proceedings

MDPI

Proceedings

Phenotypic Variation of *Castanea sativa* Mill. Ecotypes in Northern Morocco Based on Multivariate Analysis of Leaf Morphometrics †

Toujgani Ihssane [1,*], Elfatehi Salama [1,2], Ater Mohammed [1] and Hmimsa Younes [1,2]

[1] Bio-Agrodiversity Team, Laboratory of Applied Botany, Department of Biology, Faculty of Science, Abdelmalek Essaâdi University, Tetouan, Morocco; (E.S.); (A.M.); (H.Y.)
[2] Department of Life Sciences, Polydisciplinary Faculty, Abdelmalek Essaadi University, Larache, Morocco
* Correspondence: ihssanetoujgani@gmail.com
† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021.

Abstract: For decades, local and traditional species have been neglected and replaced by industrial and improved species. Sweet chestnut '*Castanea sativa* MILL.', found in a small area in northern Morocco, is no exception. Indeed, Moroccan ecotypes are neither classified nor characterized. This study aims to evaluate the local genetic resources of *Castanea sativa* MILL. via multivariate analysis of morphometric parameters of leaves. The study involved 6200 leaves from 31 villages in 3 regions; 10 trees/village and 20 leaves/tree were sampled. Then eight morphometric parameters were analyzed: lamina length (LL), lamina width (LW), petiole length (PL), distance from the base of the leaf to the widest point of the leaf (DBW), surface (S), perimeter (P), and ratios LL/LW and LL/DBW. Analysis of the descriptive statistics within and between ecotypes initially showed a large variation in the ten parameters studied. This finding was supported by analysis of variance (ANOVA) which revealed a very highly significant difference ($p < 0.0001$) for all parameters. Indeed, the analysis of agglomerative hierarchical clustering (AHC) made it possible to group the studied populations into 3 distinct groups based on the surface. Overall, the high level of variability in leaf morphometric parameters indicates that the region is an important center of genetic diversity which assessment is crucial for the implementation of conservation and enhancement strategies for this heritage.

Keywords: *Castanea sativa* MILL.; local ecotypes; phenotypic variability; morphometric analysis; conservation

Citation: Ihssane, T.; Salama, E.; Mohammed, A.; Younes, H. Phenotypic Variation of *Castanea sativa* Mill. Ecotypes in Northern Morocco Based on Multivariate Analysis of Leaf Morphometrics. *Proceedings* **2021**, 65, x. https://doi.org/10.3390/xxxxx

Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Local and traditional species have been neglected and replaced by industrial and improved species for decades. The chestnut, *Castanea sativa* MILL., present in a small area in the north-western part of Morocco, is no exception to this trend. Indeed, Moroccan ecotypes have never been studied.

However, the assessment and characterization of the diversity and populations structure is crucial for the implementation of a strategy for the enhancement, conservation and sustainable use of natural resources [1]. This study fits into this framework and aims for the first time to characterize and evaluate the local genetic resources of *Castanea sativa* MILL. via a morphometric analysis of the leaves. In fact, the study of leaves morphological traits has been frequently used by researchers to determine the genetic variability of chestnut [2–4]. Notwithstanding, blade characteristics, in particular size, shape and anatomy, are largely influenced by developmental, environmental and cultural factors [5,6]

Nevertheless, many authors confirm that leaf parameters may be appropriate variables for determining the level of genotype variability [2,6–9].

2. Materials and Methods

2.1. Study Area

The chestnut leaves were collected in the North-West of Morocco (Figure 1) at the level of 31 villages divided between three tribal groups (El Haouz, Bni Said and Bni Hozmar) and part of 7 watersheds.

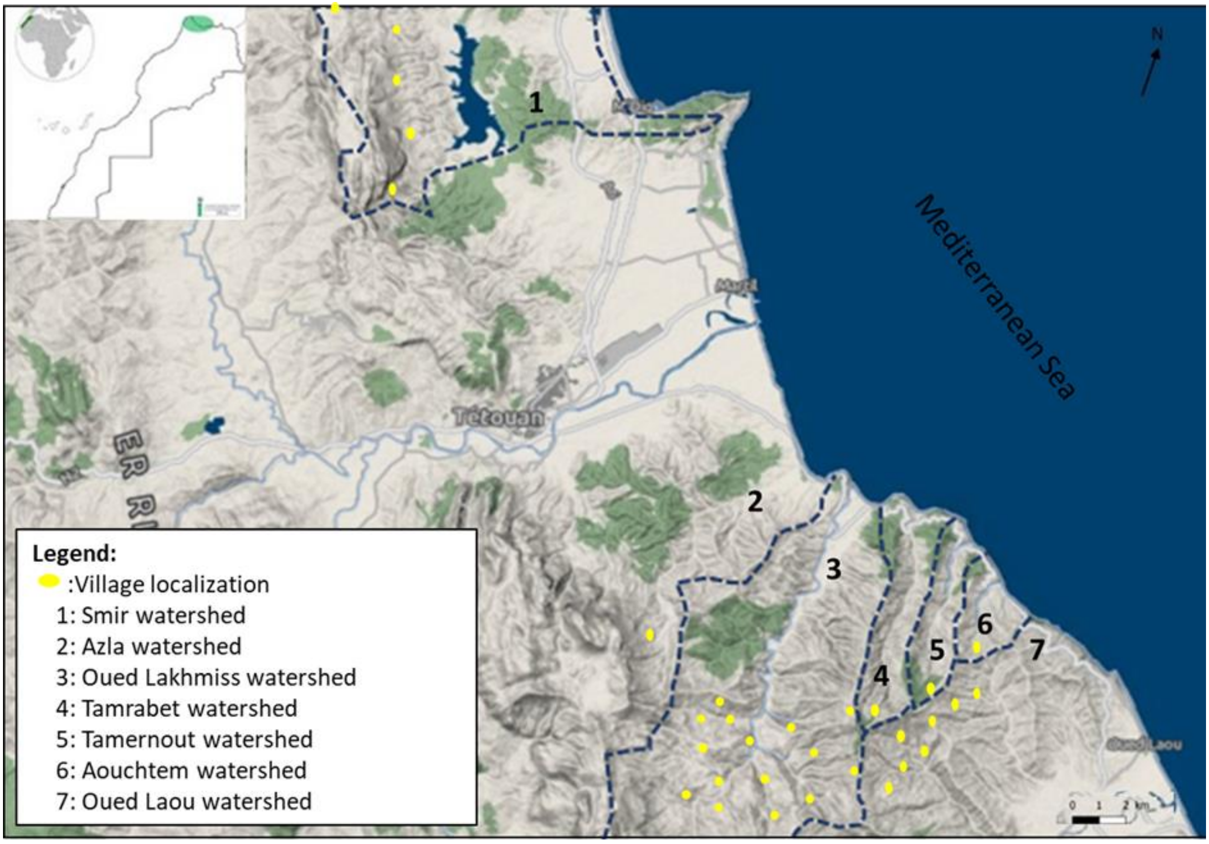

Figure 1. Distribution of villages in the seven watersheds at the study area level.

2.2. Morphometric Parameters

In order to assess the genetic variability of the Moroccan ecotypes, we used agromorphological characterization of the leaves. The collect of plant material was made from 310 trees distributed among 31 villages with a total of 10 trees/village. For each chestnut tree, 20 leaves were selected at 2 m in height, making a complete circle around the tree [7,9,10]. The 6200 leaves showed no signs of abnormal growth, mechanical damage, presence of pathogens or insect infestation. After scanning these sheets, phenotypic variability was investigated by performing a numerical analysis of the images using the Image J software (Figure 2). Morphometric measurements took into account the most relevant parameters [6,7,11]: lamina length (LL), lamina width (LW), distance from lamina base to greatest width (DBW), petiole length (PL), perimeter (P) and surface (S), then two ratios were calculated LL/LW and LL/DBW.

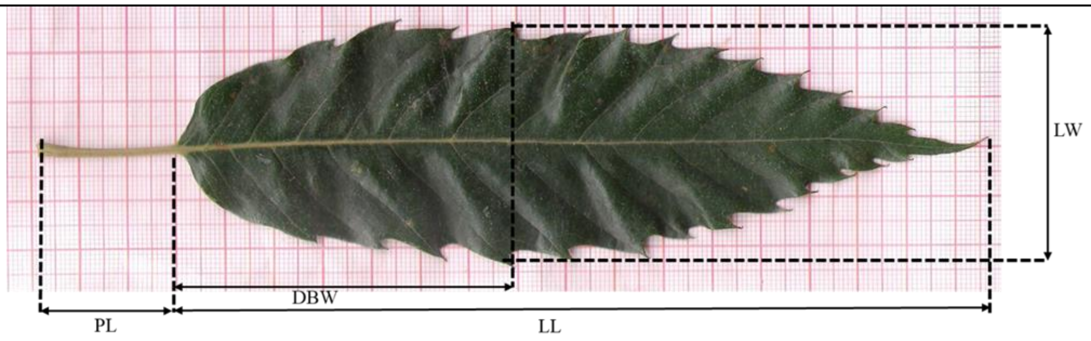

Figure 2. Diagram representing the measurements of the chestnut tree leaves.

2.3. Statistical Analysis

The data obtained were analyzed using descriptive statistics parameters, analysis of variance, comparison of means, analysis of correlation and the analysis of agglomerative hierarchical clustering (AHC) and the means comparison Student Newman Keuls test (SNK) using the statistical analysis software XLSTAT (version 2016).

3. Results

3.1. Analysis of Variability

The analysis of morphometric parameters studied at the level of the chestnut leaves of each of the seven watersheds (Table 1), allowed us to deduce that the watershed of Azla holds the leaves having the longest petioles (PL) with an average of 2.43 cm, and the highest LL/LW ratio (2.88). This ratio provides information on the shape of the leaf, the fact that it is high means that the leaves have a longer shape compared to other watersheds. However, the leaves of this watershed are the narrowest (LW) (6.31 cm). They also have the smallest distance between the base and the greatest width (DBW) (8.26 cm) and finally the smallest surface (S) (74.35 cm^2). The highest coefficient of variation of all the parameters in the seven watersheds was observed for the surface parameter (S) (34.35%) in Azla watershed.

Oued Lakhmiss watershed ecotypes leaves have the shortest blade (LL) (16.84 cm) and the smallest perimeter (38.73 cm). However, Oued Laou watershed ecotypes leaves have the highest perimeter (P) (41.65 cm) and also the longest blade (LL) (18.31 cm). The widest leaves (LW) are those of Tamrabet watershed ecotypes with an average of 7.48 cm. They also have the highest distance between the base and the greatest width (DBW) (9.10 cm), the largest surface (91.42 cm^2), and the smallest length of the petiole (PL) (1, 80 cm), and the highest LL/DBW ratio (2.22). It is at the level of this same watershed that the LL/LW ratio varies the least because it has the smallest coefficient of variation for all the parameters at the level of the seven watersheds (6.33%). In Aouchtam watershed, the leaves have the smallest LL/LW ratio with an average of 2.32.

The analysis of variance (ANOVA) for the studied morphometric parameters at the leaf level (Table 2) showed a very highly significant variation ($p < 0.0001$) for all studied parameters. These results reflect the morphological diversity between the individuals that compose the seven watersheds and indicate that the studied parameters relating to the leaves size and shape of the different ecotypes may be valid indicators for detecting phenotypic variability [6,7,12].

The comparison of the means of the leaves morphometric parameters by Student Newman and Keuls (SNK) test at the 5% threshold (Table 3), revealed 5 modes of grouping between the 7 watersheds. Thus, for the perimeter (P) and the lamina length (LL), we did not find any similarity between the means. On the other hand, the petiole length (PL) and the ratio (LL/DBW) allowed to classify the watersheds into 6 groups, suggesting the similarity between Tamernount and Aouchatam for the (PL) and between Haouz and Oued Lakhmiss for (LL/DBW). As for the surface (S) and the lamina width (LW), the ecotypes were distributed between 5 groups by associating the ecotypes of Oued Laou, Tamernount, and Aouchtam in a single group. The LL/LW ratio and the distance from base to greatest width (DBW) grouped the watersheds into 4 and 2 groups respectively.

Table 1. Morphometric parameters studied for the leaf in the 7 watersheds.

Watersheds	Parameters	S(cm²)	P(cm)	PL(cm)	LL(cm)	LW(cm)	DBW(cm)	LL/LW	LL/DBW
	Mean	88.76	41.65	2.04	18.31	7.16	8.82	2.61	2.16
Oued Laou	SD *	24.81	5.53	0.38	2.56	1.26	1.51	0.40	0.21
	CV ** (%)	28.54	13.28	19.77	13.99	18.29	16.15	15.33	9.51
	Mean	84.54	40.06	2.28	17.65	6.97	8.97	2.49	2.14
Tamernout	SD *	29.70	6.79	0.41	3.35	1.33	2.25	0.38	1.17
	CV ** (%)	25.58	13.98	10.23	14.72	12.88	16.5	33.08	11.08
	Mean	91.42	41.56	1.8	18.11	7.48	9.1	2.61	2.22
Tamrabet	SD *	32.22	7.20	0.73	3.42	1.58	2.09	0.54	0.64
	CV ** (%)	15.45	7.68	14.24	8.73	9.05	13.17	6.33	28.92
	Mean	88.95	40.34	2.05	17.35	7.45	8.85	2.32	1.98
Aouchtam	SD *	32.22	8.29	0.40	4.27	1.36	2.42	0.42	0.20
	CV ** (%)	22.06	12.65	8.97	13.22	9.39	16.2	17.87	10.27
	Mean	74.35	39.68	2.43	17.73	6.31	8.26	2.88	2.17
Azla	SD *	30.99	8.66	0.65	4.17	1.39	2.11	0.68	0.19
	CV ** (%)	34.35	19.03	20.57	20.26	16.73	22.07	23.58	8.84
	Mean	79.82	38.73	2.17	16.84	7	8.28	2.45	2.10
Oued Lakhmiss	SD *	20.12	5.20	0.37	2.50	0.96	1.48	0.32	0.20
	CV ** (%)	25.43	13.24	18.03	14.7	14.4	17.64	13.03	9.33
	Mean	84.34	39.64	2.21	17.53	7.20	9.03	2.47	1.97
Haouz	SD *	21.23	4.83	0.33	2.24	1.09	1.30	0.26	0.11
	CV ** (%)	25.17	12.18	14.82	12.76	15.21	14.41	10.73	5.71

* SD: Standard deviation ** CV: Coefficient of variation.

Table 2. Analysis of variance (ANOVA) of the studied morphometric parameters of the leaves in the ecotypes of Castanea sativa Mill.

Parameters	S	P	PL	LL	LW	DBW	LL/LW	LL/DBW
F	31.55	29.72	16.799	29.49	37.32	32.62	28.91	16.34
Pr > F	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****

* significant test, ** very significant test, *** highly significant test, **** very highly significant test.

Table 3. Comparison of the means of the leaves studied morphometric parameters in chestnut ecotypes by Student Newman Keuls test (SNK).

Watersheds	S	P	PL	LL	LW	DBW	LL/LW	LL/DBW
Tamrabet	A	A	A	A	A	A	A	A
Oued Laou	B	B	B	B	B	A	B	B
Tamernount	B	C	C	C	B	A	B	C
Aouchtam	B	D	C	D	B	A	C	D
Azla	C	E	D	E	C	A	C	E
Haouz	D	F	E	F	D	B	C	F
Oued Lakhmiss	E	G	F	G	E	B	D	F

3.2. Correlation Analysis

The analysis of the correlations between the studied parameters showed that there are links between them, which explains the very strong correlations (Table 4). Indeed, we have noticed the presence of strong correlations between the surface (S) and the lamina width (LW) (r = 0.924), as well as between the surface (S) and the distance between the base and the greatest width (DBW) (r = 0.835) and of less importance between the surface (S) and the perimeter (P) (r = 0.754). On the other hand, we also revealed strong correlations between the perimeter (P) and the lamina length (LL) (r = 0.896), as well as between the perimeter (P) and the distance between the base and the greatest width (DBW) (r = 0.610). We observed also that the surface (S) is negatively related to the LL/LW ratio (r = −0.505) which provides information on the lamina shape: This means that when the area increases, the ratio decreases which means that the sheet becomes wider than it is long. It was also noted that the petiole length (PL) is negatively correlated with the surface (S).

Table 4. Matrix of the correlation coefficients of the leaves studied parameters.

Variables	S	P	PL	LL	LW	DBW	LL/LW	LL/DBW
S	**1**	**0.754**	−0.877	0.446	**0.924**	**0.835**	−0.505	−0.025
P		**1**	−0.681	**0.896**	0.472	**0.610**	0.154	0.438
PL			**1**	−0.350	−0.867	-0.579	0.337	−0.169
LL				**1**	0.104	0.480	0.491	**0.546**
LW					**1**	**0.741**	−0.723	−0.272
DBW						**1**	−0.399	−0.119
LL/LW							**1**	**0.677**
LL/DBW								**1**

3.3. Agglomerative Hierarchical Clustering Analysis (AHC)

Agglomerative hierarchical clustering (AHC) analysis was used to produce a diagram (Figure 3) relating to the analysis of the studied parameters of the leaf of chestnut ecotypes from the seven watersheds. The topology of the dendrogram clearly shows the existence of three large groups relating to the parameters studied at the leaf level. The most similar watersheds are Tamernout and Smir on one side and Oued Laou and Aouchtem on the other one.

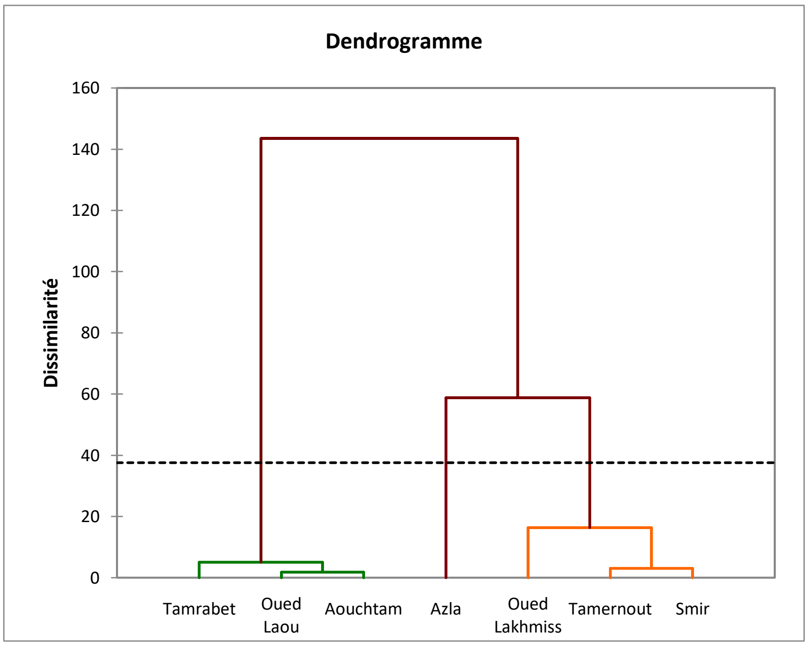

Figure 3. Grouping dendrogram of studied ecotypes based on leaf parameters.

4. Discussion

The study used the morphometric parameters of 6200 leaves to analyze the variability of Moroccan chestnut ecotypes. The results obtained confirm that the leaves morphological traits are very variable. The highest coefficient of variation was observed for the surface (S) in the Azla watershed which consists of coppice and natural populations. This same observation was made for Croatian ecotypes [13]. In contrast, the least variable parameter was the shape ratio LL/LW, which is explained by the stability of the shape ratios [6]. Furthermore, the results of the agglomerative hierarchical clustering analysis (AHC) and the comparison of the means by Student Newman and Keuls test (SNK) show a great variability within and between ecotypes that compose the seven watersheds.

5. Conclusions

This study is a first contribution to the knowledge of the Moroccan chestnut ecotypes. The results obtained show a high degree of variability among and within ecotypes. Thus, the wide range of variability in the morphometric parameters of the leaves confirms that the region is an important center of genetic diversity. The assessment of this diversity is crucial for the implementation of strategies for the conservation and enhancement of this heritage.

Funding: This work was supported by the United Nations Educational Scientific and Cultural Organization [4500 409 557-506 606], and University Abdelmalek Essâadi scholarship [2624530238]

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: The authors thank the farmers of the various villages who contributed to the realization of this study. Special thanks to Derdabi M. for his support in the field.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lang, P.; Huang, H. Genetic variation and population structure of three endemic Castanea species in China. *Act. Hort.* **1999**, *494*, 269–276.
2. Aravonoupolos, F.A. Phenotypic Variation and Population Relationships of Chestnut (*Castanea sativa*) in Greece, Revealed by Multivariate Analysis of Leaf Morphometrics. *Acta Hortic.* **2005**, *693*, 233–240.
3. Ertan, E. 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.* **2007**, *54*, 691–699.
4. Neophytou, C.H.; Palli, G.; Dounavi, A.; Aravanopoulos, F.A. Morphological differentiation and hybridization between *Quercus alnifolia* Poech and *Quercus coccifera* L. (Fagaceae) in Cyprus. *Silvae Genet.* **2007**, *56*, 271–277.
5. Bruschi, R.; Grossoni, R.; Bussotii, F. Within- and among-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. natural populations. *Trees* **2003**, *17*, 164–172.
6. Serdar, U.; Kurt, N. Some Leaf Characteristics are Better Morphometric Discriminators for Chestnut Genotypes. *J. Agric. Sci. Tech* **2011**, *13*, 885–894.
7. Aravanopoulos, F.A. Phenotypic variation and population relationships of chestnut (*Castanea sativa*) in Greece, revealed by multivariate analysis of leaf morphometrics. *Acta Hort.* **2005**, *693*, 233–240.
8. Álvarez-Álvarez, P.; Barrio-Anta, M. Ulises Diéguez-Aranda. Differentiation of sweet chestnut (*Castanea sativa* Mill.) cultivars by leaf, nut and burr dimensions. *For. Int. J. For. Res.* **2006**, *79*, 149–158.
9. Zarafshar, M.; Akbarinia, M.; Bruschi, P.; Hosseiny, S.M.; Yousefzadeh, H.; Taieby, M.; Sattarian, A. Phenotypic variation in chestnut (*Castanea sativa* Mill.) natural populations in Hyrcanian forest (north of Iran), revealed by leaf morphometrics. *Folia Oecol.* **2010**, *37*, 113–121.
10. Bruschi, R.; Grossoni, R.; Bussotii, F. Within- and among-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. natural populations. *Trees* **2003**, *17*, 164–172.
11. Serdar, Ü.; Demirsoy, H. Non-destructive leaf area estimation in chestnut. *Sci. Hortic.* **2006**, *108*, 227–230.
12. Fernández-lópez, J.; Alia, R. *Technical Guidelines for Genetic Conservation and Use for Chestnut (Castanea sativa)—Euforgen-NH (Euforgen-Noble Hardwoods)*; Regional Office for Europe (EUR): 2003.
13. Poljak, I.; Idzojtić; M; Zebec, M. Leaf morphology of the sweet chestnut (*Castanea sativa* mill.). A methodological approach. *Acta Hortic.* **2014**, *1043*, 211–218.