



RESEARCH PAPER

Open Access



Intraspecific variation of *Quercus ilex* L. seed morphophysiological traits in Tunisia reveals a trade-off between seed germination and shoot emergence rates along a thermal gradient

Nabil Amimi^{1,2*} , Hana Ghouil^{1,2} , Rim Zitouna-Chebbi³, Thierry Joët⁴  and Youssef Ammari¹

Abstract

Key message *Quercus ilex* populations from cold habitats display a large lag between seed germination and shoot emergence time, favouring avoidance of late frost events. Populations from mild habitats show the fastest seed germination and shoot emergence rates at moderate temperatures, enabling them to synchronize germination in the late winter-early spring period and a rapid seed-to-seedling transition, during the favourable rainy period.

Context *Quercus ilex* is the most abundant and representative Mediterranean oak species. Identifying and describing intraspecific variation in seed traits is necessary to characterize the germination niche, and to elucidate drivers of species' range.

Aims In order to identify adaptations to local environments that may reflect ecological strategies for stress avoidance and seed survival, we tested under common and optimal conditions whether seed functional traits vary, in *Quercus ilex* subsp. *rotundifolia* Lam., along climatic gradients within its distribution range in Tunisia.

Methods We have explored variations in seed morphological traits, desiccation sensitivity level, germination and shoot emergence rates under different controlled temperature conditions, among 15 populations of *Q. ilex* sampled throughout the Tunisian distribution of the species.

Results Significant between-populations differences were observed for morphological seed traits but no relationships could be established with the climate of the sampling sites. In contrast, key physiological traits varied significantly with elevation and temperature. Specifically, mild temperatures in lowland regions were associated with higher seed moisture content, fast germination and shoot emergence rates at moderate temperatures (13 °C) for germination. Seeds of *Q. ilex* populations from cold sites displayed the fastest germination rates at low temperatures (5 °C) as well as the greatest lag between seed germination and shoot emergence time.

Handling editor: Ignacio J. Diaz-Maroto.

*Correspondence:

Nabil Amimi

amiminabil@yahoo.fr

Full list of author information is available at the end of the article



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Conclusion Intraspecific variation in seed physiological traits is significantly associated with local climate. This functional diversity should be considered when evaluating germplasm and predicting suitability for reforestation and assisted migration programs.

Keywords Holm oak, Germination, Seed functional traits, Recalcitrant seeds, Oak acorn, Mediterranean climate

1 Introduction

Quercus ilex L. (holm oak) is the most abundant and representative *Quercus* tree species in the Mediterranean forests and is known for its widest ecological amplitude among Mediterranean oaks, from semi-arid to per-humid bioclimates, and from warm to cold conditions depending on its elevation (Barbero et al. 1992). This sclerophyllous evergreen tree species is found from Turkey to Spain on the European side of the Mediterranean Sea, and from Morocco to Tunisia on the African side, and has also colonized most of the Mediterranean islands, with large morphological and genetic variations, as well as East–West differentiation (Lumaret et al. 2002; López De Heredia et al. 2007).

Q. ilex distribution is thought to be delimited by aridity in the southern area (Terradas and Savé 1992) and by low winter temperatures and freezing stress in northern and high-altitude areas (Nardini et al. 2000). Intraspecific variations exhibited by *Q. ilex* across its circum-Mediterranean distribution have been largely documented for morphological, structural and functional traits (Barbéro et al. 1992; Gratani et al. 2003; Lumaret et al. 2002; Michaud et al. 1995; Peguero-Pina et al. 2014; Sanchez et Retuerto 2007), including those associated with tolerance to drought and cold stresses (Gimeno et al. 2009; Peguero-Pina et al. 2014). *Q. ilex* shows two main morphological types, associated with genetic differentiation, which are considered as two subspecies (Lumaret et al. 2002; Michaud et al. 1995). *Quercus ilex* subsp. *rotundifolia* Lam. (*Q. ilex* subsp. *ballota* being a heterotypic synonym, Ferrer-Galego and Sáez 2019; Le Floc’h et al. 2010) displays the ‘rotundifolia’ morphotype, characterized by small and rounded thick leaves with high vein density, and is the exclusive morphotype in Tunisia, Algeria and Morocco, and the dominant one in the Iberian Peninsula (Barbéro et al. 1992; Ferrer-Galego and Sáez 2019; Peguero-Pina et al. 2014). The ‘ilex’ morph (*Q. ilex* subsp. *ilex* L.), distinguished by elongated and large leaves with low vein density, is present in mild coastal areas from Greece to France, while an ‘intermediate’ morphotype for trees displaying intermediate characteristics between the two morphs and dominating coastal areas of eastern Spain and south-eastern France (Lumaret et al. 2002).

In the context of global climate change, circulation models predict periods of prolonged drought in the Mediterranean basin and more frequent extreme events, such

as heat waves and late-winter frosts, which have already been observed (Giorgi and Lionello 2008; Ruffault et al. 2014; Trambly et al. 2020). Mediterranean oaks are usually seen to share the capacity to cope with water deficits through specialized adaptive features such as sclerophylly, restricted leaf area, and thick cuticles. However, climate change could have dramatic consequences for the survival and distribution of Mediterranean oak species, especially on the southern edge of the Mediterranean region (Matesanz and Valladares 2014; Ruiz-Labourdette et al. 2012). Indeed, both migration and genetic adaptation are relatively slow processes in species that have a long generation time such as oaks (Bussotti et al. 2014; Garcia and Zamora 2003). Many research studies have been conducted to determine the capacity of adult oak trees to survive summer drought and to understand how vegetative functional traits contribute to the ecological ranges of the different Mediterranean oak species (Acherar and Rambal 1992; Adams et al. 2017; Castagneri et al. 2017; Cavender-Bares et al. 2005; Limousin et al. 2012; Lloret et al. 2016; Quero et al. 2011; Niinemets and Keenan 2014; Ramirez-Valiente et al., 2022; Solé-Medina et al. 2022). However, less attention has been paid to Mediterranean oak fecundity and sexual reproduction (Le Roncé et al. 2021), as well as seed traits and seedling ecology, especially in North Africa (Gomez-Aparicio et al. 2008; Gonzales-Rodrigues et al. 2011; Joët et al. 2016; Urbietta et al. 2008). Yet seed germination is a vital process in a plant life cycle affecting seedling establishment and survival, which can subsequently determine species’ distribution and population persistence (Carta et al. 2022; Cochrane et al. 2015a; Donohue et al. 2010; Rosbakh and Poschlod 2015). Sensitivity to abiotic stresses makes regeneration, which is the transition from seed to seedling, a serious bottleneck in population recruitment and the most critical stage for survival in a Mediterranean-type community (Cochrane 2016; Lloret et al. 2004; Perez-Ramos et al. 2013). For instance, changes in precipitation have been noticed to have dramatic effects on Holm oak recruitment in woodlands in southern France (Perez-Ramos et al. 2013). Considering that the seeds of Mediterranean oaks are recalcitrant, i.e. they are short-lived and desiccation-sensitive (Amimi et al. 2020; Ganatsas and Tsakaldimi 2013; Joët et al. 2013), the first type of stress they may encounter during winter is a prolonged dry spell between shedding in

autumn and the return of favourable conditions for germination in spring. Although summer drought is widely considered to be the main limiting factor for plant survival in Mediterranean ecosystems, winter stresses are crucial when interpreting species distribution (Larcher 2000). Desiccation was shown to be the major cause of in situ mortality of *Q. ilex* seeds at the end of winter and a long period of drought can lead to the loss of an entire annual seed cohort (Joët et al. 2013, 2016). The need to remain fully hydrated during winter also exposes recalcitrant seeds to a second type of hazard which is freezing.

Recently, key seed traits that govern seed persistence and germination niche breadth, and that may influence the geographical ranges and ecological strategies, have been shown to diverge significantly among four Mediterranean oak species co-occurring in Tunisia (Amimi et al. 2020). Indeed, when assessed on 4–5 different representative populations for each species, the seeds of *Q. ilex* and *Quercus canariensis* Willd., which occur at relatively high elevations where frost events are frequent, displayed the highest freezing tolerance while acorns of *Quercus coccifera* L., which is frequent in warm and arid environments, showed the highest germination rate and synchrony (Amimi et al. 2020). However, the extent of intraspecific variation for key germination traits remains to be determined for each species across a large number of populations and is the focus of the present study for *Q. ilex*.

Many studies have revealed that among-population variation in germination response to environmental conditions resulted in different responses to climate change within species, which could mitigate the species' vulnerability to changing climate and provide opportunities for species adaptation and conservation (Chamorro et al. 2017, 2018; Cochrane et al. 2015a). The sensitivity of germination to climate variability depends on the species' phenotypic plasticity, local adaptation, and geographic distribution (Cochrane et al. 2015b; Nicotra et al. 2010). However, to date, only a few studies have examined intraspecific variation in seed traits in Mediterranean oaks, and these studies have remained primarily focused on seed size. This latter is a key functional trait determining the reserves of carbohydrates and nutrients seedlings need during germination, which contributes to the ecological strategy and distribution of species (Jimenez et al. 2016; Moles and Westoby 2004). In Mediterranean *Quercus* species, the significant interspecific and intraspecific variation in seed size may facilitate their establishment in a heterogeneous environment (Quero et al. 2007). In a comparative study of *Quercus suber* L. populations, positive correlations between seed mass and xerothermic indices have been observed (Ramirez-Valiente et al. 2009). Variability, within species, in acorn

morphological and chemical traits, including starch, lipids and phenolic compounds, has also been reported for *Q. ilex* (Caliskan 2014; López-Hidalgo et al. 2021; Valero Galvan et al. 2012). Determining within-species variation in seed physiological and germination traits is essential to understand how trade-offs constrain adaptation to contrasted environmental conditions, and to elucidate the drivers of species' ranges (García-Nogales et al. 2016). Exploring how seed germination traits from different close local provenances respond to climate proxies will be helpful to understand the strategies of *Q. ilex* to adapt along local xerothermic gradients.

Here we test whether the functional traits of seeds vary along a climatic gradient within its range of distribution in Tunisia. Holm oak occurs mainly in the two orographic regions in Northern Tunisia, High-Tell and the Tunisian Ridge, and their surroundings. Influenced by the proximity of the Mediterranean Sea, the High Tell region is the wettest one, while the Tunisian Ridge is characterized by a drier climate, and low winter temperatures (Nabli 1989, 1995). To improve our understanding of the effects of climate and topographic factors on the major seed traits, the aims of the present study were to (1) inventory the distribution of the main *Q. ilex* populations in Tunisia and document the climate factors associated with their habitats; (2) measure intraspecific variations in the main seed morphophysiological traits, including seed germination and response to desiccation and assess their intraspecific variations; and (3) explore the relationships between observed variations in seed traits and climate factors in order to identify adaptations to local environments that may determine seed survival and stress avoidance in holm oak.

2 Material and methods

2.1 Study area, and climatic data

This study covered the entire Tunisian distribution area of *Q. ilex* (Fig. 1; Amimi et al. 2022). The inventory of the distribution of *Q. ilex* in Tunisia was carried out over a period of several months in 2020 using systematic transect searches on forest roads and pastoral paths, in and around the previously identified areas of occurrence in woodlands of the two Tunisian orographic regions, High-Tell and the Tunisian Ridge, and their surroundings. On the highest peaks (Chaâmbi, Kesra, Serj, Semmama) holm oak forms pure stands. Below 1000 m, under meso-Mediterranean bioclimates, holm oak mixes with *Pinus halepensis* Mill. (Aleppo pine; El Hamrouni et al. 2020). Species is also present in the thermo-Mediterranean bioclimates of the Tunisian High Tell, north of Ghardimaou (Ouled Ali forest) and north of Téboursouk (Nabli 1989). The sampling area, from 501 to 1078 m in elevation, ranged in latitude from 35° 42' 36" N to 36° 25' 48"

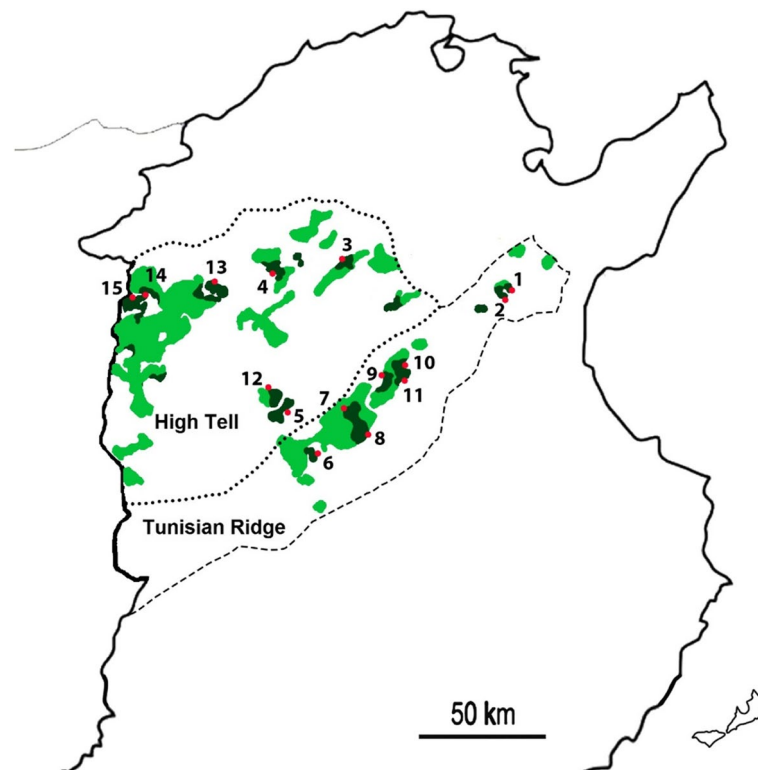


Fig. 1 Distribution map of *Quercus ilex* populations in Tunisia. The current *Q. ilex* distribution (dark green areas) was estimated in this study, while the past distribution area (light green surfaces) is based on the last national forest inventory of 1990 (Nabli 1989). All populations of *Q. ilex* are distributed in the two mountainous regions of the High Tell and the Tunisian Ridge. Locations of the 15 woodlands sampled are indicated by red dots

N and in longitude from $8^{\circ} 23' 38.4''$ E to $10^{\circ} 5' 34.8''$ E. The geographic information about each sampling site was collected by a GPS navigator upon sampling. Information on habitat was inferred from the environmental layers of 19 bioclimatic variables (bio1-19), integrated over the years 1970–2000, obtained from the Worldclim version 2 database (Fick and Hijmans 2017). The resolution used for all climatic layers was 30 arc-seconds (1 km^2).

2.2 Biological material

This study was carried out on mature acorns collected in November 2020 from 15 holm oak woodlands (*Q. ilex*). Twenty trees were randomly selected from each population, the sampled trees being located at a distance of at least 20 m from each other. The acorns were collected by shaking the tree when the acorns reached full maturity, according to the indexes of maturity described by Bonner and Vozzo (1987). In each site, about 2000 acorns were collected and pooled (100 acorns per tree), immediately enclosed in hermetically sealed plastic bags, and stored in the dark at ambient temperature for a maximum of 2 days before being transported to INRGREF (Tunis, Tunisia). In the laboratory, the acorns were first immersed in water

to identify insect-damaged or infected floating acorns. After additional visual screening for abnormal and defective acorns, sound acorns were stored in hermetically sealed plastic boxes in the dark at 4°C , as described previously (Bonner and Vozzo 1987), for a short period of a maximum of 10 days until physiological characterization was initiated.

2.3 Acorn and seed morphological traits

The seed and pericarp moisture contents, expressed on a fresh weight basis, and their dry mass were determined gravimetrically after oven-drying for 17 h at 103°C , using 50 randomly selected acorns per sampled site. The ratio of pericarp mass to acorn mass (pericarp/acorn ratio) was also calculated from these measurements. Acorn length and maximal width as well as pericarp thickness were measured on 100 randomly selected acorns per sampled site, using a digital calliper.

2.4 Germination and shoot emergence

Germination kinetics were assessed in the dark in climate chambers fixed at five different constant temperatures (5, 9, 13, 17 and 21°C) to determine the germination base

temperature (Pritchard and Manger 1990). For each condition and location sampled, the pericarp was removed and three batches of 30 seeds were placed in plastic boxes (170 × 105 × 60 mm), containing a layer of cotton moistened with distilled water. Germination and shoot emergence were surveyed daily for a period of 20 weeks and recorded when the radicle had grown at least 5 mm and curved and shoot length reached 5 mm, respectively. Time needed to achieve 50% of maximum germination (t_{50}) and the specific parameter G_a (germination asynchrony, i.e. the slope of the tangent line at the point of inflection) were determined using the following germination function and least-squares regression as computed by the quasi-Newton method:

$$G = \frac{G_{max}}{1 + \exp(G_a(t - t_{50}))}$$

where G is the percentage of germination at time t , and G_{max} is the maximum germination. The estimation of t_{50} further enabled the calculation of the germination rate, GR, defined as $1/t_{50}$. Time needed to achieve 50% of maximum shoot emergence (t_{50SE}) and the specific parameter G_aSE shoot emergence asynchrony were determined using a similar function where SE is the percentage of shoot emergence at time t , and SE_{max} is the maximum shoot emergence.

2.5 Desiccation sensitivity

To measure seed desiccation sensitivity, 10–12 batches of 50 mature acorns per population were spread on benches in a ventilated room (15 °C, 35–40% RH) and air-dried for 30 days. By using one batch every 3 days, seed water content was assessed gravimetrically on 10 seeds while seed viability was assessed on 40 others following the germination criterion, i.e. protrusion of the radicle and geotropic growth, after 2 weeks of culture at 21 °C in the dark. Desiccation sensitivity was quantified using the quantal response model (Dussert et al. 1999):

$$V = \frac{V_i}{1 + \exp(b(WC - WC_{50}))}$$

where V is seed viability, V_i is the initial viability, WC_{50} is the water content at which half of the initial viability is lost, and b is seed lot-specific parameter.

2.6 Statistical analysis

ANOVA, PCA and linear and non-linear regressions were carried out using Statistica software (Statsoft, USA). Differences in seed traits were tested using one-way ANOVA with a fixed effect and the Bonferroni post hoc test. Residuals were checked for normality and homoscedasticity using log-transformed data. Correlations

between variables were analyzed by linear regression using Pearson's correlation coefficient. A significance threshold of $P=0.05$ was retained for both ANOVA and linear regression. Principal component analysis (PCA) was performed to analyse the covariations between climatic factors and morpho-physiological traits of seeds from the different surveyed sites, latitude, longitude and elevation being supplementary variables. Hierarchical Clustering Analysis (HCA, Ward's grouping method, Euclidian distance) was then applied to factorial scores obtained from PCA analysis on climate variables in order to pinpoint clusters and similarities in bioclimatic envelopes associated with the different *Q. ilex* populations. Non-linear regressions (desiccation sensitivity and germination time) were performed using least-squares regression computed by the quasi-Newton method (Dussert et al. 1999).

3 Results

3.1 Bioclimatic conditions associated with *Quercus ilex* populations in Tunisia

The inventory of *Q. ilex* in Tunisia identified 15 major areas where holm oak is present, including 4 sites as dominant species, and 11 others in association with either Aleppo pine or other Mediterranean oaks, *Q. coccifera* and *Q. suber* (Fig. 1; Appendix Table 1; Amimi et al. 2022). The structure of climate variability among habitats was analyzed by PCA, and the first two principal components explained 81% of the overall variance (Fig. 2a). The first Principal Component (PC1) was highly correlated with average temperature T_{mean} ($R=0.981$) while negatively correlated with elevation. PC1 represented a thermal gradient associated with elevation, the average temperatures varying significantly from 13.8 °C to 16.8 °C between sites. The second component (PC2) was positively correlated with annual rainfall ($R=0.879$) and highlighted a gradient of rainfall seasonality as it separated sites with the rainiest winters and annual rainfall from sites with the broadest annual temperature range and maximal temperatures in summer. The 15 sampled locations displayed high variability in cumulative annual rainfall, ranging from 407 to 816 mm (Appendix Table 1). Each sampling population can be characterized by its factorial score co-ordinates on the first two PC, highlighting differences for bioclimatic variables associated with the different Tunisian holm oak populations (Fig. 2b). Hierarchical clustering analysis of such co-ordinates further revealed a discontinuous structure with two main clusters, I and II, which differentiate warm and cold sites, respectively (Fig. 2c). Clusters were further split into two subclusters, subclusters II-a and II-b corresponding to elevated sites associated with wet and dry climates, respectively. This analysis therefore defined a climatic

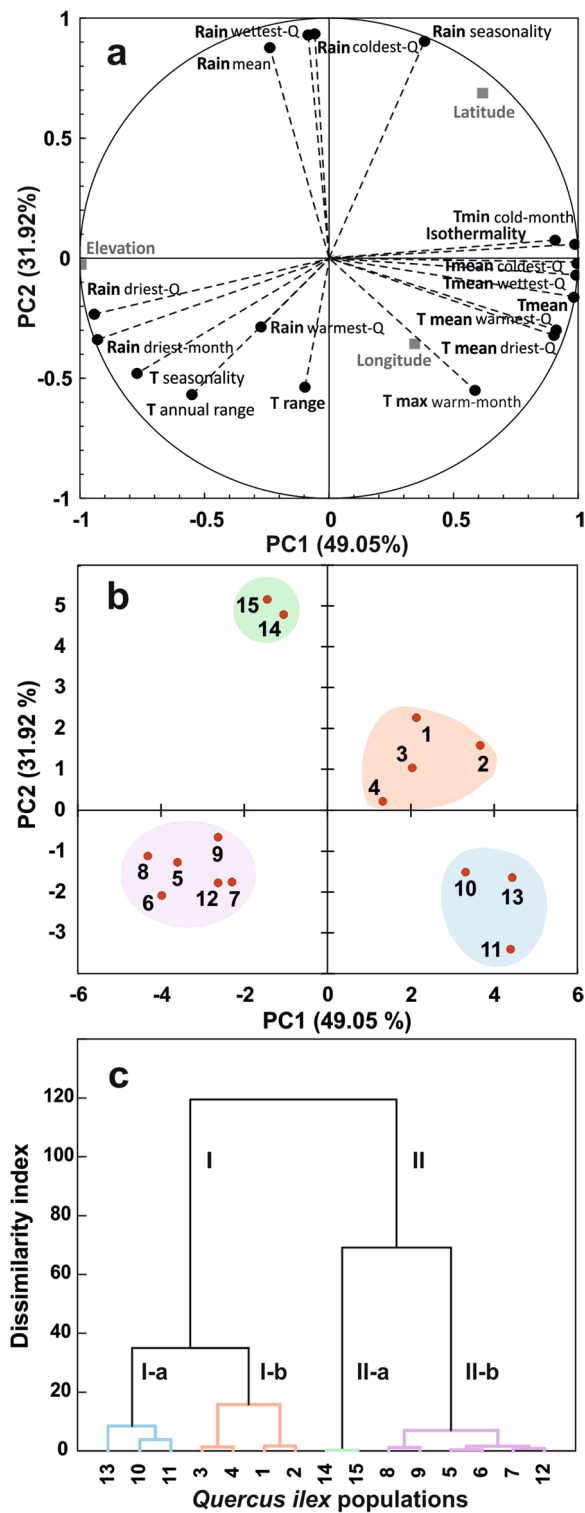


Fig. 2 Multivariate analysis of the climatic characteristics of the 15 *Quercus ilex* sampling sites. **a** Principal Component Analysis (PCA) correlations (factor loadings) of climatic variables with the first two principal components (PC). Longitude, latitude and elevation, in grey, were treated as supplementary variables. **b** PC1–PC2 score plot of the 15 populations studied. Each population sampled was characterized by its factorial score co-ordinates on the first two PCs. **c** Hierarchical Classification Ascendant (HCA) of holm oak sites according to their climatic variables. HCA analysis revealed 4 differentiated clusters, which colour code have been reported on the upper PC1–PC2 score plot

typology of holm oak woodlands, with four clusters of sites that are differentiated along the thermal and rainfall gradients.

3.2 Intraspecific variation in seed morphophysiological traits

All measured morphological traits, i.e. seed size, seed mass, seed water content, pericarp thickness, and the pericarp mass to acorn mass ratio (PAR), showed large individual variation within a population, but also statistically significant variation among sampled populations ($P < 0.05$; Appendix Table 2). The largest variation between sites was observed for seed dry mass that ranged from 1.55 to 4.45 g. The seed water content at shedding also displayed large variations and ranged from 33.82 to 41.98% (on a fresh mass basis).

Seed germination was always very high, since it ranged between 95 and 100%, independent of the population and the germination temperature within the range 5–21 °C (Fig. 3), demonstrating the high vigour of all seed lots. Tunisian holm oak displays very low temperature requirement for germination, since all populations tested were able to complete germination at 5 °C. By contrast, large intraspecific variation was observed for the germination time at such low temperatures. Indeed, for the four populations with the fastest seed germination, germination was completed in about 30 days (800 h), while 70 days (1700 h) was required for the two populations with the slowest germination (Fig. 3).

Between the two extreme populations, the difference in germination time, as quantified by t_{50} , i.e. the time to reach 50% of maximum germination, is 54 days (1316 h). A significant correlation ($P < 0.05$) was found between t_{50} and germination asynchrony G_a at 13 °C and 17 °C ($R = 0.76$ and 0.59 at 13 °C and 17 °C, respectively), indicating that populations with the longest germination time were also those with the highest seed-to-seed variability in germination time (Appendix Table 3). Time for germination at 9 °C and 17 °C was much shorter than that at 5 °C, with smaller variations in germination time between populations (Fig. 3). Furthermore, seed germination traits (t_{50} and G_a) determined at temperatures

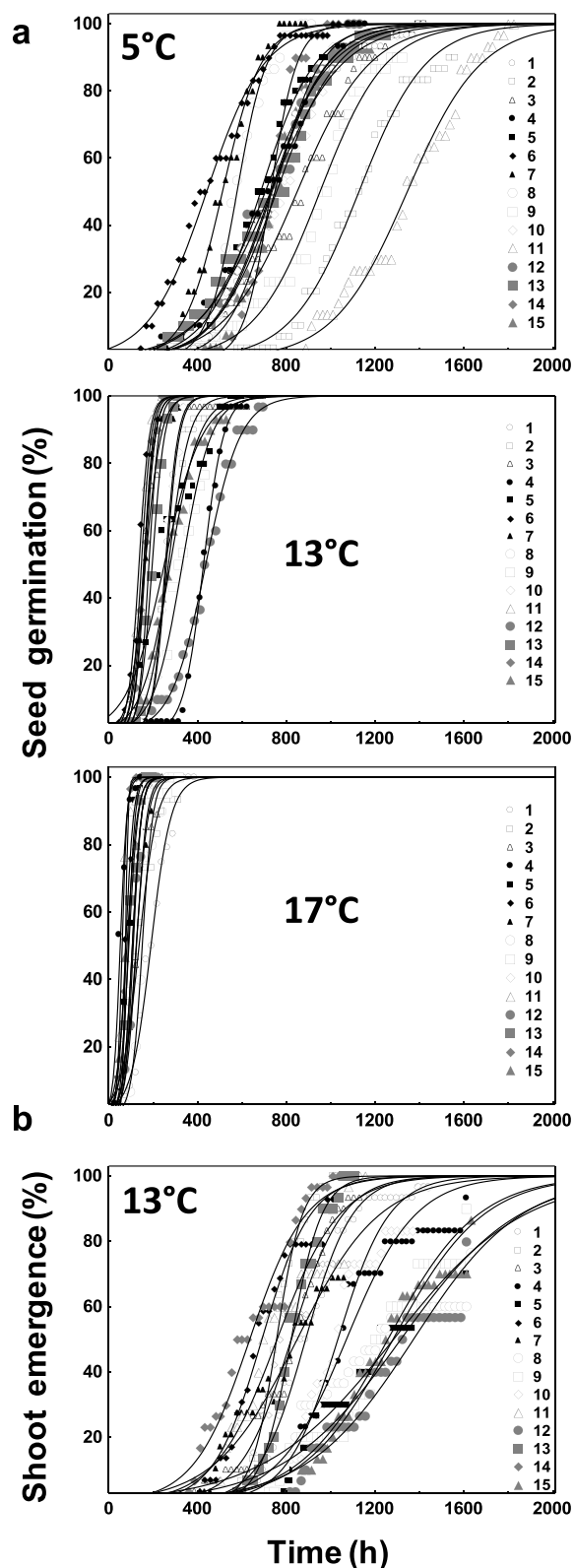


Fig. 3 Seed germination time courses at 5 °C, 13 °C and 17 °C (a) and shoot emergence time course at 13 °C (b) for the 15 Tunisian *Quercus ilex* populations sampled

higher than 5 °C (9 °C, 13 °C, 17 °C and 21 °C) displayed some positive correlations between them (Appendix Table 3).

The germination rate ($1/t_{50}$) increased linearly between 5 °C and 21 °C in all populations (Fig. 4), enabling the determination of the base temperature T_b , i.e. the minimum temperature for germination, in the different populations. *Q. ilex* showed a relatively low intraspecific variation in germination rate response to temperature, with 5.08 ± 0.91 °C average T_b , except for one population (#8) which showed a T_b of 2.5 °C. This narrow window of variation among populations for T_b nevertheless displayed significant correlations with seed mass ($R=0.57$) and germination time t_{50} at 21 °C ($R=-0.69$) (Appendix Table 3). In addition to germination time at low temperature, a large intraspecific variation was also observed for the time of emergence of shoot and primary leaves after germination at a favourable temperature of 13 °C (Fig. 3b). Indeed, the SEt_{50} , the time to reach 50% of maximum shoot emergence ranged from 26 to 57 d. This trait was positively correlated with germination time at the same temperature ($R=0.60$; Appendix Table 3). Finally, large between-population variations were observed for WC_{50} , the water content at which half of the initial viability is lost, that varied from 26.4% to 38.4% (Fig. 5). While not significantly correlated to seed mass ($R=-0.39$; $P=0.19$), WC_{50} was positively correlated with initial moisture content of mature seeds at shedding ($R=0.68$; $P=0.01$) (Appendix Table 3).

3.3 Identification of key climate-seed trait associations

PCA performed on the complete dataset, including climatic variables and seed morphophysiological traits, led to similar results to those obtained with climatic variables alone (Figs. 2a and 6). Variance of climatic variables was mainly explained by the two first principal components $PC1'$ and $PC2'$, which accounted for 47% of the overall variance. Like $PC1$, the first factor ($PC1'$) was highly correlated with average temperature T_{mean} ($R=0.971$) and represented a thermal gradient negatively associated with elevation, while the second factor ($PC2'$), as observed for $PC2$, was positively correlated with rainfall ($R=0.774$) and latitude. None of the morphological traits, including seed mass and pericarp thickness, was collinear with bioclimatic variables and correlated with either $PC1'$ or $PC2'$ (Fig. 6). As for seed physiological traits, it is worth noting that only a few of them were correlated with $PC1'$ and strikingly, none of them was correlated with $PC2'$. $PC1'$ was positively correlated with seed water content ($R=0.664$), desiccation sensitivity (0.734), and germination time at low temperatures (t_{50} at 5 °C; $R=0.631$), but negatively correlated with the time for shoot emergence when germinated at an optimal temperature of 13 °C

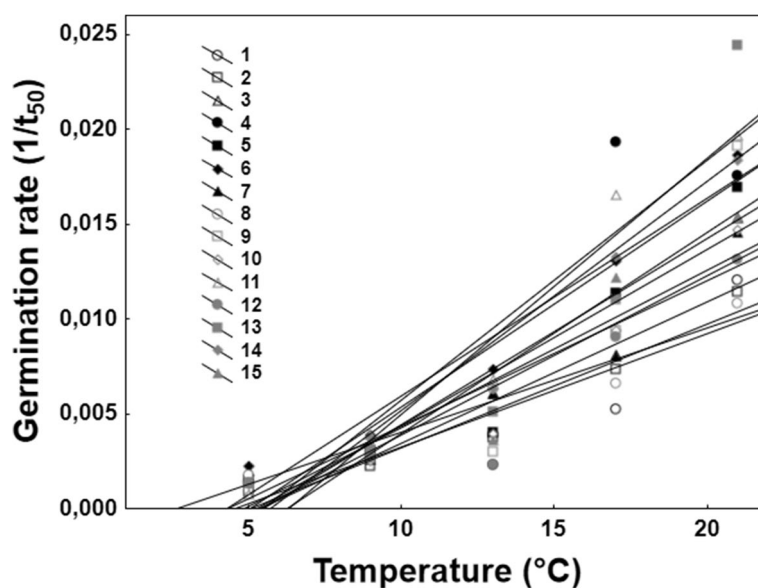


Fig. 4 Effect of germination temperature on seed germination rate ($1/t_{50}$) in the 15 Tunisian *Quercus ilex* populations studied and determination of seed base temperature, T_b (ordinate) for seed germination

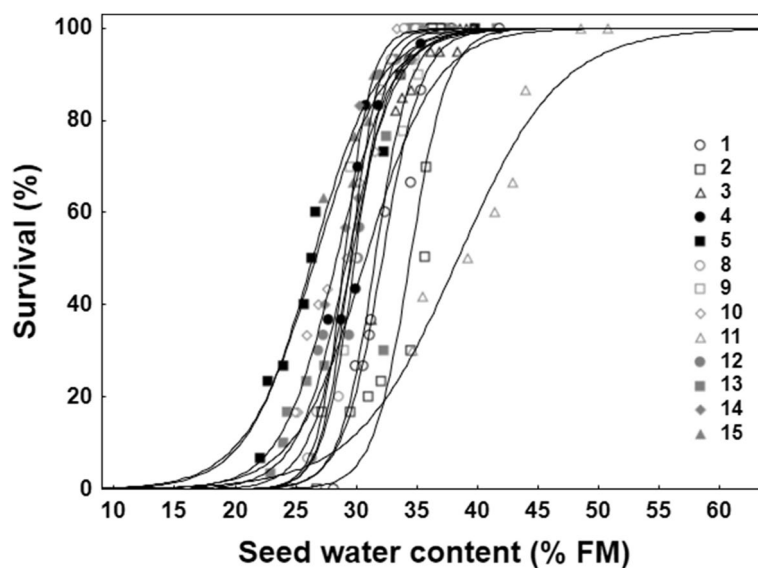


Fig. 5 Survival of seeds of the different *Quercus ilex* populations studied after desiccation to various water contents. Viability was estimated as the percentage of germination

($R = -0.736$). This result suggests an adaptive trade-off between the capacity of rapid germination at low temperatures, which is significantly higher for all populations distributed in cold climates, and seedling vigour, i.e. rapid stem emergence at more favourable temperatures ($13\text{ }^{\circ}\text{C}$) for populations distributed in areas with the mildest climates. One may also note that most of evidenced seed trait-climate associations revealed by PCA were also

significant ($P < 0.05$) when tested individually using Pearson correlation coefficients (Appendix Table 4). Indeed, germination time at low temperatures (t_{50} at $5\text{ }^{\circ}\text{C}$) and seed water content were significantly correlated with mean annual temperature ($R = 0.60$ and 0.57 , respectively) while WC_{50} was positively correlated with mean annual temperature ($R = 0.65$) and inversely correlated with annual precipitation ($R = -0.56$) (Appendix Table 4).

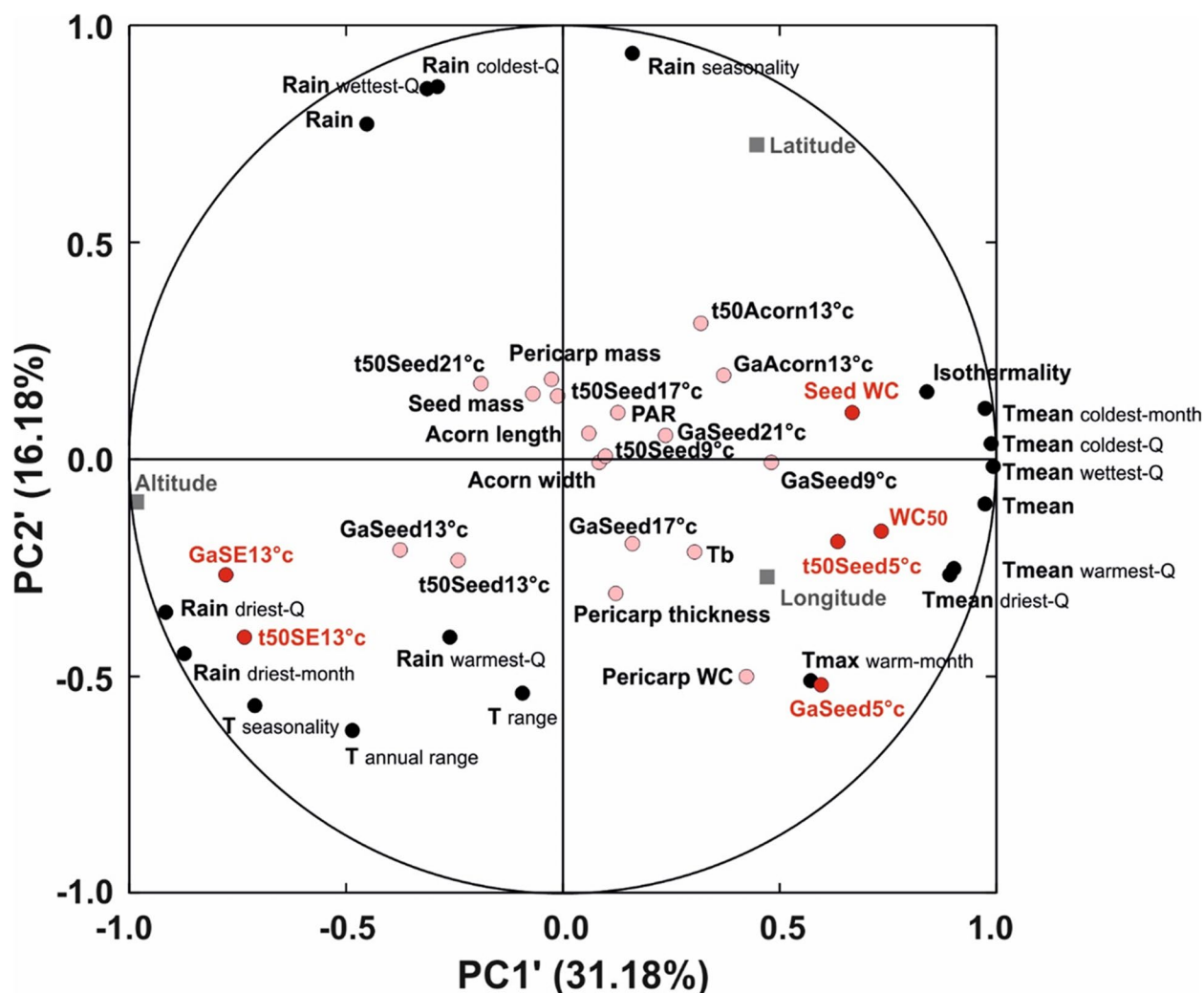


Fig. 6 Principal Component Analysis (PCA) correlations (factor loadings) of climatic variables (black circles) and seed morphophysiological traits (pink circles, red circles for correlations higher than 0.5) with the first two principal components (PC1' and PC2'). Longitude, latitude and elevation, in grey, were treated as supplementary variables

Similarly, germination asynchrony at 5 °C was positively correlated with temperature ($R=58$), while shoot emergence at 13 °C was negatively correlated ($R=-0.68$). Finally, none of the major morphological traits, including acorn size, pericarp thickness, or seed mass, showed variation among populations that correlated significantly with climate variables (Appendix Table 4).

4 Discussion

In this study, we have explored variations for seed functional traits among populations of *Q. ilex* sampled over the entire Tunisian distribution of the species. While all measured morphological traits, including acorn size, seed mass and pericarp thickness, showed within and between-site variations, no significant relationship could be established between variation in these traits and major

climatic factors along environmental gradients. When studied at large spatial scales, significant relationships have been detected between *Q. ilex* seed mass and geographical factors, such as latitude or altitude (Garcia-Nogales et al. 2016; Valero Galván et al. 2012), and similar trends have been observed in *Q. suber* along a xerothermic gradient (Ramírez -Valiente et al. 2009; Matías et al. 2018). Our study, conducted on a smaller scale, on two mountain ranges in Tunisia, did not reveal such relationships between seed mass and climate or geographical variables. In this context, it is worth noting that the seed mass of *Q. ilex* is a highly variable trait whose variations may be associated with different local factors such as selective pressure from seed predators, intensity of summer drought during seed development, or age of mother trees (Alonso-Crespo et al. 2020; Celebias and Bogdziewicz 2022; Le Roncé et al.

2021). Since variation in *Q. ilex* seed mass affects seedling fitness as well as the probability of attack by post-dispersal seed predators (Gómez et al. 2004), it has been suggested that large intraspecific variation in this trait is the result of a bet-hedging strategy facilitating seed survival and seedling establishment in a heterogeneous environment (Quero et al. 2007). Furthermore, we did not detect any significant correlation between seed mass and germination traits associated with the different *Q. ilex* populations. The absence of relationships between key morphological traits, including seed mass and pericarp thickness, and germination traits has also been observed in many other oak species (Xia et al. 2015).

By contrast, several key seed physiological traits, including seed water content at maturity and desiccation sensitivity, as well as germination time at 5 °C, displayed variations significantly correlated with mean annual temperature at the site of sampling. These results obtained on *Q. ilex* seeds corroborate several recent studies emphasizing the primary role of local climate in shaping intraspecific variation in temperate and Mediterranean plant species with respect to key seed germination traits (Carta et al. 2022; Chamorro et al. 2018; Cochrane et al. 2015a). At this stage, we cannot separate genetic and environmental aspects (involving either local genetic adaptation or phenotypic plasticity associated with maternal or environmental effects), but such seed trait variations highlight the ecological strategies adopted for adaptation to different climatic constraints. *Q. ilex* populations from the coldest locations, i.e. high elevation sites of the Tunisian ridge, displayed the fastest germination rates at low temperatures. By contrast, populations distributed within the warmest sites, i.e. low elevation sites surrounding the High-Tell and the Tunisian ridge, displayed the fastest seed germination and shoot emergence rates at mild temperatures. The observed opposite variation of these traits along a thermal gradient suggests the existence of population-specific germination niches and ecological strategies adapted to local Tunisian climates. The rapid germination rate at mild temperatures, with synchronized shoot emergence, enables seeds from mid- and low-altitude *Q. ilex* populations to align germination and seed-to-seedling transition schedules with early spring, a favourable rainy period with limited risk of a late frost. Seeds of *Q. ilex* populations from elevated sites show fast germination in the cold, enabling also germination in late winter-early spring, in mountainous climatic conditions. However, those populations display higher uncoupling between seed germination and shoot emergence time, favouring avoidance for the plantlets of the freezing stress that is associated with frequent late frost events. Early development of a large root system is characteristic of all oak species (Johnson et al. 2009), and

a temperature-dependent lag between radicle development and shoot emergence, or epicotyl dormancy, has been described for different temperate European and Asian oaks (McCartan et al. 2015; Sun et al. 2021; Xia et al. 2022). For instance, for the European temperate oak *Q. robur* under natural conditions, winter ambient temperatures are generally sufficient for seed germination, but not favourable for shoot emergence (McCartan et al. 2015). Our data also strongly suggest a population-specific variation of this key seed trait along a temperature gradient. The antagonism between populations for the ability to germinate rapidly at cold or optimal temperatures suggests physiological or molecular trade-offs for the acquisition of these traits. Specifically, we showed that mild temperatures of lowland regions are associated with higher seed moisture content and fastest germination and shoot emergence rates at optimal temperatures for germination. Being dispersed with high relative water content, these populations may have higher metabolic activity and vigour (Xia et al. 2022).

For recalcitrant seeds, air temperatures during development can influence the seed maturity state and consequently causes subtle variations in the relative level of seed desiccation sensitivity (Daws et al. 2005). In the case of *Q. ilex* in Tunisia, the variation in seed desiccation sensitivity observed in different populations correlated well with the initial seed water content at the time of dispersal and surprisingly not with xerothermic gradients. Such absence of relationships between desiccation sensitivity and climate proxy has also been observed for several Asian oaks (Xia et al. 2015, 2022), and deserves further study, including measurement of tissue water potential.

5 Conclusion

Our study provides a comprehensive picture of the seed functional diversity of *Q. ilex* in Tunisia. We highlighted the contribution of temperature regimes in shaping seed germination traits differentiation among sites. Although the genetic component of seed trait variation associated with the provenance sites remains to be determined, the evidence for large functional diversity associated with local climate supports the existence of population-specific germination niches and ecological strategies and underlines the importance of conserving the genetic resources that peripheral populations harbour at the edges of distribution (Fady et al. 2016). As previously highlighted for *Q. robur* in Europe (McCartan et al. 2015), the knowledge gained in this study will be of tremendous importance to predict the impact of reforestation and assisted migration programs on the synchronization of germination and shoot emergence of holm oak, and therefore in guiding the selection of populations to be chosen for different target areas.

Table 3 Cross-correlation matrix between seed traits. R values in bold indicate adjusted *P* values < 0.05. Ga, germination asynchrony parameter at different temperatures; PAR, pericarp/acorn mass ratio; Tb, base temperature for germination; t50, germination time at different temperatures; SE, shoot emergence; WC₅₀, seed water content for 50% mortality

	Seed mass	PAR	Seed WC	t50-5 °C	Ga-5 °C	t50-9 °C	Ga-9 °C	t50-13 °C	Ga-13 °C	t50-17 °C	G-17 °C	t50-21 °C	Ga-21 °C	t50-SE 13 °C	Ga-SE 13 °C	Tb	WC ₅₀
Seed mass	-	-0.45	-0.12	-0.08	0.15	-0.08	0.03	0.38	0.35	-0.35	0.02	-0.32	0.30	0.28	0.07	0.57	-0.39
PAR	-0.45	-	0.15	0.04	0.17	0.19	0.39	-0.03	0.16	0.42	0.47	0.29	0.01	-0.05	-0.07	-0.30	0.23
Seed WC	-0.12	0.15	-	0.46	0.27	-0.33	-0.09	-0.29	-0.40	-0.08	-0.24	-0.16	-0.13	-0.57	-0.29	0.12	0.68
t50-5 °C	-0.08	0.04	0.46	-	0.41	-0.02	0.16	0.06	0.10	-0.13	-0.09	-0.10	0.22	-0.17	-0.29	0.31	0.81
Ga-5 °C	0.15	0.17	0.27	0.41	-	0.04	0.53	0.32	0.34	-0.15	0.45	-0.32	0.44	-0.03	-0.33	0.62	0.34
t50-9 °C	-0.08	0.19	-0.33	-0.02	0.04	-	0.42	0.35	0.32	0.69	0.64	0.74	0.51	-0.23	-0.20	-0.05	-0.05
Ga-9 °C	0.03	0.39	-0.09	0.16	0.53	0.42	-	-0.06	0.15	0.29	0.79	0.17	0.33	-0.09	-0.36	-0.01	0.23
t50-13 °C	0.38	-0.03	-0.29	0.06	0.32	0.35	-0.06	-	0.76	-0.08	0.20	0.12	0.48	0.60	0.18	0.48	-0.32
Ga-13 °C	0.35	0.16	-0.40	0.10	0.34	0.32	0.15	0.76	-	-0.01	0.38	0.12	0.63	0.72	0.20	0.42	-0.46
t50-17 °C	-0.35	0.42	-0.08	-0.13	-0.15	0.69	0.29	-0.08	-0.01	-	0.59	0.77	0.22	0.07	-0.14	-0.52	0.10
Ga-17 °C	0.02	0.47	-0.24	-0.09	0.45	0.64	0.79	0.20	0.38	0.59	-	0.47	0.40	0.30	-0.17	-0.14	0.03
t50-21 °C	-0.32	0.29	-0.16	-0.10	-0.32	0.74	0.17	0.12	0.12	0.77	0.47	-	0.09	0.25	0.03	-0.69	0.05
Ga-21 °C	0.30	0.01	-0.13	0.22	0.44	0.51	0.33	0.48	0.63	0.22	0.40	0.09	-	0.10	-0.50	0.52	-0.07
t50-SE 13 °C	0.28	-0.05	-0.57	-0.17	-0.03	0.25	-0.09	0.60	0.72	0.07	0.30	0.25	0.10	-	0.66	0.05	-0.61
Ga-SE 13 °C	0.07	-0.07	-0.29	-0.29	-0.33	-0.23	-0.36	0.18	0.20	-0.14	-0.17	0.03	-0.50	0.66	-	-0.20	-0.46
Tb	0.57	-0.30	0.12	0.31	0.62	-0.20	-0.01	0.48	0.42	-0.52	-0.14	-0.69	0.52	0.05	-0.20	-	-0.12
WC ₅₀	-0.39	0.23	0.68	0.81	0.34	-0.05	0.23	-0.32	-0.46	0.10	0.03	0.05	-0.07	-0.61	-0.46	-0.12	-

Table 4 Correlation matrix between seed traits and climatic variables associated with population sites. *R* = Pearson's linear-correlation coefficients. *R* values in bold indicate adjusted *P* values < 0.05. Ga, germination asynchrony; Q, quartile; *t*₅₀, germination time, *R*, rainfall; SE, shoot emergence; T, temperature; Tb, base temperature for germination

	T mean	T range	T mean wettest-Q	T mean coldest-Q	R mean	R mean wettest-Q	R mean coldest-Q	R mean driest-Q
Acorn length	0.13	0.22	0.12	0.14	0.34	0.35	0.35	-0.09
Acorn width	0.02	0.29	0.03	0.02	0.05	0.04	0.04	0.04
Pericarp thickness	0.07	-0.25	0.11	0.10	-0.27	-0.26	-0.26	-0.06
Pericarp dry mass	-0.09	-0.06	-0.06	-0.05	0.27	0.27	0.27	-0.01
Pericarp water content	0.42	0.07	0.46	0.40	-0.32	-0.28	-0.29	-0.25
Seed dry mass	-0.07	0.23	-0.06	-0.05	0.32	0.30	0.30	0.07
Seed water content	0.57	-0.07	0.64	0.59	-0.16	-0.06	-0.06	-0.58
Pericarp/acorn mass ratio	0.02	-0.57	0.06	0.07	-0.10	-0.06	-0.06	-0.16
<i>t</i> ₅₀ -5 °C	0.60	-0.16	0.69	0.63	-0.25	-0.14	-0.14	-0.61
Ga-5 °C	0.58	0.09	0.53	0.52	-0.52	-0.48	-0.48	-0.33
<i>t</i> ₅₀ -9 °C	0.12	-0.48	0.12	0.18	-0.25	-0.20	-0.20	-0.21
Ga-9 °C	0.32	-0.31	0.29	0.32	-0.34	-0.27	-0.27	-0.38
<i>t</i> ₅₀ -13 °C	-0.03	-0.06	-0.03	0.01	0.09	0.07	0.07	0.12
Ga-13 °C	-0.19	-0.16	-0.18	-0.14	0.18	0.14	0.14	0.23
<i>t</i> ₅₀ -17 °C	-0.06	-0.66	-0.04	0.01	-0.20	-0.16	-0.15	-0.15
Ga-17 °C	0.11	-0.35	0.06	0.11	-0.39	-0.37	-0.37	-0.10
<i>t</i> ₅₀ -21 °C	-0.20	-0.64	-0.15	-0.12	-0.10	-0.07	-0.07	-0.02
Ga-21 °C	0.30	-0.30	0.29	0.36	0.10	0.14	0.14	-0.27
<i>t</i> ₅₀ -acorn-13 °C	0.23	-0.46	0.34	0.32	0.01	0.10	0.09	-0.45
Ga-acorn-13 °C	0.30	-0.13	0.36	0.34	-0.08	-0.02	-0.02	-0.36
<i>t</i> ₅₀ -SE-13 °C	-0.46	0.15	-0.47	-0.46	0.07	-0.02	-0.02	0.54
Ga-SE-13 °C	-0.68	0.34	-0.66	-0.71	0.14	0.03	0.03	0.70
Tb	0.42	0.35	0.39	0.41	0.08	0.10	0.10	-0.22
WC ₅₀	0.65	-0.20	0.72	0.66	-0.56	-0.46	-0.46	-0.62

Acknowledgements

We extend many thanks to Ridha KRIFI and Mohamed Ali ZARROUK for useful help in site prospecting and Haïfa CHROUDI for her help during the experiment.

Authors' contributions

Conceptualization: NA, TJ, HG, YA; methodology: NA, TJ; formal analysis and investigation: TJ, NA, R Z-C; writing—original draft preparation: HG, NA, TJ; writing—review and editing: TJ, HG, NA; funding acquisition: YA, NA; resources: YA, NA; supervision: TJ, YA. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the National Research Institute for Rural Engineering, Waters, and Forestry in Tunis, Tunisia.

Availability of data and materials

The datasets generated and analyzed during the current study are available in the DataSuds data repository (Amimi et al. 2022), <https://doi.org/10.23708/TYFN5U>

Declarations

Ethics approval and consent to participate

The authors declare that the study was not conducted on endangered vulnerable or threatened species.

Consent for publication

All authors gave their informed consent to this publication and its content.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Laboratory of Forest Ecology, National Institute of Research in Rural Engineering, Water and Forests (INRGREF), University of Carthage, Hédi Elkarray Street, El Menzah IV, BP 10, 2080 Ariana, Tunisia. ²Faculté Des Sciences de Bizerte, Université de Carthage, Département Des Sciences de La Vie, 7021 Jarzouna, Tunisia. ³LRVENC, National Institute of Research in Rural Engineering, Water and Forests (INRGREF), University of Carthage, Hédi Elkarray Street, El Menzah IV, BP 10, 2080 Ariana, Tunisia. ⁴DIADÉ, Univ Montpellier, IRD, CIRAD, Montpellier, France.

Received: 3 May 2022 Accepted: 30 January 2023

Published online: 23 February 2023

References

- Acherar M, Rambal S (1992) Comparative water relations of four Mediterranean oak species. In *Quercus ilex L. ecosystems: function, dynamics and management* (pp. 177–184). Springer, Dordrecht.
- Adams HD, Barron-Gafford GA, Minor RL, Gardea AA, Bentley LP, Law D, Breshears DD, McDowell NG, Huxman TE (2017) Temperature response surfaces for mortality risk of tree species with future drought. *Environ Res Lett* 12(11):115014. <https://doi.org/10.1088/1748-9326/aa93be>
- Alonso-Crespo IM, Silla F, Jiménez del Nogal P, Fernández MJ, Martínez-Ruiz C, Fernández-Santos B (2020) Effect of the mother tree age and acorn weight in the regenerative characteristics of *Quercus faginea*. *Eur J Forest Res* 139:513–523. <https://doi.org/10.1007/s10342-020-01266-8>
- Amimi N, Dussert S, Vaissayre V, Ghouil H, Doubeau S, Costantini C, Ammari Y, Joët T (2020) Variation in seed traits among Mediterranean oaks in Tunisia and their ecological significance. *Ann Bot* 125(6):891–904. <https://doi.org/10.1093/aob/mcz211>
- Amimi N, Ghouil H, Zitouna-Chebbi R, Joët T, Ammari Y (2022) Replication Data for Distribution of *Quercus ilex* subsp. *rotundifolia* in Tunisia and intraspecific variation of seed morphophysiological traits along climatic gradients. DataSuds repository, V1. <https://doi.org/10.23708/TYFN5U>
- Barbero M, Loisel R, Quezel P (1992) Biogeography, ecology and history of *Quercus ilex* ecosystems in Mediterranean region. *Vegetatio* 99–100:14–19. <https://doi.org/10.1007/BF00118207>
- Bonner FT and Vozzo JA (1987) *Seed Biology and Technology of Quercus*. Gen. Tech. Rep. SO-66. New Orleans, LA: U.S. Dept of Agriculture, Forest Service, Southern Forest Experiment Station. 21p. <https://doi.org/10.2737/SO-GTR-66>
- Bussotti F, Ferrini F, Pollastrini M, Fini A (2014) The challenge of Mediterranean sclerophyllous vegetation under climate change: From acclimation to adaptation. *Environ Exp Bot* 103:80–98. <https://doi.org/10.1016/j.envexpbot.2013.09.013>
- Çalışkan S (2014) Germination and seedling growth of holm oak (*Quercus ilex* L.): effects of provenance, temperature, and radicle pruning. *iForest-Biogeosci Forestry* 7(2):103. <https://doi.org/10.3832/IFOR0967-007>
- Carta A, Fernández-Pascual E, Gioria M, Müller JV, Rivière S, Rosbakh S, Saatkamp A, Vandeloek F, Mattana E (2022) Climate shapes the seed germination niche of temperate flowering plants: a meta-analysis of European seed conservation data. *Ann Bot* 129(7):775–786. <https://doi.org/10.1093/aob/mcac037>
- Castagneri D, Regev L, Boaretto E, Carrer M (2017) Xylem anatomical traits reveal different strategies of two Mediterranean oaks to cope with drought and warming. *Environ Exp Bot* 133:128–138. <https://doi.org/10.1016/j.envexpbot.2016.10.009>
- Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. *New Phytol* 168(3):597–612. <https://doi.org/10.1111/j.1469-8137.2005.01555.x>
- Celebias P, Bogdziewicz M (2022) Consequences of intraspecific variation in seed size: does the presence of small seeds reduce predation on large ones? *Eur J Forest Res*. <https://doi.org/10.1007/s10342-022-01508-x>
- Chamorro D, Luna B, Moreno JM (2017) Germination responses to current and future temperatures of four seeder shrubs across a latitudinal gradient in western Iberia. *Am J Bot* 104:83–91. <https://doi.org/10.3732/ajb.1600278>
- Chamorro D, Luna B, Moreno JM (2018) Local climate controls among-population variation in germination patterns in two *Erica* species across western Iberia. *Seed Sci Res* 28:112–122. <https://doi.org/10.1017/S0960258518000041>
- Cochrane A (2016) Can sensitivity to temperature during germination help predict global warming vulnerability? *Seed Sci Res* 26:14–29. <https://doi.org/10.1017/S0960258515000355>
- Cochrane A, Yates CJ, Hoyle GL, Nicotra AB (2015a) Will among-population variation in seed traits improve the chance of species persistence under climate change? *Glob Ecol Biogeogr* 24:12–24. <https://doi.org/10.1111/geb.12234>
- Cochrane A, Hoyle GL, Yates CJ, Wood J, Nicotra AB (2015b) Climate warming delays and decreases seedling emergence in a Mediterranean ecosystem. *Oikos* 124(2):150–160. <https://doi.org/10.1111/oik.01359>
- Daws MI, Garwood NC, Pritchard HW (2005) Traits of recalcitrant seeds in a semi-deciduous tropical forest in Panamá: some ecological implications. *Funct Ecol* 19(5):874–885. <https://doi.org/10.1111/j.1365-2435.2005.01034.x>
- Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG (2010) Germination, postgermination adaptation, and species ecological ranges. *Annu Rev Ecol Evol Syst* 41:293–319. <https://doi.org/10.1146/annurev-ecolsys-102209-144715>
- Dussert S, Chabrilange N, Engelmann F, Hamon S (1999) Quantitative estimation of seed desiccation sensitivity using a quantal response model: application to nine species of the genus *Coffea* L. *Seed Sci Res* 9:135–144. <https://doi.org/10.1017/S096025859900015X>
- Fady B, Cottrell J, Ackzell L, Alía R, Muys B, Prada A, González-Martínez SC (2016) Forests and global change: what can genetics contribute to the major forest management and policy challenges of the twenty-first century? *Reg Environ Change* 16:927–939. <https://doi.org/10.1007/s10113-015-0843-9>
- Ferrer-Galego PP, Sáez L (2019) Type designation of the oaks *Quercus ballota* and *Q. rotundifolia* (Fagaceae). *Nordic J Botany* 37(6):e02387. <https://doi.org/10.1111/njb.02387>
- Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int J Climatol* 37:4302–4315. <https://doi.org/10.1002/joc.5086>
- Ganatsas P, Tsakalidimi M (2013) A comparative study of desiccation responses of seeds of three drought-resistant Mediterranean oaks. *For Ecol Manage* 305:189–194. <https://doi.org/10.1016/j.foreco.2013.05.042>
- García D, Zamora R (2003) Persistence, multiple demographic strategies and conservation in long-lived Mediterranean plants. *J Veg Sci* 14(6):921–926. <https://doi.org/10.1111/j.1654-1103.2003.tb02227.x>
- García-Nogales A, Linares JC, Laureano RG, Seco JI, Merino J (2016) Range-wide variation in life-history phenotypes: spatiotemporal plasticity across the latitudinal gradient of the evergreen oak *Quercus ilex*. *J Biogeogr* 43:2366–2379. <https://doi.org/10.1111/jbi.12849>
- Gimeno TE, Pias B, Lemos-Filho JP, Valladares F (2009) Plasticity and stress tolerance override local adaptation in the responses of Mediterranean holm oak seedlings to drought and cold. *Tree Physiol* 29(1):87–98. <https://doi.org/10.1093/treephys/tpn007>
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Global Planet Change* 63(2–3):90–104. <https://doi.org/10.1016/j.gloplacha.2007.09.005>
- Gómez JM (2004) Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* 58(1):71–80. <https://doi.org/10.1111/j.0014-3820.2004.tb01574.x>
- Gómez-Aparicio L, Zamora R, Castro J, Hódar JA (2008) Facilitation of tree saplings by nurse plants: Microhabitat amelioration or protection against herbivores? *J Veg Sci* 19(2):161–172. <https://doi.org/10.3170/2008-8-18347>
- González-Rodríguez V, Villar R, Navarro-Cerrillo RM (2011) Maternal influences on seed mass effect and initial seedling growth in four *Quercus* species. *Acta Oecologica* 37(1):1–9. <https://doi.org/10.1016/j.actao.2010.10.006>
- Gratani L, Meneghini M, Pesoli P, Crescente MF (2003) Structural and functional plasticity of *Quercus ilex* seedlings of different provenances in Italy. *Trees* 17(6):515–521. <https://doi.org/10.1007/s00468-003-0269-8>
- El Hamrouni A, El Hamrouni-Aschi K, El Khorchani A (2020) Les groupements végétaux du pin d'Alep et leur dynamique en Tunisie. *Le Pin d'Alep en Tunisie: Ecologie, Gestion et Usages*, 85
- Jiménez-Alfaro B, Silveira FA, Fidelis A, Poschlod P, Commander LE (2016) Seed germination traits can contribute better to plant community ecology. *J Veg Sci* 27(3):637–645. <https://doi.org/10.1111/jvs.12375>
- Joët T, Ourcival JM, Dussert S (2013) Ecological significance of seed desiccation sensitivity in *Quercus ilex*. *Ann Bot* 111:693–701. <https://doi.org/10.1093/aob/mct025>
- Joët T, Ourcival JM, Capelli M, Dussert S, Morin X (2016) Explanatory ecological factors for the persistence of desiccation-sensitive seeds in transient soil seed banks: *Quercus ilex* as a case study. *Ann Bot* 117:165–176. <https://doi.org/10.1093/aob/mcv139>
- Johnson DM, Woodruff DR, McCulloh KA, Meinzer FC (2009) Leaf hydraulic conductance, measured in situ, declines and recovers daily: leaf

- hydraulics, water potential and stomatal conductance in four temperate and three tropical tree species. *Tree Physiol* 29(7):879–887. <https://doi.org/10.1093/treephys/tp031>
- Larcher W (2000) Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosystems* 134:279–295. <https://doi.org/10.1080/11263500012331350455>
- Le Floc'h E, Boulos L, Vela E (2010) Catalogue synonymique commenté de la Flore de Tunisie, 2nd edition. Banque Nationale de Gènes de la Tunisie, Tunis, 500 pp.
- Le Roncé I, Gavinet J, Ourcival JM, Mouillot F, Chuine I, Limousin JM (2021) Holm oak fecundity does not acclimate to a drier world. *New Phytol* 231(2):631–645. <https://doi.org/10.1111/nph.17412>
- Limousin JM, Rambal S, Ourcival JM, Rodríguez-Calcerrada J, Pérez-Ramos IM, Rodríguez-Cortina R, Joffre R (2012) Morphological and phenological shoot plasticity in a Mediterranean evergreen oak facing long-term increased drought. *Oecologia* 169(2):565–577. <https://doi.org/10.1007/s00442-011-2221-8>
- Lloret F, Penuelas J, Estiarte M (2004) Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Glob Change Biol* 10(2):248–258. <https://doi.org/10.1111/j.1365-2486.2004.00725.x>
- Lloret F, de la Riva EG, Pérez-Ramos IM, Marañón T, Saura-Mas S, Díaz-Delgado R, Villar R (2016) Climatic events inducing die-off in Mediterranean shrublands: are species' responses related to their functional traits? *Oecologia* 180(4):961–973. <https://doi.org/10.1007/s00442-016-3550-4>
- Lopez De Heredia U, Jimenez P, Collada C, Simeone MC, Bellarosa R, Schirone B, María T, Gil C (2007) Multi-marker phylogeny of three evergreen oaks reveals vicariant patterns in the western Mediterranean. *Taxon* 56:1209–1220. <https://doi.org/10.2307/25065912>
- López-Hidalgo C, Menéndez M, Jorrián-Novo JV (2021) Phytochemical composition and variability in Quercus ilex acorn morphotypes as determined by NIRS and MS-based approaches. *Food Chemistry* 338:127803. <https://doi.org/10.1016/j.foodchem.2020.127803>
- Lumaret R, Mir C, Michaud H, Raynal V (2002) Phylogeographical variation of chloroplast DNA in holm oak (*Quercus ilex* L.). *Molecular Ecology* 11(11):2327–2336. <https://doi.org/10.1046/j.1365-294x.2002.01611.x>
- Matesanz S, Valladares F (2014) Ecological and evolutionary responses of Mediterranean plants to global change. *Environ Exp Bot* 103:53–67. <https://doi.org/10.1016/j.envexpbot.2013.09.004>
- Matías L, Abdelaziz M, Godoy O, Gómez-Aparicio L (2018) Disentangling the climatic and biotic factors driving changes in the dynamics of Quercus suber populations across the species' latitudinal range. *Divers Distrib* 00:1–12. <https://doi.org/10.1111/ddi.12873>
- McCartan SA, Jinks RL, Barsoum N (2015) Using thermal time models to predict the impact of assisted migration on the synchronization of germination and shoot emergence of oak (*Quercus robur* L.). *Ann Forest Sci* 72(4):479–487. <https://doi.org/10.1007/s13595-014-0454-5>
- Michaud H, Toumi L, Lumaret R, Li TX, Romane F, Di Giusto F (1995) Effect of geographical discontinuity on genetic variation in Quercus ilex L. (holm-oak). Evidence from Enzyme Polymorphism *Heredity* 74:590–606. <https://doi.org/10.1038/hdy.1995.83>
- Moles AT, Westoby M (2004) Seedling survival and seed size: a synthesis of the literature. *J Ecol* 92(3):372–383. <https://doi.org/10.1111/j.0022-0477.2004.00884.x>
- Nabli MA (1989) Essai de synthèse sur la végétation et la phyto-écologie tunisiennes. I-Élément de botanique et de phyto-écologie [Synthesis essay on the Tunisian vegetation and phytocology. I-Botany and phytocology element] (vol. 4–6). Faculté des Sciences, Tunis, Tunisia, 247.
- Nabli MA (1995) Essai de synthèse sur la végétation et la phyto-écologie tunisiennes. II/III-Le milieu physique et la végétation. *Ecologie végétale appliquée*. (vol. 5–6). Faculté des Sciences, Tunis, Tunisia, 542.
- Nardini A, Salleo S, Gullo ML, Pitt F (2000) Different responses to drought and freeze stress of Quercus ilex L. growing along a latitudinal gradient. *Plant Ecology* 148(2):139–147. <https://doi.org/10.1023/a:1009840203569>
- Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, Van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. *Trends Plant Sci* 15(12):684–692. <https://doi.org/10.1016/j.tplants.2010.09.008>
- Niinemetts Ü, Keenan T (2014) Photosynthetic responses to stress in Mediterranean evergreens: mechanisms and models. *Environ Exp Bot* 103:24–41. <https://doi.org/10.1016/j.envexpbot.2013.11.008>
- Peguero-Pina JJ, Sancho-Knapik D, Barrón E, Camarero JJ, Vilagrosa A, Gil-Pelegrín E (2014) Morphological and physiological divergences within Quercus ilex support the existence of different ecotypes depending on climatic dryness. *Ann Bot* 114(2):301–313. <https://doi.org/10.1093/aob/mcu108>
- Pérez-Ramos IM, Rodríguez-Calcerrada J, Ourcival JM, Rambal S (2013) Quercus ilex recruitment in a drier world: a multi-stage demographic approach. *Perspectives in Plant Ecology, Evolution and Systematics* 15(2):106–117. <https://doi.org/10.1016/j.ppees.2012.12.005>
- Pritchard HW, Manger KR (1990) Quantal response of fruit and seed germination rate in Quercus robur L. and Castanea sativa Mill. to constant temperatures and photon dose. *J Exp Bot* 41:1549–1557. <https://doi.org/10.1093/jxb/41.12.1549>
- Quero JL, Villar R, Marañón T, Zamora R, Poorter L (2007) Seed-mass effects in four Mediterranean Quercus species (Fagaceae) growing in contrasting light environments. *Am J Bot* 94(11):1795–1803. <https://doi.org/10.3732/ajb.94.11.1795>
- Quero JL, Sterck FJ, Martínez-Vilalta J, Villar R (2011) Water-use strategies of six co-existing Mediterranean woody species during a summer drought. *Oecologia* 166(1):45–57. <https://doi.org/10.1007/s00442-011-1922-3>
- Ramírez-Valiente JA, Valladares F, Gil L, Aranda I (2009) Population differences in juvenile survival under increasing drought are mediated by seed size in cork oak (*Quercus suber* L.). *Forest Ecology Manage* 257(8):1676–1683. <https://doi.org/10.1016/j.foreco.2009.01.024>
- Ramírez-Valiente JA, Santos del Blanco L, Alia R, Robledo-Arnuncio J, Climent J (2022) Adaptation of Mediterranean forest species to climate: Lessons from common garden experiments. *J Ecol* 110(5):1022–1042. <https://doi.org/10.1111/1365-2745.13730>
- Rosbakh S, Poschlod P (2015) Initial temperature of seed germination as related to species occurrence along a temperature gradient. *Funct Ecol* 29:5–14. <https://doi.org/10.1111/1365-2435.12304>
- Ruffault J, Martin-StPaul NK, Duffet C, Goge F, Mouillot F (2014) Projecting future drought in Mediterranean forests: bias correction of climate models matters! *Theoret Appl Climatol* 117(1):113–122. <https://doi.org/10.1007/s00704-013-0992-z>
- Ruiz-Labourdette D, Nogués-Bravo D, Ollero HS, Schmitz MF, Pineda FD (2012) Forest composition in Mediterranean mountains is projected to shift along the entire elevational gradient under climate change. *J Biogeogr* 39(1):162–176. <https://doi.org/10.1111/j.1365-2699.2011.02592.x>
- Sánchez-Vilas J, Retuerto R (2007) Quercus ilex shows significant among-population variability in functional and growth traits but maintains invariant scaling relations in biomass allocation. *Int J Plant Sci* 168(7):973–983. <https://doi.org/10.1086/518943>
- Solé-Medina A, Robledo-Arnuncio JJ, Ramírez-Valiente JA (2022) Multi-trait genetic variation in resource-use strategies and phenotypic plasticity correlates with local climate across the range of a Mediterranean oak (*Quercus faginea*). *New Phytol* 234(2):462–478. <https://doi.org/10.1111/nph.17968>
- Sun XQ, Song YG, Ge BJ, Dai XL, Kozłowski G (2021) Intermediate Epicotyl Physiological Dormancy in the Recalcitrant Seed of Quercus chungii FP Metcalf with the Elongated Cotyledonary Petiole. *Forests* 12(3):263. <https://doi.org/10.3390/f12030263>
- Terradas J, Savé R (1992) The influence of summer and winter stress and water relationships on the distribution of Quercus ilex L. In Quercus ilex L. ecosystems: function, dynamics and management (pp. 137–145). Springer, Dordrecht.
- Tramblay Y, A, Samaniego L, Vicente-Serrano SM, Volaire F, Boone A, Le Page M, Llasat MC, Albergel C, Burak S, Cailleret M, Kalin KC, Davi H, Dupuy JL, Greve P, Grillakis M, Hanich L, Jarlan L, Martin-StPaul N, Martínez-Vilalta J, Mouillot F, Pulido Velazquez D, Quintana-Seguí P, Renard D, Turco M, Türkeş M, Trigo R, Vidal JP, Vilagrosa A, Zribi M, Polcher P (2020) Challenges for drought assessment in the Mediterranean region under future climate scenarios. *Earth-Sci Rev*. 210:103348. <https://doi.org/10.1016/j.earscirev.2020.103348>
- Urbieta IR, Perez-Ramos IM, Zavala MA, Maranon T, Kobe RK (2008) Soil water content and emergence time control seedling establishment in three co-occurring Mediterranean oak species. *Can J Res* 38(9):2382–2393. <https://doi.org/10.1139/x08-08-09>
- Valero Galván J, Jorrián Novo JJ, Cabrera AG, Ariza D, García-Olmo J, Cerrillo RM (2012) Population variability based on the morphometry and chemical composition of the acorn in holm oak (*Quercus ilex* subsp. ballota

[Desf.] Samp.). *Eur J Forest Res* 131(4):893–904. <https://doi.org/10.1007/s10342-011-0563-8>

Xia K, Daws MI, Zhou ZK, Pritchard HW (2015) Habitat-linked temperature requirements for fruit germination in *Quercus* species: a comparative study of *Quercus* subgenus *Cyclobalanopsis*. Asian evergreen oaks, and *Quercus* subgenus *Quercus*. *S Afr J Bot* 100:108–113. <https://doi.org/10.1016/j.sajb.2015.04.015>

Xia K, Daws MI, Peng LL (2022) Climate drives patterns of seed traits in *Quercus* species across China. *New Phytol* 234(5):1629–1638. <https://doi.org/10.1111/nph.18103>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

