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LEAF CHARACTERISTICS AS IMPORTANT MORPHOMETRIC DISCRIMINATORS FOR CHESTNUT (*CASTANEA SATIVA* MILL.) GENOTYPES

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This study was carried out in order to determine the leaf characteristics of some chestnut (*Castanea sativa* Mill.) genotypes in the M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) and also to determine whether the leaf morphometric characteristics could be used for differentiation of genotypes. In this study, 9 chestnut genotypes (CS-01 – CS-09) were used. Some leaf parameters such as lamina length, lamina width, petiole length, petiole width, petiole thickness, teeth length, teeth width, stomata length, stomata width were studied. Morphometric parameters were following: leaves length from 85.0 to 250.0 mm, leaves width from 26.0 to 100.0 mm, petiole length from 8.07 to 36.18 mm, petiole width from 0.91 to 2.60 mm, petiole thickness from 0.81 to 2.44 mm, teeth length from 1.27 to 5.05 mm, teeth width 0.87 to 2.82 mm. The shape indexes of leaves were found ranging from 2.45 to 4.26. Analysis of coefficient of variation (CV) showed a high variability in morphometric characteristics between *Castanea sativa* samples. Data showed that the teeth width is the most variable signs (from 15.30 to 22.18%). Other studied characteristics have an average level of variability. Collected quantitative data were subjected to principal hierarchical cluster analysis. The cluster analysis of morphometric parameters exhibited that broad morphologic diversity was found in *Castanea sativa* genotypes examined in this study. Most of the chestnut genotypes could be differentiated easily by using leaf morphometric characteristics.

Keywords: *Castanea sativa*, leaf, morphometric characteristic, discrimination, genotype

Introduction

The study of morphological traits of leaf have been used frequently in practice by scientists to study genetic variability (Dickinson et al., 1986; Aravanopoulos et al., 2001; Kremer et al.,

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2002; Ertan, 2007; Neophytou et al., 2007), since the leaf location, size, shape and anatomical characteristics of leaf plates vary widely depending on environmental conditions. These signs reflect the degree of plant resistance, because the leaves are the most important organs of photosynthesis and transpiration (Sahin and Soylu, 1991; Bruschi et al., 2003; Pinto et al., 2011; Mensah, 2012). These characteristics are important for genotypes identification base on the observation of morphological characteristics of which expressions are largely influenced by developmental, environmental and cultivation factors (Serdar and Kurt, 2011; Poljak et al., 2014). Many authors use morphological analyses to estimate the variability of *Castanea sativa* and which confirm that the leaf parameters can be appropriate variables for establishing the level of genotype variability (Aravanopoulos et al., 2001; Aravanopoulos, 2005; Bolvansky and Uzik, 2005; Álvarez-Álvarez et al., 2006; Ertan, 2007; Zarafshar et al., 2010; Serdar and Kurt, 2011; Mujagić-Pašić and Ballian, 2012; Poljak et al., 2014; Atefe et al., 2015).

Identification of genotypes based on morphological traits allows us to find valuable specimens for further selection, which is very valuable, especially for woody plants (Skvortsov et al., 2005; Brindza et al., 2007; Monka et al., 2014; Grygorieva et al., 2014, 2018; Kucelova et al., 2016; Vinogradova et al., 2017).

The objective of this research was to evaluate the leaf characteristics of seven *Castanea sativa* Mill. genotypes and also to determine whether leaf morphometric characteristics could be used for differentiation of genotypes.

Material and methodology

Locating trees and data collection

The objects of the research were 45-year-old plants of *Castanea sativa* from seed origin, which are growing in Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG). Seeds were brought from Czech, Carpathians, Kyrgyzstan. They are well adapted to the climatic and soil conditions (Grygorieva et al., 2017; Klymenko et al., 2017). Field studies were carried out in August, 2017 on mature leaves of 9 chestnut genotypes (CS-01 – CS-09). In the study, chestnut genotypes with superior fruit characteristics were preferred. Trees in the same exposure were selected.

Morphometric characteristics

Leaf morphological parameters were determined on the 1-year-old shoots (middle part of shoot). It was selected from the tree of each genotype 30 leaves from the four sides of crown. The total number of selected leaves was 1080. For each leaf, seven characteristics were measured. In this study, leaf length (LL) in cm; leaf width (LW) in cm; petiole length (PL) in mm; petiole width (PW) in mm; petiole thickness (PT) in mm; teeth length (TL) in mm; teeth width (TW) in mm. The measurements were made in each leaf element as shown in Figure 1.

Statistical analyses

Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between phenotypes were computed on the basis of the Bray-Curtis similarity

index; multi-dimensional scaling (MDS) analyses were performed in PRIMER (Clarke and Gorley, 2006). Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by Stehlíková (1998).

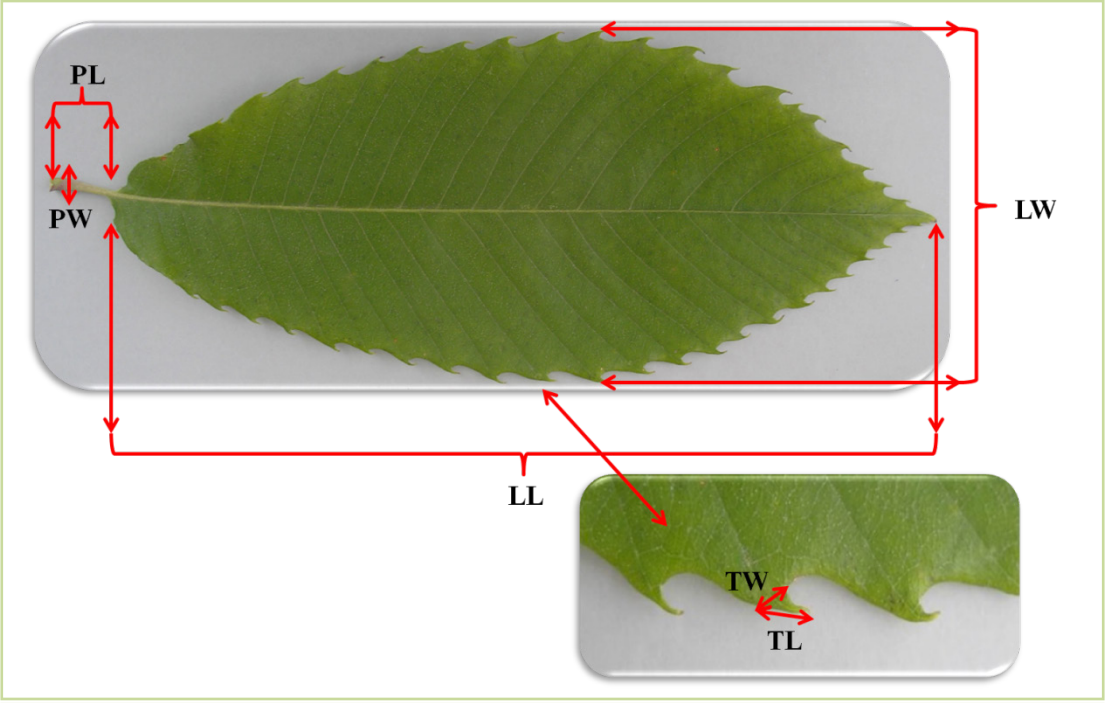

Figure 1 Illustration of measuring process: leaf length, leaf width, petiole length, petiole width, teeth length, teeth width

Results and discussion

Castanea sativa leaves arranged alternately, are simple, shiny in appearance and dark green in color. The leaf coloration changes within the chestnut genotypes. However, there is a clear distinction among the upper surface coloration (green-dark) and the lower surface (light green) coloration. The margins of leaf are toothed. In the collection several types of leaves could be found lanceolate, narrow elliptic, broad elliptic. The shape of apex narrow acuminate, broad acuminate, acute. The shape of base acute, obtuse, cordate. The incisions of margin mucronate and dentate. The petiole colour is yellowed or red.

The images of sweet chestnut leaves of various genotypes are shown on Figures 2, 3, 4, 5 and 6.

Figure 2 Variability in the shape of *Castanea sativa* Mill. leaves

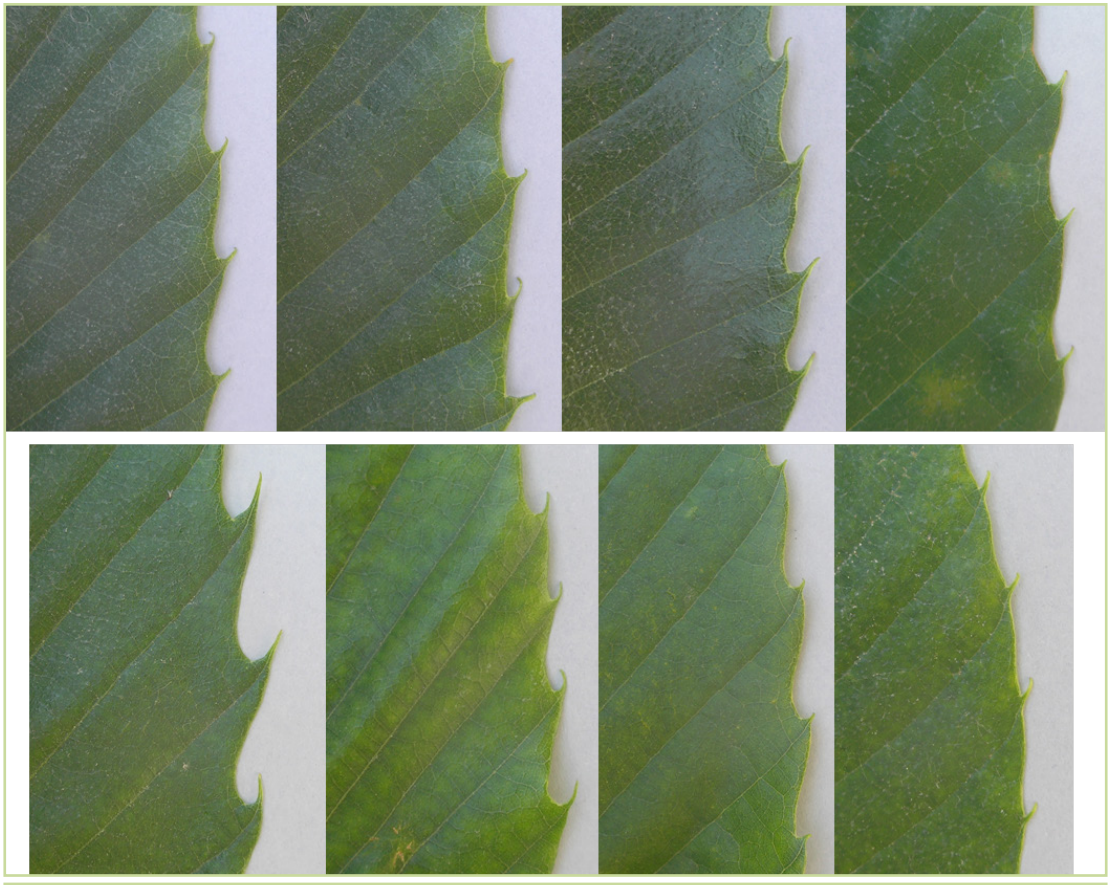

Figure 3 Variability in the teeth of *Castanea sativa* Mill.

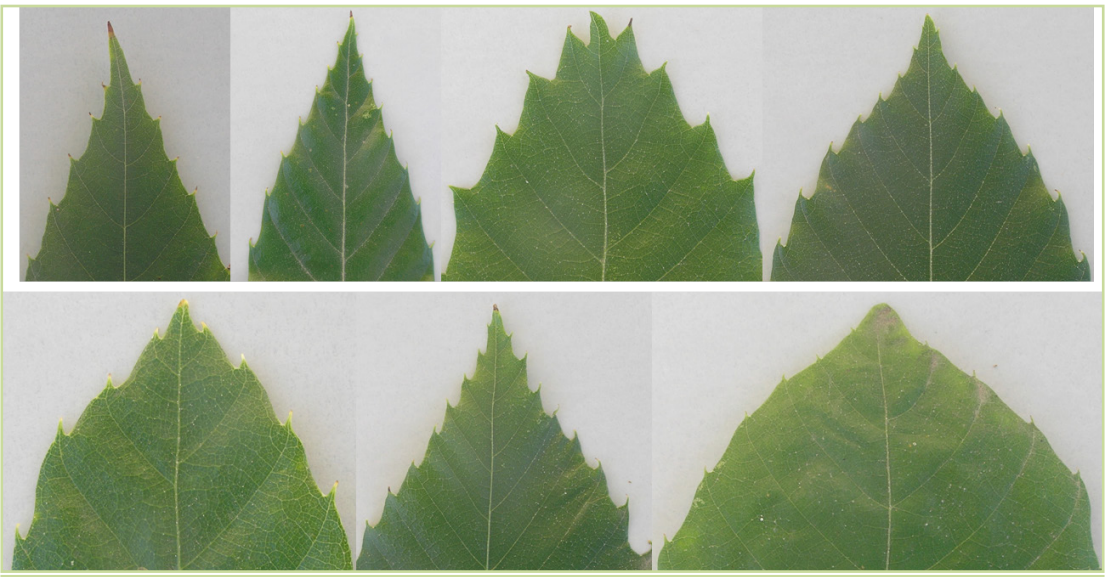

Figure 4 Shape of leaf apex of *Castanea sativa* Mill.

Figure 5 Shape of leaf blade bases of *Castanea sativa* Mill.

Figure 6: Variability in the petiole of *Castanea sativa* Mill.

The length of sweet chestnut leaves of present study was in the range of 85.0 (CS-08) to 250.0 (CS-06) mm (Table 1). Significant differences in leaves were reaffirmed a lot of authors from different countries (Table 2). The leaves length was determined in range from 9.20 to 30.80 cm by Mujagić-Pašić and Ballian Dalibor (2012), from 14.21 to 24.08 cm by Álvarez-Álvarez et al. (2006), from 14.50 to 21.20 cm by Poljak et al. (2014), from 17.70 to 23.40 cm by Serdar and Kurt (2011), from 21.30 to 23.40 cm by Zarafshar et al. (2010), from 22.44 to 25.05 cm by Atefe et al. (2015). Data comparison shows a high consistency with our results.

Table 1 The variability of some morphometric parameters of leaves of *Castanea sativa* Mill. genotypes

Genotypes	min	max	S_x	CV%	Genotypes	min	max	S_x	CV%
Leaf length					**Petiole width**				
CS-01	108	189	14.85	10.76	**CS-01**	0.91	1.42	0.11	10.55
CS-02	119	229	23.47	13.37	**CS-02**	1.13	1.56	0.11	8.20
CS-03	148	215	14.90	8.44	**CS-03**	1.63	2.24	0.14	7.62
CS-04	103	167	17.15	13.14	**CS-04**	1.02	1.38	0.10	8.44
CS-05	98	187	18.60	12.11	**CS-05**	1.25	2.20	0.20	12.54
CS-06	155	250	23.83	11.75	**CS-06**	1.61	2.60	0.23	11.74
CS-07	148	206	15.78	9.00	**CS-07**	1.34	2.17	0.20	10.94
CS-08	85	210	23.30	13.91	**CS-08**	1.41	2.08	0.18	10.42
CS-09	114	237	28.02	16.63	**CS-09**	1.33	2.25	0.20	12.37
Leaf width					**Petiole thickness**				
CS-01	28	47	4.76	12.89	**CS-01**	0.81	1.20	0.12	12.16
CS-02	30	57	7.00	16.85	**CS-02**	1.05	1.66	0.14	11.12
CS-03	52	77	5.14	7.88	**CS-03**	1.19	1.87	0.16	10.64
CS-04	26	42	4.76	13.84	**CS-04**	0.85	1.39	0.14	13.07
CS-05	28	54	6.73	15.75	**CS-05**	1.00	1.62	0.15	11.51
CS-06	54	100	10.40	12.58	**CS-06**	1.46	2.44	0.24	13.09

Continue the Table 1

Genotypes	min	max	S_x	CV%	Genotypes	min	max	S_x	CV%
	Leaf width					**Petiole thickness**			
CS-07	59	75	4.24	6.37	**CS-07**	1.03	2.15	0.25	15.60
CS-08	35	65	7.32	14.18	**CS-08**	1.20	1.90	0.19	12.84
CS-09	38	63	6.18	12.54	**CS-09**	1.13	2.11	0.21	14.12
	Petiole length					**Teeth length**			
CS-01	18.24	28.06	2.44	10.22	**CS-01**	1.27	2.93	0.35	16.76
CS-02	18.31	34.56	4.55	17.80	**CS-02**	1.61	3.15	0.43	18.95
CS-03	10.03	19.96	2.81	19.49	**CS-03**	1.76	4.19	0.60	19.90
CS-04	19.32	35.85	3.89	13.86	**CS-04**	1.62	3.67	0.56	20.56
CS-05	16.15	23.92	2.26	11.10	**CS-05**	2.03	3.88	0.49	16.82
CS-06	14.03	24.89	2.95	15.73	**CS-06**	2.07	4.41	0.52	16.77
CS-07	8.07	13.97	1.66	15.08	**CS-07**	1.86	3.96	0.53	17.60
CS-08	17.91	36.18	4.92	17.97	**CS-08**	1.80	5.05	0.69	20.68
CS-09	13.02	19.07	1.58	10.28	**CS-09**	1.49	3.83	0.48	18.12

Teeth width				
Genotypes	**min**	**max**	S_x	**CV%**
CS-01	0.87	2.03	0.22	16.07
CS-02	1.04	2.12	0.23	16.83
CS-03	1.10	2.38	0.32	18.61
CS-04	1.01	2.12	0.27	18.76
CS-05	1.02	2.30	0.30	17.42
CS-06	1.20	2.45	0.36	21.69
CS-07	1.39	2.82	0.33	15.30
CS-08	1.13	2.65	0.39	22.18
CS-09	1.03	2.71	0.33	18.04

Note: min, max – minimal and maximal measured values; S_x – standard deviation; CV – coefficient of variation (%)

The leaves width in our analyses was determined in the range of 26.0 (CS-04) to 100.0 (CS-06) mm (Table 1). The leaves width was determined in range from 2.50 cm by Mujagić-Pašić and Ballian Dalibor (2012) to 14.80 g by Zarafshar et al. (2010) (Table 2).

The petiole length, width and thickness in our analyses was determined in the range of 8.07 (CS-07) to 36.18 (CS-08) mm, from 0.91 (CS-01) to 2.60 (CS-06) mm and from 0.81 (CS-01) to 2.44 (CS-06) mm, respectively (Table 1). Maximal signs of petiole length were 6.70 cm (Table 2) found in study Mujagić-Pašić and Ballian Dalibor (2012).

Table 2 Variability of some morphometric characteristics on *Castanea sativa* Mill. leaves according to the authors from different countries

Authors	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)
Álvarez-Álvarez et al. (2006)	14.21–24.08	4.46–8.68	1.02–2.21
Zarafshar et al. (2010)	21.30–23.40	14.10–14.80	1.30–1.50
Serdar and Kurt (2011)	17.70–23.40	4.10–6.77	1.90–2.90
Mujagić-Pašić and Ballian Dalibor (2012)	9.20–30.80	2.50–12.0	0.50–6.70
Poljak et al. (2014)	14.50–21.20	4.70–8.50	1.90–2.80
Atefe et al. (2015)	22.44–25.05	6.98–8.66	1.33–1.92

The teeth length and width in our analyses were determined in the range of 1.27 (CS-01) to 5.05 (CS-08) mm and of 0.87 (CS-01) to 2.82 (CS-07) mm, respectively (Table 1). Mean values of morphological parameters of leaves demonstrated on Figure 7. There is a significant difference between the morphological parameters of leaves size that confirmed by results from multi-dimensional scaling visual distribution of studied genotypes (Figure 8). The sample CS-04 (green ellipse) with the smallest leaves size and the sample CS-06 (light green ellipse) with the largest leaves size differ each another with the probability of 95% (the ellipses in the figure do not overlap).

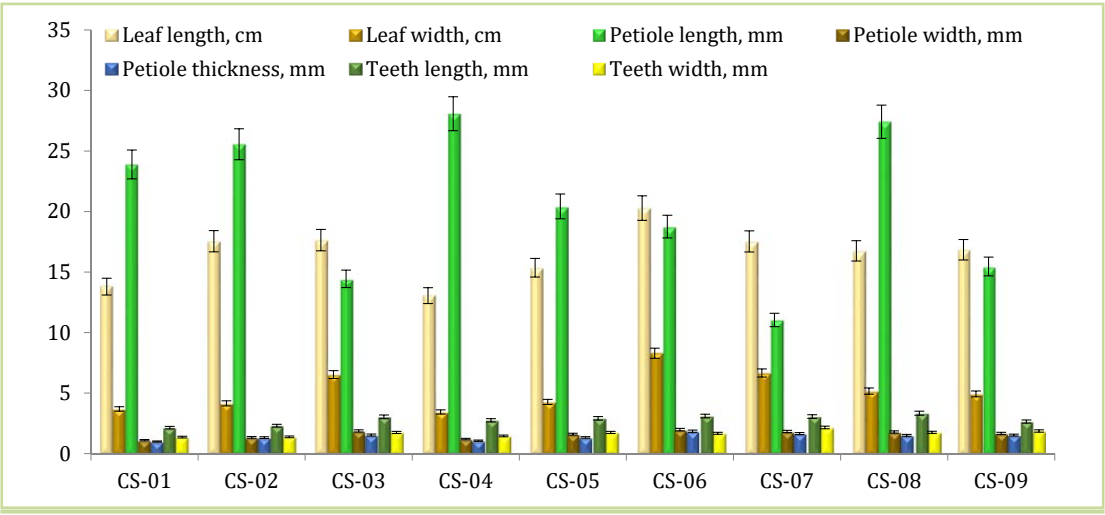

Figure 7: Mean values for leaf of *Castanea sativa* Mill. genotypes

The coefficient of variation showed the difference in variability of morphological signs between *Castanea sativa* samples (Table 1). Data showed that the most variable signs are the teeth width from 15.30 to 22.18%. Other studied characteristics have an average degree of variability.

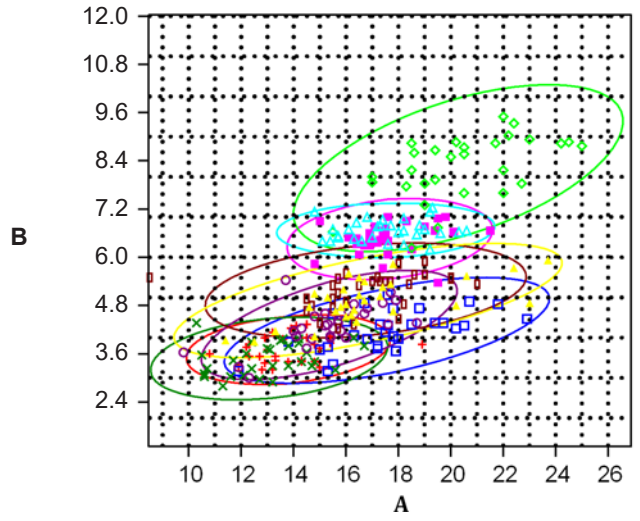

Figure 8: The scatter plot of leaf resulted from PCA in space coordinate axes based on the two components

The shape of each object can be characterized by the shape index, i.e. the length to width ratio. Figure 9 represents the shape indexes of leaves. The shape index of the leaves was found in the range from 2.45 (CS-06) to 4.26 (CS-02), so the genotypes collection demonstrates significant variability in the shape of the leaves, as seen in Figure 2. These parameters can be used for the identification of the genotypes.

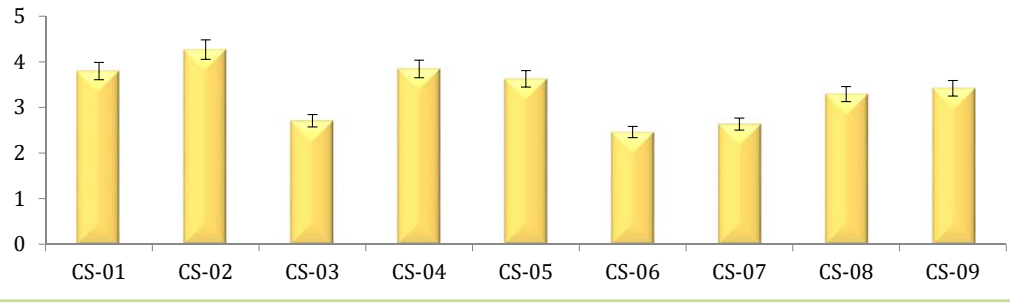

Figure 9: Comparison of the tested *Castanea sativa* Mill. genotypes in the shape index of leaves

The bivariate Pearson correlations revealed very strong correlation between PW/PT ($r = 0.92$), LW/PT and LL/PT ($r = 0.92$), strong correlation revealed between LW/PW ($r = 0.89$), LL/LW ($r = 0.87$), PW/TL ($r = 0.82$), LL/PW ($r = 0.81$), PW/TW ($r = 0.74$). Moderate positive correlations were between the PT/TL, PT/TW, TL/TW, LW/TL, and LW/TW.

Table 3 The matrix of Pearson correlation coefficients for 21 pairs of variables leaf *Castanea sativa* Mill.

Parameters	Lamina length	Lamina width	Petiole length	Petiole width	Petiole thickness	Teeth length
Lamina width	0.87*					
Petiole length	-0.49	-0.65				
Petiole width	0.81*	0.89*	-0.62			
Petiole thickness	0.92*	0.92*	-0.62	0.95*		
Teeth length	0.45*	0.64	-0.26*	0.82	0.68*	
Teeth width	0.42	0.58*	-0.77	0.75*	0.69	0.66*

Note: Significant according to the *t*-test (p <0.05)

The cluster analysis was performed according to the hierarchical cluster analysis method using the mean value to distinguish similar groups among the various leaves morphometric parameters.

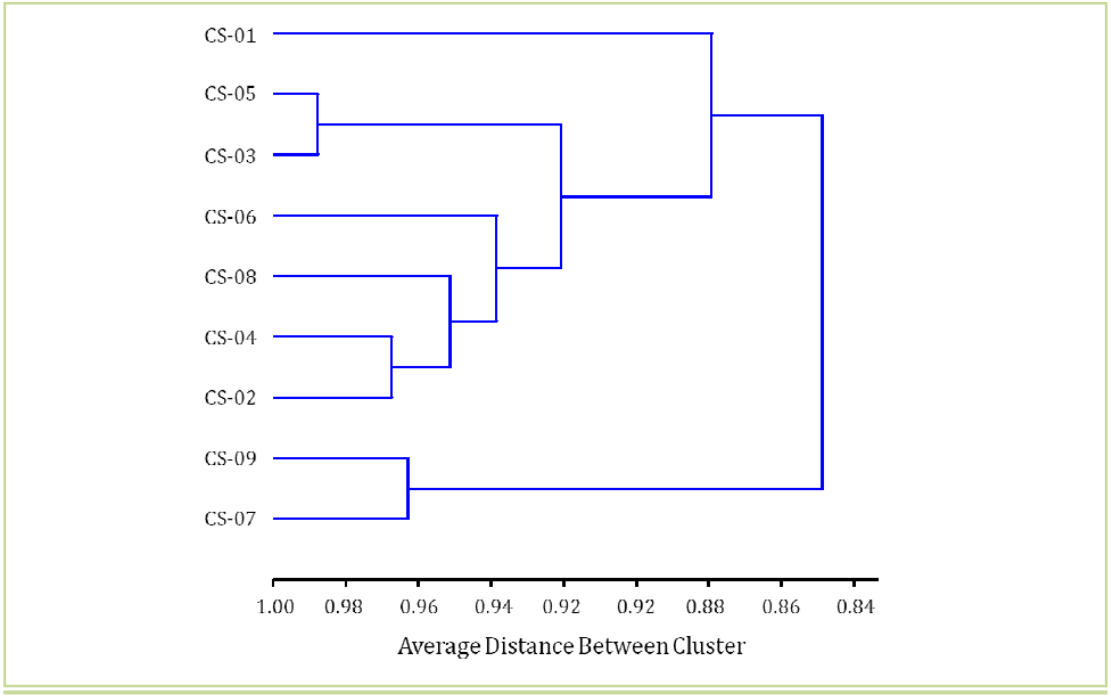

Figure 10: Cluster dendrogram based on morphometrics parameters of *Castanea sativa* Mill. leaves genotypes

The Figure 10 clearly identified significant differences between tested *Castanea sativa* genotypes. In this study, nine genotypes were grouped into four main clusters based on highest similarities. Cluster I contained the genotype (CS-01) only, which differs from other

genotypes of collection by lamina length and width. Cluster II contained only two (CS-03 and CS-05) *Castanea sativa* genotypes. In the cluster III included 4 genotypes and in the cluster IV – 2 genotypes, which had similar mean values of leaves morphological parameters. Figure 10 confirms results from the evaluated variability of morphometric characteristics (Table 1).

Conclusions

In general, our results indicate considerable leaf variation in the collections studied. The leaf parameters are suitable variables to detect levels of variability. The high diversity observed in the introduction population *Castanea sativa* studied is very important for the conservation of the species genetic resources. The high variability of the chestnut collection, created in the Grishka Botanical Garden, is a guarantee of its stability in the conditions of introduction (Skvortsov et al., 2005). In the course of further breeding work with this crop, it will be possible, based on this study, to select specimens with the most economically valuable traits in the first year of life of the seed offspring.

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