southern Europe, Northeast Turkey and the Caucasus. It has a discontinuous, scattered range, occupying hundred thousands of hectares in coppices and orchards on acid soils. Sweet chestnut is an important multipurpose tree species. It is important to ensure the conservation and sustainable use of chestnut genetic resources.

In Croatia sweet chestnut grows in very diverse ecological conditions, in various forest communities, on an area of about 35000 ha. Stands with sweet chestnut as management class cover about 15000 ha. Sweet chestnut provides direct and indirect benefits (high-quality wood, edible fruits, honey, firewood, preventing soil erosion, maintaining the watershed etc.) and it also contributes to the distinctive character of landscapes. It is among the priority species for the conservation of genetic resources in Croatia. In the last 50-60 years it has been seriously threatened due to chestnut blight. In addition, the sweet chestnut distribution range is under considerable influence of human activities, so that the favouring of particular genotypes also contributes to the loss of genetic diversity.

It is spread in two main, split areas in different climate zones. The larger part of the area spreads along central Croatia, from the Slovenian to the Bosnian-Herzegovinian border. Sweet chestnut can be found on all massifs of this region, whereas the biggest and most beautiful forests grow on the Zrinska and Petrova gora, as well as Medvednica. This is where we can find sweet chestnut mixed with oak, beech or hornbeam trees. Due to the continental climate, sweet chestnut most readily grows in warm, well-lit positions in the hilly region, avoiding dry, cold and foggy sites. On plateaus, mountain ridges and generally mildly sloping terrains it often builds pure stands, whereas on larger steeps and more open terrains it grows individually or in groups. It grows on deep, acidic and decalcified soil, basically on a siliceous substrate. The second part of the sweet chestnut distribution area belongs to the sub-Mediterranean region, including Istria and the islands of Cres and Krk. Apart from forest stands, in this part of Croatia the sweet chestnut orchards for fruit growing are located. In this region of warmer climate sweet chestnut grows on fresh terrains with greater precipitation, as well as on deep, leached soils with a carbonate substrate. It grows well in positions protected from strong winds, whereas in places exposed to wind it chooses the most protected positions (ANIĆ 1940, ANIĆ 1943, MEDAK 2004, MEDAK & PERIĆ 2007, NOVAK-AGBABA et al. 2000, 2005).

Material and methods

Sweet chestnut fruits were collected during October 2007 in 10 natural populations in Croatia (Bosiljevo, Cres, Gvozd, Eastern Medvednica, Western Medvednica, Moslavačka gora, Ozalj, Topusko, Učka and Vojnić), as shown in Figure 1.

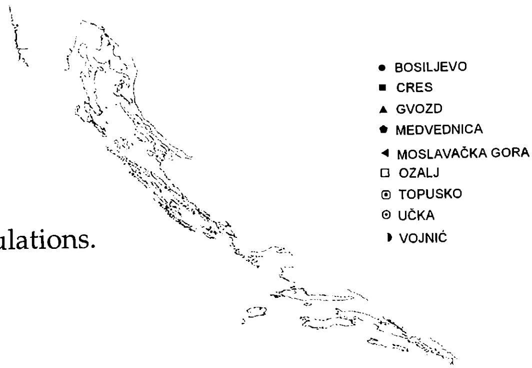

Fig. 1: Sampled populations.

In each population 10 trees were chosen growing more than 50m away from each other, and from each tree 30 healthy nuts, with the outer position in the bur were collected. The fresh fruits were weighted (m = weight), after which the following morphological traits were digitally measured (Figure 2): height (h), width (w), thickness (t), scar length (sl), scar width (sw), seeds per nut (ns), number of intrusions at transversal section (ni), as well as the length of intrusions (li). In total 3000 fruits were analyzed. From the measured traits the following indices were calculated, i.e. the following variables established: nut height/width (h/w), nut thickness/height (t/h), nut thickness/width (t/w), scar length/nut width (sl/w), scar width/nut thickness (sw/t), scar width/ scar length (sw/sl) and length of intrusions (li)/nut thickness (li/t).

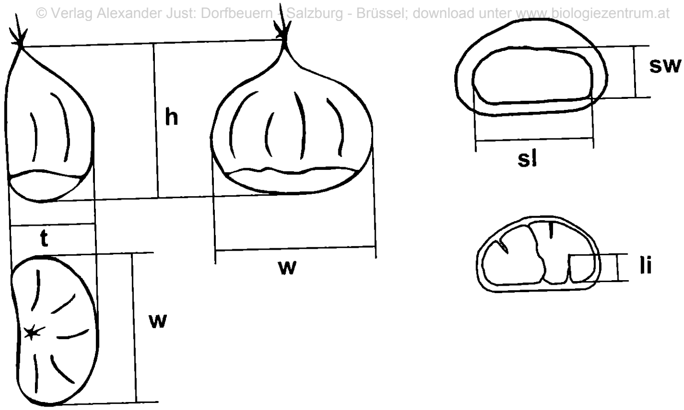

Fig. 2: Measured traits: h = nut height, w = nut width, t = nut thickness, sl = scar length, sw = scar width, li = length of intrusions.

Descriptive statistical methods were used to calculate arithmetic mean values ($\bar{x}$), standard deviations (s) and coefficients of variability (CV). To determine intra- and interpopulational variability, univariate (ANOVA) and multivariate analyse of variance (MANOVA) were used. The analysed factors were population and tree (tree factor nested inside the population factor). To determine the degree of similarities between analysed populations, according to the morphology of fruits, cluster analysis (tree clustering algorithm, Euclidean distance, complete linkage method) was used. Discriminated analysis was used to find out the variables which best discriminate the groups obtained by cluster analysis. Statistical analyses were carried out using the program packages Statistica (STATSOFT INC. 2001) and SAS System for Windows 6.12 (SAS 1990).

Results

The results of descriptive statistics are shown in Table 1. The average nut weight for ten populations was 7.1 g. From the examined populations, the highest weight on the average was found in the population Bosiljevo (8.8 g), then Cres (8.6 g), Učka (8.2 g), Topusko (8.0 g), Western Medvednica (7.7 g), Ozalj (7.1 g), Eastern Medvednica (6.9 g), Gvozd (6.5 g) and Vojnić (5.7 g). The lowest nut weight was found in the population of Moslavačka gora (3.8 g). The coefficients of variability for this trait ranged from 22 to 33%.

Tab. 1: Descriptive statistical parameters.

Variable	Parameter	Bosiljevo	Cres	Gvozd	Eastern Medved.	Western Medved.	Mosl. gora	Ozalj	Topusko	Učka	Vojnić	All Populations
m	$\bar{x}$ (g)	8.8	8.6	6.5	6.9	7.7	3.8	7.1	8.0	8.2	5.7	7.1
	s (g)	2.4	2.4	1.6	1.6	2.2	1.3	1.7	2.1	2.7	1.4	2.5
	CV (%)	27.5	27.7	25.2	22.9	28.5	33.3	23.6	26.8	33.1	24.0	34.4
h	$\bar{x}$ (mm)	26.1	29.3	24.4	25.1	26.1	19.6	24.9	26.7	26.3	24.4	25.3
	s (mm)	2.3	3.3	2.5	2.5	3.0	1.9	2.5	2.8	3.4	1.9	3.5
	CV (%)	8.9	11.2	10.4	10.1	11.4	9.8	10.2	10.4	12.8	8.0	14.0
w	$\bar{x}$ (mm)	30.0	27.8	26.3	26.9	27.6	21.5	27.6	28.4	29.1	24.9	27.0
	s (mm)	3.2	3.8	2.6	2.2	3.2	2.6	2.4	3.1	4.0	2.5	3.8
	CV (%)	10.6	13.5	9.8	8.2	11.6	11.9	8.7	10.9	13.8	10.1	14.0
t	$\bar{x}$ (mm)	18.5	18.3	15.9	16.6	17.3	14.3	17.7	17.3	17.8	15.9	17.0
	s (mm)	2.5	2.8	2.2	2.3	2.5	2.3	2.6	2.5	2.8	2.3	2.8
	CV (%)	13.7	15.3	13.6	14.0	14.2	16.0	14.5	14.2	15.9	14.8	16.3
sl	$\bar{x}$ (mm)	23.9	20.5	21.2	20.4	19.5	17.0	21.5	20.5	19.8	19.2	20.3
	s (mm)	3.7	4.0	3.1	2.8	4.1	3.1	2.8	2.6	3.2	2.7	3.7
	CV (%)	15.5	19.6	14.7	13.6	21.2	18.4	13.1	12.9	16.0	14.3	18.0
sw	$\bar{x}$ (mm)	11.8	11.2	10.6	10.6	9.9	9.1	11.1	10.1	9.8	10.4	10.4
	s (mm)	2.1	1.9	1.9	1.5	2.0	2.0	2.2	1.9	1.5	1.9	2.0
	CV (%)	17.8	17.2	17.7	14.0	20.5	22.0	19.9	18.6	15.3	18.1	19.6
ns	$\bar{x}$	1.0	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.0
	s	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.2
	CV (%)	17.4	21.4	15.7	8.1	18.2	5.8	5.8	18.2	21.4	9.9	15.6
ni	$\bar{x}$	1.5	2.3	1.8	1.6	1.5	0.3	1.8	1.6	0.8	1.3	1.4
	s	1.2	1.3	1.4	1.6	1.4	0.7	1.4	1.3	1.1	1.3	1.4
	CV (%)	80.6	56.9	80.0	99.2	91.4	210.9	75.7	83.3	137.0	100.8	95.8
li	$\bar{x}$ (mm)	3.3	4.2	2.7	2.4	2.7	0.9	2.8	2.7	1.9	2.3	2.6
	s (mm)	2.3	2.1	2.0	2.0	2.2	1.7	1.9	1.9	2.5	1.9	2.2
	CV (%)	70.0	50.3	72.4	83.4	81.9	196.9	69.2	71.1	128.7	84.8	85.9
h/w	$\bar{x}$	0.9	1.1	0.9	0.9	1.0	0.9	0.9	0.9	0.9	1.0	0.9
	s	0.1	0.6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
	CV (%)	8.7	58.1	11.7	10.4	11.7	9.1	11.3	9.7	10.2	11.3	24.2
t/h	$\bar{x}$	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
	s	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.5	0.1	0.2
	CV (%)	13.9	12.5	43.7	14.8	16.4	14.3	16.3	15.8	72.2	16.1	30.8
t/w	$\bar{x}$	0.6	0.7	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6
	s	0.1	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
	CV (%)	11.9	63.1	12.8	12.7	12.1	12.1	12.9	12.4	11.5	13.5	24.8
sl/w	$\bar{x}$	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.7	0.7	0.8	0.8
	s	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	CV (%)	9.0	44.7	14.7	10.4	12.9	12.3	10.9	11.2	11.6	11.7	18.7

sw/t	$\bar{x}$	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.6
	s	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	CV (%)	14.8	14.9	15.2	13.4	16.1	17.6	16.6	16.8	14.1	15.0	16.5
sw/sl	$\bar{x}$	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5
	s	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
	CV (%)	18.7	28.2	19.7	16.9	16.9	16.6	21.1	22.3	16.3	18.7	20.5
li/t	$\bar{x}$	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.2
	s	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	CV (%)	67.7	45.5	70.5	81.2	79.9	194.5	67.1	71.1	126.8	83.7	83.2

From the established variables conclusions about the fruit shape can be made. The nut width is larger than nut height in the majority of populations, the ratio being h/w = 0.9. Two populations (Western Medvednica and Vojnić) have averagely equally high and wide fruits (h/w = 1.0). The population Cres is the only one in which averagely the fruits are higher than wider (h/w = 1.1). The fruit thickness/height ratio (t/h) is 0.7 in most populations, whereas in the population Cres this ratio is 0.6. Equally, the fruit thickness/width ratio (t/w) is mostly 0.6, whereas in the populations Cres and Moslavačka gora it is 0.7 (Table 1). Since in all the three mentioned ratios the population Cres occurs as an exception, it can be concluded that the fruit shape of this population is on the average different than in the others.

The variables scar length/width were compared with nut width, i.e. thickness (sl/w, i.e. sw/t). The ratio sl/w was 0.8 or 0.7, and the ratio sw/t 0.6 or 0.7 for all the examined populations. These two variables were put into mutual relationship (sw/sl), from which it was apparent that the scar of the nuts in most of the populations were averagely twice longer than their width (sw/sl = 0.5), and in two populations this ratio was 0.6 (Table 1).

In the fruits of most populations there was one seed on the average (ns = 1.0), and for the populations Cres and Učka ns = 1.1. The coefficients of variability for this trait range from 6 to 21% (Table 1). The average number of intrusions ranged from 0.3 for the population of Moslavačka gora to 2.3 for Cres. The length of these intrusions for all populations amounted to 10-20% of the fruit thickness (li/t = 0.1 do 0.2). The coefficients of variability for variables ni, li and li/t were comparatively large, since the values of these variables can also be zero.

In Table 2 the results of ANOVA and MANOVA are presented. Univariate ANOVA indicates that variation between populations was significant, at the level of significance 0.01, for most traits, except for ns, ni, t/h, t/w and sw/sl. It can be seen that the variation within populations differed at a high level of significance by all traits. Results of MANOVA showed the significant variation between as well as within studied populations in respect to all traits jointly.

Tab. 2: Results of ANOVA and MANOVA.

Variable	Population		Tree/Population	
	ANOVA			
	df = 9		df = 90	
	F	p	F	p
m	9.8	< 0.01	36.5	< 0.01
h	13.1	< 0.01	46.3	< 0.01
w	10.1	< 0.01	42.7	< 0.01
t	7.2	< 0.01	16.7	< 0.01
sl	5.3	< 0.01	32.6	< 0.01
sw	3.9	< 0.01	23.2	< 0.01
ns	1.3	0.24	3.7	< 0.01
ni	5.0	< 0.01	16.1	< 0.01
li	5.1	< 0.01	14.4	< 0.01
h/w	4.6	< 0.01	5.6	< 0.01
t/h	2.6	0.01	3.2	< 0.01
t/w	2.4	0.02	3.4	< 0.01
sl/w	5.9	< 0.01	5.3	< 0.01
sw/t	4.0	< 0.01	17.0	< 0.01
sw/sl	1.9	0.06	11.1	< 0.01
li/t	4.7	< 0.01	12.4	< 0.01
	MANOVA			
	df = 126		df = 154	
	F	p	F	p
	40.4	< 0.01	6.7	< 0.01

The dendrogram (Figure 3) shows similarities between ten Croatian sweet chestnut populations based on 9 analysed fruit traits. The most similar populations were Gvozd and Vojnić, then Eastern Medvednica, and finally Ozalj and Topusko. To all these populations Western Medvednica could be added, so that these six populations made one group (Cluster 2). The second group (Cluster 1) was made of the populations Bosiljevo and Učka, to which the population Cres was added. The population of Moslavačka gora made the third group (Cluster 3).

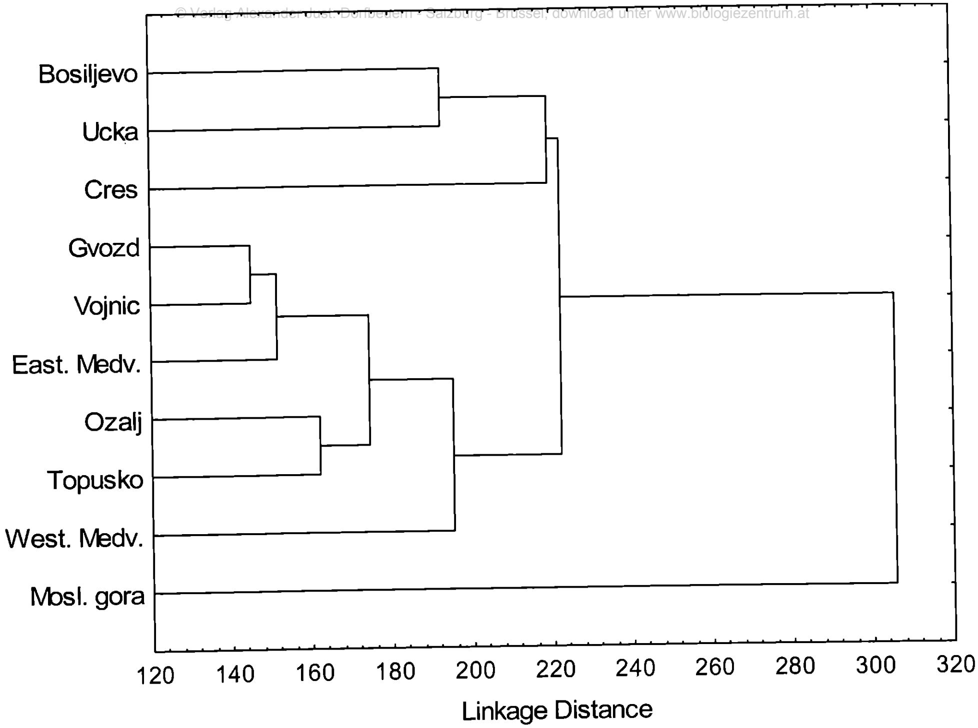

Fig. 3: Horizontal dendrogram.

To determine the traits in which the previously mentioned groups (clusters) differ the most, the discriminative analysis was used.

From the means of canonical variables (Table 3) it can be seen that the first discriminant function (root 1), which explained 91% of variability, best discriminated cluster 3 (population Moslavačka gora) from cluster 1 (populations Bosiljevo, Učka and Cres), as well as from cluster 2 (other populations), since the means for these groups were most distant (Figure 4, axis x).

Table 3: Means of canonical variables.

Group	Root 1	Root 2
Cluster 1	0.7398	0.3172
Cluster 2	0.0130	-0.2085
Cluster 3	-2.2977	0.2993

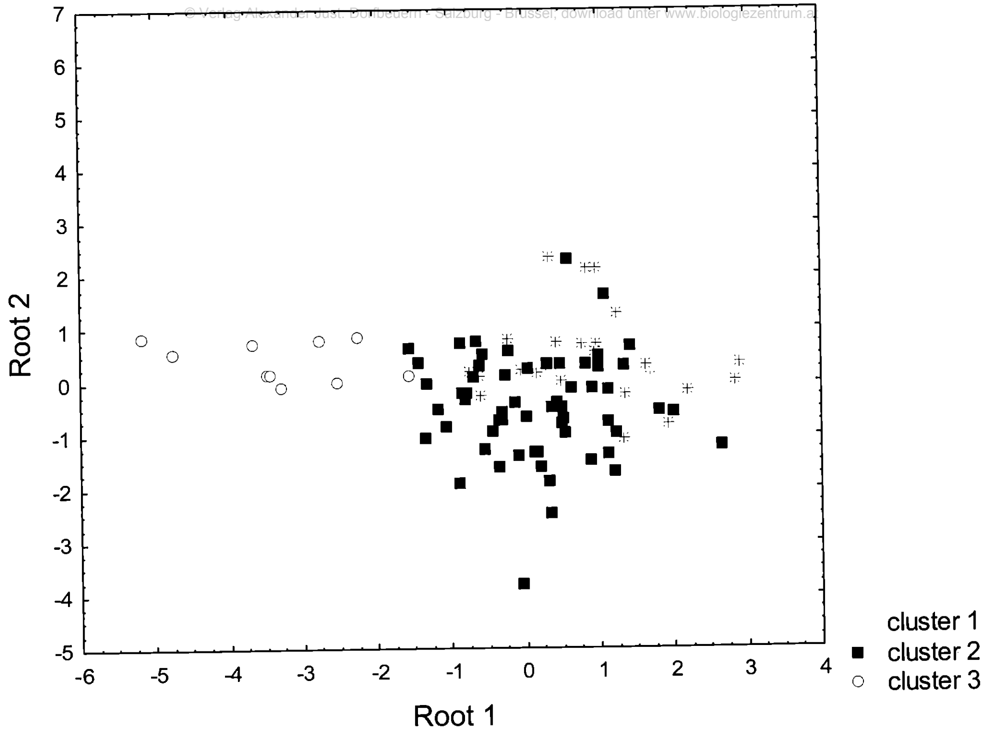

Fig. 4: Scatterplot of the canonical scores for three population groups (clusters).

Standardized coefficients for canonical variables (Table 4) show that the discrimination explained by the first function was mostly determined by variables nut width (w), height (h) and weight (m).

Table 4: Standardized coefficients for canonical variables.

Variable	Root 1	Root 2
m	-0.589	1.752
h	0.797	-0.379
w	0.809	-0.836
t	0.223	-0.202
sl	-0.074	-0.192
sw	0.064	-0.179
ns	0.023	0.062
ni	0.026	-1.022
li	0.010	0.628
Eigenvalue	0.693	0.065
Cumulative Proportion	0.91	1.00

Discussion and conclusion

The population with the highest average nut weight and size was Bosiljevo. It is a population located in the central Croatia, but through its morphological fruit characteristics it could be grouped into the cluster of coastal populations, Učka and Cres. The common feature of these populations is that they grow on the carbonate substrate, covered with a deep soil layer, i.e. they grow in similar pedological conditions. Apart from that, the populations Bosiljevo, Učka and Cres were the only ones with an average nut weight larger than 8 grams. The results of morphometric analysis of sweet chestnut fruits in Croatia can be compared with similar research results in other European countries. Here we will only consider the trait of nut weight, which is in positive correlation with fruit and scar size (nut height, width and thickness, as well as scar length and width). In Slovakia BOLVANSKY & UŽIK (2005) carried out research on fruit variability in 4 populations. The average nut weight of these populations (6.9 g) was a little lower than the average weight of 10 examined Croatian populations (7.1 g). For 3 examined Slovenian populations (SOLAR et al. 2005) the average fruit weight was 12.6 g, i.e. it was significantly higher than in the Croatian and Slovakian populations. Fruit weight is the characteristic with highest variability (CV = 34%), compared with other characteristics describing fruit and scar size (CV = 14-19%) - similar results being found in Slovenia as well. In most other European countries pomological research was carried out mainly on selected cultivars (marrons), which cannot be compared with Croatian natural populations. An additional problem is human influence on natural populations, as well as the question of autochthonous of some populations. The research of variability is important for preparation and implementation of measures for the conservation of sweet chestnut genetic resources, whereas the morphological research of fruits is, along with chemical analysis, important as the first step in selecting trees as candidates for future autochthonous best-quality sweet chestnuts cultivars.

Acknowledgements

This research was carried out with the financial support of the Ministry of Science, Education and Sports of the Republic of Croatia. Grant No. 068-0242108-2773.

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