Genetic variation among and within *Quercus suber* L. populations in survival, growth, vigor and plant architecture traits

Teresa Sampaio [a],[*], Elsa Gonçalves [b], Carla Faria [a], M. Helena Almeida [a]

[a] *Centro de Estudos Florestais (CEF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal*
[b] *Centro de Investigação em Agronomia, Alimentos, Ambiente e Paisagem (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal*

ARTICLE INFO

ABSTRACT

Cork oak is an important forest tree species in the Western Mediterranean region due to the high economic value of its renewable cork and its ecological and social services. However, studies regarding the genetic variation within cork oak populations are scarce, and this gap of knowledge is contributing to the delay of the set-up of a breeding and conservation strategy for the species. In this study, the genetic variation in fitness (survival, height, and stem diameter) and plant architecture traits (apical dominance, stem straightness, stem inclination degree, branchiness), and tree vigor was evaluated among and within cork oak populations in two progeny field trials established in Portugal. Measurements were carried out in each trial in two different periods: ages 9 and 14 years at Monte da Fava trial and 8 and 14 years at Herdade da Caniceira trial. A significant genetic variation among and within cork oak populations was detected for survival, growth, and form traits (stem straightness and inclination). Growth traits presented high values of heritability estimates at the family mean level ($\geq$0.75), and genetic gains were obtained when families with superior growth were selected. Additionally, results highlighted that early selection of families with superior performance could be performed, and it is possible to achieve improvement in both growth and form traits simultaneously, with implications on the profitability and sustainability of cork oak stands.

1. Introduction

Cork oak (*Quercus suber* L.) is an evergreen forest tree species naturally distributed in the western Mediterranean basin, occurring in a wide range of geographic and climatic conditions (Natividade, 1950; Aronson et al., 2009). Around the region, and particularly in Portugal, where the species occupy the most significant area (719,000 ha) (ICNF, 2019a) and is protected by law (DL 169/2001 of 25 May), cork oak stands are important multipurpose ecosystems that combine environmental and socio-economic services. They contribute to maintaining high levels of biodiversity (Bugalho et al., 2009), are a source of income for landowners and rural populations, support diverse agroforestry practices, and provide the raw material for the cork industry. Cork, the renewable bark of cork oak trees, is a remarkably versatile product that is being used in many applications (e.g., stoppers for wine bottles, pavements, coverings, insulation products). It accounts for 1% of total Portuguese exports, which was equivalent to a trade balance of 816 million euros in

2019 (APCOR, 2020).

The economic interest in cork oak has led to the research on this species' silvicultural practices (Cañellas and Montero, 2002; Cortina et al., 2009; Paulo and Tomé, 2017; Faias et al., 2018), growth and yield modeling (Paulo and Tomé, 2010; Paulo et al., 2011), and the development of new plant production techniques, such as the long-term acorn storage that facilitates planning and enhances seed and seedling quality (Almeida et al., 2009) or the vegetative propagation by somatic embryogenesis (Bueno et al., 1992; Hernández et al., 2003; Álvarez et al., 2004; Pérez et al., 2013). The Forest Reproductive Materials (FRM) of cork oak marketed for forestry purposes are controlled by EU Directive 1999/105/CE. This directive was transposed to the Portuguese legislation in 2003. Consequently, cork oak was included in the National Catalogue of Reproductive Base Materials, that ensures that the species' reproductive material must comply with certain minimum requirements regarding genetic characteristics and external quality, ensuring the quality of all products in the cork oak production chain. This catalogue

---

* Corresponding author.
E-mail addresses: tsampaio@isa.ulisboa.pt (T. Sampaio), elsagoncalves@isa.ulisboa.pt (E. Gonçalves), carlafaria@isa.ulisboa.pt (C. Faria), nica@isa.ulisboa.pt (M.H. Almeida).

includes 102 cork oak stands classified under the category of "selected" distributed along five different provenance regions (divided according to major edaphoclimatic conditions). To meet legislation requirements, only materials from the "selected" (or higher) category should be commercialized. Based on the current legislation, about 1.1 million plants are certified every year (ICNF, 2019b).

In the 1930s, Natividade (1934) highlighted the need for genetic studies to improve cork oak for cork quality and quantity in Portugal; however, no conventional breeding program has been developed yet. The main reasons are the absence of public and private investment, justified with the long reproductive cycle of this species (having a long juvenile phase) and its complex reproductive biology (with self-incompatibility and a high degree of heterozygosis). Another reason is the time required to obtain accurate assessments for valuable economic traits, such as cork quality. Indeed, the most valuable cork obtained is from the third extraction onwards, usually stripped in trees over 40 years of age. The first genetic initiatives occurred in 1998, under the framework of the Concerted Action, FAIR 1 CT 95-0202 (Varela, 2000). An international series of genetic trials, including 17 provenance and progeny common-garden field trials and covering the natural distribution range of the species, was established to evaluate the genetic variation for appropriate use in (re)forestation actions, breeding, and gene conservation.

Significant genetic variation in fitness and functional traits (e.g., survival, growth, budburst phenology, drought tolerance, cold resistance) has been found among cork oak populations (Aranda et al., 2005; Gandour et al., 2007; Ramírez-Valiente et al., 2009a, b; 2010; 2011; 2014a, b, c; Sampaio et al., 2016; 2019). However, cork oak intra-population variation, which could be used to improve the vitality, productivity, and resilience of stands, is not well-studied. The few studies in progeny trials were conducted at an early tree age in a single field trial and pointed out the existence of significant cork oak intra-population genetic variation and genetic control of several traits (Ramírez-Valiente et al., 2011; 2014b; 2015). Additionally, research using isozymes (Toumi and Lumaret, 1998; Jiménez et al., 1999) and microsatellites (SSRs) (Ramírez-Valiente et al., 2009b) reported high genetic variation within cork oak populations.

For several forest tree species, the existence of genetic variation of form traits among populations, families, and clones has been widely documented (Codesido and Fernández-López, 2008; Mwase et al., 2008; Luechanimitchit et al., 2017; Wang et al., 2018). However, the information available for cork oak is limited (Gandour et al., 2007; Ramírez-Valiente et al., 2014a). Plant architecture traits and tree vigor are particularly relevant not only for the economic value of timber products, but primarily because they determine the quantity of light capture, and thus tree height growth, and promote the mechanical stability of trees (Poorter et al., 2006). Therefore, the desired trees are those with large volumes, straightened boles, good crown form, high wood quality, and that appear to be healthy (without signs of disease, insect attack, or damage from drought or frost). For cork oak, if these traits are addressed, then more quantities of valuable cork planks can be obtained. Thus, it is evident that evaluating cork oak traits such as growth, plant architecture (including stem form and branching), and tree vigor at a genetic level will be essential to leverage economic returns and related silvicultural benefits.

Adaptive, growth, and form traits, when taken together, reflect the quality of individual trees. These traits can provide a reliable basis for the identification and selection of superior genetic material suitable for future forest restoration and management activities and the development of an efficient breeding strategy. Therefore, the main goal of this study was to assess the genetic variation in survival, growth, form traits, and tree vigor among and within cork oak populations, leading to, the identification and selection of families with superior performance. For this purpose, two progeny trials were used to evaluate inter- and intra-population genetic variation of the traits. These trials were established in different sites where different populations were tested; each

population was present in places with different silvicultural management techniques. The ultimate goal was to contribute to the knowledge base for the establishment of a cork oak breeding program in Portugal and other countries, where the species is commercially relevant, leading to an efficient management and conservation of the cork oak genetic resources.

2. Materials and methods

2.1. Plant material, trial sites, and experimental design

The study was conducted in two sites of Portuguese cork oak progeny trials, Monte da Fava and Herdade da Caniceira (Fig. 1), that are part of an international network of cork oak genetic trials. Both field trial sites are characterized by a Mediterranean climate with hot and dry summers, cold and mildly wet winters, with precipitation mainly concentrated between October and May (Varela, 2000). Long-term (1971–2000) mean annual temperature is 16.1 °C and 15.8 °C, mean annual precipitation is 550 mm and 708 mm, and mean summer precipitation is 17 mm and 48 mm in the Monte da Fava and Herdade da Caniceira trials, respectively (National Meteorological Agency). The Monte da Fava trial is at an altitude of 79 m, whereas the Herdade da Caniceira trial is at an altitude of 108 m. Soils are predominantly of sandy type in both trials.

At Monte da Fava, four cork oak populations were studied: two Portuguese, one Spanish, and one Italian. The Portuguese populations studied are: a local population (PT35) and the Quinta da Serra population (PT19), which is a population extensively studied for its reproductive behavior (Varela and Valdiviesso, 1996). The Spanish population is La Almoraima (ES8), referred to as a high cork quality stand; and the Italian population of Catania (IT14), an example of a population from the eastern natural range of the species. At Herdade da Caniceira, five Portuguese populations were tested (PT18, PT20, PT21, PT22, PT-ES25). They are representative of populations from areas where cork is economically relevant and were included in our study to investigate the genetic variation within the Portuguese cork oak populations. Regarding the climate and geographic origin of these populations, it can be highlighted the high range of altitudes. The mean annual precipitation of the PT18 and PT22 is lower than the other Portuguese tested populations. Detailed information on location, climate, and geographic variables for these populations is presented in Sampaio et al. (2019).

In both trials, each population was represented by 22 open-families. Mother trees were randomly selected within each population and assessed based on the following criteria: trees over 50 years (to minimize human impact on gene flow due to afforestation), a good acorn production, and a healthy sanitary status. Selected trees were at least 50–100 m apart to avoid a family structure. The seeds were collected in cork oak stands during the autumn of 1996. The sowing process was carried out at the beginning of 1997 in a nursery, with water and nutrient controlled conditions, and seedlings were planted in field trials in March 1998 at Monte da Fava and in February 1999 at Herdade da Caniceira. The experimental design was a split-plot with 22 blocks at Monte da Fava and 20 blocks at Herdade da Caniceira, each one with four and five plots (corresponding to the populations tested in each trial, respectively). Each plot was divided into 22 subplots, where each family was randomly assigned in a single-tree plot replicate. Trees were spaced in a 6 × 6 m manner.

2.2. Phenotypic measurements: survival, growth, and plant architecture traits

Trait measurements included plant survival (S), total height (HT), aboveground diameter at root collar (10 cm) (D), and diameter at breast height (130 cm) (DBH). These traits were assessed in all plants of each field trial (22 blocks × 22 families per population × 4 populations at Monte Fava; 20 blocks × 22 families per population × 5 populations at
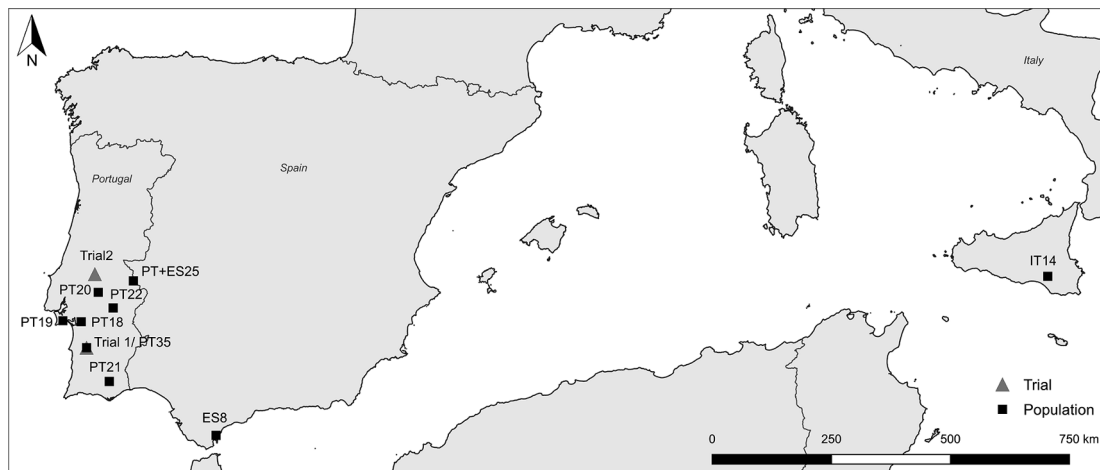
Fig. 1. Location of the sampled cork oak populations within the natural distribution range of the species (represented by a square), and the two cork oak progeny field trials (represented by a triangle) in Portugal - Monte da Fava (Trial 1) and Herdade da Caniceira (Trial 2).

Herdade da Caniceira) at different tree ages. At Monte da Fava, S, HT, and D were assessed in 2006 and 2011 (trees were 9 and 14 years old, respectively), whereas at Herdade da Caniceira these traits were evaluated in 2005 and 2011 (trees were 8 and 14 years old, respectively). DBH was assessed in both trials in 2011. This parameter is particularly relevant for cork oak silviculture as it determines the starting point for the debarking. For this reason, particular reference will be given to the results obtained for age 14 years. Total height and diameter (D and DBH) were recorded in meters and centimeters, respectively, and survival was scored as *0* or *1* for dead and alive plants, respectively. Traits were measured in autumn after the end of the vegetative season; in Portugal, the growth period most frequently extends from spring to midsummer.

Plant architecture traits were assessed before tree pruning, in each plant of each trial at 9 years of age. The evaluated metrics, apical dominance (AD), stem straightness (SS), stem inclination (SI), branchiness (B), and vigor (V), were scored according to the criteria detailed in Table 1.

2.3. Data analysis

Data analysis was based on the theory of mixed models (McCulloch et al., 2008; Stroup, 2013). Statistical analyses were performed separately for each experiment, given that there were different populations represented in each progeny trial.

2.3.1. Growth data

For the analysis of growth traits (HT, D, and DBH), the general linear mixed model formulation considered is described as follows:

$$y_{ijk} = \mu + \tau_i + b_j + (\tau b)_{ij} + f(\tau)_{ki} + e_{ijk},$$

where y_{ijk} is the response variable (HT, D, or DBH), μ is the overall mean, τ_i represents the population effects (assumed as fixed effects), b_j the block effects, $(\tau b)_{ij}$ the block × population interaction effects, $f(\tau)_{ki}$ the family within-population effects, and e_{ijk} represents the random errors. The effects b_j, $(\tau b)_{ij}$, $f(\tau)_{ki}$, and e_{ijk} were assumed to be normal random variables, mutually independent. Consequently, y_{ijk} was assumed to be a normal random variable.

The first step in the analysis of growth traits was to evaluate whether the variability among families is different within each population. Three linear mixed models were fitted to achieve this objective:

Model 1 (M1) – Model with homogeneous variances for family within-population effects and for random errors within-populations. The effects b_j, $(\tau b)_{ij}$, $f(\tau)_{ki}$, and e_{ijk} were assumed to be independent and identically distributed normal random variables with expected value zero and variances $\sigma_b^2, \sigma_{\tau b}^2, \sigma_{f(\tau)}^2$, and σ_e^2, respectively. That is, the genetic variability of the families within each population is equal for all the studied populations, as well as, the error variances (homogeneous variances).

Model 2 (M2) – Model with heterogeneous variances for family within-population effects and homogeneous variances for random errors within-populations. The effects b_j, $(\tau b)_{ij}$, and e_{ijk} were assumed to be independent and identically distributed normal random variables with expected value zero and variances $\sigma_b^2, \sigma_{\tau b}^2$, and σ_e^2, respectively. The effects $f(\tau)_{ki}$, were assumed to be independent, but heterogeneous variances for family within-population were considered. Denoting $\sigma_{f(\tau)_i}^2$ the variance for family within-population i, 4 and 5 family within-population variance components were considered for Monte da Fava and Herdade da Caniceira, respectively.

Model 3 (M3) – Model with heterogeneous variances for family within-population effects and for random errors within-populations. The effects b_j and $(\tau b)_{ij}$ were assumed to be independent and identically distributed normal random variables with expected value zero and variances σ_b^2 and $\sigma_{\tau b}^2$, respectively. The effects $f(\tau)_{ki}$ and e_{ijk} were assumed to be independent but heterogeneous variances among populations were considered for families within-population effects, and different error variances were assumed for each population. Denoted by $\sigma_{f(\tau)_i}^2$ the variance for family within-population i, 4 and 5 family within-population variance components were considered for Monte da Fava and Herdade da Caniceira, respectively. Denoted by $\sigma_{e_i}^2$ the error variance for population i, 4 and 5 error variance components were considered for Monte da Fava and Herdade da Caniceira, respectively.

The variance parameters were estimated by the residual maximum likelihood (REML) method. Empirical best linear unbiased estimators (EBLUEs) of the fixed effects and empirical best linear unbiased predictors (EBLUPs) of the random effects were obtained from mixed model equations (Henderson, 1975).

Model selection was performed by conducting a residual likelihood

Table 1
Plant architecture traits (apical dominance - AD, stem straightness - SS, stem inclination - SI, branchiness - B, vigor - V) assessed at Monte da Fava and Herdade da Caniceira progeny trial sites.

Trait	Score	Description
AD	1	No clear main stem since soil level (multi-stemmed from the base) – shrub-like form
	2	No clear main stem since soil level but with a trend for some branches to stand as axes
	3	No clear main stem exists from the soil level but with one branch standing out as the main axis
	4	A clear main trunk can be identified up to a certain height from which dominance is lost to a cluster of branches
	5	A clear main trunk can be identified up to a certain height from which dominance is lost to one or two main branches
	6	A clear main trunk can be singled out from base to apex
SS	1	Markedly sinuous bole, twisted and with diverse deformities along its entire length – absent rectitude and cylindricity
	2	Sinuous bole, and with diverse deformities along its entire length – low rectitude and cylindricity
	3	Bole of average rectitude and cylindricity, with moderate sinuosity and deformities along its length
	4	Bole of average rectitude and cylindricity, with marked sinuosity and deformities in part of its length
	5	Bole with good rectitude and cylindricity, with light sinuosity and deformities in part of its length
	6	Bole of excellent rectitude and cylindricity along its entire length
SI	1	The degree of inclination is in relation to ground level 0° (shrub-like form)
	2	1–22.5°
	3	23–45°
	4	46–67.5°
	5	68–89°
	6	90° (tree-like form)
B	1	Highly closed tree crown, with very high branch density and disordered architecture, very thick branches (compared to bole) with bad insertion angle. Characteristics observed in the entire tree crown.
	2	Slightly more open crown, with high branch density and disordered architecture in its entire extension, thick branches (compared to bole) with bad insertion angle.
	3	High branch density but with distinguishable bole and/or main branches. Existence of some thick branches with a bad insertion angle.
	4	Average branch density with ordered architecture in part of the tree crown, mainly thin branches (in relation to the bole) and with a good insertion angle
	5	Low branch density with simple and ordered architecture in most of the tree crown, mainly thin branches (in relation to the bole) and with a good insertion angle
	6	Low branch density with simple and well-shaped architecture, mainly thin branches (in relation to the bole) and with a good insertion angle. Characteristics observed in the entire tree crown.
V	1	Low vigor
	2	Low-middle vigor
	3	High-middle vigor
	4	High vigor

ratio test (LRT) and using the Akaike Information Criterion (AIC).

Variance components were tested using LRTs. The asymptotic distribution of the LRT statistic was assumed to be a chi-squared distribution, with the number of degrees of freedom equal to the increase in the number of parameters between the two compared models. A Wald-type F test was used to test growth differences among population means in each test site. Based on the predicted mean values of families within-population of HT and DBH, at age 14 years, Pearson correlation coefficients were determined to assess the correlation between traits for both sites.

As the selection units are families, rather than individuals, the heritability of family means and genetic gain from family selection were computed. For each trait, the heritability of family means (h_f^2) was

calculated according to Carrasquinho et al. (2018):

$$h_f{}^2 = \frac{\sum_{k=1}^{a}\left(1 - \frac{\widehat{PEV_k}}{\sigma^2_{f(\tau)}}\right)}{a - 1},$$

where a is the total number of half-sib families studied ($a = 88$ at Monte da Fava and $a = 110$ at Herdade da Caniceira), $\widehat{PEV_k}$ is the prediction error variance estimate of the effect for family k, and $\sigma^2_{f(\tau)}$ is the variance component estimate for the family within-population effects.

For each site, the expected genetic gain for HT and DBH was calculated, dividing the average of the five highest EBLUPs of family within-population effects for a given trait by the respective overall mean. The expected genetic gain obtained for each trait was expressed as a percentage.

The ASREML-R package (Butler et al., 2007) was used to fit the linear mixed models previously described.

2.3.2. Survival and plant architecture data

Survival data (binary response) was analyzed using a generalized linear mixed model with a logit link function. The logit model considered is described as follows:

$$\ln\left(\frac{\pi_{ijk}}{1 - \pi_{ijk}}\right) = \mu + \tau_i + b_j + (\tau b)_{ij} + f(\tau)_{ki},$$

where π_{ijk} is the probability of survival success for family k within-population i in the block j, μ is the intercept, τ_i represents the population effects (considered as fixed), b_j the block effects, $(\tau b)_{ij}$ the block × population interaction effects, and $f(\tau)_{ki}$ represents the family within-population effects. The effects b_j, $(\tau b)_{ij}$, and $f(\tau)_{ki}$ were assumed to be independent and identically distributed normal random variables with expected value zero and variances $\sigma^2_b, \sigma^2_{\tau b}$, and $\sigma^2_{f(\tau)}$, respectively.

From the inverse link, the probability of survival success for family k within-population i in block j (π_{ijk}) is given as follows:

$$\pi_{ijk} = \frac{1}{1 + e^{-\ln\left(\frac{\pi_{ijk}}{1 - \pi_{ijk}}\right)}}.$$

For the interpretation of the results, the mean of the predicted survival percentage for each family within-population was computed.

To evaluate the relation of survival and the studied growth traits, at age 14 years, in both sites, Pearson correlation coefficients were determined using the predicted mean percentage of survival of families within-population and the predicted mean values of families within-population of HT and DBH.

For each plant architecture trait evaluated (Table 1), several categories with a logic order were considered. The lower categories, i.e., the categories 1 and 2 for vigor and the categories 1, 2, and 3 for the form traits, represent plants with lower performance (i.e., with no clear apical dominance, sinuous bole, shrub form, high branch density, and poor vigor). Thus, they will need more cultural and silvicultural actions to return the same economic benefits compared to plant classified in superior classes. This data (the response variable with ordinal multinomial distribution) was analyzed with a generalized linear mixed model, considering a cumulative logit link function. In this proportional odds model, the linear predictor is defined as:

$$\eta_{c_{ijk}} = \eta_c + \tau_i + b_j + (\tau b)_{ij} + f(\tau)_{ki},$$

where $\eta_{c_{ijk}}$ denotes the link for category c for the combination of family k in the population i in block j, η_c is the intercept for the link c, τ_i represents the population effects (assumed as fixed effects), b_j the block effects, $(\tau b)_{ij}$ the block × population interaction effects, and $f(\tau)_{ki}$ represents the family within-population effects. The effects b_j $(\tau b)_{ij}$, and

$f(\tau)_{ki}$ were assumed as random (independent and identically distributed normal random variables with expected value zero and variances $\sigma_b^2, \sigma_{\tau b}^2$, and $\sigma_{f(\tau)}^2$, respectively). All random effects were assumed to be mutually independent.

For c categories, there are $c - 1$ link functions, one for each boundary:

$$\eta_{1_{ijk}} = ln\left(\frac{\pi_1}{1-\pi_1}\right), \eta_{2_{ijk}} = ln\left(\frac{\pi_1+\pi_2}{1-(\pi_1+\pi_2)}\right), \cdots, \eta_{(c-1)_{ijk}} = ln\left(\frac{\pi_1+\pi_2+\cdots\pi_{(c-1)}}{1-(\pi_1+\pi_2+\cdots\pi_{(c-1)})}\right),$$

where π_c denotes the probability that an observation belongs to category c. The inverse links are given as follows:

$$\frac{1}{1+e^{-\eta_{1_{ijk}}}} = \pi_1, \frac{1}{1+e^{-\eta_{2_{ijk}}}} = \pi_1+\pi_2, \cdots, \frac{1}{1+e^{-\eta_{(c-1)_{ijk}}}} = \pi_1+\pi_2+\cdots\pi_{(c-1)}.$$

Therefore, the probability that an observation belongs to a given category is given by:

$$\pi_1 = \frac{1}{1+e^{-\eta_{1_{ijk}}}}, \ \pi_2 = \frac{1}{1+e^{-\eta_{2_{ijk}}}} - \frac{1}{1+e^{-\eta_{1_{ijk}}}}, \ \cdots, \ \pi_c = 1 - \frac{1}{1+e^{-\eta_{(c-1)_{ijk}}}}.$$

The odds ratios were estimated to evaluate the relative differences among populations. Generically, denoting $\widehat{\tau}_i$ as the estimate of the increment of a population A compared to a population B for a given trait, the odds ratio was calculated as $e^{\widehat{\tau}_i}$. Thus, an odds ratio of 1 indicates that being in a lower level category is equally likely to occur in both populations; an odds ratio greater than 1 indicates that being in a lower level category is more likely to occur in population A; an odds ratio <1 indicates that being in a lower level category is less likely to occur in

population A. The predicted probability of a family within-population belonging to category c was also computed.

For the generalized linear mixed models described, parameters estimation was based on maximum likelihood by Laplace approximation. The GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., 2015) was used for these analyses.

3. Results

3.1. Growth and survival traits

In general, when comparing models M1 and M2, lower values for AIC were found for model M1, and no significant differences ($p > 0.01$) between these models were observed (see Table S1 in the supplementary material for models comparison). Thus, genetic variability of families was not different among populations. When comparing models M1 and M3 at the Monte da Fava trial, model M1 showed the lowest AIC value, and no significant differences were detected between these models ($p > 0.01$). At the Herdade da Caniceira trial site, although model M3 always showed lower AIC values, no significant differences were found for a significance level of 0.01. Therefore, the option of considering a more complex covariance matrix structure, i.e., heterogeneous variances among populations (for family and error variances) is not justified. That is, considering the previous models' comparison, family genetic variability among populations was not significantly different, as well as error variances among populations for all the evaluated traits.

Table 2
Results for the test of population effects (F-value and respective *p-value*), and variance components estimates and heritability values of family means (h_f^2) for growth traits (total height - HT, aboveground diameter at root collar – D, and diameter at breast height – DBH) obtained for model M1, at different ages for the test sites Monte da Fava and Herdade da Caniceira; the respective *p-values* for the tests of variance components are given in parentheses. The bold type indicates significant results for variance components estimates and heritability for a significance level of 0.05.

Trial	Age	Trait	F-value	Variance Components				h_f^2
				Block	Block × Population	Family(Population)	Error	
Monte da Fava	9	HT	1.489 ($p = 0.224$)	**0.2095** ($p < 0.001$)	**0.0923** ($p < 0.001$)	**0.0159** ($p < 0.001$)	0.3867	**0.77**
		D	0.9056 ($p = 0.443$)	**2.9744** ($p < 0.001$)	**1.3381** ($p < 0.001$)	**0.1429** ($p = 0.003$)	5.0923	**0.75**
	14	HT	**4.817** ($p = 0.004$)	**0.9280** ($p < 0.001$)	**0.2899** ($p < 0.001$)	**0.0319** ($p = 0.009$)	1.2853	**0.75**
		D	2.413 ($p = 0.074$)	**8.6839** ($p < 0.001$)	**3.2543** ($p < 0.001$)	**0.4759** ($p < 0.001$)	14.0113	**0.76**
		DBH	**5.778** ($p = 0.001$)	**6.0733** ($p < 0.001$)	**1.9627** ($p < 0.001$)	**0.4860** ($p < 0.001$)	11.8528	**0.76**
Herdade da Caniceira	8	HT	1.666 ($p = 0.165$)	**0.2852** ($p < 0.001$)	**0.1134** ($p < 0.001$)	**0.0308** ($p < 0.001$)	0.7563	**0.80**
		D	**3.583** ($p = 0.010$)	**1.5667** ($p < 0.001$)	**0.9807** ($p < 0.001$)	0.1550 ($p = 0.080$)	8.2613	0.77
	14	HT	1.851 ($p = 0.126$)	**0.7580** ($p < 0.001$)	**0.2672** ($p < 0.001$)	**0.1006** ($p < 0.001$)	1.9799	**0.80**
		D	2.129 ($p = 0.085$)	**5.1765** ($p < 0.001$)	**2.9675** ($p < 0.001$)	**0.7874** ($p = 0.024$)	30.1528	**0.78**
		DBH	2.417 ($p = 0.056$)	**4.8387** ($p < 0.001$)	**2.5144** ($p < 0.001$)	**0.6347** ($p = 0.028$)	24.9450	**0.78**

Considering this, the results presented for growth traits were based on the fitting of model M1.

At both trials and for the respective evaluated ages, significant family within-population variability for growth traits was observed, except for diameter (D) at age 8 years at Herdade da Caniceira (Table 2). The importance of considering the experimental design effects was confirmed by the significant detected variability associated with the block and block-population interaction (Table 2). This result, accomplished with the high number of blocks, justified the high values obtained for heritability of family means. They ranged between 0.75 and 0.80, and they were always significant, except for D at age 8 years at Herdade da Caniceira (Table 2).

The ranking of the EBLUPs of all family within-population effects of growth traits is presented in Tables S2 and S3 of the supplementary material. From the ranking of the five higher and lower EBLUPs of family within-population effects of HT and DBH at age 14 years, it can be highlighted that in general families with higher performance remain in the five top-ranked families for HT and DBH (Table 3). This result is particularly evident for the Portuguese families studied at Herdade da Caniceira. This outcome was expected as the predicted mean values of these traits are highly and significantly correlated ($r = 0.77$, $p < 0.001$; and $r = 0.90$, $p < 0.001$, at Monte da Fava and Herdade da Caniceira, respectively).

The predicted genetic gains, calculated as the ratio between the average of the five highest EBLUPs of family within-population effects and the respective overall mean, are 4% and 7.6% for HT and DBH, respectively, at Monte da Fava. The five families with the highest values for HT were PT19-F44, IT14-F24, IT14-F9, PT19-F41, and ES8-F11, and the five families with the highest values for DBH were ES8-F2, PT19-F25, ES8-F8, IT14-F11, and IT14-F24. At Herdade da Caniceira, the predicted genetic gain is 8.1% for HT, considering the selection of PT20-F27, PT20-F39, PT-ES25-F34, PT18-F20, and PT22-F37, and 7% for DBH, considering PT-ES25-F34, PT18-F20, PT18-F27, PT20-F39, and PT20-F31.

As observed for growth, a significant variability associated with the

Table 4
Result for the test of population effects (F-values and respective *p-values*), and variance components estimates obtained under the generalized linear mixed model for survival, at different ages and for the test sites Monte da Fava and Herdade da Caniceira; the respective *p-values* for the tests of variance components are given in parentheses. The bold type indicates significant results for variance components estimates for a significance level of 0.05.

Trial	Age	F-value	Variance Components		
			Block	Block × Population	Family (Population)
Monte da Fava	9	1.80 ($p = 0.157$)	**1.3191** ($p < 0.001$)	0.2085 ($p = 0.058$)	**0.1942** ($p = 0.025$)
	14	1.49 ($p = 0.227$)	**0.7403** ($p < 0.001$)	**0.6562** ($p < 0.001$)	**0.2096** ($p = 0.008$)
Herdade da Caniceira	8	1.25 ($p = 0.299$)	**0.6920** ($p < 0.001$)	**0.3397** ($p < 0.001$)	0.0391 ($p = 0.362$)
	14	2.44 ($p = 0.053$)	**0.4011** ($p < 0.001$)	**0.2223** ($p < 0.001$)	0.0405 ($p = 0.279$)

experimental design was observed for survival (Table 4). Survival variability differed among families within each population at Monte da Fava, but not at Herdade da Caniceira (Table 4). The ranking of the predicted mean percentage of survival of family within-population effects is presented in Tables S4 of the supplementary material. Overall, high values of predicted mean percentage of survival were observed, and the range of these values was low among populations and families within-population. Indeed, except for family F21 of ES8, which presented the lower value (66.5%), the predicted mean percentage of survival was greater than 82%. The families with higher survival rates were F1, F9, F12, F14, and F20 in ES8, F18 and F21 in the IT14 population,

Table 3
Ranking of the five families within populations with the higher and lower performance for growth traits (total height – HT (m) and diameter at breast height (130 cm) – DBH (cm)) at age 14 years after planting, based on the empirical best linear unbiased predictors (EBLUPs) of family effects, obtained for test sites Monte da Fava and Herdade da Caniceira.

	Monte da Fava							
Ranking	ES8		IT14		PT19		PT35	
	HT	DBH	HT	DBH	HT	DBH	HT	DBH
1	F11	F2	F24	F11	F44	F25	F24	F18
2	F8	F8	F9	F24	F41	F44	F17	F24
3	F2	F11	F16	F14	F42	F43	F12	F11
4	F1	F14	F21	F17	F28	F42	F11	F12
5	F23	F19	F11	F9	F27	F27	F18	F25
18	F9	F20	F20	F22	F29	F37	F22	F13
19	F21	F6	F22	F7	F36	F45	F6	F7
20	F7	F17	F25	F12	F37	F46	F20	F9
21	F6	F21	F2	F19	F23	F31	F13	F10
22	F5	F5	F19	F25	F32	F23	F10	F20

|	Herdade da Caniceira									
	PT18		PT20		PT21		PT22		PT-ES25	
	HT	DBH	HT	DBH	HT	DBH	HT	DBH	HT	DBH
1	F20	F20	F27	F39	F42	F43	F37	F37	F34	F34
2	F27	F27	F39	F31	F37	F42	F40	F38	F23	F22
3	F29	F29	F24	F27	F23	F37	F38	F34	F22	F2
4	F36	F23	F33	F42	F25	F28	F34	F20	F24	F29
5	F41	F41	F42	F33	F34	F25	F20	F33	F29	F12
18	F28	F7	F32	F32	F27	F22	F30	F35	F11	F14
19	F22	F22	F36	F29	F44	F38	F35	F24	F27	F27
20	F26	F28	F29	F30	F45	F26	F21	F25	F26	F10
21	F7	F26	F30	F36	F26	F45	F23	F23	F10	F35
22	F21	F21	F35	F35	F30	F30	F25	F21	F33	F26
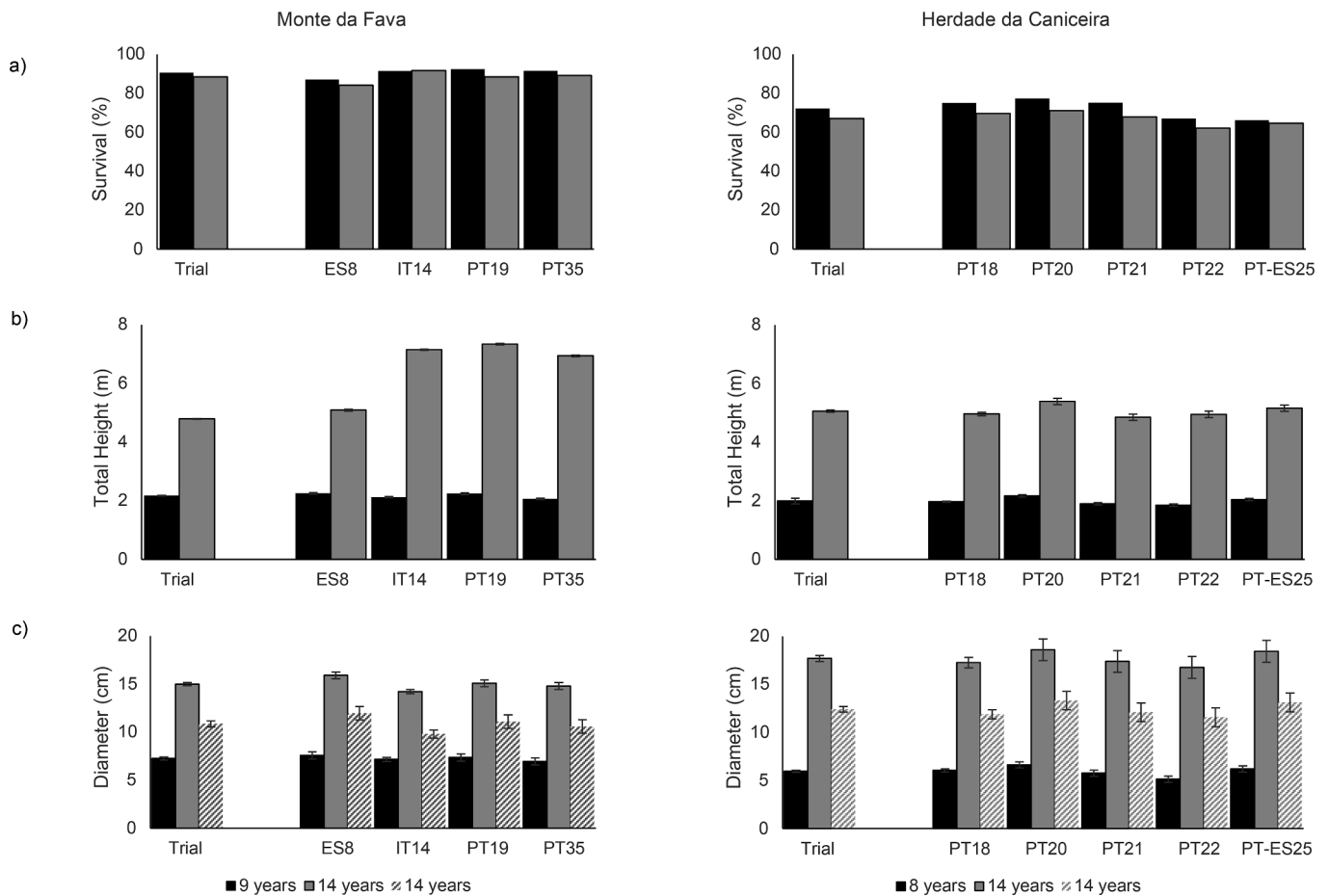
Fig. 2. Predicted mean percentage of population survival (a), obtained with the generalized linear model, and overall mean estimates and respective standard errors for total height (b) and diameter (c) (aboveground diameter at root collar in grey color and diameter at breast height in line pattern) obtained with model M1 at different ages for the test sites Monte da Fava and Herdade da Caniceira.

F26 in the PT19 population, and F10 and F14 in the PT35 population.

At Monte da Fava, significant differences among populations for HT and DBH at age 14 years were found; At Herdade da Caniceira, differences were only observed at age 8 years for D (Table 2). The results for survival and growth traits at the population level are presented in Fig. 2. Populations with high mean growth performance were La Almoraima (ES8) at Monte da Fava and Ponte de Sôr (PT20) at Herdade da Caniceira. For survival, no differences were observed at the population level at either of the field trial sites (Table 4). A slight decrease in the overall predicted survival rate was observed with age Fig. 2. Survival is not significantly correlated with HT ($r = 0.04$, $p = 0.686$; $r = 0.04$, $p = 0.702$, at Monte da Fava and Herdade da Caniceira, respectively) and DBH ($r = 0.06$, $p = 0.554$; $r = 0.03$, $p = 0.739$, at Monte da Fava and Herdade da Caniceira, respectively) and the correlation is very low.

3.2. Plant architecture traits and tree vigor

A significant variability associated with the block for vigor and form traits was detected in both sites (Table 5). The variability of the block-population interaction was significant for all traits except for stem inclination at Monte da Fava. At Herdade da Caniceira, this variability was only significant for branchiness and vigor (Table 5). Significant differences among the studied populations at Monte da Fava were found

for stem straightness, stem inclination, and tree vigor; whereas, the results are less certain for apical dominance. At Herdade da Caniceira, only stem straightness differed significantly among populations (Table 5). The probability estimates of each population belonging to a given class of tree form and vigor, are shown in Fig. 3a, b, and c (in the case of the Monte da Fava trial) and Fig. 3d (in the Herdade da Caniceira trial). For stem straightness (Fig. 3a), population IT14 was found to have a higher proportion of plants in classes SS1 and SS2 and a lower proportion of plants in classes SS4 and SS5, being the population with more sinuosity and deformities. PT35 showed an opposite trend, revealing higher rectitude and cylindricity along the entire bole length. For this trait, most of the plants are classified as middle stem straightness (SS3). No tree was classified as class SS6. For stem inclination at the Monte da Fava trial (Fig. 3b), IT14 showed a higher proportion of plants in class SI4 (compared with other populations) but a lower proportion of plants in class SI5. Higher the degree of inclination in relation to ground level, the higher the tree-like form. For this trait, most of the trees were classified as high stem inclination (SI5). For vigor (Fig. 3c), it was evident that population ES8 showed higher vigor compared to other populations, with a lower proportion of plants at class V2 and a higher proportion at V4. For this trait, most of the plants were classified as high middle vigor (V3). At Herdade da Caniceira, population PT21 exhibited more sinuosity and deformities, whereas population PT20 appeared to

Table 5

Result for the test of population effects (H_0: $\tau_i = 0$ vs. H_1: $\tau_i \neq 0$; F-values and respective p-values), and variance components estimates for plant architecture traits (apical dominance – AD, stem straightness – SS, stem inclination – SI, branchiness – B, and vigor – V), at age nine years old for the test sites Monte da Fava and Herdade da Caniceira; the respective *p-values* for the tests of variance components are given in parentheses. The bold type indicates significant results for variance components estimates for a significance level of 0.05.

Trial	Trait	F-value	Variance Components		
			Block	Block × Population	Family (Population)
Monte da Fava	AD	3.95 ($p = 0.051$)	**0.2550** ($p = 0.009$)	**0.2205** ($p = 0.030$)	0
	SS	**4.42** ($p = 0.007$)	**0.1726** ($p = 0.001$)	**0.1388** ($p = 0.001$)	0.0618 ($p = 0.068$)
	SI	**12.75** ($p < 0.001$)	**0.2137** ($p < 0.001$)	0.0123 ($p = 0.777$)	**0.1581** ($p < 0.001$)
	B	2.41 ($p = 0.075$)	**0.1772** ($p = 0.025$)	**0.2998** ($p < 0.001$)	**0.3814** ($p < 0.001$)
	V	**2.96** ($p = 0.034$)	**0.6505** ($p < 0.001$)	**0.3445** ($p < 0.001$)	0.0349 ($p = 0.467$)
Herdade da Caniceira	AD	1.17 ($p = 0.331$)	**0.2020** ($p < 0.001$)	0.02875 ($p = 0.538$)	0.0688 ($p = 0.177$)
	SS	**7.36** ($p < 0.001$)	**0.3123** ($p < 0.001$)	0.0141 ($p = 0.718$)	**0.1160** ($p = 0.002$)
	SI	2.14 ($p = 0.084$)	**0.1205** ($p = 0.005$)	0.0843 ($p = 0.121$)	0.0093 ($p = 0.841$)
	B	1.76 ($p = 0.146$)	**0.3154** ($p < 0.001$)	**0.1403** ($p = 0.004$)	0.0665 ($p = 0.086$)
	V	1.80 ($p = 0.173$)	**0.1866** ($p < 0.001$)	**0.1206** ($p = 0.006$)	0

have higher stem straightness quality (Fig. 3d). More than 65% of the trees of population PT20 were assessed as having stem straightness superior or equal to SS4.

Relative differences among populations were evaluated more objectively by the estimate of odds ratio (Table 6). At Monte da Fava, the odds of the population IT14 being in a lower stem straightness category is 1.965 times that of the population PT35. The odds of the population IT14 being in lower stem inclination and tree vigor category is 2.823 and 1.975 times those of the population ES8, respectively. At Herdade da Caniceira the odds of population PT21 being in a lower stem straightness category is 1.906 times that of the population PT-ES25.

At the family level, significant variability was observed with respect to stem inclination and branchiness at Monte da Fava and with straightness at Herdade da Caniceira (Table 5). The proportion of each category/class for each family is represented in Fig. 4, demonstrating the magnitude of that variability. For stem inclination at Monte da Fava (Fig. 4a), there were no plants in any family categorized in class SI1. It was evident that families within population IT14 (F10, F18, F19, F25, F24, and F9) presented the lower proportion of SI5, being those with a lower degree of inclination. Families F23, F8, F9, F11, and F12 in the ES8 population showed a higher degree of inclination (SI5). In the

population PT19, families with a higher stem inclination degree were F27, F36, F44, F31, and F37. Families F4, F24, F8, F15, and F17 were on the top of a superior class of inclination for the PT35 population. In Fig. 4b, it can be seen that there were no families of the studied populations classified in the B6 class. Some families presented a high proportion of plants with high branch density and disordered architecture, mainly F7 and F21 of the ES8 population and F20 from the IT14 population. At Herdade da Caniceira (Fig. 4c), families of the PT21 population presented a higher proportion of classes SS1 and SS2, particularly families F28, F32, F27, and F26. Families F27, F33, and F28 of PT20 population can be preferred for their higher rectitude and cylindricity form.

4. Discussion

4.1. Growth and survival performance among and within cork oak populations

Plant populations can adjust to new and heterogeneous environmental conditions by migrating to areas closer to their ecologic optimal, through phenotypic plasticity, or evolve through natural selection (Aitken et al., 2008; Alberto et al., 2013; Davis and Shaw, 2001; Gratani, 2014; Valladares et al., 2007). Thus, the existence of genetic variation in fitness and functional traits, among and within tree populations, constitutes a relevant mechanism to cope with the ongoing increasing land-use and environment pressures (Hamrick, 2004).

Mediterranean forest ecosystems have to cope with recurrent periods of environmental stress (drought and temperature) that are expected to increase in intensity and duration (IPCC, 2014). During these events, photosynthesis can be strongly reduced, with significant impacts on carbon allocation. In such conditions, as a process that is not vital to the tree, radial growth can be reduced and restricted in time. The relation between cork oak physiological responses to drought conditions and tree growth has been reported in the literature (Ramírez-Valiente et al., 2010). For instance, it was found that cork oak trees with more sclerophyllous leaves grew more during a dry year, as a result of avoiding an excessive water use. Indeed, both tree growth and crown transparency were reported as adequate tree vitality indicators under environmental constraints caused by drought and pest attacks (Dobbertin, 2005). In this sense, the cork oak populations and/or families identified with superior performance for survival and growth traits, i.e., well-adapted to the environmental study conditions, can be used in future (re)forestation actions, contributing to increase the species productivity and stands resilience. Nevertheless, an integrative approach also considering survival and other adaptive traits, namely those related to drought, pests, and disease tolerance, is required to increase the adaptation potential of cork oak and support sustainable management decisions. Additionally, the high levels of genetic variation obtained in this study aid the development of strategies for breeding practices (not yet developed in the country), which will result in economic gains for the cork chain.

The significant growth difference we observed among populations, both in terms of HT and DBH, at the Monte da Fava trial site agrees with the results already observed for these traits in other cork oak common garden experiments (Gandour et al., 2007; Ramírez-Valiente et al., 2009b, 2014c, Sampaio et al., 2019). The results obtained for populations with higher (ES8) and lower (IT14) growth performance are also in agreement with previous studies (Sampaio et al., 2019). No growth differences were found among the populations (all Portuguese) at the Herdade da Caniceira trial site. Although the specimens are from a high range of altitudes, this variable does not seem to be correlated with cork oak growth traits. These findings agree with a previous study in which the Portuguese populations showed a similar intermediate ranking based on genetic effects, suggesting that they do not differ in their growth performance (Sampaio et al., 2019). In terms of survival rate, no significant population variation was detected which suggests that it is more related to environmental effects.
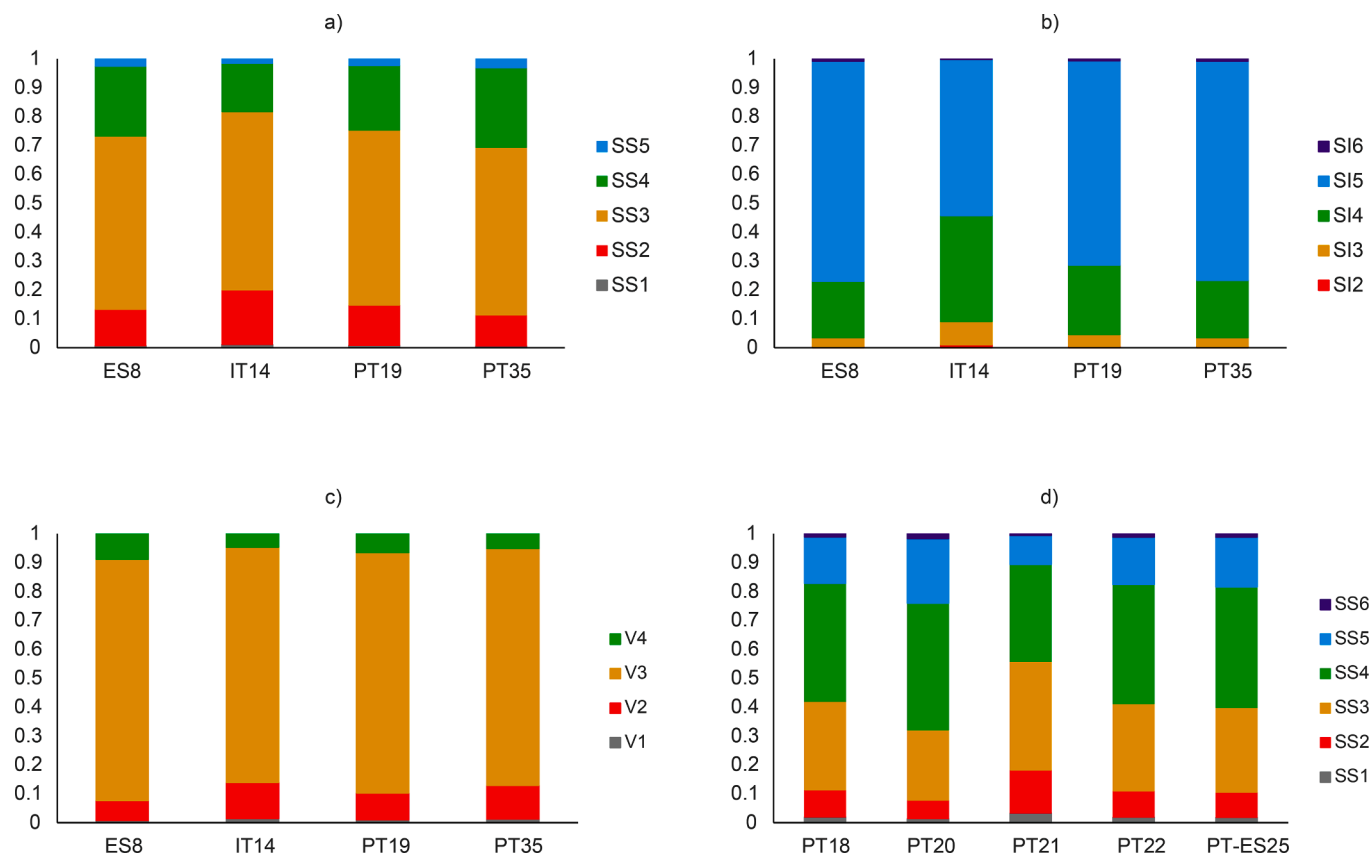
Fig. 3. Probability estimates of each population belonging to each stem straightness class (a), stem inclination class (b), vigor class (c) at Monte da Fava, and stem straightness class (d) at Herdade da Caniceira.

It should be noted that these trials were established to assess the magnitude of genetic variation within cork oak populations and to obtain preliminary estimates of the genetic control of relevant traits at the family level. A significant genetic variation in growth traits among cork oak families was detected in both of the progeny trial sites. These results suggest that for growth, superior families can be selected across the natural geographic range of the species. In contrast, significant variation in survival was only detected at the Monte Fava trial, specifically for family F21 of ES8 population, which demonstrated simultaneous lower growth and survival performance. The results are in agreement with the significant genetic variation detected at the family level for growth traits of other forest species (Zas et al., 2017; Carrasquinho et al., 2018; Nickolas et al., 2020), as well as for cork oak (Ramírez-Valiente et al., 2011, 2015). These latter authors also reported significant intra-population genetic variation in drought-tolerance traits (leaf size and thickness, specific leaf area, and carbon isotopic discrimination). Additionally, Ramírez-Valiente et al. (2014b), using molecular markers, found high genetic differentiation among maternal families within populations. Our study results do not allow for a more integrative approach of survival and growth traits since they were not significantly correlated.

The heritability of family means for growth traits was high (ranging between 0.75 and 0.80) and slightly high for the Portuguese families tested at Herdade da Caniceira. These results indicate that cork oak families are under genetic control and reveal that genetic differences among families explain a high proportion of phenotypic variability on the family mean basis. The heritability values were similar among growth traits, in contrast to previous studies on other forest species, in which higher values were found for height compared to diameter (Codesido and Fernández-López, 2008; Mwase et al., 2008). Although heritability is site- and age-specific, the present study highlights that selection for growth traits remains an appropriate criterion (genetic

gains were determined based on the ratio between the average of the five highest EBLUPs of family within-population effects and the respective overall mean). The heritability estimates reported by Ramírez-Valiente et al. (2011) for height and diameter in cork oak families are not comparable with those reported in this study. In their research, different methodologies for data analysis and heritability computation were applied; narrow-sense heritability was estimated and calculated individually for each population tested in the Spanish progeny trial (Alcácer do Sal – Portugal, La Almoraima – Spain, and Ain Rami – Morocco). Based on the results of Model 3, we concluded that for the Portuguese trials, the choice of considering different within-population variances was not justified. In this sense, a heritability value for each of the tested populations was not estimated. From the present study, it can be concluded that superior families can be identified earlier and selected, and genetic gains can be expected. The present results are promising, as the genetic variation detected at the family level can serve as the starting point in designing an adaptive breeding strategy for cork oak.

4.2. Tree vigor and architecture traits

From the perspective of a forest manager, a balance among survival, growth, and form traits should be taken into account to ensure the sustainability of cork oak and its economic benefits. We investigated, for the first-time, tree vigor and architecture traits (apical dominance, stem straightness and inclination, and branchiness) of cork oak families. This information is relevant to management of the species, given that higher cork oak straightness of the bole (i.e., the lower sinuosity, malformation, damaged leader, and forking) results in i) a reduction of pruning practices, ii) higher stem volume and lower cork wastes, and iii) lower costs of extraction and transport. Currently, cork extraction is done manually with high labor costs; however, future developments allowing

Table 6

Estimates of the odds ratio of population effect for plant architecture traits and tree vigor (stem straightness – SS, stem inclination – SI and vigor – V), at age nine years after planting, for the test sites Monte da Fava and Herdade da Caniceira.

Trial	Trait	Population	Population	Odds estimate
Monte da Fava	SS	IT14	PT19	1.456
		IT14	PT35	1.965
		IT14	ES8	1.633
		PT19	PT35	1.350
		PT19	ES8	1.122
		PT35	ES8	0.831
	SI	IT14	PT19	2.102
		IT14	PT35	2.763
		IT14	ES8	2.823
		PT19	PT35	1.314
		PT19	ES8	1.343
		PT35	ES8	1.022
	V	IT14	PT19	1.421
		IT14	PT35	1.095
		IT14	ES8	1.975
		PT19	PT35	0.771
		PT19	ES8	1.390
		PT35	ES8	1.804
Herdade da Caniceira	SS	PT18	PT20	1.526
		PT18	PT21	0.572
		PT18	PT22	1.033
		PT18	PT-ES25	1.091
		PT20	PT21	0.375
		PT20	PT22	0.677
		PT20	PT-ES25	0.715
		PT21	PT22	1.805
		PT21	PT-ES25	1.906
		PT22	PT-ES25	1.056

widespread mechanization of this operation will require trees with higher straightness. On the other hand, a decline of cork oak stands has been documented in the last several decades, resulting in a decrease in tree vigor and physiological status and subsequent attack by diseases and pathogens (Costa et al., 2010). Thus, the identification and selection of superior individuals for tree architecture traits and tree vigor, to be used in (re)forestation actions, is very important to increase the vitality and resilience of cork oak stands. Trees with higher vigor can be expected to present an adequate physiological status, which reduces the susceptibility to pathogens and diseases. Also, trees with higher vigor have a higher photosynthetic capacity and, consequently, can enhance carbon allocation, favoring tree growth and increasing the economic cork benefits. Tree architecture determines the spatial distribution of leaves and stems and the hydraulic architecture of trees, i.e., the structure and function of the water-conducting system in the flow path from roots to leaves (Cruiziat et al., 2002). Cork oak has been found to have a highly sectored main axis and crown, that is, to have xylem sections of the tap root and the trunk dedicated to transport water to specific branches (David et al., 2012; 2013). Hence, sinuous boles and unevenly distributed crowns might present defects causing hydraulic constraints that reduce the supply of water to parts of the crown or render sections of the trunk xylem redundant and, therefore, increase carbon use, reduce tree vigor and the potential for growth. Also, a cork oak tree with excessive brunch density might promote micro-climatic conditions close to the tree base favorable to the development of diseases.

A significant cork oak population variation was detected for stem form traits (straightness and inclination) and tree vigor, which agrees with the previous results obtained in a Tunisian cork oak common garden (Gandour et al., 2007). Since a significant correlation was found between the temperature at the origin of the population and the evaluated architecture traits in the Spanish and Tunisian cork oak genetic trials, it should be noted that climate factors seem to influence the

evolution of cork oak architecture traits. The significant block variation observed for most of the studied form traits reinforces the utility of an adequate experimental design in forest genetic trials that usually occupied large areas with environmental heterogeneity (water and soil nutrients availability, wind exposition). For example, sodden blocks might affect tree growth performance and, consequently, its apical dominance and vigor. Also, trees that are most exposed to wind effects might suffer from sinuosity in the bole.

In the present study, we highlight the following results: i) PT35 (i.e., the "local" population of Monte Fava trial) tended to show superior stem straightness, ii) ES8 showed simultaneous superior growth, stem inclination, and tree vigor, and iii) PT-ES25, a population on the border of Portugal and Spain, demonstrated high-quality stem inclination class. Although the performance of these populations, with regard to these traits, constitutes an advantage in terms of silvicultural economic gains, the results in terms of growth and survival cannot be ignored. Indeed, in a previous study, conducted at the Portuguese cork oak provenance trials where 35 cork oak populations were evaluated, it was found that population PT35 presented an intermediate growth and survival performance, whereas the ES8 population was one of the populations with lower survival performance (Sampaio et al., 2019). Therefore, a compromise among these traits needs to be considered to ensure higher economic gains.

Recently, researchers investigated whether the significant population genetic variation in tree form traits and vigor is the effect of cork oak local adaptation or the result of the evolutionary history of the species (Ramírez-Valiente et al., 2014a). The results indicated that these traits were associated with the cork oak lineages reported in the literature (Magri et al., 2007), and that populations from the eastern lineages presented the lowest apical dominance, highest branchiness, and lowest vigor. In addition, population ES8 showed higher vigor, similar to what we found in the present study.

Patrício et al. (2013) evaluated tree architecture traits in a large sample of cork oak populations, verifying that, before pruning, most of the studied Moroccan populations and some from Portuguese and Spanish origins presented higher values for straightness, apical dominance, and tree vigor. Among the populations with the poorest performance for straightness and tree vigor were the Italian, French, and Tunisian. Moreover, in the present study, the Italian population (IT14) showed lower stem straightness quality.

A marked family genetic variation in stem form (straightness and inclination degree) and branchiness was observed. These results are in agreement with many forest studies in which significant variation in form traits at a family level was detected (Doede and Adams, 1998; Codesido and Fernández-López, 2008; Callister et al., 2011; Blackburn et al., 2013). It should be noted that in the present study families F27 and F33 of PT20 were top-ranked, simultaneously, for stem straightness and growth performance and that F24 and F17 presented superior height growth, associated with a superior classification of stem inclination. These results are promising because they indicate that the selection of superior families of cork oak for growth and form traits will be possible, contributing to economic benefits from this species.

It is recognized that the evaluation of tree form traits and vigor is difficult to quantify because a subjective categorical scale is usually used. Thus, trait evaluation is highly dependent on the operator, and a single value assigned to each tree may not be an adequate measure of the trait. However, in this study, the experimental design of the field trials and appropriate data analysis (i.e., a generalized linear mixed model with multinomial response) allowed the detection of significant variability among cork populations and families within populations, for some of the studied traits. Finally, it should be noted that the evaluation of architecture traits and tree vigor was conducted in trees in a juvenile phase. Thus, the effect of inter-tree competition was neglected. The entire bole and crown were easily accessible from the ground, reducing the time and cost required for evaluation, which constitutes an advantage compared to the assessment of these traits in older trees (Doede and
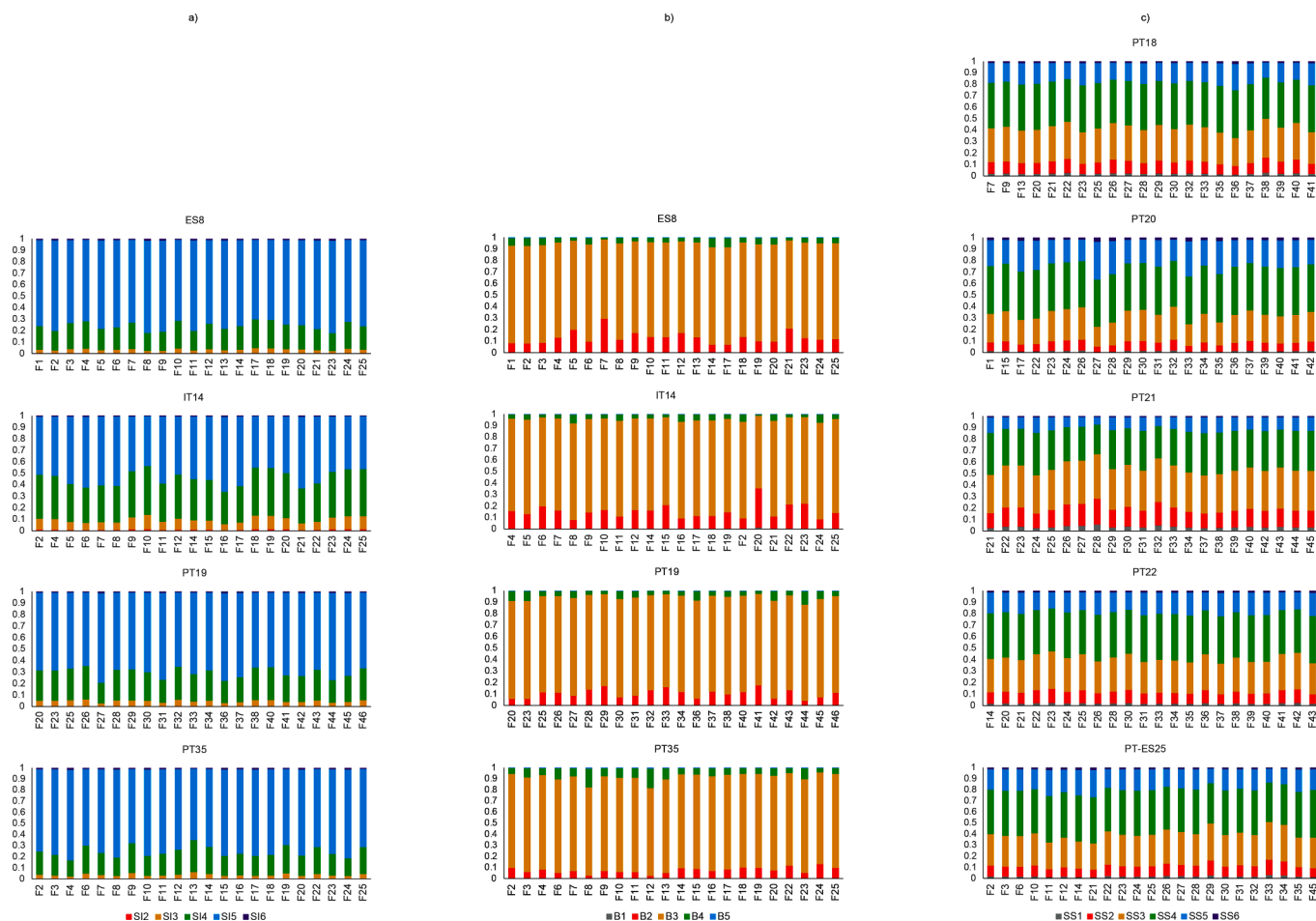
Fig. 4. Probability estimates of each family within-population belonging to each stem inclination class (a), branchiness class (b) at Monte da Fava, and stem straightness class (c) at Herdade da Caniceira.

Adams, 1998). Additionally, the traits were evaluated before tree formation pruning, and hence not to underestimate the results. This silvicultural treatment, applied in juvenile cork oak trees from 1 m to 1.5 m in height, is used to improve tree apical dominance and rectify deformed trunks and branch distribution (DGRF, 2006). Nevertheless, the evaluation of the formation pruning treatment on the quality of the form traits in cork oak populations indicated that this practice seems to have no effect or has a minimal impact, as previously reported, in which the pruning treatment not only revealed the worst morphological characteristics, but also enhanced those populations that showed superior form traits (Patrício et al., 2013).

In the future, the study of the relationship among architecture traits and tree vigor and other relevant morphological, physiological, and hydraulic traits, will improve the scientific knowledge of this highly relevant species. Additionally, because cork is the most economically valuable product of cork oak, there is an urgent need to assess the genetic variation and heritability of the species traits.

5. Conclusions

A significant genetic variation in survival and growth traits was found within cork oak populations. Heritability estimates revealed that growth traits are under high genetic control, and this finding highlights the suitability of these traits for the selection and improvement of the cork oak. Genetic gains from selection are possible for these traits.

This study provides the first evaluation of architecture traits at a family level. Stem straightness and stem inclination showed significant genetic variability for both inter- and intra-population levels. Overall, trees showed a propensity to be in a moderate-to-high class for the studied form traits.

From these results, we conclude that it is possible to achieve simultaneous improvements for both growth and form traits by selecting superior families. Thus, economic gains are expected as a consequence of higher stem volume and lower cork wastes, reduction of pruning practices, and decreasing extraction and transport costs.

The levels of inter- and intra-population genetic variation identified in this study will increase the adaptation potential of cork oak trees under the current environmental pressures. The allocation of more adapted reproductive material, i.e., plants with higher vigor, survival, growth, and form performance, that results from an adequate physiological status, allows trees to be less susceptible to drought stress events or diseases and pest attacks, thus, improving the vitality and resilience of cork oak stands. Additionally, it will also contribute to ensure the economic sustainability of forest-related activities.

CRediT authorship contribution statement

Teresa Sampaio: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Elsa Gonçalves:** Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing. **Carla Faria:** Investigation, Visualization, Writing - review & editing. **M. Helena Almeida:** Conceptualization, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

Acknowledgments

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118715.

References

Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S., 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evol. Appl. 1, 95–111. https://doi.org/10.1111/j.1752-4571.2007.00013.x.

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change – evidence from tree populations. Glob. Chang. Biol. 19, 1645–1661. https://doi.org/10.1111/gcb.12181.

Almeida, M.H., Merouani, H., Costa e Silva, F., Cortina, J., Trubat, R., Chirino, E., Vilagrosa, A., Khaldi, A., Stiti, B., El Alami, L., Vallejo, V.R., 2009. Germplasm Selection and Nursery Techniques. In: Aronson, J., Pereira, J.S., Pausas, J.G. (Eds.), Cork Oak Woodland son the Edge: Ecology, Adaptive Management, and Restoration of an Ancient Mediterranean Ecosystem. Island Press, Washington DC, pp. 129–137.

Álvarez, R., Alonso, P., Cortizo, M., Celestino, C., Hernández, I., Toribio, M., Ordás, R.J., 2004. Genetic transformation of selected mature cork oak (*Quercus suber* L.) trees. Plant Cell Rep. 23, 218–223. https://doi.org/10.1007/s00299-004-0810-2.

APCOR, 2020. APCOR's cork yearbook 19/20. Portuguese Cork Association. Santa Maria de Lamas, Portugal, pp. 128 Online at https://www.apcor.pt/wp-content/uploads/2019/12/boletim_estatistico_apcor_2019.pdf.

Aranda, I., Castro, L., Alia, R., Pardos, J.A., Gil, L., 2005. Low temperature during winter elicits differential responses among populations of the Mediterranean evergreen cork oak (*Quercus suber* L.). Tree Physiol. 25, 1085–1090. https://doi.org/10.1093/treephys/25.8.1085.

Aronson, J., Pereira, J.S., Pausas, J., 2009. Cork Oak Woodlands on the Edge: Ecology, Biogeography, and Restoration of an Ancient Mediterranean Ecosystem. Island Press, Washington DC, p. 315.

Blackburn, D.P., Hamilton, M.G., Harwood, C.E., Baker, T.G., Potts, B.M., 2013. Assessing genetic variation to improve stem straightness in Eucalyptus globulus. Ann. For. Sci. 70, 461–470. https://doi.org/10.1007/s13595-013-0277-9.

Bueno, M.A., Astorga, R., Manzanera, J.A., 1992. Plant regeneration through somatic embryogenesis in *Quercus suber*. Physiol. Plant 85, 30–34. https://doi.org/10.1111/j.1399-3054.1992.tb05259.x.

Bugalho, M., Plieninger, T., Aronson, J., Ellatifi, M., Crespo, D.G., 2009. Open woodlands: a diversity of uses (and overuses). In: Aronson, J., Pereira, J.S., Pausas (Eds.), 1999, Cork Oak Woodlands on the Edge: Conservation, Adaptive Management and Restoration. Island Press, Washington DC, pp. 33–47.

Butler, D., Cullis, B.R., Gilmour, A.R., Gogel, B.J., 2007. Analysis of mixed models for S language environments. ASReml-R reference manual. Training Series QE02001, Queensland Department of Primary Industries and Fisheries.

Callister, A.N., England, N., Collinsc, S., 2011. Genetic analysis of Eucalyptus globulus diameter, straightness, branch size, and forking in Western Australia. Can. J. For. Res. 41, 1333–1343. https://doi.org/10.1139/x11-036.

Cañellas, I., Montero, G., 2002. The influence of cork oak pruning on the yield and growth of cork. Ann. For. Sci. 59, 753–760. https://doi.org/10.1051/forest:2002061.

Carrasquinho, I., Lisboa, A., Inácio, M.L., Gonçalves, E., 2018. Genetic variation in susceptibility to pine wilt disease of maritime pine (*Pinus pinaster* Aiton) half-sib families. Ann. For. Sci. 75, 85. https://doi.org/10.1007/s13595-018-0759-x.

Codesido, V., Fernández-López, J., 2008. Juvenile genetic parameter estimates for vigour, stem form, branching habit and survival in three radiata pine (Pinus radiata D.Don) progeny tests in Galicia, NW Spain. Eur. J. Forest Res. 127, 315–325. https://doi.org/10.1007/s10342-008-0207-9.

Cortina, J., Pérez-Devesa, M., Vilagrosa, A., Abourouh, M., Messaoudène, M., Berrahmouni, N., Sousa, L.N., Almeida, M.H., Khaldi, A., 2009. Field Techniques to improve cork oak establishment. In: Aronson, J., Pereira, J.S., Pausas, J.G. (Eds.), Cork Oak Woodlands on the Edge: Ecology, Adaptive Management, and Restoration of an Ancient Mediterranean Ecosystem. Island Press, Washington DC, pp. 141–149.

Costa, A., Pereira, H., Madeira, M., 2010. Analysis of spatial patterns of oak decline in cork oak woodlands in Mediterranean conditions. Ann. For. Sci. 67, 204. https://doi.org/10.1051/forest/2009097.

Cruiziat, P., Cochard, H., Améglio, T., 2002. Hydraulic architecture of trees: main concepts and results. Ann. For. Sci. 59, 723–752. https://doi.org/10.1051/forest:2002060.

David, T.S., David, J.S., Pinto, C.A., Cermak, J., Nadezhdin, V., Nadezhdina, Na., 2012. Hydraulic connectivity from roots to branches depicted through sap flow: analysis on a *Quercus suber* tree. Funct. Plant Biol. 39, 103–115. https://doi.org/10.1071/FP11185.

David, T.S., Pinto, C.A., Nadezhdina, N., Kurz-Besson, C., Henriques, M.O., Quilhó, T., Cermak, J., Chaves, M.M., Pereira, J.S., David, J.S., 2013. Root functioning, tree water use and hydraulic redistribution in Quercus suber trees: A modeling approach based on root sap flow. For. Ecol. Manag. 307, 36–146. https://doi.org/10.1016/j.foreco.2013.07.012.

Davis, M.B., Shaw, R.G., 2001. Range shifts and adaptive responses to Quaternary climate change. Science 292, 673–679. https://doi.org/10.1126/science.292.5517.673.

DGRF – Direção Geral dos Recursos Florestais, 2006. Boas práticas de gestão em sobreiro e azinheira. Barros, M.C. and Sousa, E. (Eds.), pp. 104.

DL 169/2001 of 25 May. https://data.dre.pt/eli/dec-lei/169/2001/05/25/p/dre/pt/html.

Dobbertin, M., 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. Eur. J. Forest. Res. 124, 319–333. https://doi.org/10.1007/s10342-005-0085-3.

Doede, D.L., Adams, W.T., 1998. The genetics of stem volume, stem form, and branch characteristics in sapling noble fir. Silvae Genet. 47, 177–183.

Faias, S.P., Paulo, J.A., Palma, J.H.N., Tomé, M., 2018. Understory effect on tree and cork growth in cork oak woodlands. For. Syst. 27, 202S. https://doi.org/10.5424/fs/2018271-11967.

Gandour, M., Khouja, M.L., Toumi, L., Triki, S., 2007. Morphological evaluation of cork oak (*Quercus suber* L.): Mediterranean provenance variability in Tunisia. Ann. For. Sci. 64, 549–555. https://doi.org/10.1051/forest:2007032.

Gratani, L., 2014. Plant phenotypic plasticity in response to environmental factors. Adv. Botany 2014, 1–17. https://doi.org/10.1155/2014/208747.

Hamrick, J.L., 2004. Response of forest trees to global environmental changes. Dynamics and conservation of genetic diversity in forest ecology. Forest Ecol. Manage. 197, 323–335. https://doi.org/10.1016/j.foreco.2004.05.023.

Henderson, C.R., 1975. Best linear unbiased estimation and prediction under a selection model. Biometrics 31, 423–447. https://doi.org/10.2307/2529430.

Hernández, I., Celestino, C., Toribio, M., 2003. Vegetative propagation of *Quercus suber* L. by somatic embryogenesis I. Factors affecting the induction in leaves from mature cork oak trees. Plant Cell Rep. 21, 759–764. https://doi.org/10.1007/s00299-003-0605-x.

ICNF – Instituto da Conservação da Natureza e das Florestas, 2019a. 6° Inventário Florestal Nacional (IFN6), 2015 Relatório final. Instituto da Conservação da Natureza e das Florestas. Lisboa, pp. 284.

ICNF – Instituto da Conservação da Natureza e das Florestas, 2019b. Produção e comercialização de material florestal de reprodução (MFR). Relatório da Campanha 2018/2019. Instituto da Conservação da Natureza e das Florestas. Lisboa, pp. 31.

IPCC, 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. In: Field, C. B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), Part A: Global and Sectorial Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1132.

Jiménez, P., Agundez, D., Alia, R., Gil, L., 1999. Genetic variation in central and marginal populations of *Quercus suber* L. Silvae Gen. 48, 278–284.

Luechanimitchit, P., Luangviriyasaeng, V., Laosakul, S., Pinyopusarerk, K., Bush, D., 2017. Genetic parameter estimates for growth, stem-form and branching traits of *Casuarina junghuhniana* clones grown in Thailand. For. Ecol. Manage. 404, 251–257. https://doi.org/10.1016/j.foreco.2017.08.030.

Magri, D., Fineschi, S., Bellarosa, R., Buonamici, A., Sebastiani, F., Schirone, B., Simeone, M.C., Vendramin, G.G., 2007. The distribution of *Quercus suber* chloroplast haplotypes matches the palaeogeographical history of the western Mediterranean. Mol. Ecol. 16, 5259–5266. https://doi.org/10.1111/j.1365-294X.2007.03587.x.

McCulloch, C.E., Searle, S.R., Neuhaus, J.M., 2008. Generalized Linear and Mixed Models, 2nd edn. John Wiley & Sons, New York.

Mwase, W.F., Savill, P.S., Hemery, G., 2008. Genetic parameter estimates for growth and form traits in common ash (Fraxinus excelsior, L.) in a breeding seedling orchard at

Little Wittenham in England. New Forest. 36, 225–238. https://doi.org/10.1007/s11056-008-9095-6.

Natividade, J.V., 1934. Cortiças – Contribuição para o estudo do melhoramento da qualidade. Publ. Direcção dos Serviços Florestais e Aquícolas, I (1). Lisboa.

Natividade, J.V., 1950. Subericultura. Direccao-Geral dos Servicos Florestais e Aquicolas, Lisboa, p. 387.

Nickolas, H., Williams, D., Downes, G., Tilyard, P., Harrison, P.A., Vaillancourt, R.E., Potts, B., 2020. Genetic correlations among pulpwood and solid-wood selection traits in *Eucalyptus globulus*. New For. 51, 137–158. https://doi.org/10.1007/s11056-019-09721-0.

Patrício, M.S., Nunes, L., Almeida, M.H., 2013. Efeito da Intervenção Cultural na Caracterização Morfológica de Diferentes Proveniências de *Quercus suber*. Silva Lus. 21, 87–98.

Paulo, J.A., Tomé, M., 2010. Predicting mature cork biomass with t years of growth based in one measurement taken at any other age. For. Ecol. Manage. 259, 1993–2005. https://doi.org/10.1016/j.foreco.2010.02.010.

Paulo, J.A., Tomé, J., Tomé, M., 2011. Nonlinear fixed and random generalized height-diameter models for Portuguese cork oak stands. Ann. For. Sci. 68, 295–309. https://doi.org/10.1007/s13595-011-0041-y.

Paulo, J.A., Tomé, M., 2017. Does debarking intensity in the first cork extraction affect future cork thickness? Ann. For. Sci. 74, 66. https://doi.org/10.1007/s13595-017-0662-x.

Pérez, M., Bueno, M.A., Escalona, M., Toorop, P., Rodríguez, R., Cañal, M.J., 2013. Temporary immersion systems (RITA®) for the improvement of cork oak somatic embryogenic culture proliferation and somatic embryo production. Trees 27, 1277–1284. https://doi.org/10.1007/s00468-013-0876-y.

Poorter, L., Bongers, L., Bongers, F., 2006. Architecture of 54 moist-forest tree species: traits, trade-offs and functional groups. Ecology 87, 1289–1301. https://doi.org/10.1890/0012-9658(2006)87[1289:AOMTST]2.0.CO;2.

Ramírez-Valiente, J.A., Alia, R., Aranda, I., 2014a. Geographical variation in growth form traits in *Quercus suber* and its relation to population evolutionary history. Evol. Ecol. 28, 55–68. https://doi.org/10.1007/s10682-013-9660-0.

Ramírez-Valiente, J.A., Lorenzo, Z., Soto, A., Valladares, F., Gil, L., Aranda, I., 2009a. Elucidating the role of genetic drift and natural selection in cork oak differentiation regarding drought tolerance. Mol. Ecol. 18, 3803–3815. https://doi.org/10.1111/j.1365-294X.2009.04317.x.

Ramírez-Valiente, J.A., Sanchez-Gomez, D., Valladares, F., Aranda, I., 2010. Phenotypic plasticity and local adaptation in leaf ecophysiological traits of thirteen contrasting cork oak populations under different water availabilities. Tree Physiol. 30, 618–627. https://doi.org/10.1093/treephys/tpq013.

Ramírez-Valiente, J.A., Valladares, F., Delgado, A., Nicotra, A.B., Aranda, I., 2015. Understanding the importance of intrapopulation functional variability and phenotypic plasticity in *Quercus suber*. Tree Genet. Genomes 11, 35. https://doi.org/10.1007/s11295-015-0856-z.

Ramírez-Valiente, J.A., Valladares, F., Gil, L., Aranda, I., 2009b. Population differences in juvenile survival under increasing drought are mediated by seed size in cork oak (*Quercus suber* L.). For. Ecol. Manage. 257, 1676–1683. https://doi.org/10.1016/j.foreco.2009.01.024.

Ramírez-Valiente, J.A., Valladares, F., Huertas, A.D., Granados, S., Aranda, I., 2011. Factors affecting cork oak growth under dry conditions: local adaptation and contrasting additive genetic variance within populations. Tree Genet. Genomes 7, 285–295. https://doi.org/10.1007/s11295-010-0331-9.

Ramírez-Valiente, J.A., Valladares, F., Huertas, A.D., Granados, S., Aranda, I., 2014b. Exploring the impact of neutral evolution on intrapopulation genetic differentiation in functional traits in a long-lived plant. Tree Genet. Genomes 10, 1181–1190. https://doi.org/10.1007/s11295-014-0752-y.

Ramírez-Valiente, J.A., Valladares, F., Sanchez-Gomez, D., Huertas, A.D., Aranda, I., 2014c. Population variation and natural selection on leaf traits in cork oak throughout its distribution range. Acta Oecol. 58, 49–56. https://doi.org/10.1016/j.actao.2014.04.004.

Sampaio, T., Branco, M., Guichoux, E., Petit, R.J., Pereira, J.S., Varela, M.C., Almeida, M.H., 2016. Does the geography of cork oak origin influence budburst and leaf pest damage? For. Ecol. Manage. 373, 33–43. https://doi.org/10.1016/j.foreco.2016.04.019.

Sampaio, T., Gonçalves, E., Patrício, M.S., Cota, T.M., Almeida, M.H., 2019. Seed origin drives differences in survival and growth traits of cork oak (*Quercus suber* L.) populations. For. Ecol. Manage. 448, 267–277. https://doi.org/10.1016/j.foreco.2019.05.001.

SAS Institute Inc., 2015. SAS® 9.4 In-Database Products: User's Guide, Sixth Edition. SAS Institute Inc., Cary, NC.

Stroup, W.W., 2013. Generalized Linear Mixed Models: Modern Concepts. CRC Press, Boca Raton, Methods and Applications.

Toumi, L., Lumaret, R., 1998. Allozyme variation in cork oak (*Quercus suber* L.): the role of phylogeography and genetic introgression by other Mediterranean oak species and human activities. Theo. Appl. Genet. 97, 647–656. https://doi.org/10.1007/s001220050941.

Valladares, F., Gianoli, E., Gomez, J.M., 2007. Ecological limits to plant phenotypic plasticity. New Phytol. 176, 749–763. https://doi.org/10.1111/j.1469-8137.2007.02275.x.

Varela, M., Valdiviesso, T., 1996. Phenological phases of *Quercus suber* L. Flowering For. Genet. 3, 93–102.

Varela, M.C., 2000. European Network for the Evaluation of Genetic Resources of Cork Oak for Appropriate use in Breeding and Gene Conservation Strategies, Handbook. Lisboa, pp. 126.

Wang, J.J., Weng, Y.H., Krasowski, M., Yan, G.H., Fullarton, M., 2018. Genetic parameters of growth and stem forking for black spruce progeny tested in New Brunswick, Canada. New For. 49, 265–277. https://doi.org/10.1007/s11056-017-9618-0.

Zas, R., Björklund, N., Sampedro, L., Hellqvist, C., Karlsson, B., Jansson, S., Nordlander, G., 2017. Genetic variation in resistance of Norway spruce seedlings to damage by the pine weevil *Hylobius abietis*. Tree Genet. Genomes 13, 111. https://doi.org/10.1007/s11295-017-1193-1.