

# Morphological evaluation of cork oak (*Quercus suber*): Mediterranean provenance variability in Tunisia

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**Abstract** – Twenty-six provenances (2 340 plants) of cork oak (*Quercus suber* spp.) originating from Portugal, Spain, Italy, Morocco, Algeria, and Tunisia were tested for genetic variation among and within provenances by growth traits. Seven morphometrical characters were measured in 90 plants from each provenance. Analysis of variance showed highly significant differences for all characters. The phenotypic coefficient of differentiation reached 0.24 for the form and 0.22 for height, thus revealing a strong structuring between the provenances. Comparative study of growth among the provenances revealed more vigorous growth and better survival rate for those from Morocco, Spain, and Portugal, which may constitute better materials for afforestation. Furthermore, this variability appeared to be geographically structured and would be mainly genetically controlled, as cork oak provenances were cultivated under the same environmental conditions. Our results should be helpful for guide forest managers in afforestation.

*Quercus suber* / genetic variability / afforestation / Mediterranean plants

**Résumé** – Évaluation morphologique chez le chêne liège (*Quercus suber*) : variabilité des provenances méditerranéennes en Tunisie. La variabilité génétique inter et intra-provenances a été étudiée pour des caractères de croissance chez *Quercus suber* spp. 26 provenances (2 340 individus) de chêne liège originaires d'Espagne, du Portugal, d'Italie, du Maroc, d'Algérie et de la Tunisie ont été implantées et testées. Sept caractères morphométriques, jugés discriminants, ont été mesurés chez 90 individus par provenance. L'analyse statistique des résultats a montré des différences hautement significatives entre ces provenances. Le plus haut coefficient de différenciation  $Q_{st}$  est trouvé pour les caractères forme (0,24 %) et hauteur (0,22 %). L'étude comparative a en outre révélé une croissance plus vigoureuse associée à une moindre mortalité chez les provenances originaires du Portugal, d'Espagne et du Maroc, leur conférant ainsi un intérêt potentiel pour le reboisement. La variabilité observée chez le chêne liège apparaît structurée selon l'origine géographique et serait essentiellement de nature génétique, puisque toutes les provenances ont été cultivées dans les mêmes conditions pédoclimatiques.

*Quercus suber* / variabilité génétique / reforestation / plantes méditerranéennes

## 1. INTRODUCTION

The conservation of genetic diversity within species of economic importance is important in order to insure the potentialities of populations for future adaptation and future breeding programs [8, 21, 39]. Information on genetic variation within and between populations, and about existing adaptability, can therefore be valuable both in prioritizing populations for conservation, and for developing sustainable management practices [20, 40, 44]. Characteristics such as high levels of phenotypic plasticity [14, 15, 42] and genetic variation contribute to the success of the genus *Quercus* [11, 13, 17, 24, 32, 34, 45]. Studies on European white oaks (subgenus *Quercus*) showed that most genetic variation was found within populations rather than between populations or between species. It was reported that 74% of isoenzyme diversity was found within populations, whereas inter-populations and inter-species variations were 3% and 23% respectively [17]. Exceptionally,

the allozyme diversity between populations of *Quercus suber* (11%) was relatively high when compared to other oak species [41].

General interest comes mainly from the need to preserve genetic resources and to use them in rehabilitation programs. Nevertheless, few information regarding the performance and adaptation traits of *Quercus suber* are available. In order to limit the progressive reduction of cork oak distribution areas, a multi-locality provenance trial was established in 1997 at the site of Tebaba mountain (Tunisia), as a part of EUFORGEN Network. It is worth mentioning that the study of morphological differentiation among populations is a first step for the estimation of the evolutionary forces promoting or preventing differentiation. Population differentiation may be promoted by either natural selection or genetic drift. Intense natural selection may favor different phenotypes in each population in response to differences in selective regimes among localities.

In the present study we aim to: (1) examine the level of genetic variation among 26 local and introduced provenances

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**Table I.** Geographical, edaphic and climatic characteristic for the sample populations of cork oak (*Quercus suber* L.) [10].

Code	Country	Locality	Altitude (m)	P (mm)	Substrate	Latitude
DZ 1	Algeria	Guerbes	–	–	–	–
ES-PT 2	Portugal-Spain	Basteros+ Sierra de San Pedro Tojera1	450–515	778	Siliceous	39° 12' N
TN 3	Tunisia	B. Fernana	270	1 610	Siliceous	36° 35' N
TN 4	Tunisia	Makna	12	948	Siliceous	36° 57' N
IT 5	Italia	Lazio Tuscania	160	937	Eruptive	42° 25' N
IT 6	Italia	Puglia Prindizi	45	588	Clayey	40° 34' N
IT 7	Italia	Sicilia Catania	250	448	Terra rosa	37° 07' N
IT 8	Italia	Sardegna Cagliari	200	883	Granite	40° 27' N
IT 9	Italia	Sardegna Sassari	300	910	Eruptive	39° 05' N
MA10	Morocco	Bousafi	150	574	Clayey	35° 11' N
MA11	Morocco	Ain Rami	300	1 280	Siliceous	35° 07' N
MA12	Morocco	Canton d' Ain Johra	150	479	Clayey	34° 07' N
MA13	Morocco	Oulmes Piles 65	1 115	673	Schistose	33° 46' N
MA14	Morocco	Bab Azhar pile202	1 130	970	Schistose	34° 12' N
ES15	Spain	Montes de Toledo Ca Amero (V)	600–800	1 063	Siliceous	39° 22' N-39° 25' N
ES16	Spain	Morena oriental Fuencaliente (CR)	700–900	719	Siliceous	38° 24' N-38° 33' N
ES17	Spain	Sierra Morena Occidental Jerez de Los Caballeros	400–500	666	Siliceous	38° 13' N
ES18	Spain	Parque de Los Alcornocales la Almorena (ALM)	20–120	993	Siliceous	36° 16' N
ES19	Spain	Catalu a Littoral Santa Colomba de Farnes 66	200–500	802	Siliceous	41° 51' N-41° 53' N
ES20	Spain	Sierra Gouadarrama el Pardo (PAR)	680–740	455	Siliceous	40° 31' N
ES21	Spain	J. Alpujarras Haza de lino-H	1 300	742	Schistose	36° 50' N
PT22	Portugal	Chamusca	75	829	Schistose	36° 35' N
PT23	Portugal	Ponte de Sor	70	710	Siliceous	36° 35' N
PT24	Portugal	Sain Bras Alportel	440–485	874	Siliceous	36° 35' N
PT25	Portugal	Evorra Azurrija	360	564	Siliceous	36° 35' N
PT26	Portugal	Santiago de Cacem	140	736	Siliceous	36° 35' N

P: average annual rainfall.

of *Quercus suber*, (2) examine the pattern of genetic variation among these populations and (3) estimate some genetic parameters for each of the studied characteristic.

## 2. MATERIALS AND METHODS

### 2.1. Plant material and sampling method

Twenty-six provenances of cork oak were sampled from six countries (Tab. I). The latter were evaluated in the INRGREF station in the region of Nefza (140 km to the west of Tunis), where 30 blocks were defined. The pre-planting started in 1997. Cork oak seedlings were grown for 4 months in monospecific conditions without fertilizer in order to eliminate any source of variation. Then, they were planted in 30 randomized complete blocks at Nefza station. Each block contained 3 rows, so that 78 trees of the 26 provenances were planted as follows: 1 tree of each provenance per row, at 3 × 2 m spacing. No treatment had been undertaken until the date of sampling.

### 2.2. Description of the site

The experimental site of Nefza is one of the several plantations of varied *Quercus suber* provenances, which have been set up by INRGREF in northern Tunisia. This plantation is located in the southern

side of Tebaba mountain (8° 52' E, 36° 58' N), zone corresponding to the numidian flysh, that extends from Oligocene to early Miocene [3, 4, 29], on clay and sandstone flysh soils. The site is located at an altitude of 250 m under a humid bioclimate. Before planting, the dominant species were *Quercus suber*, *Pistacia lentiscus* L., *Olea europaea* L., *Calycotome spinosa* L., *Cistus monspeliensis* L., *Inula viscosa*, *Phyllerea angustifolia*, *Lavandula stoechas*, and *Erica arborea*.

### 2.3. Morphological characters

At the study site, seven response variables were measured on each surviving tree in each block: total tree height (HT), diameter at base (DB), crown width at two perpendicular directions, form (on a subjective scale from 1 (poor quality) to 3 (good quality), according to straightness and verticality), vigour (on a subjective scale from 1 (poor state) to 3 (good state)) [1], and the survival percentage (SUR) for each provenance per block.

### 2.4. Statistical analyses

Prior to statistical analysis, the percentages of survival rate were transformed with angular (arcsin) transformation. Variation among provenances in adaptive traits was analyzed using both univariate

**Table II.** Descriptive statistics of characters measured in 26 provenances within 7 populations of *Quercus suber* (Means, coefficients of variation (%), *F* statistic for characters which gave a significant result using ANOVA and Level of population differentiation in quantitative characters as determined by  $Q_{st}$  and its 95% lower confidence limit (*CL*)). Standard errors (*SE*) of  $Q_{st}$  are in parentheses.

N	Variable	Average	Provenances			Populations			Qst (SE)	CL
			F	C.V.%	Df	F	C.V.%	Df		
1	Total tree height	45.07 (cm)	4.90**	59.13	25	11.8**	59.13	6	0.22 (0.18)	-0.076
2	Diameter at base	2.036 (cm)	1.62*	68.3	25	2.61**	68.3	6	0.13 (0.09)	-0.018
3	Crown width1	52.44 (cm)	2.84**	58.86	25	4.07**	58.8	6	0.148 (0.16)	-0.11
4	Crown width2	71.51 (cm)	2.10**	51.09	25	2.09**	51.09	6	0.143 (0.13)	-0.07
5	Vigour	2.07 (score)	2.51**	40.57	25	4.61**	40.57	6	0.18 (0.105)	0.007
6	Form	2.14 (score)	3.83**	36.44	25	10.45**	36.44	6	0.247 (0.145)	0.008
7	Survival	11.18 (score)	1.50*	43.8	25	2.77*	43.8	6	0.18 (0.129)	-0.032

\*\* F significant at 0.1 %.  $CL = Q_{st} - (1.645 \times SE)$ .

**Table III.** Descriptive statistics (mean, standard deviation and rank) for each morphological trait measured in 26 provenances of *Quercus suber*. Homogenous groups at 95% confidence (SNK) are presented by the same letter for each trait.

Prov	Total tree height	Diameter at base	Crown width1	Crown width2	Vigour	Form	Survival
	mean, (SD), SNK	mean, (SD), SNK	mean, (SD), SNK	mean, (SD), SNK	mean, (SD), SNK	mean, (SD), SNK	mean, (SD), SNK
1	44.04 (25.82) af	1.76 (0.95) ab	47.12 (27.76) ac	65.38 (35.71) ac	1.98 (0.80) ab	2.21 (0.80) ae	1.05 (0.58) ab
2	44.41 (27.57) af	2.06 (1.22) ac	49.31 (29.22) ac	68.26 (34.37) ac	2.08 (0.82) ab	2.30 (0.73) be	1.45 (0.29) b
3	37.34 (19.28) ad	1.79 (1.00) ab	46.70 (27.30) ac	66.54 (33.06) ac	1.89 (0.81) ab	1.92 (0.80) ac	0.96 (0.58) a
4	47.44 (24.85) bf	2.08 (1.03) ac	60.15 (31.52) bc	77.28 (35.74) ac	2.10 (0.82) ab	2.05 (0.79) ad	1.14 (0.55) ab
5	37.51 (19.88) ad	1.88 (0.78) ac	46.10 (29.79) ab	64.16 (33.94) ac	1.79 (0.81) a	1.94 (0.77) ac	1.25 (0.51) ab
6	35.00 (22.16) ab	1.91 (1.18) ac	51.16 (31.49) ac	69.40 (36.85) ac	1.86 (0.90) ab	1.86 (0.74) ab	0.95 (0.55) a
7	36.79 (17.63) ac	1.97 (1.04) ac	51.44 (31.96) ac	72.17 (36.36) ac	1.82 (0.84) a	1.80 (0.83) a	0.99 (0.51) ab
8	31.74 (15.72) a	1.69 (0.83) a	40.41 (23.92) a	59.13 (31.90) ab	1.86 (0.84) ab	2.04 (0.76) ad	1.12 (0.46) ab
9	40.78 (21.32) ae	2.02 (1.11) ac	49.86 (30.35) ac	71.04 (38.66) ac	2.16 (0.79) ab	2.10 (0.78) ae	1.14 (0.55) ab
10	52.50 (24.18) ef	2.19 (1.10) ac	62.96 (35.47) c	81.58 (38.49) c	2.09 (0.81) ab	2.22 (0.77) ae	0.92 (0.65) a
11	52.76 (29.59) ef	2.04 (1.18) ac	61.31 (32.61) bc	78.24 (35.63) bc	2.06 (0.85) ab	2.34 (0.76) ce	1.32 (0.42) ab
12	49.77 (25.80) cf	1.93 (0.95) ac	51.74 (28.78) ac	70.80 (37.39) ac	2.25 (0.77) ab	2.47 (0.75) de	1.08 (0.56) ab
13	57.13 (31.42) f	2.30 (1.16) bc	59.54 (32.15) bc	79.60 (34.82) c	2.13 (0.82) ab	2.52 (0.60) e	1.17 (0.55) ab
14	49.47 (32.26) cf	2.14 (1.30) ac	50.85 (33.74) ac	69.70 (41.02) ac	2.09 (0.86) ab	2.27 (0.78) be	1.17 (0.56) ab
15	47.14 (32.96) bf	2.16 (1.18) ac	54.37 (33.04) ac	74.80 (41.56) ac	2.11 (0.82) ab	2.21 (0.81) ae	1.24 (0.48) ab
16	42.84 (22.49) ae	2.00 (0.93) ac	50.42 (29.82) ac	67.52 (34.75) ac	2.22 (0.79) ab	2.30 (0.70) be	1.08 (0.52) ab
17	46.33 (24.74) bf	2.58 (4.63) ac	57.02 (32.83) bc	75.34 (35.85) ac	2.20 (0.86) ab	2.12 (0.78) ae	1.19 (0.53) ab
18	52.61 (31.16) ef	2.40 (1.12) c	56.90 (29.03) bc	78.82 (35.70) c	2.19 (0.81) ab	2.26 (0.77) be	1.20 (0.55) ab
19	52.19 (30.03) ef	2.19 (1.12) ac	55.50 (33.37) ac	75.19 (43.58) ac	2.33 (0.86) b	2.16 (0.79) ae	1.07 (0.57) ab
20	37.15 (19.43) ad	1.83 (1.06) ac	45.46 (28.41) ab	64.16 (34.95) ac	2.08 (0.89) ab	1.94 (0.76) ac	1.27 (0.48) ab
21	40.41 (24.86) ae	1.88 (0.97) ac	39.82 (22.59) a	58.44 (29.57) a	2.16 (0.87) ab	2.06 (0.79) ad	1.19 (0.53) ab
22	46.42 (27.38) bf	2.26 (1.26) ac	54.68 (28.64) ac	75.05 (34.56) ac	2.26 (0.82) ab	2.08 (0.81) ad	1.16 (0.49) ab
23	51.00 (31.57) df	2.18 (1.11) ac	56.59 (33.06) bc	76.02 (37.22) ac	2.16 (0.83) ab	2.13 (0.76) ae	1.25 (0.41) ab
24	42.79 (26.19) ae	1.84 (0.97) ac	51.26 (29.60) ac	70.97 (34.89) ac	1.80 (0.83) a	2.00 (0.80) ac	1.08 (0.52) ab
25	40.63 (21.32) ef	2.00 (0.97) ac	54.36 (32.93) ac	74.77 (39.65) ac	1.96 (0.82) ab	2.03 (0.70) ad	1.26 (0.44) ab
26	53.58 (31.75) ef	2.17 (1.24) ac	58.03 (29.96) ab	77.32 (34.82) ac	2.24 (0.82) ab	2.25 (0.72) be	1.26 (0.44) ab

and multivariate methods. A two-way ANOVA was conducted for all traits including population and provenance effect using the following model:  $Y_{ijk} = \mu + P_i + B_{ij} + E_{ijk}$ .

Where  $Y_{ijk}$ : the phenotypic value of the trait  $Y$  in the  $K$ th plant of the  $j$ th provenance in the  $i$ th population,  $\mu$ : overall mean,  $P_i$ : population effect,  $B_{ij}$ : the effect of provenance  $j$  in population  $i$ , and  $E_{ijk}$ : error term specified by the effect of plant  $k$  of the provenance  $j$  from population  $i$ . The variability of the morphological characters among provenances was measured by calculating the coefficient of variation (CV) [37]. The levels of genetic variance within ( $V_G$ ) and between populations ( $V_B$ ) were evaluated using ANOVA. Estimated  $V_B$  and  $V_G$  were used to quantify the level of population differentiation ( $Q_{st}$ ) for each trait [27, 28, 38]:  $Q_{st} = \frac{V_B}{V_B + 2V_G}$ . This parameter is analogous to Weir and Cockerham parameter (Fst) (1984) [43] and therefore provides an opportunity to compare population divergence in phenotypic characters and allozyme markers. This calculation assumes that the differences between populations are genetically controlled. This assumption is plausible when plants are grown in the same environment; it is the case of this study. Product moment and Spearman's correlations between each trait and the origin site characteristics were calculated following appropriate procedures [35]. It is worth noting that Spearman's rank correlation is the best-known procedure for studying the degree of relationship between 2 variables when there is sub-normality in both pairs of variables. Geographic differentiation among provenances in growth traits was explored by means of cluster analysis using an unweighted pair-group method with arithmetic averaging (UPGMA; [36]), and Euclidian distances as the criterion for clustering (STATISTICA for windows version 3.10). Finally, in order to test the correlation between genetic and geographical distances among populations, a Mantel test [25] was performed using the program GENETIX (version 4.02). The null hypothesis refers to absence of association between the elements of the pairs of matrices. The matrix of geographical distances was calculated by converting geographical coordinates to Cartesian coordinates ( $x$ ,  $y$  and  $z$ ) and the Euclidian distance (dab) between two points ( $a$  and  $b$ ) was calculated as follow:

$$dab = \sqrt{(xa - xb)^2 + (ya - yb)^2 + (za - zb)^2}. \quad (1)$$

### 3. RESULTS

Growth traits analyzed between provenances (total tree height, diameter at base, crown width at two perpendicular directions, form, vigour and the survival percentage) showed coefficients of variation (C.V.), ranging from 36% for the form to 59% for total tree height (Tab. II). The most variable characters, total height and crown width1, were 1.6-fold more variable than form trait, characterized by the lowest coefficients of variation (Tab. II). In spite of the observed moderate variation, the two-way ANOVA revealed statistically significant differences in mean and score values among provenances for each examined trait (Tab. II). Similarly, on the population basis, coefficients of variation ranged from 36% to 59% (Tab. II).

Mean traits and provenances ranking are shown in Table III. In the whole, Moroccan provenance (Oulmes: 13) was exceptionally excellent in regard to height and stem straightness. Spanish provenance (la Almorema: 18) had a thick diameter and a regular crown width and form. Furthermore, total height was the most discriminate variable between populations.

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

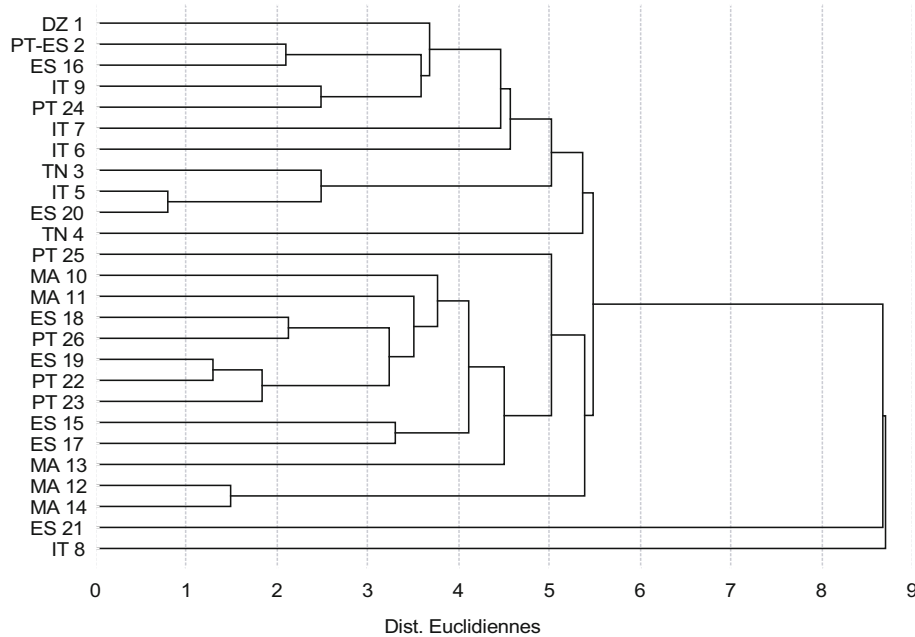
Variable	T	P	Altitude	Longitude	Latitude
Total tree height	-0.57**	0.18	0.07	0.63***	-0.5**
Diameter at base	-0.30	0.14	0.03	0.46**	-0.28
Crown width1	-0.40**	0.20	-0.22	0.36	-0.39**
Crown width2	-0.36	0.16	-0.25	0.36	-0.39
Vigour	-0.27	0.01	0.18	0.56**	-0.16
Form	-0.64***	0.06	0.34	0.56**	-0.54**
Survival	-0.04	0.21	0.23	0.47**	0.15

\* Significant at the 5% level. \*\* Significant at the 1% level. \*\*\* Significant at the 0.1% level.

Except the survival-remaining traits, where no correlation was found, all the morphological characters were positively correlated, and correlation coefficients among all characters were statistically significant. On the other hand, a negative correlation was observed between characteristics of the origin sites (temperature and latitude) and growth traits in 6 year-old *Quercus suber* trees. Seedlings especially originating from high temperature sites displayed the lowest growth traits in this experimental site characterized by a low altitude (250 m). The highest correlations were found between height-longitude, height-temperature, and form-temperature of the origin sites (0.63, -0.57, and -0.64 respectively) (Tab. IV).

Survival rate showed a significant difference among provenances ( $P = 0.01$ ). It varied from 95% for Spain-Portugal provenance (No. 2) to 70% for the Morocco one (No. 10). Estimates of  $Q_{st}$  differed widely between traits (Tab. II).  $Q_{st}$  values, as well as the mean estimate over traits, differed significantly from zero. They ranged from 0.137 to 0.31. The greatest  $Q_{st}$  is found for form (0.24,  $P < 0.001$ ) and height (0.22,  $P < 0.001$ ). Survival and vigour (0.18,  $P < 0.001$ ), exhibited also significant differences among population, although at a reduced level. Diameter, crown width1 and crown width2 displayed the least population differentiation (0.137, 0.148, and 0.143 respectively) although values were significantly greater than zero.

In order to examine if the differentiation is a consequence of genetic drift and/or of natural selection, we have performed a cluster analysis (UPGMA), which groups the provenances on the basis of traits similarities. Two groups were revealed: The first group was constituted by the provenances originating from Morocco, Spain, and Portugal, while the second included those from Italy, Algeria and Tunisia (Fig. 1). A high correlation was found between genetic distances and geographic distances ( $r = 0.699$ ;  $P = 0.039$ ) based on the Mantel test. The one-tail probability indicated that the null hypothesis could be rejected, suggesting a clear geographical pattern of isolation-by-distance in the distribution of the species genetic variability.



**Figure 1.** Spearman's coefficient correlation ( $r$ ) between measured traits and geographic parameters of origin site. (T°, P, Altitude, Longitude, and Latitude).

#### 4. DISCUSSION

This study showed that cork oak populations have significant levels of phenotypic differentiation as indicated by their morphological traits. Differentiation was evident for growth traits from both univariate and multivariate comparisons based on the matrix distances between provenances and populations. From an analysis of early trials of Mediterranean cork oak provenances, Moroccan ones were found to be better than the remaining material [23]. It is now accepted that the best provenances come from Morocco, especially the Oulmes pile 65. No provenance-block interaction was found in this study, which includes multi-locality provenances and a wide geographical range. It suggests absence of local effects on the specific adaptability of provenances. The evolutionary history of a region determines the distribution of the genetic variability within and among populations of a given species [2, 9, 33]. In other plant, important clinal effects have been found e.g. *Pinus sylvestris* [16, 30, 31]. In our case, we suggest that differences among populations can be related to the climate of the seedling sources [10], since correlation between phenotypic traits and characteristics of origin sites was significant. Although it is relatively easy to demonstrate that inter-populations and interspecific variation for quantitative traits is different from zero [7], this is not the appropriate null hypothesis for detecting the action of natural selection in leading to population differentiation [18, 19, 22]. Indeed it has been proven difficult to demonstrate that variation among species exceeds the minimum rate of neutral phenotypic evolution [20]. Comparison of  $Q_{st}$  and  $G_{st}$  can provide insight on the relative importance of natural selection and random genetic drift in population differentiation. In theory, the value of  $Q_{st}$  for a selectively neu-

tral trait should be the same as that of  $G_{st}$ . That is, if  $Q_{st}$  is significantly different from  $G_{st}$ , the null hypothesis of the trait arising by genetic drift alone can be rejected [26, 38]. In cork oak, 17.8% of the total genetic variation measured over the growth traits is attributable to differentiation among populations. This value was higher than Toumi and Lumaret (1998) value (11%) and was relatively higher than the mean value (7%) obtained over 25 oaks species studied for enzyme polymorphism [17], this suggests that natural selection has favoured different phenotypes in different populations. Indeed, Merilä and Crnokrak (2001) reviewed results from several taxa for which estimates of both  $G_{st}$  and  $Q_{st}$  were available, and found that  $G_{st}$  exceeded rarely  $Q_{st}$ , suggesting that natural selection is a widespread source of differentiation between populations. There are at least two alternative explanations for this pattern of variation: (i) populations of *Quercus suber* have become differentiated as a result of an heterogeneous (and non-clinal) regime of selection, which has led each population to its local optima; and/or (ii) differentiation is a consequence of genetic drift produced by the lack of gene flow between populations, and by founder events. It could be expected for geographically adjacent populations to have similar environmental conditions and, possibly, similar abiotic selective pressures [6]. Such a selective hypothesis would predict a tendency of closer populations to share similar morphologies. Thus, we would expect a negative relationship between growth similarity among provenances and the distance separating their origin. In contrast, the genetic drift hypothesis does not predict any relationship. This analysis (Fig. 1) supports the selective hypothesis: provenances from each region tend to be clumped.

The UPGMA and  $Q_{st}$  results suggest that selection affected the discrete traits in such a way that unifying selection has

acted within the geographic groups but this unifying selection has differed between the geographic groups. This diversity among populations in morphological characters, as generally reported for many forest tree species, may result from typical out crossing reproductive system and the intense gene flows between species, this was shown particularly between Holm oak and cork oak [5, 41]. It has been, also, well established that survival and regeneration in cork oak is deeply affected by predation, particularly by *Sus scrofa*. Indeed, the biotic effect has been implicated as a factor responsible for cork oak regeneration loss [12] since the intensity of these different types of predation can vary spatially and temporally among populations. This source of regression in regeneration of cork oak can act as a potent source of diversifying selection among *Quercus suber* populations.

In conclusion, according to the relative high genetic polymorphism of this species in comparison with others oaks species [39], this study allowed us to distinguish between provenances and to validate the morphometrical approach as a tool for early selection of provenances for afforestation. In addition, this work provides useful information about *Quercus suber* spp. variability, either in this project or in future ecological and genetic investigations.

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