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# Variability of *Castanea sativa* MILL. Ecotypes in Northern Morocco Based on Morphometric Leaf Analysis †

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**Abstract:** Over time, farmers have always made chestnut selections by multiplying the most interesting plants. Indeed, a good knowledge of the phenotypic and genotypic variability of ecotypes is the basis of plant breeding. However, Moroccan chestnut ecotypes are neither classified nor characterized. This study aims to evaluate the genetic resources of Castanea sativa MILL. in northern Morocco through the morphometric characterization of leaves. Thirty-one villages in three regions were involved, ten trees per village and twenty leaves per tree were sampled. Then ten 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), area (S), perimeter (P), and ratios LL/LW, LL/LP, LL/DBW, and DBW/LP. Analysis of the descriptive statistics within and between ecotypes initially showed a large variation in the parameters studied. This finding was supported by analysis of variance (ANOVA) which revealed a very highly significant difference ($p < 0.0001$) for parameters. Indeed, the analysis of agglomerative hierarchical clustering (AHC) made it possible to group the studied populations into three distinct groups based on the leaf area. Thus, the wide variability of the morphometric parameters of the leaves constitutes an important group of traits useful for conventional breeding programs, providing a knowledge base for the exploitation of local genetic resources and the in-situ conservation of this heritage.

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

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## 1. Introduction

Plant genetic resources (PGR) are part of agrobiodiversity and include all cultivated forms, spontaneous and sub-spontaneous, species related to cultivated forms and species not cultivated but used by humans for specific purposes [1,2]. In this context, traditional agroecosystems are known to shelter a great agrobiodiversity [3–5], practiced on the basis of species cultivated by humans including a great genetic diversity, the study of which would help to understand the genetic resources and the dynamic processes of past and present domestication as well as subsequent diversification.

In Morocco, the common chestnut, *Castanea sativa* MILL., Like other natural species, is a good example of exploited plant genetic resources, despite its very restricted geographical distribution in the western north. Indeed, Rif peasants, throughout history, have always made selections to preserve and multiply the most interesting plants. This finding testifies to a good knowledge of the phenotypic and genotypic variability of ecotypes which is the basis of plant selection. However, the ecotypes of the Moroccan chestnut tree are neither classified nor characterized. However, the assessment and characterization of

the diversity and structure of populations is crucial for the implementation of a strategy for the enhancement, conservation and sustainable use of natural resources [6].

For this, and in order to better characterize these resources and determine the genetic variability of the chestnut tree, the study of the morphological features of the leaves has been frequently used by researchers [7–9]. However, characteristics related to the lamina including size, shape and anatomy are largely influenced by developmental, environmental and cultural factors [10,11].

However, many authors confirm that leaf parameters may be appropriate variables to establish the level of genotype variability [7,8,11–14]. This contribution falls within this context and aims to characterize and evaluate local genetic resources of *Castanea sativa* MILL. via morphometric analysis of leaves for the first time in Morocco.

## 2. Materials and Methods

### *2.1. Data Collection*

The collection of chestnut leaves was carried out in the North-West of Morocco (Figure 1) at 31 villages in three regions: Bni Said, Bni Hozmar and Haouz, recognized by the presence of the chestnut tree. It concerned 310 trees with an average of 10 trees/village. For each chestnut, 20 leaves were selected at 2 m in height by making a complete turn around the tree [10,13,14]. The 6200 leaves sampled showed no signs of abnormal growth, mechanical damage, presence of pathogens or insect infestation.

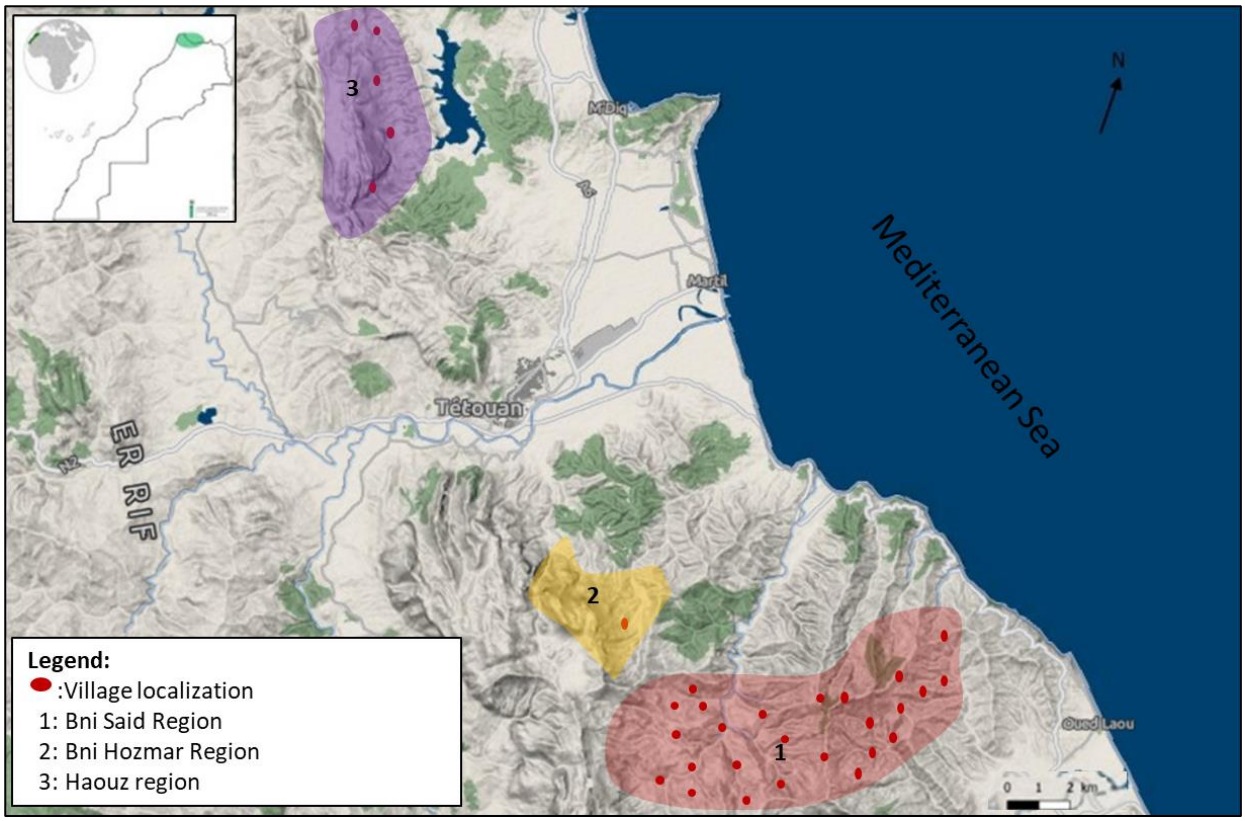


**Figure 1.** Distribution of villages by region in the study area.

### *2.2. Morphometric Description*

For the morphometric analysis of the leaves, we scanned the different leaves and then studied the phenotypic variability of the leaves by performing a digital analysis of the images using the ImageJ software (Figure 2). The morphometric measurements took into account the most relevant parameters [11,12,15]: the lamina length (LL), the lamina width

(LW), the petiole length (PL), the distance from base to the longest width (DBW), the perimeter (P), the surface (S). Then four shape ratios were calculated: LL/LW, LL/PL, DBW/PL and LL/DBW.

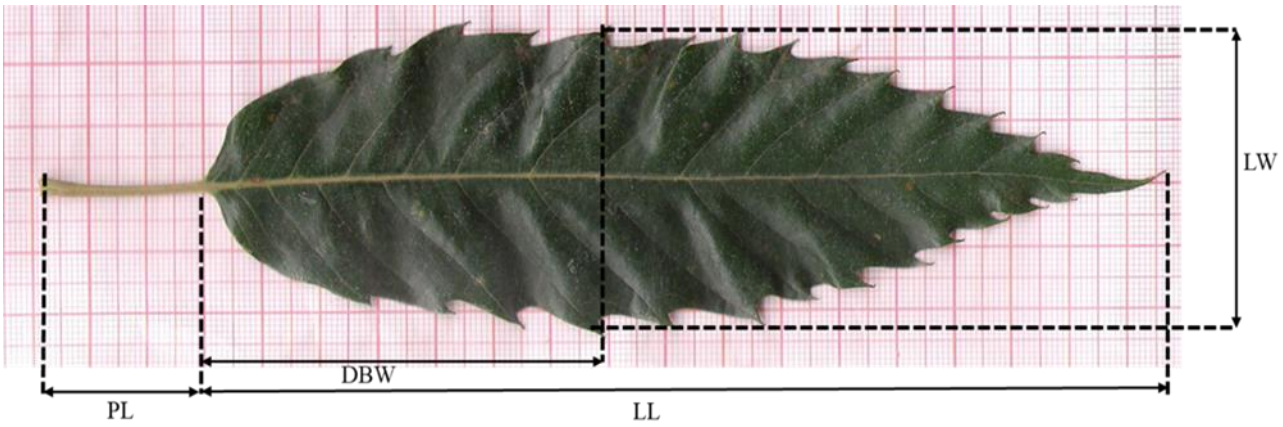


**Figure 2.** Measurements of the chestnut leaves.

*2.3. -Statistical Analysis*

The data obtained was analyzed using the parameters of descriptive statistics, analysis of variance (ANOVA) as well as analysis of hierarchical clusters (CAH). The calculations were computed using the XLSTAT statistical analysis software (version 2016).

## 3. Results

*3.1. Analysis of Variability*

The parameters of the descriptive statistics between the different ecotypes as well as the coefficients of variation of the parameters studied for leaves in the three regions revealed a high level of variability (Table 1).

The highest's perimeter mean (P) which is 39.89 cm is observed for Bni Said leaves ecotypes. These ecotypes have also the smallest petiole (PL) (mean = 2.10 cm) as well as the two highest DBW/PL and LL/PL ratios with respective means of 4.31 and 8.80.

The Haouz's leaves ecotypes are the shortest (LL) (mean = 17.32 cm) and the widest (LW) (mean = 7.16 cm) and have the lowest LL/LW and LL/DBW ratios with respective means of 2.45 and 1.95. They also have the highest means in terms of area (S) (mean = 83.73 $cm^2$) and distance between base and width (DBW) (mean = 9.03 cm). The lowest perimeter's mean (P) (39.42 cm) is encountered in this region.

We also note that ecotypes from the Bni Hozmar region have leaves with the longest lamina (LL) (mean = 17.73 cm) and petioles (PL) (mean = 2.43 cm). They also have the lowest area's mean (S) (74.35 $cm^2$). However, they are the narrowest (LW) with a mean of 6.31 cm and have the smallest distance between the base and the width (DBW) (mean = 8.26 cm). They also have the smallest DBW/PL and LL/PL ratios with respective means 3.49 and 7.48, as well as the maximum means of the LL/DBW and LL/LW ratios with 2.17 and 2.88.

Note also that the highest coefficient of variation of all the parameters and at the level of the three regions is observed for the surface (S) (CV = 41.69%) in this region (Bni Hozmar). In contrast, the smallest coefficient of variation of all parameters is encountered in the same region for the LL/DBW ratio with 8.84%.

**Table 1.** Morphometric parameters studied in the three regions.

| Regions | Parametrs | S ($cm^2$) | P (cm) | PL (cm) | LL (cm) | DBW (cm) | LW (cm) | LL/DBW | DBW/PL | LL/LW | LL/PL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bni Said** | Mean | 83.26 | 39.89 | 2.10 | 17.47 | 8.53 | 7.05 | 2.09 | 4.31 | 2.53 | 8.80 |
| | SD * | 31.97 | 7.77 | 0.54 | 3.71 | 2.19 | 1.52 | 0.28 | 1.58 | 0.50 | 2.85 |
| | CV(%) ** | 38.40 | 19.47 | 25.90 | 21.26 | 25.62 | 21.49 | 13.21 | 36.68 | 19.74 | 32.43 |
| | Max | 227.70 | 67.14 | 4.24 | 31.12 | 17.66 | 11.51 | 3.86 | 17.53 | 5.34 | 35.96 |
| | Min | 20.40 | 16.41 | 0.71 | 7.65 | 3.02 | 2.14 | 0.99 | 1.41 | 1.38 | 3.78 |
| **Haouz** | Mean | 83.73 | 39.42 | 2.22 | 17.32 | 9.03 | 7.16 | 1.95 | 4.25 | 2.45 | 8.13 |
| | SD * | 30.52 | 7.57 | 0.55 | 3.62 | 2.15 | 1.43 | 0.22 | 1.24 | 0.41 | 2.14 |
| | CV(%) ** | 36.44 | 19.21 | 24.93 | 20.91 | 23.85 | 19.95 | 11.48 | 29.27 | 16.76 | 26.29 |
| | Max | 194.77 | 64.02 | 4.08 | 29.25 | 16.26 | 11.16 | 2.98 | 9.50 | 4.30 | 20.83 |
| | Min | 22.22 | 18.44 | 0.93 | 8.50 | 4.05 | 3.45 | 1.39 | 1.86 | 1.62 | 4.06 |
| **Bni Hozmar** | Mean | 74.35 | 39.68 | 2.43 | 17.73 | 8.26 | 6.31 | 2.17 | 3.49 | 2.88 | 7.48 |
| | SD * | 30.99 | 8.66 | 0.65 | 4.17 | 2.11 | 1.39 | 0.19 | 0.78 | 0.68 | 1.46 |
| | CV(%) ** | 41.69 | 21.81 | 26.59 | 23.55 | 25.56 | 22.07 | 8.84 | 22.35 | 23.58 | 19.48 |
| | Max | 159.24 | 59.89 | 3.91 | 27.17 | 13.42 | 9.86 | 2.63 | 5.59 | 4.74 | 11.60 |
| | Min | 24.33 | 25.59 | 1.25 | 11.30 | 4.83 | 3.45 | 1.72 | 2.11 | 1.85 | 4.71 |

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

The analysis of variance (ANOVA) for the morphometric parameters studied for the leaves (Table 2) showed a very highly significant variation ($p < 0.0001$) for the surface (S), the petiole's lenght (PL), the distance from the base to the longest width (DBW), the width (LW) as well as for the ratios: LL/LW, LL/PL, LL/DBW and DBW/PL. These results reflect the morphological diversity among the individuals that make up the ecotypes of the three regions. However, we cannot state the difference in terms of perimeter (P) and lamina's length (LL) between the three regions ($p > 0.050$). These results can be explained by the fact that some parameters may be better than others in indicating the genotypic variability between the different ecotypes [12] and that the variability of certain traits in chestnut leaves may be more or less pronounced in some cultivars than in others [11].

**Table 2.** Analysis of variance (ANOVA) of parameters studied for chestnut ecotypes leaves.

| | S | P | PL | LL | DBW | LW | LL/DBW | DBW/PL | LL/LW | LL/PL |
|---|---|---|---|---|---|---|---|---|---|---|
| F | 27,745 | 1356 | 30,391 | 1151 | 22,679 | 25,250 | 53,844 | 26,963 | 59,908 | 39,884 |
| Pr > F | <0.0001 **** | 0.258 | <0.0001 **** | 0.317 | <0.0001 **** | <0.0001 **** | <0.0001 **** | <0.0001 **** | <0.0001 **** | <0.0001 **** |

* test significatif, ** test très significatif, *** test hautement significatif, **** test très hautement significatif.

*3.2. Agglomeration Hierarchical Grouping Analysis (AHC)*

Agglomerative hierarchical clustering (AHC) analysis is used to produce a diagram (Figure 3) relating to the analysis of the studied parameters of the leaf of chestnut ecotypes from the three regions. The topology of the dendrogram clearly shows the existence of three groups.

The two closest groups are those of Haouz and Bni Said which are composed of ecotypes of varying ages, ranging from young plants grown from seedlings to century-old trees planted by the ancestors. In these two regions, the chestnut trees are for the most part maintained, and the peasants multiply their plants according to several methods (grafting, sowing and rejection). It should be noted that the Haouz ecotypes benefit from a richer substrate than that of Bni Said, impoverished by the cultivation of cannabis. The third group is made up of Bni Hozmar ecotypes which are made up only of old abandoned chestnut trees that are geographically isolated from all other ecotypes.

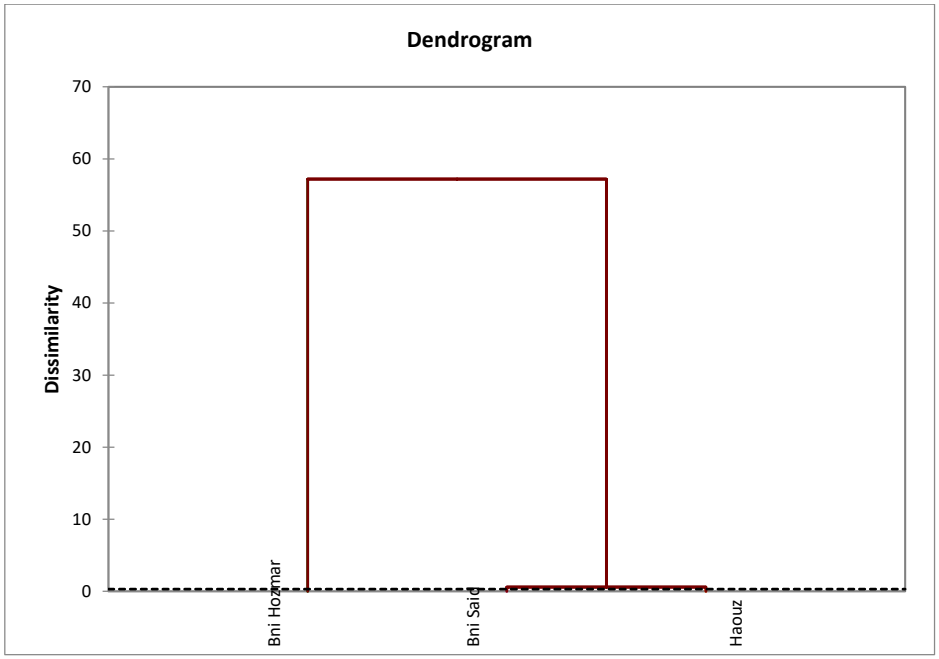


**Figure 3.** Distance dendrogram between tree regions of *C. sativa* obtained by Euclidian distance.

## 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 morphological features of the leaves are very variable. The highest coefficient of variation was observed for the area (S) in the Bni Hozmar region which consists of coppice and abandoned ecotypes that multiply freely and without any control. This same observation was made for ecotypes from Croatia [16]. On the other hand, the least variable parameter was LL/DBW, which is explained by the stability of the shape ratios [6]. In addition, the results of analysis of variance (ANOVA) and agglomerative hierarchical clustering (AHC) show great variability within and between the ecotypes that make up the three regions.

## 5. Conclusions

This study contributed, for the first time, to the characterization of the ecotypes of the chestnut *Castanea sativa* MILL. in northern Morocco. The great variability in morphometric parameters of the leaves constitutes an important group of useful traits for conventional breeding programs. This will constitute a knowledge base for the exploitation of underutilized local genetic resources as well as for the in-situ conservation of this heritage. The results obtained deserve to be supplemented by a morphometric study of fruits of different ecotypes and molecular characterization.

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

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