Australian Journal of Crop Science

AJCS

AJCS 16(10):1170-1176 (2022) ISSN:1835-2707
doi: 10.21475/ajcs.22.16.10.p3649

Phenotypic polymorphism of leaves among the populations of Moroccan chestnut (*Castanea sativa* Mill.)

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Abstract

Moroccan chestnut populations cover a small area in northern Morocco. They occupy great environmental and socio-economic interests. Nonetheless, such interests remained unvalued and little studied. This study aims to characterize and assess phenotypic similarities (or disparities) among the Moroccan populations of *C. sativa* via univariate and multivariate analyses of leaves traits. The experiments involved 6200 leaves from 31 populations, 10 trees per population, and 20 leaves per tree. Six morphometric parameters [blade area (S), perimeter (P), lamina length (LL), lamina width (LW), distance between the base and largest width (DBW), petiole length (PL)] and four ratios [LL/LW, LL/LP, LL/DBW, DBW/LP] were analyzed. Analysis of descriptive statistics within and between populations showed large variations among the studied parameters. This trend was supported as well by the analysis of variance (ANOVA) which revealed a highly significant differences (P <0.0001). Furthermore, the statistical analysis revealed a high phenotypic plasticity. The correlation analysis showed a significant relationship between most of the studied parameters. The principal component analysis (PCA) assigned the studied populations into two groups. We conclude that the studied parameters have great potential to determine the phenotypic polymorphism of *C. sativa* populations.

Keywords: Moroccan Chestnuts, populations, leaves, phenotypic variability, morphometric analysis.

Introduction

The genus *Castanea* is widely spread in the Boreal Hemisphere: Southeastern of North America, Southern Europe, Northwestern Africa, and Western East Asia (Fernandez-Lopez and Alia, 2003). According to botanical classification, it includes 12 to 13 species (Bounous, 2014). *Castanea sativa* is found in central Europe and around the Mediterranean basin (Mellano et al., 2018), where it is among the most cultivated tree species (Aravonopoulos et al., 2005; Pollegioni et al., 2020). Its phylogenetic evolution has induced a great genetic diversity in mountainous regions (Mellano et al., 2018). However, few decades ago, chestnut has lost its importance as a source of subsistence food without losing its cultural importance for many agroforestry systems (Tumpa et al., 2021). Furthermore, the historical investigations of chestnut revealed a complex stress involved biogeographic and anthropogenic factors (Pollegioni et al., 2020). Several populations and varieties are under multiple sanitary problems and suffer from years of carelessness (Mellano et al., 2007). The Moroccan chestnut undergoes similar scenario. In fact, due to its environmental and socio-economic importance (Toujgani et al., 2021), this tree is undervalued and less studied. Located in a small area in northern part of the country, Moroccan chestnut populations (Toujgani et al., 2021) are neither classified nor characterized. Nonetheless, the assessment and the characterization of chestnut population diversity and structuring are crucial for conservation strategies development and implementation, bearing in mind the sustainable use of natural resources (Lang and Huang, 1999). In this regard, morphological and phenological characterization is conventionally used for estimating genetic similarities and relationships (Atefe et al., 2015; Ertan, 2007; Mujagić-Pašić and Ballian, 2012; Poljak et al., 2013; Serdar and Kurt, 2011; Zarafshar et al., 2010). It is the official method used and accepted to register new cultivars (Pereira-Lorenzo et al., 1996; Serdar and Kurt, 2011; UPOV, 1989).

Due to its availability and its large range of morphological variation, leaves have frequently been used to study genetic variability (Aravanopoulos et al., 2005; Dickinson, 1986; Ertan, 2007; Kremer et al., 2002; Neophytou et al., 2007). Several authors have used leaf morphometric analysis to assess the genetic variability of *C. sativa*. They stated that leaf parameters are appropriate indicators to estimate the level of phenotypic variability/polymorphism (Álvarez-Álvarez et al., 2006; Aravanopoulos et al., 2001; Aravanopoulos et al., 2005; ; Bolvansky and Uzik, 2005; Ertan, 2007; Mujagić-Pašić and Ballian, 2012; Poljak et al., 2013; Serdar and Kurt, 2011; Zarafshar et al., 2010).

Moreover, the multivariate data analysis allowed the characterization and the assessment of plants genetic resources classification, as far as many enough characteristics were considered in a large sample (Cruz and Regazzi, 1994; Ertan, 2007).

The present study fits into this framework and contributes to the characterization and the assessment of phenotypic variabilities between Moroccan populations of *C. sativa* through univariate and multivariate analyses of leaf morphometric.

Results

Univariate analysis

The analysis of variance (ANOVA) for the 10 leaf morphometric parameters (Table 1S) revealed a very high significant variation (P<0.0001).

The morphometric trait differences between the studied populations were revealed by the coefficient of variation (CV%). In fact, the parameters S, DBW/PL, LL/PL, and PL showed a medium to a large variation (CV% between 30 and 40%), while the other parameters showed a small to a medium variation (CV% less than 30%).

However, we noticed that the average of the blade area (S) of all studied populations was (82.99 ± 31.83 cm²) with the highest blade area (108 ± 39.47 cm²) measured in Tazka population and the lowest blade area (53.43 ± 21.21 cm²) measured in Aazfa population.

The petiole length (PL) average was (2.14 ± 0.65 cm) with the longest length (2.56 ± 1.21 cm) noted in the Chouibene population while the shortest petioles (1.78 ± 0.42 cm) recorded in Zemmouret. The lamina length average (LL) was about (17.43 ± 3.76 cm). The longest laminas correspond to the leaves of Tazka population (20.43 ± 4.20 cm) and the smallest one recorded in Aazfa (13.57 ± 2.67 cm). Concerning the perimeter of the leaves (P), the average was about (39.79 ± 7.79 cm), while the largest perimeter was noted in Tazka (46.48 ± 8.27 cm) and the smallest (31.98 ± 6.01 cm) in Aazfa. The lamina width average (LW) was about (7.04 ± 1.54 cm) with the larger one (7.92 ± 1.87 cm) measured in Tazka, and the smaller one (5.63 ± 1.29 cm) measured in Aazfa. The distance between the base and the largest width average (DBW) was about (8.58 ± 2.21 cm), with the largest DBW (10.28 ± 2.23 cm) found in Bni Aasseme, and the smallest DBW (6.72 ± 1.57 cm) noted in Hrazma.

Phenotypic plasticity

Regarding the phenotypic plasticity, we noticed a rather high plasticity for all the studied parameters (Fig. 1).

Thus, the highest plasticity indices were observed for the four ratios: LL/DBW (between 0.326 and 0.955), LL/LW (between 0.467 and 0.838), LL/PL (between 0.573 and 0.969), and DBW/P (between 0.605 and 0.984).

The average LL/LW ratio was about (2.52 ± 0.51), with the highest ratio (3.07 ± 0.71) noted in Aanane, and the lowest one (2.26 ± 0.40) noted in Iaajanene. Similarly, the average LL/PL ratio was about (8.63 ± 2.76) with the highest one (11.06 ± 4.76) recorded in Tazka population and the lowest one (5.94 ± 1.34) in Aazfa population. The average LL/DBW ratio was about (2.08 ± 0.43) with the maximum value (2.41 ± 0.38) recorded in Hrazma population and the minimum value of (1.85 ± 0.19) in Bni Aasseme. The average DBW/PL ratio was (4.26 ± 1.53) with the maximum value (5.43 ± 2.20) found in Bni Aasseme, and the minimum value (2.97 ± 0.73) noted in Aazfa.

Multivariate Analysis

The correlation matrix analysis between the 10 studied parameters showed that there is a significant correlation between most of the studied parameters (Table 1).

Thus, positive correlations were identified between the blade area (S) and the perimeter (P) (r=0.945), the lamina length (LL) (r=0.893), the lamina width (LW) (r=0.923), the distance between the base and the largest width (DBW) (r=0.803) as well as with the ratios DBW/PL (r=0.804) and LL/PL (r=0.791).

In addition, the perimeter (P) was correlated with the lamina length (LL) (r=0.983), the lamina width (LW) (r=0.763), the distance between the base and the largest width (DBW) (r=0.787), as well as with the ratios DBW/PL (r=0.751) and LL/PL (r=0.790).

The lamina length (LL) was correlated with the lamina width (LW) (r=0.689), the distance between the base and the largest width (DBW) (r=0.792) and both ratios DBW/PL (r=0.738) and LL/PL (r=0.780). The blade width (LW) was correlated with the distance between the base and the largest width (DBW) (r=0.673) and the ratios DBW/PL (r=0.759) and LL/PL (r=0.724).

The distance between the base and the largest width (DBW) was correlated with the ratios: DBW/PL (r=0.787) and LL/PL (r=0.581). Finally, the DBW/PL ratio is correlated with LL/PL (r=0.921).

Similarly, negative correlations were identified between the lamina width (LW) and the petiole length (PL) (r=-0.526) as well as with the LL/LW ratio (r=0.524). Furthermore, the distance between the base and the largest width (DBW) was correlated with LL/DBW (r= -0.619), the ratio DBW/PL with the petiole length (PL) (r=-0.744), and finally, the petiole length (PL) with LL/PL (r=-0.800).

Principal component analysis (PCA) was applied on a matrix including the 31 populations of *C. sativa* considering the ten studied leaf morphometric parameters.

The analysis shows that most of the information is correlated with the two first factorial axes that hold 77.37% of the total variance (Table 2). The first Axis explains 61.39% of the total variance.

The first factorial axis (F1) is highly correlated with the blade area (S) (14.99%), the perimeter (P) (13.71%), the lamina length (LL) (12.89%), the lamina width (LW) (12.76%), and with the two ratios LL/PL (13.21) and DBW/PL (14.21%). The second factorial axis (F2), which explains 15.99% of the total variance, is correlated with the petiole length (PL) (23.74%) and the two shape ratios LL/LW (45.41%) and LL/DBW (0.98%).

The projection of the populations on the two-dimensional factorial plane (Fig. 2) shows a structuring gradient along the F1 axis. As a result, we noticed the existence of two large groups according to the value in terms of blade area (S), perimeter (P), lamina length (LL), lamina width (LW), distance between base and largest width (DBW), DBW/PL, and LL/PL.

Discussion

Our comparative study with previous similar works revealed similarities with *C. sativa's* population of Turkey in terms of blade area (S) (81. 6 cm²) (Serdar and Kurt, 2011), lamina length (17.63) (Ertan, 2007), lamina width (7.19) (Ertan, 2007), petiole length (2.11) (Ertan, 2007) and (2.13) (Serdar and Kurt, 2011), and LL/PL ratio (8.92) (Serdar and Kurt, 2011).

Table 1. Correlation coefficients matrix of all leaf measured parameters.

Variables		P	LL	LW	DBW	LL/LW	LL/DBW	DBW/PL	PL	LL/PL
S	1	0.945	0.893	0.923	0.803	-0.186	-0.167	0.804	-0.429	0.791
P		1	0.983	0.763	0.787	0.132	-0.051	0.751	-0.325	0.790
LL			1	0.689	0.792	0.246	-0.036	0.738	-0.297	0.780
LW				1	0.673	-0.524	-0.180	0.759	-0.526	0.724
DBW					1	0.018	-0.619	0.787	-0.223	0.581
LL/LW						1	0.199	-0.150	0.365	-0.051
LL/DBW							1	-0.275	-0.129	0.107
DBW/PL								1	-0.744	0.921
PL									1	-0.800
LL/PL										1

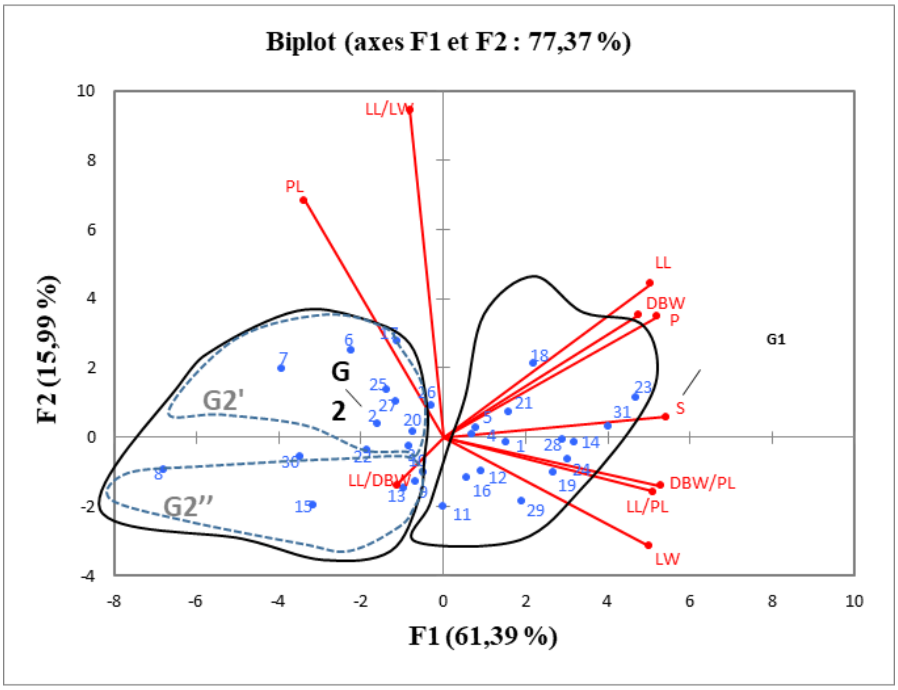

Figure 1. The plot of plasticity (PI) for all leaf measured parameters of 31 chestnut populations.

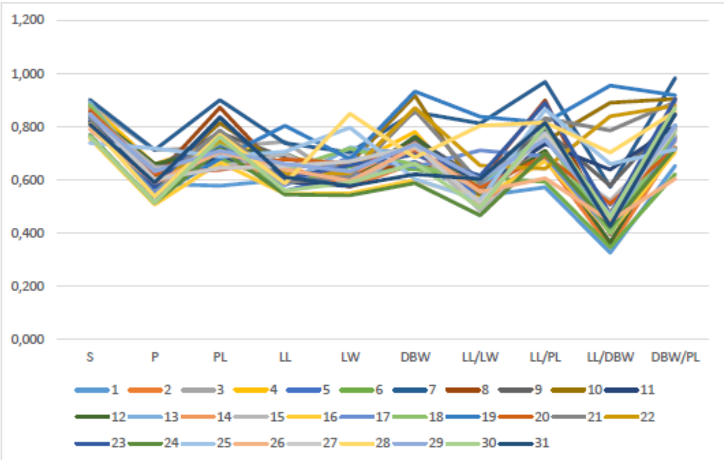

Figure 2. Projection of the plan [1, 2] of the principal component analysis (PCA). The blue dots represent the studied chestnut populations. The red lines represent the projection of the measured parameters (The corresponding codes are given in Table 3).

Table 2. PCA analysis results showing the contribution of the two main axes to the total variation (61.39%).

	F1	F2
Eigen values	6.14	1.69
Variability (%)	61.39	15.99
Cumulative %	61.39	77.37

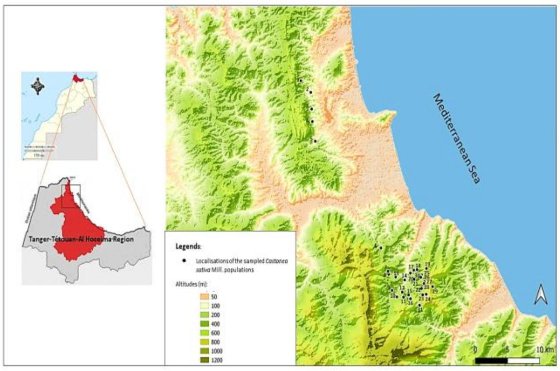

Figure 3. Localization of the sampled populations of *C. sativa.*

Table 3. List of the measured leaf morphometric parameters of *C. sativa.*

Morphometric parameter	Code	Unit of measure
Blade area	S	cm^2
Perimeter	P	cm
Lamina length	LL	cm
Lamina width	LW	cm
Distance between the base and the largest width	DBW	cm
Petiole length	PL	cm
Lamina length/lamina width	LL/LW	--
Lamina length/petiole length	LL/PL	--
Lamina length/Distance between the base and the largest width	LL/DBW	--
Distance between the base and the largest width/petiole length	DBW/PL	--

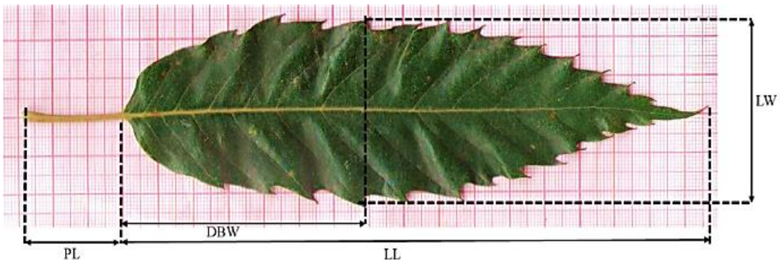

Figure 4. Presentation of the measured leaf morphometric parameters (PL: Petiole length; LL: Lamina length; DBW: Distance between the base and the largest width; LW: Lamina width).

The distance between the base and the largest width (DBW) as well as the LL/DBW ratio of the studied populations were close to the results found in Croatia; 8.5 cm for DBW and 2 for LL/DBW (Poljak et al., 2013). Regarding the LL/LW shape ratio, our result showed a similarity with the variety *'Judia'* from Portugal (2.8) (Dinis et al., 2011). The descriptive statistics of the studied morphometric parameters of 6200 leaves from the 31 populations and the analysis of variance (ANOVA) showed a high intra and inter-population variability. Therefore, we believe that the studied parameters related to size and shape could be used as valid indicators to detect phenotypic variability (Aravanopoulos et al., 2005; Furones and Fernandez Lopez, 2005; Serdar and Kurt, 2011). Our study revealed a high phenotypic plasticity for all the studied parameters. Gianoli and Gonzalez-Teuber, 2005 reported that high plasticity is related to physiological, morphological, or the development of the same genotype in different environments. As a result, phenotypic plasticity allows the plants in natural populations to cope with environmental heterogeneity/variability (Gianoli and Gonzalez-Teuber, 2005; Pigliucci et al., 1999). However, the study of the plasticity index of phenotypic traits does not necessarily imply that the response is adaptive (Sultan, 1995; Zarafshar et al., 2010). Nonetheless, authors have reported high plasticity even in controlled field trial conditions (Zarafshar et al., 2010). Although morphometric parameters can be influenced by environmental conditions, the genetic effect is expected to be more important (Atefe et al., 2015; Mujagić-Pašić and Ballian, 2012; Poljak et al., 2013; Serdar and Kurt, 2011; Zarafshar et al., 2010).

In addition, morphological and phenological characterizations are traditionally used to elaborate and estimate genetic similarities and relationships (Bruschi et al., 2003; Ertan 2007; Zarafshar et al., 2010). It is also the official method used and accepted for the registration of new cultivars (Aravanopoulos et al., 2001; Aravonopoulos et al., 2005; Pereira-Lorenzo et al., 1996; Serdar and Kurt, 2011; UPOV, 1989). Thus, our study revealed a high genotypic variability of Moroccan chestnut populations over a restricted area in the northern part of the country.

Principal component analysis (PCA) showed that the group structure was essentially related to the factorial axis F1 (61.39%). This was strongly correlated with the blade area (S), the perimeter (P), the lamina length (LL), the lamina width (LW), and LL/PL and DBW/PL ratios.

In this way, the parameters with the strongest correlations along the F1 axis are considered to be the most important factors for group differentiation (Atefe et al., 2015; Bruschi et al., 2003). In this sense, we can conclude that the lamina variables have a more important role than the petiole length in population differentiation. Similar statement has been reported by other authors (Aravonoupolos et al., 2005; Serdar and Kurt, 2011).

Therefore, the studied populations were divided into two main groups according to the parameters correlated to F1.

The first group 1 (G1) is composed by the populations from: Imftahene, Tikinziouine, Tazka, Oued Lille, Boulouazene, Oued Zarjoune, Slimatene, Oued Lkhmisse, Ilahmrene, Zaouya Sidi kacem, Imkouarene, Iaajanene, Ibraquene, Zemmouret and Bni Aasseme. This group is characterized by a large value in terms of blade area (S), perimeter (P), lamina length (LL), lamina width (LW), distance between base and largest width (DBW), DBW/PL and LL/PL.

At the opposite side of F1 axis, the group 2 (G2) is characterized by a small value for abovementioned parameters. However, G2 can be divided into two subgroups: a first G2' composed by populations issued from: Chouibene, Mokdassene, Aanane, Ihamdanene, Chouitene, Kouf, Imoudnene and Iaaboutene. These populations have high LL/LW ratio and high petiole length (PL). The second subgroup G2'' is composed by populations from: Aazfa, Tiglharoune, Hrazma, Aaychoune, Bayine, Iquzazene, Toughza and Sidjiwine. This subgroup is featured by high values of LL/DBW.

Previous studies have revealed that geographic origin and altitude factors may be important in discriminating different populations (Atefe et al., 2015; Babaaei et al., 2010; Mujic et al., 2010; Mujagić-Pašić and Ballian, 2012). Our results did not reveal a separation of groups based on these factors; rather confirm the findings of Bruschi and his team (2003) and Zarafshar and his team (2010). These findings highlighted the heterogeneity between different populations that predict very significant phenotypic plasticity.

Material and methods

Study area

The sampling of chestnut leaves involved 31 populations in Northwest part of Morocco (Fig.3; Table2S).

This is the only area where chestnut occurs in Morocco (Toujgani et al., 2021). It is characterized by a Mediterranean climate (hot and dry in summer, warm and humid in winter). The average annual values are 634 mm for the precipitation, 18.3°C for the temperature, and 36 Km/h for the wind (Gauché, 2006; HCP, 2020). The topography is marked by rugged terrain with soil featured with a fine silty-clay texture as found in a mountainous area.

Plant material

Leaf sampling was conducted between October and December 2019. The sampling involved 310 trees with an average of 10 trees/ population. The sampled trees had various ages and are found in open fields, orchards and/or matorral. About 20 leaves were sampled from each tree, selected at a height of 2 meters, by making a complete circle around the tree (Alvarez-Alvarez et al., 2006; Zarafshar et al., 2010). The 6200 sampled leaves were well-developed and collected from the current year's shoots (UPOV, 1989). They showed no signs of growth anomalies, mechanical damage, presence of pathogens, or insect infestation.

Morphometric characteristics

To study the morphometric parameters, leaves were scanned and measurements were made using Image J software (Fig. 4).

To examine the phenotypic variability of leaves, six morphometric parameters were used (Table 3) and four ratios representing the independent variables of leaf shape (Aravonopoulos et al., 2005; Atefe et al., 2015; Serdar and Demirsoy, 2006; Serdar and Kurt, 2011; UPOV, 1989).

Data analysis

The morphometric data were used to calculate standard descriptive statistical parameters (means, standard deviation (SD), and coefficient of variation (CV%)). Intra and inter-population statistical analyses were performed using analysis of variance (ANOVA). In addition, total plasticity within the population (PI) was calculated for each parameter as follows (Ashton et al., 1998; Bruschi et al., 2003; Zarafshar et al., 2010): $PI = 1 - (x/X)$, where x is the smallest value and X is the largest value for any given leaf measure. This was complemented by a principal component analysis (PCA) to identify the contribution of each variable to discriminate the chestnut populations. Furthermore, the PCA allows to plot the ordination of the variable groups in the main space and to find the most important traits among the studied features. All computing was performed by XLSTAT (2016).

Conclusion

This study is the first contribution to characterize the Moroccan chestnut populations, pointing out the existence of a great phenotypic variability. Our study involved the analysis of 10 morphometric parameters of 6200 leaves belonging to 31 populations located in a restricted area in northern part of Morocco. Our finding shows that the studied variables can be appropriate to determine the phenotypic variability of *C. sativa* populations. On the other hand, the region is a hotspot for biodiversity conservation. Therefore, the assessment of this natural heritage is crucial for the development and the implementation of conservation strategies to safeguard chestnut genetic resources. In conclusion, future studies should focus on the fruit morphometric and pomological studies as well as on the assessment of chestnut populations' genetic diversity in Morocco via molecular markers.

Acknowledgments

The authors thank the farmers of the various visited villages for their contribution to realize this study. Special thanks go to Mr. M. Derdabi for his eminent support in the field.

Funding

This work was supported by the United Nations Educational Scientific and Cultural Organization, Men and Biospher Programme (MAB) [4500 409 557-506 606] and by a scholarship from the Faculty of Sciences of Tetouan.

References

Álvarez-Álvarez P, Barrio-Anta M, Diéguez-Aranda U (2006) Differentiation of sweet chestnut (*Castanea sativa* Mill.) cultivars by leaf, nut and burr dimensions. Forestry.79(2): 149-158.

Aravanopoulos F A, Drouzas A D, Alizoti P G (2001) Electrophoretic and quantitative variation in chestnut (*Castanea sativa* Mill.) in Hellenic populations in old-growth natural and coppice stands. Forest Snow and Landscape Research. 76(3): 429-434.

Aravanopoulos F A, Bucci G, Akkak A, Blanco Silva R, Botta R, Buck E, Cherubini M, Drouzas A D, Fernández-López J., Mattioni C, Marinoni D, Papadima A, Russell K, Zas R, Villani F (2005) Molecular population genetics and dynamics of chestnut (*Castanea sativa*) in Europe: Inferences for gene conservation and tree improvement. Acta Hortic. 693: 403-412.

Ashton P M S, Olamder L P, Berlyn G P, Thadani R, Cameron I R (1998) Change in leaf structure in relation to crown position and tree size of *Betula papyrifera* within fire region stands of interior cedar-hemlock. Can J Bot. 76(7): 1180-1187.

Atefe K, Kambiz T A, Javad T (2015) Variations in leaf and fruit morphological traits of sweet chestnut (*Castanea sativa*) in Hyrcanian Forests, Iran. International Journal of Plant Research. 1(4), 155-161.

Babaaei F, Jalali G, Azadfar D (2010) Investigation of genetic variation in *Zelkova carpinifolia* (Pallas) K. Koch, by use of leaf peroxidase isozyme in three lowland habitats in North of Iran. Journal of Wood and Forest Science and Technology. 19 (1): 121-133.

Bolvansky M, Uzik M (2005) Morphometric variation and differentiation of European chestnut (*Castanea sativa*) in Slovakia. Biologia. 60(4): 423-429.

Bounous G (2014) Il castagno: risorsa multifunzionale in Italia e nel mondo. Bologna, Italy: Il Sole 24 Ore Edagricole, pp.420.

Bruschi R, Grossoni R, Bussotii F (2003) Within-andamong-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. Natural populations. Trees. 17: 164–172.

Cruz C D, Regazzi A J (1994) Modelos biométricos aplicados ao melhoramento genético Viçosa: UFV, 309p. ISBN 8572692878.

Dickinson T A (1986) Topodeme differentiation in Ontario taxa of Crataegous (Rosaceae: Maloideae): leaf morphometric evidence. Can J Bot. 64: 2738-2747.

Dinis L T, Peixoto F, Pinto T, Costa R, Bennett R N, Gomes-Laranjo J (2011) Study of morphological and phenological diversity in chestnut trees ('Judia'variety) as a function of temperature sum. Environmental and Experimental Botany. 70(2-3): 110-120.

Ertan E (2007) Variability in leaf and fruit morphology and in fruit composition of chestnuts (*Castanea Sativa* Mill.) in the Nazilli region of Turkey. Genet Resour Crop Evol. 54: 691–699.

Fernández-López J, Alía R (2003) EUFORGEN technical guidelines for genetic conservation and use for chestnut *(Castanea sativa)*. Bioversity International. ISBN 92-9043-606-9.

Furones P, Fernández-López J (2005) Differentiation among chestnut cultivars using adaptative and morphological traits. Acta Hortic. 693 : 497-504

Gauché E (2006) La crise environnementale des bassins versants des Beni Saïd (Rif oriental, Maroc). Géomorphologie: Relief, Processus, Environnement. 12(2): 141-156.

Gianoli E, González-Teuber M (2005) Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). Evolutionary Ecology. 19(6): 603-613.

Goulão L, Valdiviesso T, Santana C, Oliveira CM (2001) Comparison between phenetic characterisation using RAPD and ISSR markers and phenotypic data of cultivated chestnut (*Castanea sativa* Mill.). Genetic Resources and Crop Evolution. 48(4): 329-338.

HCP (Haut-Commissariat au Plan du Maroc) (2020), Monographie de la région Tanger-Tetouan-Alhoceima, available at : https://www.hcp.ma/region-tanger/attachment/2099765/, consulted on December, 2020.

Kremer A, Dupouey J L., Deans, J D. Cottrell J, Csaikl U, Finkeldey R, Espinel S, Jensen J, Kleinschmit J, Van Dam B, Ducousso A, Forrest I, Lopez de Heredia U, Lowe A J, Tutkova M, Munro R C, Steinhoff S, Badeau V (2002) Leaf morphological differentiation between *Quercus robur* and *Quercus petraea* is stable across western European mixed oak stands. Ann Forest Sci. 59: 777–787.

Lang P, Huang H (1999) Genetic variation and population structure of three endemic Castanea species in China. Act. Hort. 494: 269–276.

Mellano M G, Beccaro G L, Bounous G, Trasino C, Barrel A (2007) Morpho-biological and sensorial quality evaluation of chestnut cultivars in Aosta Valley (Italy). In International Workshop on Chestnut Management in Mediterranean Countries-Problems and Prospects. 815 (pp. 125 132).

Mellano M G, Torello-Marinoni D, Boccacci P, Donno D, Cerutti A K, Beccaro G L (2018) Ex situ conservation and characterization of the genetic diversity of Castanea spp. Acta Horticulturae. (1220): 1-5.

Mujagić-Pašić A, Ballian D (2012) Variability of the sweet chestnut (*Castanea sativa* Mill.) based on the morphological properties of the leaf in natural populations of Bosanska Krajina. Works of the Faculty of Forestry University of Sarajevo. 1: 57-69.

Mujić I, Alibabić V, Živković J, Jahić S, Jokić S, Prgomet Ž, Tuzlak Z (2010) Morphological characteristics of chestnut *Castanea sativa* from the area of Una-Sana canton orfološke karakteristike pitomog kestena *Castanea sativa* sa područja unsko-sanskog kantona. Journal of Central European Agriculture. 11(2): 185-190.

Neophytou CH, Palli G, Dounavi A, Aravanopoulos FA (2007) Morphological differentiation and hybridization between *Quercus alnifolia* Poech and *Quercus coccifera* L. (Fagaceae) in Cyprus. Silvae Genet. 56 (6): 271– 277.

Pereira-Lorenzo S, Fernandez-Lopez J, Moreno-Gonzalez J (1996) Variability and grouping of northwestern Spanish chestnut cultivars. I. Morphological traits. Journal of the American Society for Horticultural Science. 121(2): 183-189.

Pigliucci M, Cammell K, Schmitt J (1999) Evolution of phenotypic plasticity a comparative approach in the phylo-genetic neighborhood of *Arabidopsis thaliana*. Journal of Evolutionary Biology. 12: 779– 791. ISSN: 1010-061X.

Poljak I, Idzojtić M, Zebec M (2013) Leaf morphology of the sweet chestnut (*Castanea sativa* Mill.): a methodological approach. In II European Congress on Chestnut 1043 pp. 211-218.

Pollegioni P, Lungo SD, Müller R, Woeste KE, Chiocchini F, Clark J, Hemery GE, Mapelli S, Villani F, Malvolti ME, Mattioni C (2020) Biocultural diversity of common walnut (*Juglans regia* L.) and sweet chestnut (*Castanea sativa* Mill.) across Eurasia. Ecology and Evolution. 10(20): 11192-11216.

Serdar Ü, Demirsoy H (2006) Non-destructive blade area estimation in chestnut. Sci Hortic. 108: 227–230.

Serdar U, Kurt N (2011) Some leaf characteristics are better morphometric discriminators for chestnut genotypes. Journal of agricultural science and technology (JAST). 13(6): 885-894.

Sultan SE (1995) Phenotypic plasticity and plant adaptation. Acta botanica neerlandica. 44(4): 363–383.

Toujgani I, El Fatehi S, Elmaraghi H, Ater M, Hmimsa Y (2021) Traditional knowledge and use value for sustainability “*Castanea sativa* Mill.’ as a model of socio-economic recovery in northern Morocco. Current Research in Environmental Sustainability. 3: 100075.

Tumpa K, Vidaković A, Drvodelić D, Šango M, Idžojtić M, Perković I, Poljak I (2021) The Effect of Seed Size on Germination and Seedling Growth in Sweet Chestnut (*Castanea sativa* Mill.). Forests. 12(7):858.

UPOV (International Union for the Protection of New Varieties of Plants) (1989) Draft guidelines for the conduct of tests for distinctness, homogeneity and stability (Chestnut).TG/124/1(proj.).

Zarafshar M, Akbarinia M, Bruschi P, Hosseiny S M, Yousefzadeh H, Taieby M, Sattarian A (2010) Phenotypic variation in chestnut (*Castanea sativa* Mill.) natural populations in Hyrcanian forest (north of Iran), revealed by leaf morphometrics. Folia Oecologica. 37(1): 113.