

Analysis of dendrometric diversity among natural populations of cork oak (*Quercus suber* L.) from Morocco

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Abstract: The cork oak (*Quercus suber* L.) has been the focus of research dealing with the conservation and reforestation of this species due to its economic importance and the problem of deforestation affecting it. The genetic diversity of this tree species, its main aspect of adaptation, has not been sufficiently studied. The Moroccan cork oak tree is found in the northern part of the country, where the fruits of the tree are soft corns. This forest tree species has undergone a strong decline due to many factors, including a significant loss of its biological diversity. While working within the national framework of protection and enhancement of this tree species, our research aimed to analyze and assess the phenotypic diversity of different provenances, using qualitative and quantitative dendrometric traits and geographical characteristics such as the total height of the tree (H), the height to the first branch (Hbr), girth (Gir), surface coefficient of the bole (K) ($K = (H \times Gir/200)$), number of branches (NbrBr), vigor (V), foliage density (D), and altitude. The population of trees studied included 390 individuals from 6 regional provenances: the central plateau, Mamora, the Middle Atlas, the western Rif, the eastern Rif, and the Atlantic Rif. Univariate analysis showed a highly significant variability among these provenances. The highest coefficient of variation concerned K (62.79%) and Gir (42%), followed by NbrBr and Hbr with 32% and 30%, respectively. Hierarchical clustering led to the identification of 2 major groups, with the central plateau and eastern Rif representing the first group, and the Middle Atlas, western Rif, Atlantic Rif, and the Mamora forest representing the second group. The assembling of different groups as explained by dendrometric variation is mainly based on geographical traits.

Key words: Dendrometric diversity, genetic conservation, principal component analysis, provenance, *Quercus suber* L.

1. Introduction

Quercus is one of the endemic genera of the northern hemisphere, found in habitats ranging from temperate and tropical forests to dry thorn scrubs and semideserts (Nixon, 1993). It includes several species and has a high level of genetic variability. Some 450 species are known worldwide, of which 6 are found in Morocco (Belhabib et al., 2005). Among these species, the cork oak tree (*Quercus suber* L.) is an important part of Moroccan forest species. Its area, however, has declined over the last decades. The total area decreased from 1.53 million ha in 1947 to 425,000 ha in 1980 (Hammoudi, 2002). Currently the Mamora forest, considered the largest cork oak forest in the world, is only 60,000 ha (AAFI, 2005a). There was severe deforestation due to climate change, anarchic exploitation, and parasitic attacks. Preservation of the genetic diversity of this species

is therefore important in terms of adaptation options to climate change, to reduce ecosystem vulnerability, and to support future breeding programs (Gandour et al., 2007).

Genetic variability in *Quercus suber* L., using biochemical and molecular markers, has been investigated in several countries, including France (Lumaret et al., 2005), Spain (Lopez-Aljorna et al., 2007), Portugal (Coelho et al., 2006), and Italy (Simeone et al., 2009). These studies showed a high level of differentiation among the populations of this species. In Morocco cork oak forests have been the subject of several studies such as vegetation mapping (Aafi et al., 2005), genetic introgression (Belhabib et al., 2005), artificial regeneration (Belghazi et al., 2001), and forest health and technology by cork quality mapping (El Antry Tazi and Abourouh, 2008). However, no study has been conducted on the biodiversity of this species. The

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phenotypic trait variability across large populations with components of geography and climate as selective factors often mirrors the distribution of genetic variation at the geographical level.

The aim of the present study was to analyze the phenotypical variability of *Quercus suber* L. using qualitative and quantitative dendrometric traits. An interpopulation comparison was also carried out to provide basic information for conservation and improvement of cork oak forests by analyzing the structure of the phenotypic diversity specific to each Moroccan geographic territory.

2. Materials and methods

2.1. Prospected sites

The study covered the distribution area of natural cork oak tree populations in Morocco, stretching from the coastal plains to the Middle Atlas and the western, Atlantic, and eastern Rif, with an altitude ranging from 40 m to 1290 m (Figure 1). The main geographical and climatic characteristics of the six prospected regions distributed through 17 sites are shown in Table 1.

Three hierarchical levels were included in our sampling strategy: region, site, and individual. The choice of sites was made on the basis of a catalog made in 1997 as part

of a Moroccan–German cooperation (GTZ) to select the seed stands for seedling production. We enriched this collection with samples of trees from the eastern Rif (Jbal Karn), which were not included in that catalog.

In 2010 and 2011, with the exception of small groups of trees and some areas showing a mixture with *Quercus ilex* L., 17 sites (35 populations), scattered over all the natural cork oak tree areas in Morocco mentioned above, were selected. Each of these populations was represented by a number of individuals depending on the surface area and the density of the site (Table 1). Thus, a total of 390 trees were sampled from the six most representative regions of the cork oak forests in Morocco (Figure 1). The selected sites included 25 populations in Mamora, 5 in the central plateau, 2 in the oriental Middle Atlas, 2 in the Atlantic Rif, 2 in the western Rif (Rif Occidental), and 1 in the eastern Rif (Rif Oriental).

The trees were randomly sampled with interspaces among individuals of at least 50 m. Sampled trees were mapped using the Geolocation Geographic Information System.

A set of dendrometric characteristics of forest interest, including the total height of the tree (H), the height to the first branch (Hbr), girth (Gir) at a height of 130 cm, surface

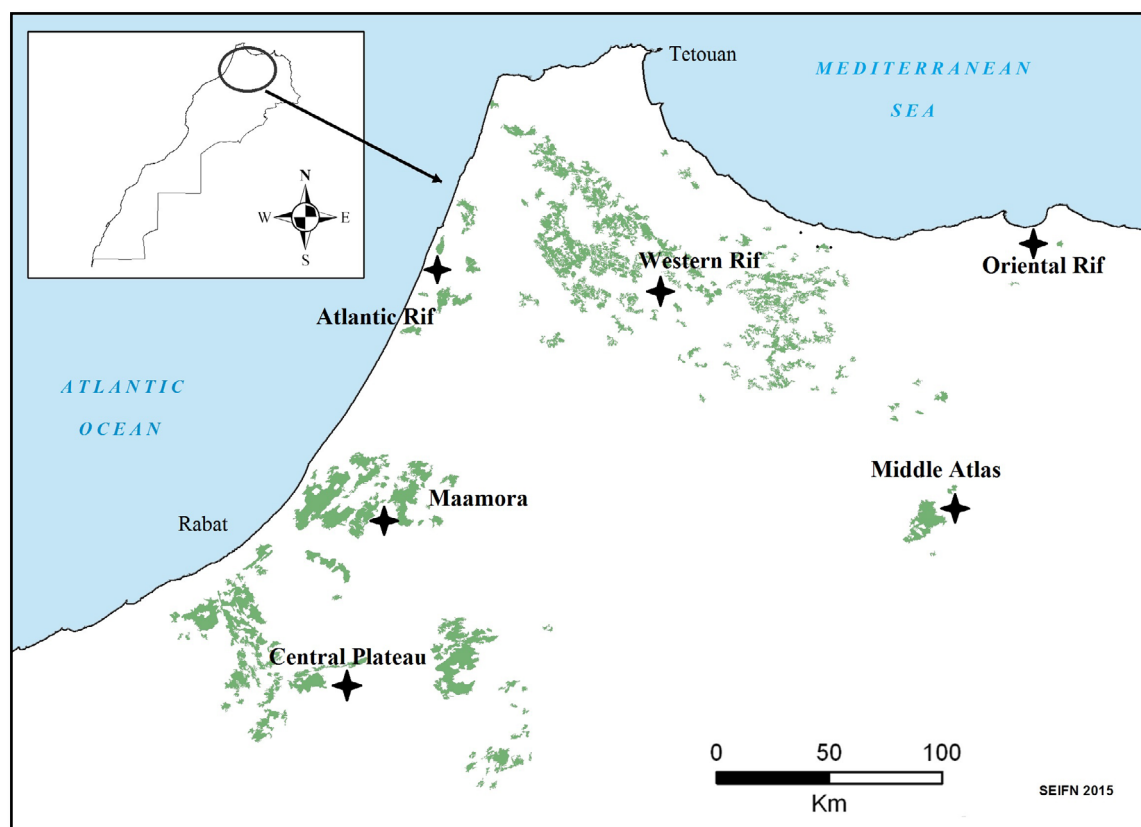


Figure 1. Locations of sampled populations of *Quercus suber* L. in Morocco.

Table 1. Principal characteristics of the *Quercus suber* L. regions [A.E.F.C.S 1973, GTZ 1997, Climatology Service at the Center for Forest Research in Rabat-Agdal (1997–2007)].

Provenances = Regions	Sites	Number of populations	Size (ha)	Sample size	Alt. (m)	Lat. N (°)	Rainfall (mm)	T (°) Min–max	Texture of the soil
Central Plateau (PC III2)	Zitchouine	1	65	5	1113	33°27'	378–673	(7.6–19.8)	Loamy slimy
	Timksaouine	1	65	5	410	33°36'			
	Tiliouine	1	145	15	760	33°33'			
	Beni Abid	1	309	30	415	33°42'			
	Temara	1	50.8	5	150	33°51'			
Mamora	Canton A	5	23.868	50	161	34°03'	362 - 537	(12–25)	Sandy clay
	Canton B	7	28.460	55	180	34°17'			
	Canton C	3	17.220	25	140	34°23'			
	Canton D	8	32.464	60	190	34°14'			
	Canton E	2	30.059	50	261	34°12'			
Oriental Middle Atlas (Mao Iv2)	Bab Azhar I	1	76	10	1250	34°05'	1002	-	Loamy slimy
	Bab Azhar Ii	1	111	10	1290	34°03'			
Atlantic Rif (Rif At1)	Larache	1	–	10	40	35°07'	683	(11.8–23.9)	Sandy clay
	Boussafi	1	175	10	80	35°13'			
Western Rif (Rif Oci)	Bouhachem	1	–	10	1220	35°16'	1482	(8.7–21.1)	Sandstone with the root of the sandstone marly flysch
	Ain Rami	1	86	10	300	35°07'			Sandy clay
Orrietal Rif (Rif)	Jbal Karn	1	1.200	30	1070	35°07'	-	-	Stony schist

Alt.: Altitude; Lat.: Latitude; Long.: Longitude; Annual mean rainfalls; Tmax: annual mean maximal temperatures; Tmin: annual mean minimal temperatures.

coefficient of the bole (K) ($K = (H \times \text{Gir}/200)$), number of branches (NbrBr), vigor (V), and foliage density (D) were noted. Some of these characteristics (H, Hbr, K, Gir, and V) were assessed in a subjective way by other researchers (Gracia-Valdecantos et al., 1993; Gandour et al., 2007).

Geographical and climatic factors such as altitude, latitude, longitude, rainfall, the normalized difference vegetation index (NDVI), and temperature were also taken into consideration. The remote sensing datasets used in the analyses were: the Land Processes Distributed Active Archive Center of NASA, NDVI, and the Tropical Rainfall Measuring Mission project of NASA. Data for the period 2005–2014 were downloaded, converted into a unique coordinate system (WGS84/UTM/zone 30), and analyzed by ENVI software 4.5.

2.2. Statistical analysis

The ANOVA of morphological traits among regions relied on both univariate and multivariate methods. The data were analyzed using SAS (SAS Institute Inc. 2004. SAS/STAT 9.1 User's Guide. Cary, NC, USA: SAS Institute Inc.) and GenStat v.10.2 (Payne et al., 2007). Descriptive statistics were used to describe the main features of the variables studied. The variability of the phenotypical

characters among regions was measured calculating their coefficients of variation (CV%). The Pearson correlation coefficients were used to examine the relationship among and between all dendrometric traits. For studying the degree of relationship between each trait and the origin site characteristics, we used Spearman's rank correlation procedure. Spearman's correlation was calculated as follows:

$$r = 1 - \frac{6 \sum d^2}{n^3 - n}$$

where r is the rank value, d^2 is the difference squared, and n is the number of data.

Discriminant analysis was used to assess the degree of separation of the provenances through multivariate measurements. In order to find the main variation between individuals, data were processed according to principal component analysis (PCA). The geographical structure among regions was established using the unweighted pair groups method arithmetic averages (UPGMA) and Darwin method. The Manhattan coefficient of similarity (Faith et al., 1987) was used to calculate the distance between individuals.

3. Results

3.1. Descriptive analysis

The descriptive study of the measured variables and their contributions to the total variation shows a highly significant variability among the 390 individuals for altitude and the five quantitative traits studied. The CV% values obtained for all traits are shown in Table 2. High CV values were obtained for the following traits: K (62.79%) and Gir (42%). The CV of K was transformed into a square root value ($K = 4.46$) and that of the Gir was transformed into a logarithmic value ($Gir = 8.66$). The CV% was 4.46% for K, 32% for NbrBr, and 30% for Hbr, indicating a high variation of these traits in the studied phenotypes. The majority of measured traits were positively correlated ($P < 0.01$) and the correlation coefficients among the majority of traits were significant as shown in Table 3. Negative correlations were found between characters of the origin site and morphological traits. Some significant correlations were found between four dendrometric variables (Gir,

NbrBr, D, and K) and precipitation and NDVI (Table 4). No correlations were found between characters of the origin site and morphological characters. Table 5 shows highly significant differences in all six variables in terms of dendrometric variability within and among provenances. Comparison of averages also leads to a high level of significance among the different regions.

Moreover, according to the descriptive analysis of two qualitative characteristics (V and D) mentioned in Figure 2, we found that 45% of sampled individuals were vigorous and had a good health status, 40% of these individuals were moderately vigorous, and 15% were stunted or had a poor health status.

Regarding D, 42% of individuals were moderately dense, 26% had a low D, and 32% had a high D. The observed values of chi-square for V and D were 92.48 and 33.67 ($P < 0.05$), and their critical values were 88.25 and 18.29, respectively.

Table 2. Analysis of variance of 6 traits measured for 390 individuals sampled from Moroccan *Quercus suber* L.

Variables					Regions			Sites		
	Mean	Min	Max	SD	CV%	F1	DF	CV%	F2	DF
Alt	330.90	11.0	1263.0	335.95	13.901	3372.25***	5	13.901	124 ***	34
H	10.77	2.50	24.00	3.32	25.94	25.14***	5	25.94	2.43***	34
Gir	150.38	28.0	700.00	70.92	8.66	10.39***	5	8.66	2.75***	34
HBr	2.65	1.00	6.00	0.97	30.70	9.04***	5	30.70	2.49***	34
NbrBr	2.58	1.00	8.00	1.00	32.27	16.66***	5	32.27	2.63***	34
K	0.09	0.00	0.49	0.061	4.46	1.79***	5	4.46	2.05***	34

Min: minimum; Max: maximum; SD: standard deviation; CV: coefficient of variation; F1: F statistic between provenances; F2: F statistic among sites (regions); ***: highly significant.

Table 3. The Pearson coefficients of correlation for altitude (Alt), total height of the tree (H), height to the first branch (HBr), girth (Gir), number of branches (NbrBr), and surface coefficient of the bole (K) ($K = Hbr \times Gir/200$).

	Alt	H	Gir	HBr	NbrBr	K
Alt	1.00000					
H	-0.03934	1.00000				
Gir	0.10696	0.59691**	1.00000			
HBr	-0.16832	0.36880**	0.18731**	1.00000		
NbrBr	0.6802	0.38771**	-0.43851**	0.15493**	1.00000	
K	0.10419*	0.76817**	0.93203**	0.23688**	0.41625**	1.00000

*Correlation is significant at the $P \leq 0.05$ level; **correlation is significant at the $P \leq 0.01$ level.

Table 4. Spearman's coefficient correlation (r) between measured traits and geographic parameters of origin site.

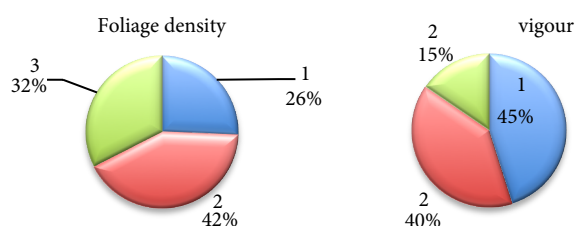
	Alt	Pmin	Pmax	Tmin	Tmax	Latitude	Longitude
H	0.066	-0.017	0.057	0.450	0.505*	-0.098	-0.283
Circf	0.135	0.339	0.339	0.270	0.270	0.294	-0.439
HBr	-0.258	-0.177	-0.238	0.426	0.385	0.032	0.021
NbrBr	0.250	0.405	0.295	0.363	0.284	0.377	-0.637
Vigor	0.192	-0.228	-0.386	0.114	0.012	-0.132	0.234
Foliage density	0.278	-0.427	-0.427	0.104	0.104	-0.440*	-0.068
K	0.248	0.314	0.242	0.455	0.418	0.257	-0.465*

*: significant at the 5% level; **: significant at the 1% level; ***: significant at the 0.1%.

Table 5. Descriptive statistics (mean, standard deviation, and rank) using Duncan's method for each morphological trait of Moroccan *Quercus suber* L. measured in 6 provenances.

Regions	Alt			H			HBr			Gir			NbrBr			K		
	M	SD	SNK	M	SD	SNK	M	SD	SNK	M	SD	SNK	M	SD	SNK	M	SD	SNK
PC III2	574.93	37	c	9.67	0.3	c	2.4	0.1	a	118.5	6.4	C	2.5	0.1	b	0.1	0.01	d
Mamora	139.02	4	d	11.2	0.2	b	2.8	3.4	a	153.7	3.4	b	2.7	0.1	ab	0.1	0.1	c
MAO IV2	1133.1	24	a	14.6	1	a	2.5	0.2	a	179.7	15.7	b	2.8	0.2	ab	0.1	0.02	a
Rif	754.8	24	b	6.5	0.5	d	1.8	0.1	b	113.13	23.12	c	1.3	0.1	c	0.05	0.02	d
Rif Atl	49.6	6	e	11.5	0.7	b	2.6	0.3	a	170.9	15.7	b	3	0.3	ab	0.1	0.01	bc
Rif Oci	744.7	71.7	b	10.7	0.5	bc	2.8	0.2	a	212.2	27.4	a	3.1	0.3	a	0.1	0.02	ab
Critical value	23.6			1.4			0.4			32.4			0.4			0.03		

M: Mean, SD: standard deviation, SNK: Student–Newman–Keuls test.


Figure 2. Frequency distribution of the two quality characteristics (vigor and foliage density)

Vigor: 1 = vigorous; 2 = moderately vigorous; 3 = slightly vigorous.

Foliage density: 1 = weakly dense; 2 = moderately dense; 3 = very dense.

3.2. Morphological diversity (multivariate analysis)

The PCA provides a brief description of total variability that exists among the six Moroccan provenances studied

(Figure 3). The first ACP axis (F1: 49% of the total variability) was mostly related to K, H, NbrBr, and Gir. The second (F2: 20% of the variance) was related to altitude. It should be noted that all phenotypical variables were positively correlated with axis F1.

This axis enabled the distinction between two main groups: the first contains groups A, B, C, and D; and the second contains groups E and F.

Group A: perfectly described by two dendrometric characteristics (Gir and altitude); it brings together individuals from the Middle Atlas, western Rif, eastern Rif, and some individuals from the central plateau and Mamora (Canton D).

Group B: especially distinct with respect to the altitude trait, it contains individuals from the eastern Rif, central plateau, and some individuals from Mamora.

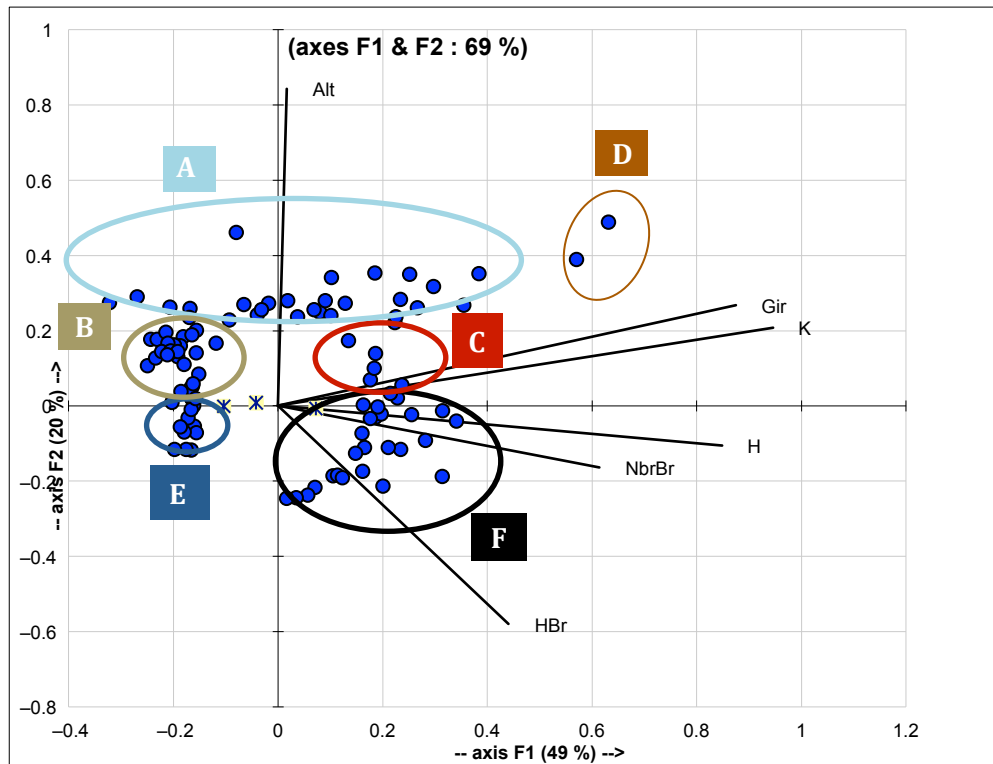


Figure 3. Distribution of the 390 individuals for the dendrometric traits in the plan of axes F1 and F2 of PCA.

Group C: defined by two traits (K and Gir) and including individuals from Mamora (Cantons A, B, and C).

Group D: it includes two individuals with the highest Gir: QSM343 from the eastern Rif with a total height of 7.00 m and QSM389 from the western Rif with a height of 5.83 m.

Group E: designated by Gir, includes individuals from Mamora (Cantons E and B).

Group F: includes individuals from Mamora (Cantons A and B) and the Atlantic Rif, described by altitude, H, NrBr, and HBr.

Figure 4 shows the dendrogram of the 390 individuals of Moroccan cork oak tree (*Quercus suber* L.) based on the simple matching dissimilarity matrix of 8 phenotypic characteristics. It was drawn using Darwin's method with the unweighted neighbor joining tree. The dendrogram of Darwin scores revealed two main groups, I and II, as shown in Figure 4. Group I includes two subgroups (I-1 and I-2). Subgroup I-1 contains individuals from the central plateau, the eastern Rif, and the western Rif, while subgroup I-2 includes individuals from Mamora and the Atlantic Rif. Group II comprises four subgroups (II-3, II-4, II-5, and II-6). Subgroup II-3 contains two individuals, one from the western Rif (QSM389) and one from the eastern Rif (QSM343). Subgroups II-4 and

II-5 contain individuals from the Middle Atlas and the western Rif. Subgroup II-6 contains individuals of the same provenances as Subgroup I-1.

Based on Euclidean distances obtained by UPGMA, the hierarchical classification of the groups enabled the aggregation of individuals at different levels of increasing distances and allowed the arrangement between the groups.

Figure 5 shows a 60% similarity between the two main groups of cork trees. The central plateau and eastern Rif belong to the first group, while the second group includes the Middle Atlas, the western Rif, the Atlantic Rif, and Mamora. Low genetic distance was observed between the Atlantic Rif and Mamora (0.69), between the Oriental Rif and PCIII2 (5.94), and between the western Rif and PCIII2 (3.16). Sixty-five of the 390 individuals sampled were shared by the Atlantic Rif and Mamora, 14 individuals by PCIII2 and the eastern Rif, and 6 by the western Rif and PCIII2.

The cophenetic correlation between the dendrogram and the similarity matrix revealed a good degree of fit ($r = 0.80863$, $P < 0.002$, $SD = 0.0155$). Both distances appeared significantly more remote and were between 6.7% and 5.8%.

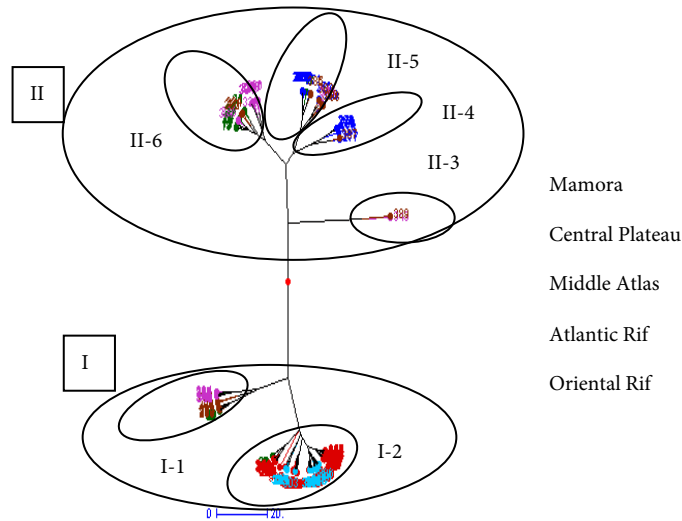


Figure 4. Darwin's method (Perrier et al. (2003); Perrier and Jacquemoud-Collet (2006); DARwin software (<http://darwin.cirad.fr/darwin>)).

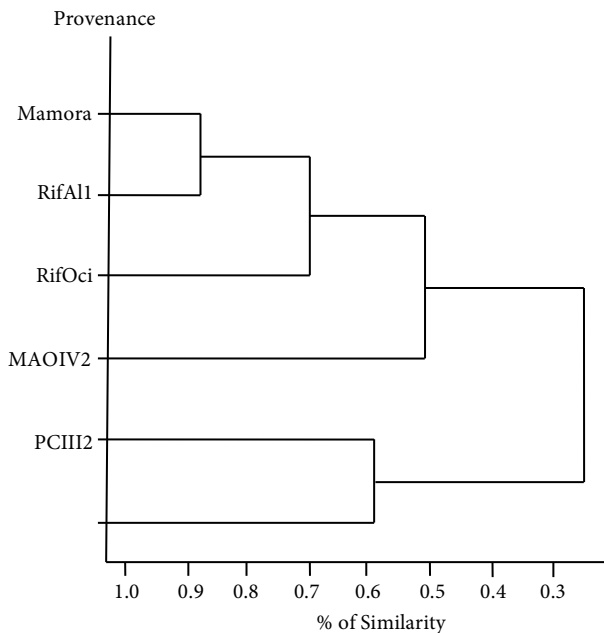


Figure 5. UPGMA cluster analysis of *Quercus suber* L. (Moroccan provenances) based on genetic distances evaluated using both quantitative and qualitative dendrometric traits.

4. Discussion

4.1. Structure of morphological diversity: univariate analysis

For all traits studied, we found high correlations with tree provenances. The highest correlations were observed between K and H ($r = 0.77$), K and Gir ($r = 0.93$), and Gir

and H ($r = 0.59$). These results confirm those obtained by Cermak et al. (1998) and Hirakawa et al. (2002). Furthermore, the four characteristics (K, Gir, HBr, and H) can be perfectly used to differentiate among the cork populations. Considering the biological dependence of these traits, the correlations probably indicate the presence of genetic factors controlling the traits by pair.

On the other hand, the cork oak tree populations have significant levels of phenotypic differentiation, which was also observed in natural populations of other tree species in Morocco such as the maritime pine (Wahid et al., 2006).

It was found that, compared with other regions, individuals from the eastern Rif (Jbal Karn) have the lowest values of all variables measured. The populations of the western Rif have a high crown (HBr), a large Gir, and significant NbrBr. When compared with other populations, the individuals of the eastern Middle Atlas, located in a high altitude site, have the highest K and the highest H. We observed that individuals from different geographic origins (Mamora, Atlantic Rif, and eastern Middle Atlas) showed dendrometric similarities. Therefore, the variations between regions are not influenced by altitude, topography, or temperature (Table 4). A study by Ohsawa et al. (2008) on the impact of geographical factors on the genetic diversity of *Quercus serrata* populations revealed that altitude is not a useful variable for estimating the genetic diversity of plant populations and there was no correlation between phenotypic diversity and altitude. Only rainfall and vegetation indices were significantly correlated with the four dendrometric variables (Gir, NbrBr, D, and K).

The critical values of chi-square for the V and D of trees revealed that V was not appropriate for differentiating populations.

Several authors have shown remarkable differences among Mediterranean provenances of this species (Jiménez et al., 1999; Bellarosa et al., 1996). In fact, our results confirmed this and showed a high phenotypic variability among and within Moroccan cork oak tree populations. Trees from the eastern Rif had the lowest values for the traits measured compared with those in the other populations.

4.2. Structure of morphological diversity (multivariate analysis)

We observed individuals from different geographic origins (Mamora, Atlantic Rif, and eastern Middle Atlas) with dendrometric similarities. We also noted that there was no homogeneous geographic distribution of the morphological data. This suggests that differences between individuals and the dendrometric variability within and among populations are determined by the evolutionary history of the regions, climate, and human activities (Faith, 1992; Frison et al., 1995; Schaal et al., 1998; Avise, 2000). This variability was related to genetic heterogeneity and may have resulted from gene flow among populations and species, particularly among populations of the cork oak tree (Elena-Rossello et al., 1992; Toumi and Lumaret, 1998).

Mahalanobis distances between different regions and the dendrogram using the dendrometric variables (Figure 5) enabled the appreciation of similarities and differences among the regions. This analysis confirmed the results of the PCA and may lead to the conclusion that there is a possible adaptation of the species in relation to the environmentally predefined regions.

A Moroccan study using isozyme markers led to results different from those drawn from the use of molecular markers. In the same populations, five genetic groups were distinguished using isozyme markers and four genetic groups were identified using molecular markers (Belhabib et al., 2007). Our results showed that the analysis of morphological traits allowed for the distinction of two

groups: the first is related to the eastern Rif (named Rif) and the central plateau, and the second is related to the populations of Mamora, the western Rif, the Atlantic Rif, and the Middle Atlas.

Oak species are very variable morphologically and they show a great deal of convergent evolution with regard to phenotypical characters. The evaluation of phenotypic diversity within the 390 trees of *Quercus suber* proved that variations in dendrometric characteristics existed among and within the Moroccan populations of this forest species. Morphological and dendrometric characteristics can be used for conservation programs and the selection of provenances for reforestation schemes (Mona et al., 2004). The statistical analysis of the studied dendrometric characteristics demonstrated a large diversity justified by the observed differences between individuals of different provenances of cork oak trees. Several allometric relationships were highlighted between traits. Moreover, we emphasize that most of the variability of dendrometric characteristics of trees are due to changes in environmental conditions. Furthermore, morphological characterization is an important tool for the management and selection of provenances, and can be used to prioritize material for use in breeding programs. However, in order to optimize the conservation programs for this species, additional research is needed to confirm the phenotypic variability observed in our work using molecular markers, which are independent from the environmental factors at different sites. This will shed light on the structure of genetic diversity between and within the different provenances of Moroccan cork oak trees. It would be important to know the genetic structure in order to determine the location, number, and size of sites for in situ conservation, and to plan samples for ex situ conservation.

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