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Seasonal evolution of water status after outplanting of two provenances of Holm oak nursery seedlings

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Abstract Forest restoration programs using Holm oak (*Quercus ilex* ssp. *ballota* [Desf.] Samp.) have had limited success. The effect of plant provenance on plantation success is uncertain, although some previous studies suggest that some provenances may be better able to tolerate stress. We studied the tolerance to drought in seedlings from two Spanish provenances of Holm oak before and after outplanting. One provenance was from a continental climate with cold winters (*GR*) and the other was from a xeric climate (*HU*). Seedlings were subjected to a water stress test in the nursery during the summer and survival was visually assessed after 2 weeks. In addition, 35 healthy seedlings of each provenance that were not subjected to the water stress tests were used for outplanting experiment. In these plants the seasonal changes in water potential at dawn (Ψ), specific leaf area (SLA), cuticular transpiration (E_c), and loss of xylem hydraulic conductance of twigs (PLC) were measured over 18 months. After the water stress test in summer, mortality was 44.3 % for *GR* seedlings and 12.6 % for *HU* seedlings. In addition there were differences between the two provenances in plant water status after planting. The *HU* provenance had a better water status and was more water conservative in the summer (higher Ψ, lower E_c, lower PLC), but not in the winter. The different drought tolerance and water relations parameters of these two provenances indicate that provenance should be considered in forest restoration and conservation programs involving Holm oak.

Keywords Water stress · Drought · Field performance · Physiological traits · Adaptation

Introduction

Mediterranean forest species undergo water stress during the summer, during which there are high temperatures, little or no rainfall, and high levels of solar radiation. Previous studies have examined the physiological responses of mature Holm oak (*Quercus ilex* L.)

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to drought and the adaptations and acclimation to water stress (Baquedano and Castillo 2006; Ogaya and Peñuelas 2006; Ogaya et al. 2003; Paço et al. 2009; Salleo and Lo Gullo 1993). Young Holm oak seedlings have poorer field performance than other Mediterranean species in reforestation programs (Andivia et al. 2011; del Campo et al. 2010; Oliet et al. 2011; Villar-Salvador et al. 2004a, b). Water stress due to summer drought is the main factor that limits establishment of this species. Additionally, Holm oak is often exposed to long periods of low temperature and frost in continental areas (Aranda et al. 2005; García-Plazaola et al. 1999; Gimeno et al. 2009), which further hinders establishment.

Holm oak can colonize diverse environments, suggesting that it has high phenotypic plasticity (Gratani 1995; Valladares et al. 2000). Studies of the tolerance of different provenances of Holm oak to drought and frost (Andivia et al. 2012; Aranda et al. 2005; Gimeno et al. 2009; Gratani et al. 2003; Morin et al. 2007; Sánchez-Vilas and Retuerto 2007) have reported that the physiological responses to these stressors may be under strong genetic control. The exposure to drought and frost lead to cell dehydration of plants exposure to these abiotic stresses (Welbaum et al. 1997). The underlying mechanisms for drought and frost tolerance both involve reduction of cell dehydration and preservation of membrane integrity (Gimeno et al. 2009; Larcher 2000); hence, the study of water status parameters could provide an accurate approach to plant tolerance to drought, and even to frosts.

Analysis of the variability of ecologically important traits, such as tolerance to drought and frost, among populations may provide valuable information on the selection criteria for breeding and reforestation programs. Climate change models for the Mediterranean area predict an increase in temperature, a decrease in rainfall, and an increase in the frequency of extreme events such as late season frosts and extreme droughts (Christensen et al. 2007). Thus, knowledge of provenance variability is needed to predict how species and ecosystems will respond to environmental change.

The objective of this study was to compare the water status in seedlings from two Spanish provenances of *Quercus ilex* subsp. *ballota* (Desf.) Samp. after outplanting, in order to determine if physiological responses to drought or frost are under genetic control. Specific objectives were to: (1) examine the differences in drought tolerance between Holm oak seedlings from two Spanish provenances subjected to an experimental drought in the nursery, (2) study the seasonal evolution of water relations parameters of Holm oak seedlings in two Spanish provenances after outplanting.

Materials and methods

Plant material and growing conditions

In autumn of 2006, acorns of *Quercus ilex* ssp. *ballota* were collected from two Spanish provenances: *Sierra de Segura* (*GR*) (UTM, zone 30: X, 542930; Y, 4204237; altitude 1353 m) and *Sierra Morena Occidental* (*HU*) (UTM, zone 29: X, 644288; Y, 4161376; altitude 160 m). At each location, acorns were collected from 25 trees that were more than 80 m apart. The *GR* provenance had a continental climate with cold winters (3 months of frost) and 1–2 months of summer drought; the *HU* provenance had a more xeric climate without winter frosts and with 4 months of summer drought. Climatic data (Table 1) were obtained from weather stations of the Murcia Regional Government [“Casas del Rey” (UTM, zone 30: X, 579314; Y, 4219140; altitude 1,110 m)] for *GR* and stations of the

Table 1 Climatic conditions of the "Sierra de Segura" (*GR*) and "Sierra Morena" (*HU*) provenances

Provenance	*GR*	*HU*
Mean annual precipitation (mm)	490	564
Mean summer (Jun–Sep) precipitation (mm)	124	46
Mean temperature (°C)	12.7	16.2
Absolute maximum temperature (°C)	36.0	42.7
Mean of maximum temperatures of the hottest month (°C)	28.5	33.5
Absolute minimum temperature (°C)	−10.9	−3.6
Mean of minimum temperatures of the coldest month (°C)	−2.3	4.8

Andalusia Regional Government ["La Puebla de Guzman" (UTM, zone 29: X, 654836; Y, 4157771; altitude 288 m)] for *HU*.

In February 2007, acorns were pre-germinated in a growth chamber at 20 °C, on wet perlite. Acorn mass were 3.89 ± 0.60 g [*HU*] and 4.28 ± 0.97 g [*GR*], without significant differences between provenances. During the first week of March 2007, 440 healthy acorns (220 per provenance) were randomly sown in 300 cm^3 Plasnor® containers that contained sphagnum peat Kekkilä® B0 (pH adjusted to 6.5 with 2 $kg\ m^{-3}$ Dolokal®). Pre-germination was performed to reduce germination time and to ensure that each cell in the trays contained a plant. The 440 acorns were sown in 11 trays, each one with 20 acorns from each provenance. Seedlings were grown in a nursery (UTM, zone 29: X, 684708; Y, 4119184; altitude 23 m) under a shade-cloth that reduced radiation by ~50 %. All trays were well watered with tap water, moved weekly, and rotated to eliminate microsite effects.

A constant fertilization regimen was applied, in which the doses of N, P, and K were similar to those typically used for commercial cultivation of Holm oak (Navarro-Cerrillo et al. 2009). Fertilization started on March 19, 2007 and finished on December 10, 2007 with each seedling received a single weekly dose of 2.5 mg N (0.2 mg of ammoniacal nitrogen and 2.3 mg of nitrate nitrogen), 1.088 mg P, and 2.075 mg K from a water-soluble fertilizer (Peters professional® 20-20-20) at a rate of 125 ppm N, 54 ppm P, and 104 ppm K. The total fertilizer applied to each seedling over 39 weeks was: 97.5 mg N, 55.4 mg P and 70.2 mg K. Seedlings were then subjected to one of the following tests: water stress or outplanting.

Nursery water stress test

In mid-August, 280 seedlings (140 per provenance) were randomly selected and moved to 7 trays (20 seedlings per provenance in each tray). Seedling height on this date was 10.92 ± 3.31 cm, with no significant difference between the provenances. These seedlings had been well watered, with a mean leaf water potential at dawn (Ψ) higher than −0.4 MPa. Then seedlings were deprived of water and fertilizer until mean Ψ went down below −3.3 MPa. This happened the 10th day. Afterwards, mortality was visually assessed after 2 weeks.

Outplanting test

Plants not subjected to drought stress (20 seedlings per provenance in each of 4 trays, 160 in total) were watered and fertilized as described previously (subsection "Plant material and growing conditions"). Afterwards 70 of these seedlings (35 per provenance) were randomly selected for outplanting. Planting was conducted in a 12 × 16 m flat and homogenous experimental plot at the University of Huelva (UTM, zone 29: X, 684959; Y, 4119139; altitude 9 m) in February 2008. Seedlings were planted in 7 lines (5 plants per provenance and per line, randomly distributed), with a separation of 1 m among consecutive plants and 2 m among lines. Seedling height at the time of planting was 14.6 ± 2.9 cm for HU and 15.7 ± 2.7 cm for GR. For the period Jan-2008 to Dec-2009, the mean annual temperature was 16.5 °C with maximum and minimum temperatures of 36.5 and −3.0 °C, respectively. Mean annual rainfall was 484 mm with 4 months of summer drought period.

Water status was measured every 6–7 weeks from April 2008 to September 2009 on 16 planted seedlings per provenance. At each measurement date, the 16 plants per provenance were randomly selected in order to avoid an excessive damage to seedlings due to the sampling. One mature leaf per seedling was for measuring leaf water potential (Ψ), using a pressure chamber (Model 1000; PMS Instruments, Corvallis, OR) as previously described (Scholander et al. 1965). Cuticular transpiration (E_c) and relative water content at the point of stomatal closure (RWC_c) were assessed using the method of Quisenberry et al. (1982). Hydraulic conductivity (K) and percent loss of hydraulic conductivity (PLC) were determined as described by Sperry et al. (1988), beginning in June (not April) 2008 because newly developed twigs with at least 4–5 leaves were needed for these measurements. Specific hydraulic conductivity (K_s) as the ratio of K and the cross-section of the debarked stem segment, and leaf specific hydraulic conductivity (K_l), expressed in terms of leaf area above the cut segment, were also calculated. The dry weight of each leaf and the specific leaf area (SLA, $m^2\ kg^{-1}$) were also measured. Survival of outplanted seedlings was assessed after the dry summer period in 2008 and 2009 (Sep-2008 and Sep-2009) and growth was assessed by measuring height (H) and diameter (D) in four dates: Jun-2008, Dec-2008, Jun-2009 and Dec-2009.

Data analysis

Provenance effect on seedling survival was analyzed using a Chi-square test. Water relation parameters and growth of outplanted seedlings were analyzed using the following general linear model (GLM):

$$y_{ijl} = \mu + DATE_i + PRO_j + (DATE * PRO)_{ij} + e_{ijl} \quad (1)$$

where y_{ijl} is the value of the dependent variables in plant l of provenance j, at measurement date i; μ is the overall mean; *DATE* is the measurement date fixed effect, *PRO* is a provenance fixed effect, *DATE*PRO* is the interaction between measurement date and provenance fixed effects, and e is the error term for the hypothesis $e_{ijl} \sim N(0, \sigma_e^2)$. The relations between water relation parameters were assessed by calculation of Pearson's correlation coefficient. For all analysis significant differences were considered with $p \leq 0.05$; although in Fig. 1, differences with $p \leq 0.10$ have also been shown.

Results

Nursery water stress test

Mortality after the water stress test was 44.3 % in the *GR* provenance and 12.6 % in the *HU* provenance. The Chi-square test showed significant differences between provenances

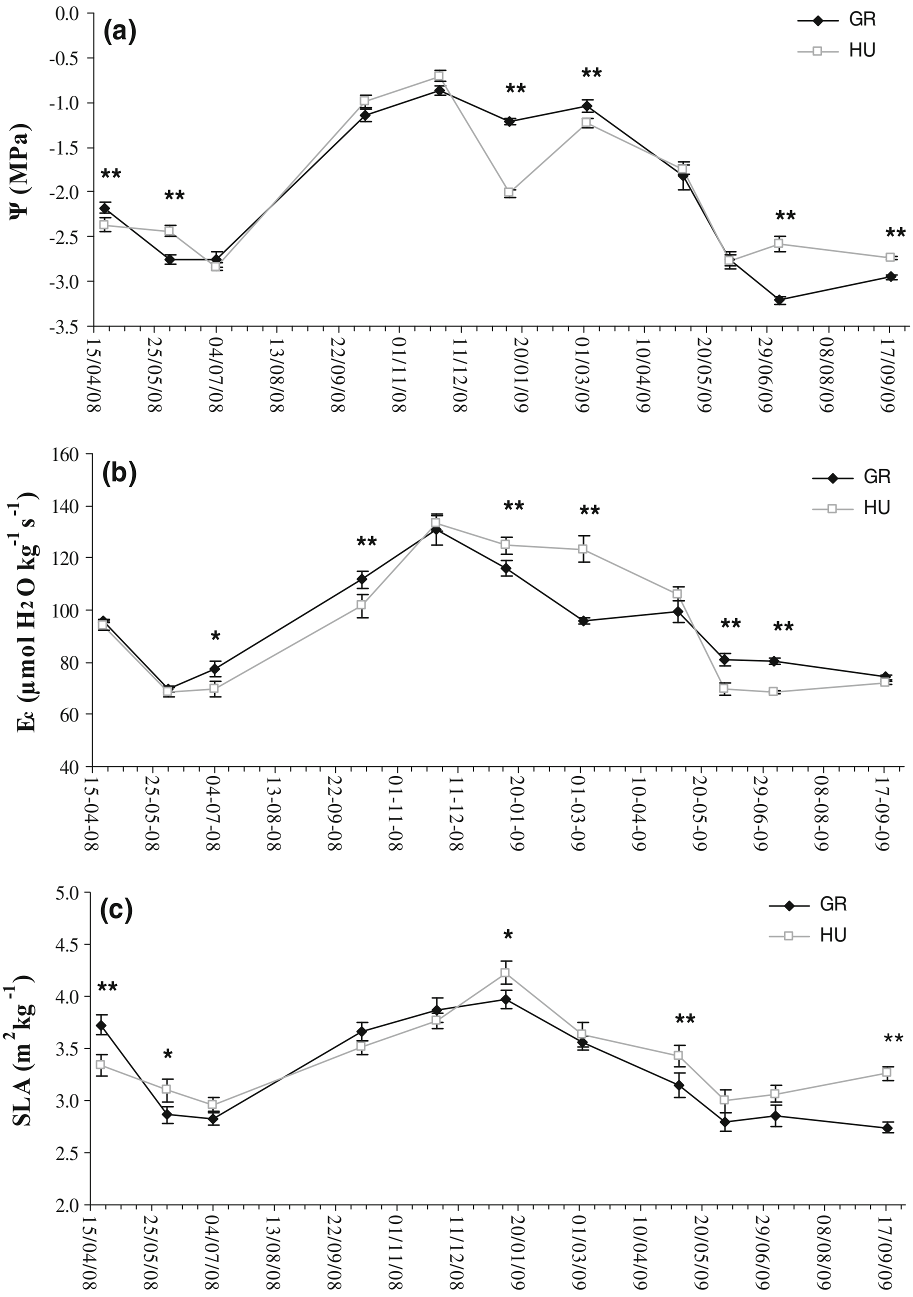

Fig. 1 Seasonal variation (mean ± SE) of: **a** predawn leaf water potential (Ψ), **b** cuticular transpiration (E_c), **c** specific leaf area (SLA), **d** relative water content at the point of stomatal closure (RWC_c), **e** specific hydraulic conductivity (K_s), and **f** loss of hydraulic conductivity (PLC) of outplanted *HU* and *GR* Holm oak seedlings. *Double asterisks* indicate significant differences ($p < 0.05$) and *single asterisk* differences ($p < 0.10$) between provenances at individual dates

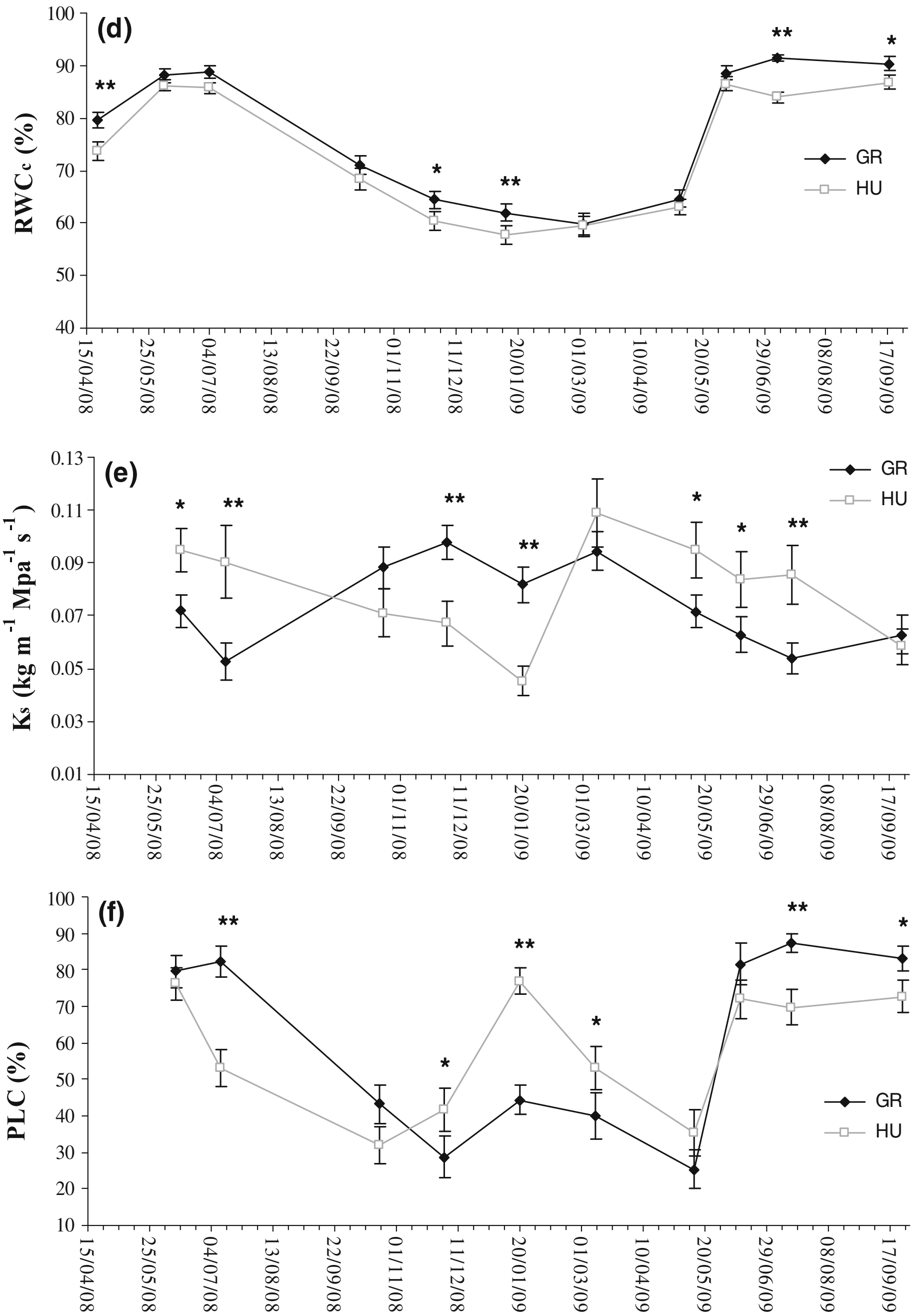

Fig. 1 continued

in survival ($\chi^2 = 33.880$; $p < 0.001$). Leaf water potential at dawn (Ψ) of the surviving seedlings after the test was between −3.5 and −4.0 MPa, with no significant difference between provenances.

Outplanting performance

There was a significant interaction between date and provenance for all parameters except RWC$_c$ and K_l (Table 2). The pattern of Ψ, E$_c$ and SLA over time was similar for both provenances (Fig. 1), with lower values in summer and higher values in winter. In summer, *HU* plants had higher Ψ values than *GR* plants, but during winter, when the minimum temperature reached −3 °C for at least three days, *GR* plants had higher Ψ values. *HU* plants had lower E$_c$ values than *GR* plants during the summer and higher E$_c$ values during the winter. From the second winter after planting, *GR* plants had lower SLA values. RWC$_c$ had the same seasonal pattern as Ψ for both provenances, but *HU* plants had lower values at most measurement times (Fig. 1d).

In winter 2009, *HU* plants had the lowest K_s and the highest PLC; *GR* plants had minimal K_s during the period of greatest water stress, also coinciding with the highest PLC values (Fig. 1d). The overall K_l mean value for *GR* plants was $7.97 \times 10^{-5} \pm 0.45 \times 10^{-5}$ and the overall mean value for *HU* plants was $8.33 \times 10^{-5} \pm 0.69 \times 10^{-5}$ kg m^{-1} MPa^{-1} s^{-1}.

Analysis of correlations between parameters indicated negative relationships for Ψ and PLC ($n = 320$; $r = -0.555$; $p < 0.001$), Ψ and RWC$_c$ ($n = 320$; $r = -0.773$; $p < 0.001$), SLA and RWC$_c$ ($n = 320$; $r = -0.625$; $p < 0.001$), and RWC$_c$ and E$_c$ ($n = 320$; $r = -0.728$; $p < 0.001$), and positive relationships for Ψ and SLA ($n = 320$; $r = 0.615$; $p < 0.001$), Ψ and E$_c$ ($n = 320$; $r = 0.699$; $p < 0.001$), SLA and E$_c$ ($n = 320$; $r = 0.595$; $p < 0.001$) and RWC$_c$ and PLC ($n = 320$; $r = 0.535$; $p < 0.001$).

Survival of outplanted seedlings on Sep-2008 was higher than 90 % without significant differences between provenances. From Sep-2008 to Sep-2009 no mortality occurred. We did not found significant differences in growth between provenances, mean values of height and diameter varied from 24.82 ± 4.64 cm [*H*] and 5.38 ± 1.10 mm [*D*] on Jun-2008, to 67.65 ± 27.15 cm [*H*] and 14.43 ± 3.52 mm [*D*] on Dec-2009.

Discussion

Although results of previous studies have been contradictory, some suggest that physiological responses of *Quercus* species to drought and frost are due to genetic adaptations of

Table 2 *F* and *p* values derived from the general lineal model of physiological parameters measured at different dates in the field of *HU* and *GR* Holm oak seedlings

	Provenance		Date		Provenance*date	
	F	*p*	*F*	*p*	*F*	*P*
E$_c$ (μmol H$_2$O kg^{-1} s^{-1})	0.001	0.974	113.536	<0.001	7.863	<0.001
SLA (m^2 kg^{-1})	8.618	0.004	40.925	<0.001	3.562	<0.001
RWC$_c$ (%)	34.084	<0.001	150.622	<0.001	0.961	0.478
Ψ (MPa)	0.550	0.459	278.988	<0.001	14.333	<0.001
K_s (kg m^{-1} MPa^{-1} s^{-1})	2.648	0.105	3.839	<0.001	4.772	<0.001
K_l (kg m^{-1} MPa^{-1} s^{-1})	0.377	0.540	3.043	0.002	0.956	0.477
PLC (%)	0.336	0.336	31.892	<0.001	6.786	<0.001

E$_c$: cuticular transpiration, SLA: specific leaf area, RWC$_c$: relative water content at the point of stomatal closure, Ψ: predawn leaf water potential, K_s: specific hydraulic conductivity, K_l: leaf specific hydraulic conductivity, PLC: loss of hydraulic conductivity individual provenances (Andivia et al. 2012; Aranda et al. 2005; Gratani et al. 2003; Morin et al. 2007; Sánchez-Vilas and Retuerto 2007). Our results indicated that *HU* seedlings had less mortality than *GR* seedlings after the water stress test, possibly because *HU* seedlings were from a region that had less favorable summer conditions. Holm oak seedlings from a geographic location with marked summer drought might lead to morphological and physiological adaptations that allow the seedlings to better tolerate this stress.

Our study of water relations parameters after planting provided another perspective on the responses of the two provenances to stress. The interaction of provenance × sampling date significantly affected all measured parameters except RWC_c and K_l. This indicates that seasonal variation of seedling traits allows acclimation of this species through the year. Moreover, the different responses of *GR* and *HU* seedlings are indicative of inter-population variability of this species (Rodríguez-Estévez et al. 2007).

Most of the observed differences between the *HU* and *GR* provenances were during the specify measurement dates in summer and winter. The decrease of hydraulic conductivity during the summer was probably related to xylem vessel cavitation and decreased Ψ (Salleo and Lo Gullo 1993; Tognetti et al. 1998). *HU* plants had higher values of Ψ and K_s and lower values of E_c and PLC during both summers, indicating better water status than *GR* plants. Once the stomata are closed, cuticular transpiration (E_c) determines the rate of water loss (Kerstiens 1996; Smith and Hinckley 1995). This probably allowed *HU* plants to maintain higher Ψ values, indicating that seedlings from a more xeric geographic region were better able to tolerate water stress. We also found differences between the two provenances in winter. The lower values of PLC and higher values of K_s in *GR* plants during winter indicate that these seedlings (from a geographic region with a longer frost period) are more tolerant to cold stress after outplanting. Previous studies have reported the presence of reduced hydraulic conductivity and increased PLC during winter, with temperatures below −2 °C, which induce xylem cavitation (Cochard and Tyree 1990; Tognetti et al. 1998). In January 2009, there were 6 days with temperatures below 0 °C, and the absolute minimum was −3.7 °C in our study area.

The relative water content at the point of stomatal closure (RWC_c) is an important indicator of leaf water status under drought conditions (Kerstiens 1996). RWC_c is closely related to cell volume and therefore more accurately reflects the balance between water content, water supply to the leaf, and transpiration rate. We observed seasonal changes of this parameter that were indicative of water conservation during the summer in both provenances (with maximum values close to 90 %), but the decrease of RWC_c during the wet season suggests a "water-spender" strategy when water is available. SLA is related to water stress conditions and crown position in relation to light and nutrient availability (Mousseau 1999). Taken together, the differences in RWC_c, SLA and E_c between our two provenances of Holm oak during the dry season indicate that *HU* seedlings could withstand greater dehydration before stomatal closure (lower RWC_c), and that *GR* seedlings (more permeable than the other provenance in summer because of its higher E_c) tries to avoid and excessive water loss through the epidermis by a lower SLA (Larcher 2003). However, during the wet season, *HU* seedlings tended to be less water conservative than *GR*, probably related to the wetter and warmer climate of *HU* provenance in this time.

Our study of two *Q. ilex* provenances indicated different physiological responses to seasonal abiotic stress that were suggestive of adaptation to the unique climatic conditions of these provenances. Seedlings from the drier summer provenance (*HU*) were more tolerant to water stress. Our planting experiments, which showed that *HU* plants were more effective in maintaining water potential and xylem hydraulic conductance and in reducing permeability of the leaf epidermis, confirming the greater water conservation of *HU* plants during summer. By contrast, plants from the cooler provenance (*GR*) had better frost tolerance during the winter.

Consideration of seedling provenance in the design of reforestation programs could help management decisions in order to reduce the high mortality of *Q. ilex* plantings. Climate change scenarios for Mediterranean basin predict an increase in water stress for plants, especially in summer, as a consequence of predicted temperature increase and rainfall decrease. Our results suggest that the consideration of plants from provenances more resistant to water stress, together with plants from the local provenance, in the design of future reforestation programs could lessen global warming impact on long-term survival and growth of plantations. Nevertheless, future studies with a greater number of provenances taken into account SLA and water relations parameters (such as E_c, vulnerability to xylem cavitation and water consumption) are needed.

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