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Original article

Water relations of cork oak (*Quercus suber* L.) seedlings in response to shading and moderate drought

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Abstract – The interactive effects of light and drought on water relations and soluble sugars were addressed on *Quercus suber* L. seedlings grown under the combination of four irradiances and two soil water contents. Leaf water potentials at predawn and midday were lower for water stressed seedlings, independently of light environment. Osmotic potentials at full ($\Psi\pi_{100}$) and zero turgor ($\Psi\pi_0$) decreased with drought, under the four light treatments. However, the decline was greater with the development of water stress under the two treatments of higher light availability. The higher soluble sugar concentrations in seedlings grown under higher irradiances provoked a decrease of $\Psi\pi_0$. The decrease in $\Psi\pi_{100}$ and $\Psi\pi_0$ in moderate stressed seedlings was accompanied by an increase in ε_{max}. The lower ability for osmotic adjustment in seedlings grown under moderate water stress and low light environment suggested a lower efficiency in developing physiological mechanisms for drought tolerance in shade-grown seedlings.

cork oak / drought / light / osmotic adjustment / soluble sugars

Résumé – Relations hydriques des semis de chêne-liège (*Quercus suber* L.) en réponse à l'ombre et à une sécheresse modérée. Les effets interactifs de la lumière et de la sécheresse sur les relations hydriques et les sucres solubles ont été étudiés sur des plantules de *Quercus suber* L. qui ont poussé sous la combinaison de quatre éclairements différents et de deux contenus en eau du sol. Les potentiels hydriques foliaires mesurés l'un avant l'aube et l'autre à midi étaient plus bas pour les plantules subissant un stress hydrique indépendamment du niveau d'éclairement. La pression osmotique à pleine turgescence ($\Psi\pi_{100}$) et la pression osmotique à turgescence nulle ($\Psi\pi_0$) ont diminué avec la sécheresse sous les quatre éclairements. Cependant, les diminutions étaient plus grandes pour les plantules soumises à un stress hydrique et à un éclairement élevé. La plus grande concentration de sucres solubles des plantules ayant poussé sous un plus grand éclairement a provoqué la diminution de $\Psi\pi_0$. La diminution de $\Psi\pi_{100}$ et de $\Psi\pi_0$ pour les plantules ayant poussé sous une sécheresse modérée a été accompagnée d'une augmentation de ε_{max}. La moindre capacité pour l'ajustement osmotique des plantules ayant poussé sous un stress hydrique modéré et une faible intensité lumineuse a montré une plus faible efficacité pour le développement des mécanismes physiologiques de tolérance à la sécheresse de ces plantules ayant poussé à l'ombre.

chêne-liège / sécheresse / lumière / ajustement osmotique / sucres solubles

1. INTRODUCTION

Light [10, 48, 52] and soil water moisture [32, 34] are among the major factors constraining primary productivity of Mediterranean species and, thus, that may contribute significantly to the future stand composition. Acclimation to different light environments occurs at both whole-plant and leaf levels [24]; leaf acclimation being associated with morphological, anatomical and physiological changes [31]. It is suggested that plants genotipically adapted to open sunny habitats have the ability to acclimate and grow under shaded conditions [42]. In particular, seedlings growing under high irradiances are able to maintain the turgor when water is limitant by decreasing the osmotic potential, through the accumulation of osmotically active solutes or by changing the bulk leaf modulus of elasticity [1, 5, 9]. However, Meletiou-Christou et al. [30] comparing sun and shade leaves of four evergreen sclerophylls, did not find any significant differences in the soluble sugar concentration.

Light levels in the understory of mature cork oak stands in Spain, can vary from 12 to 70% of incident radiation, depending on the structure of the stand [33]. Mortality of cork oak germinants in open areas can reach up to 90 to 100% due to the joint effect of rodents seed predation and summer drought [54]; but once the first growing season is overcome, seedlings are able to persist for 2 to 4 years and live in a suppressed state, through the dieback of the shoot during the droughty summers, followed by resprouting in the fall. The result is a recruitment of seedlings of variable age, not more than 30 cm high, with a deep root system, that in most cases are not able to perform adequately, as long as favourable conditions for growth do not

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occur [29, 54]. Thus, although mature cork oak is a shade-intolerant evergreen species, which shows a water-saver strategy under water stress [14, 34, 45], some canopy protection during the seedling state favours its establishment and early growth.

In Mediterranean-type climates, plants are typically subjected to water stress during summer. The scarcity of rainfall during this season is generally associated with large solar radiation loads [52]. When soil water is gradually depleted, physiological mechanisms of drought tolerance, including stomatal control of water loss, osmotic adjustment [35] and development of hydraulic systems resistant to cavitation [39, 47], bring into play turgor maintenance and prevention of water loss [13, 25, 28, 49, 50]. Indeed, drought tolerance in Mediterranean areas has a relevant impact on the physiological response of seedlings, even under shade conditions [53].

Because it is unlikely that tree growth is limited by deficiencies of only one resource in nature, comparative responses to multiple resources and their interactions are particularly relevant to understand adaptative strategies [8, 17, 26]. In particular, the interaction of light and water stresses may be a compromise between contradictory patterns on seedlings' physiological response. The question that arises is whether the mechanisms of drought tolerance are modified by leaf acclimation to long-term irradiance conditions [31]. According to the *trade-off hypothesis* mentioned by Holmgren [23] and Sack and Grubb [44], drought has a stronger impact on individuals grown in deep shade (< 5% of full-light) than on those grown under high irradiances. Thus, lower ability for osmotic adjustment in leaves grown under increasing shade conditions within the canopy has been reported [6]. In addition, π_0 may decrease because of active osmolyte accumulation when seedlings of drought-tolerant species are submitted to water stress [13]. There are some studies concerned with water relations in response to light and drought and their interactions, for mediterranean species [11, 21, 31, 53, 55], but little is known about cork oak. The aim of this work was to investigate leaf water relations of containerised cork oak seedlings under changing light and water conditions. The specific objectives were: (1) to determine if the occurrence of osmotic and elastic adjustment under moderate stress was similar in seedlings grown under different light environments; (2) to determine if shade can decrease seedlings' acclimation to water stress.

2. MATERIALS AND METHODS

2.1. Plant material and experimental design

A factorial experiment of two factors (light and water) of four and two levels, respectively, with treatments replicated in three blocks, was designed to test for main effects and interactions on water relation variables measured throughout two drying cycles. The four light levels varied from high to low PFD; the two water levels were well watered versus moderate stress. Twenty plants were grown under each combination and five plants per treatment combination and date were used for construction of P-V curves.

Acorns of cork oak (*Quercus suber* L.) were collected from trees of the Valle del Tiétar Iberian provenance in the fall of 2000, and stored in moist plastic bags at 4 °C until germination in mid-April 2001. One germinated acorn was planted in each 3-L pot (truncated square pyramid containers, 25 cm height, 169 cm^2 and 64 cm^2, upper and lower cross-sectional area, respectively), filled with a mixture of fine sand and peat moss (1:3, v/v). Five grams per litre of a six months controlled-release fertilizer (N:P:K, 20:10:20 + micronutrients) was added to the growing media. Seedlings were grown in the greenhouse (30 °C day/10 °C night temperature, under natural photoperiod, 750–850 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of radiation intensity at midday) and kept well watered twice a week. On 27 May 2001 seedlings were placed under a transparent plastic shelter (15 m long × 6.8 m wide × 2.3 m high), with the sides and ends opened to facilitate circulation of air. Seedlings were randomly divided in four groups, according to the four light environments. Plants were grown under metal frames with different layers of neutral shade white cloth (Polysack Plastic Industries Ltd., Israel) to produce the four light environments. The design of the frame was optimized to avoid any effect on the temperature of the air in contact with the plants. The average photosynthetic photon flux density (PFD) under each light environment during a sunny day was: HL1: 66–70% (34.66 $mol \cdot m^{-2} \cdot day^{-1}$); HL2: 44–50% (23.22 $mol \cdot m^{-2} \cdot day^{-1}$), LL1: 13.5–16% (7.92 $mol \cdot m^{-2}.day^{-1}$) and LL2: 5–6% (2.59 $mol \cdot m^{-2}.day^{-1}$) of full sunlight.

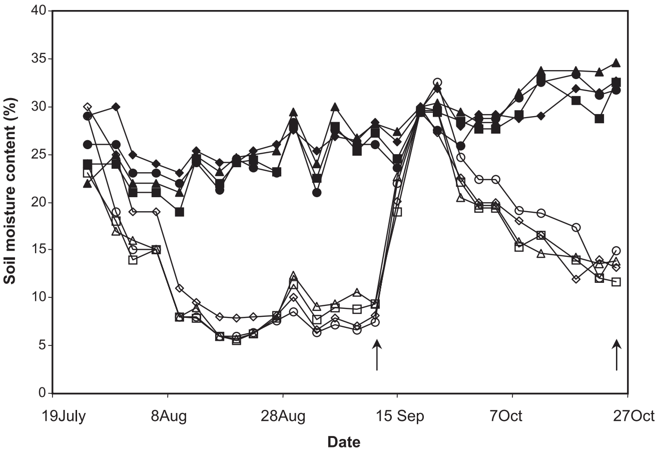


Figure 1. Variation on volumetric soil moisture content throughout the study on seedlings grown under different light environments (HL1: square; HL2: triangle; LL1: circle; LL2: diamond) and watered to field capacity (solid symbols) or submitted to two soil-drying cycles (open symbols). Volumetric soil water content in the W+ seedlings was maintained between 25% and 35%, while W– seedlings were allowed to dry to a water content between 7% and 10%. At the end of the first soil-drying cycle W– seedlings were watered to runoff on 20 and 23 Sept, before beginning the second soil-drying cycle. Arrows showed date of P-V curves construction. Analysis of variance in every measuring date showed no significant differences between light levels for seedlings in W– or W+ treatment ($P > 0.05$).

Seedlings under each frame were randomly divided in two groups, one of which was watered to field capacity twice per week (W+) and the other group was subjected to a series of two soil-drying cycles of 51 and 38 days (W–), respectively, beginning on 23 July and extending to 24 October. Moderate water-stressed seedlings (W–) were watered to runoff at the end of the first drying cycle. Volumetric soil water content was monitored twice per week at 15 cm depth with time domain reflectometry (TDR, Trase System I, Soil Moisture Equipment Corp., USA). Volumetric soil water content in the well-watered seedlings (W+) was maintained between 25% and 35%, while seedlings under moderate stress (W–) were allowed to dry to a water content between 7% and 10% (end of the first soil-drying cycle) (Fig. 1). The exact amount of water supplied in the W– treatment was a function of the volume of water lost under the lowest PFD (LL2), which had the minimum evaporative demands. By this means, a slow rate of imposition of the water stress conditions was assured. Analysis of variance of water content in every measuring date showed no significant differences between light levels for seedlings in the W– treatment ($P > 0.05$).

2.2. Measurements

At the end of each soil-drying cycle, five plants per each light × water regime combination were harvested for determination of tissue water relation parameters. Previous to construction of pressure-volume (P-V) curves, predawn water potential (Ψ_{pd}) was measured in one leaf of the same seedlings, with a pressure chamber (PMS 1000, PMS Instrument Co., Corvallis, OR, USA). In addition, predawn (Ψ_{pd}) and midday water potential (Ψ_{min}) were measured between July and October (Ψ_{pd} and Ψ_{min}: July 23, Aug 13, Aug 27; Oct 3; Ψ_{pd}: Sep 9, Oct 24) to assess the water status of the seedlings. At each measurement date, five seedlings were randomly selected from each light × water regime combination.

For construction of P-V curves, three fully expanded leaves were sealed with parafilm and the base of the petiole was placed in distilled water in a beaker after recutting under water. Leaves were allowed to rehydrate for an hour at room temperature. Special care was taken to prevent oversaturation of apoplasmic and intercellular spaces in leaves because of inmersion. Oversaturation during the first steps of dehydration causes the shift in leaf saturation deficit due to water losses without changes in measured water potential [16, 27]. The repeat pressurization technique [22] was used to construct PV curves from a series of parallel fresh weight and pressure chamber Ψ measurements. After each Ψ measurement, leave samples were removed from the chamber, weighed again inmediately and allowed to air dry between consecutive Ψ determinations. When approximately 7–8 data points on the apparent linear portion of the PV curves were obtained, samples were placed in an oven at 70 °C for 48 h to obtain leaf dry weight (DW). Sample relative water content (RWC) was calculated as (fresh weight – dry weight) / (turgent weight – dry weight). Weight at turgor was derived from the relationship between fresh weight and water potential. Data points above loss of turgor were identified and turgent weight determined by linear regression ($r^2 > 0.98$) [27].

For the derivation of PV parameters, paired observations of Ψ and RWC were plotted using ($1/\Psi$) transformations [51] to identify data points to be included in simple regression analysis of the linear portion of PV curves. For the regression analysis, $1/\Psi$ and RWC were used as the dependent and independent variables, respectively. The x- and y-intercepts yielded estimates of the water content in the symplast at full turgor (RWC_a) and inverse of osmotic potential at full turgor ($\Psi\pi_{100}$). The x- and y-coordinates of the first data point of the linear portion of the PV curve corresponded to relative water content at the turgor-loss point (RWC_0) and inverse of osmotic potential at the turgor-loss point ($\Psi\pi_0$). From the relationship between $1/\Psi$ and RWC, the relationship between turgor pressure (P) and RWC was calculated. Values for the bulk tissue elastic modulus (ε) were calculated from this latter relationship. The bulk tissue elastic modulus is defined as the change in tissue turgor pressure for a given fractional change in symplastic content ($\varepsilon = dP/dRWC\ (RWC - RWC_a)$). Osmotic and elastic adjustments, defined as the decrease in osmotic potential at full turgor or in ε, respectively, in response to water deficits were calculated. Estimates of $\Psi\pi_{100}$ and ε were used to characterize osmotic and elastic adjustments.

After construction of P-V curves, five to six leaves per seedling were oven-dried at 70 °C during 48 h and ground in a mill fitted with a 0.7 mm screen. Samples were analysed for soluble sugar concentration (mmol). Approximately 0.1 g of ground tissue was extracted three times in boiling 80% (v/v) ethanol, centrifuged and the supernatants pooled. Soluble sugars were quantified spectrophotometrically following reaction with anthrone [46].

2.3. Statistical analysis

A factorial arrangement of sample date, water regime and light was employed in a completely randomized design. Analysis of variance was conducted using the SAS GLM procedure. Comparison of mean treatment differences was done by Tukey test. As sample date was not significant, data from the two harvests were pooled, and a two-way fixed effects general linear model with the treatments of water regime and light as the main effects was used. The relationship between variables was assessed using the Pearson (*r*) coefficient, and considering the individual values of all seedlings.

Linear regressions of osmotic potential at full turgor ($\Psi\pi_{100}$, MPa) and at the turgor-loss point ($\Psi\pi_0$, MPa) with predawn water potential (Ψ_{pd}) were made. In a first step, functions were fitted to data from each light treatment independently (1). In a second step, a unique fit was made for all the data (2). In a third step, data from the four light treatments were grouped in two groups, high light (HL = HL1 + HL2) and low light (LL = LL1 + LL2), and two new functions were fitted to them (3). Once all regressions were proved significant, we determined whether significant differences exist among light treatments by a *F*-test for detecting simultaneous homogeneity among parameters of the regressions. The method requires the fitting of a full and a reduced model. The full model fitted a function for each light treatment. When comparing (1) and (2), the reduced model made a unique fit to all four treatments in the data set. When comparing (1) and (3), the reduced model fitting was done by calculating a different set of parameters for HL and LL groups

The F-test uses the following statistic:

$$F = \frac{\dfrac{SS_r - SS_f}{df_r - df_f}}{\dfrac{SS_f}{df_f}}$$

where SS_f = sum of squares error for full model; SS_r = sum of squares error for reduced model; df_f = degrees of freedom for full model; df_r = degrees of freedom for reduced model. The statistical decision rule at the given significance α level is:

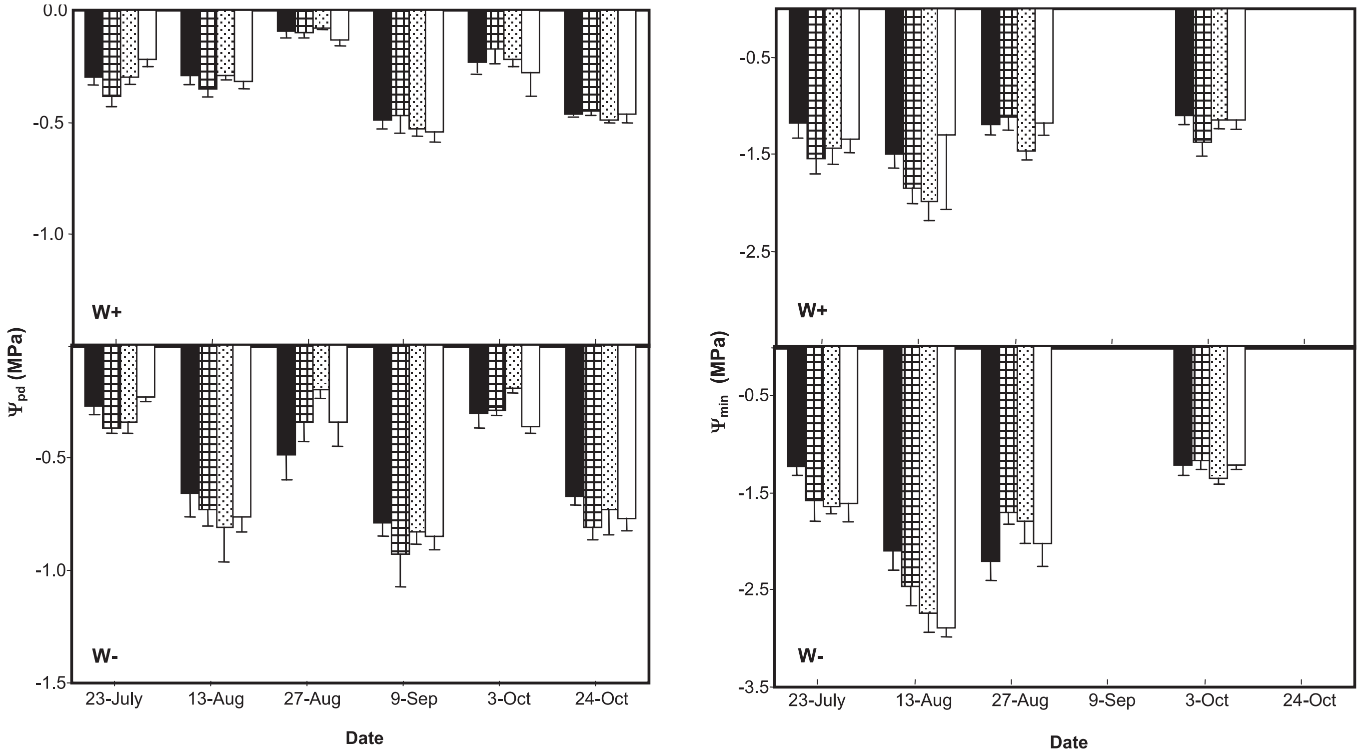
If $F > F_{\text{Fisher-Snedecor}}$ ($1\text{-}\alpha$; $dfr\text{-}df_f$; df_f), the separate models are required.

If $F < F_{\text{Fisher-Snedecor}}$ ($1\text{-}\alpha$; $dfr\text{-}df_f$; df_f), the reduced model is appropriate.

3. RESULTS

Significant differences between water regimes were found for Ψ_{pd} and Ψ_{min} in all measurement dates ($P < 0.001$), except at the beginning of the two soil-drying cycles (July 23 and Oct 3), when soil water moisture was high and similar in all seedlings (Fig. 2). Well-watered seedlings had higher values of Ψ_{pd} and Ψ_{min} compared to seedlings submitted to moderate water stress (Fig. 2). The lowest Ψ_{pd} values in the moderate water stressed seedlings were shown at the end of each soil-drying cycle. Predawn water potential (Ψ_{pd}) and midday water potential (Ψ_{min}) were not affected by light ($P > 0.05$); this result elucidated a similar imposition of the water regime, independently of the irradiance under which seedlings were grown (Fig. 2).

Soluble sugar concentration expressed on a leaf area basis was significantly higher under high light environments (HL1 and HL2), for both water regimes ($P < 0.001$, Fig. 3). The concentration under the HL1 treatment was between 1.8 (for W+) and 2.8-fold (for W–) the value under the LL2 treatment.



(a) **(b)**

Figure 2. Time course of **(a)** predawn (Ψ_{pd}, MPa) and **(b)** midday leaf water potential (Ψ_{min}, MPa) on cork oak seedlings watered to field capacity (W+) or submitted to a moderate water stress (W–) and grown under different light environments [HL1 (66–70%): ■; HL2 (44–50%): ⊞; LL1 (13.5–16%): ⊡; LL2 (5–6%): □].

Both the osmotic potential at full turgor ($\Psi\pi_{100}$) and at the turgor-loss point ($\Psi\pi_0$) were significantly affected by water and light treatments, although their variation was mostly explained by water availability (Tab. I). Thus, moderate water-stressed seedlings showed significant lower $\Psi\pi_{100}$ and $\Psi\pi_0$ under the four light treatments (Fig. 4). Seedlings grown under LL1 and LL2 light levels showed higher $\Psi\pi_{100}$ and $\Psi\pi_0$, than seedlings grown under HL1 and HL2. Bulk modulus of elasticity at maximum turgor (ε_{max}) was affected by water regime, but only under the two highest light environments (HL1 and HL2) (Fig. 4). High cell-wall rigidity was related to high RWC_0 and low RWC_a, Ψ_{pd}, $\Psi\pi_{100}$ and $\Psi\pi_0$ (Tab. II). The rest of parameters derived from the P-V curves (relative water content at the turgor loss point, RWC_0, and relative symplastic water content, RWC_S) showed no evident trend related to treatment. Estimates of $\Psi\pi_{100}$ are used to characterize osmotic adjustment, and decreases of 0.48, 0.46, 0.28 and 0.28 MPa were recorded for the HL1, HL2, LL1 and LL2, respectively.

At a significant level $\alpha = 5\%$, when comparing (1) and (2), we reject the null hypothesis of parameters homogeneity, which means that separate models were required for each of the four light treatments. When comparing (1) and (3), we cannot reject the null hypothesis, accepting that the reduced model was appropriate for the light treatment. Thus, a different model was fitted for the high light group (HL = HL1 + HL2) and the low light group (LL = LL1 + LL2). Values of F statistic and results of fitting the functions for the HL and LL groups are shown in Table III. For both light groups, $\Psi\pi_{100}$ and $\Psi\pi_{0\ values}$ in seedlings submitted to a moderate water stress were significantly lower than values in the well irrigated seedlings (Fig. 5). Low Ψ_{pd} induced low $\Psi\pi_{100}$ and $\Psi\pi_0$ values, irrespective of light environment.

4. DISCUSSION

Tissue water relation parameters in cork oak seedlings changed in response to water availability and light environment. Water potential (Ψ_{pd} and Ψ_{min}) was higher in well-irrigated seedlings, for all light levels, suggesting a generally small effect of light on water potential, as previously reported by Rhizopoulou et al. [42] in four Mediterranean evergreen sclerophylls. At the end of the second soil-drying cycle, there was a significant trend for higher Ψ_{pd} values in well-irrigated seedlings grown under the high light environments (–0.45 MPa in HL1 and HL2 vs. –0.49 MPa in LL1 and LL2, $P = 0.0129$, Fig. 2). Similarly, Abrams [1] and Kloeppel et al. [25] found higher Ψ_{pd} under sunny conditions, although it is not a general pattern [5, 17, 41, 53].

The decrease in osmotic potential ($\Psi\pi_{100}$ and $\Psi\pi_0$) as water availability decreased and PFD increased, allows seedlings to maintain a water potential gradient from the soil to the plant and to facilitate water uptake. Although no interaction was

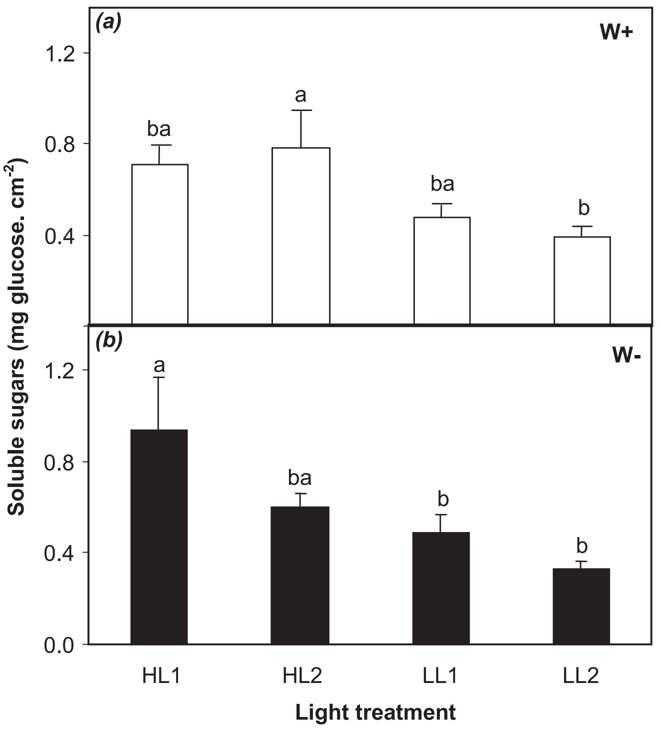


Figure 3. Sugar soluble concentration (mg glucose·cm^{-2}) in leaves of cork oak seedlings grown under four light environments (HL1: 66–70%; HL2: 44–50% , LL1: 13.5–16% and LL2: 5–6% of full sunlight) and watered to field capacity (W+) **(a)**, or subjected to a moderate water stress (W–) **(b)**. Means separation between light environments by *t*-test, $P < 0.05$.

Table I. Analysis of variance for pressure-volume curve parameters. Factors are light (*d.f.* 3), water (*d.f.* 1), and their interaction (*d.f.* 3).

Variable	Factor	*F*	*P*
$\Psi\pi_0$	Light	9.34	< 0.0001
	Water	43.09	< 0.0001
	Light × Water	0.16	0.9246
$\Psi\pi_{100}$	Light	6.32	0.0008
	Water	35.83	< 0.0001
	Light × Water	0.67	0.5750
Ψ_{pd}	Light	0.48	0.6964
	Water	45.00	< 0.0001
	Light × Water	0.54	0.6534
$\Psi_{pd} - \Psi\pi_0$	Light	6.26	0.0008
	Water	3.72	0.0196
	Light × Water	0.23	0.8773
RWC_0	Light	0.30	0.8234
	Water	0.25	0.6177
	Light × Water	0.79	0.5039
RWCa	Light	1.36	0.2621
	Water	0.03	0.8739
	Light × Water	0.10	0.9624
ε_{max}	Light	0.74	0.5307
	Water	3.70	0.0500
	Light × Water	2.25	0.0909

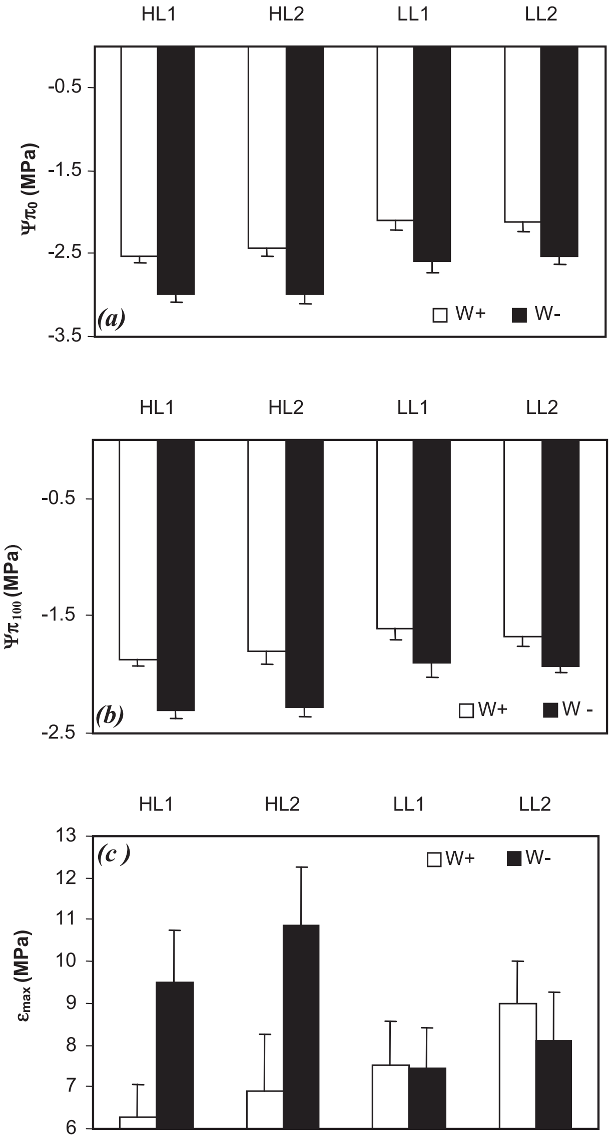


Figure 4. Osmotic potential at full ($\Psi\pi_{100}$, MPa) **(a)** and at turgor-loss point ($\Psi\pi_0$, MPa) **(b)**, and bulk modulus of elasticity (ε_{max}, MPa) **(c)** in cork oak seedlings watered to field capacity, W+ (white symbols) or submitted to two soil-drying cycles, W– (black symbols), under different light environments (HL1: 66–70%, HL2: 44–50%, LL1: 13.5–16% and LL2: 5–6% of full sunlight).

shown between light and water availability, $\Psi\pi_0$ was lower under high light (HL1 and HL2) for both water regimes. In addition, $\Psi\pi_{100}$ was lower under high light (HL1 and HL2), but only for moderate water-stressed seedlings. As suggested by Aranda et al. [7] the additive effect of high irradiance and low water availability can start mechanisms of drought tolerance such as a decrease on $\Psi\pi_0$. Active accumulation of solutes decreases $\Psi\pi_0$, acting to sustain a gradient in Ψ between plant protoplasm and the soil solution [8]. In this way, osmotic adjustment may facilitate water uptake, turgor potential maintenance, and tissue survival at the low tissue Ψ that results from water stress. For both water treatments, seedlings grown at high irradiances

Table II. Correlation values (Pearson's *r*) of different water relation parameters considering all seedlings ($n = 80$). Probability values are shown in parenthesis (values in bold: $P < 0.05$), ns: non-significant. Abbreviations: predawn leaf water potential (Ψ_{pd}, MPa), leaf water potential al the turgor-loss point ($\Psi\pi_0$, MPa), osmotic potential at full turgor ($\Psi\pi_{100}$, MPa), relative water content at turgor-loss point (RWC_0), relative apoplastic water content (RWC_a), turgent to dry weight ratio (TW/DW), bulk modulus of elasticity at maximum turgor (ε_{max}, MPa).

	Ψ_{pd}	$\Psi\pi_0$	$\Psi\pi_{100}$	RWC_0	RWC_a
$\Psi\pi_0$	0.52 (< **0.0001**)				
$\Psi\pi_{100}$	0.51 (< **0.0001**)	0.92 (< **0.0001**)			
RWC_0	ns	0.29 (**0.0114**)	ns		
RWC_a	ns	0.22 (0.0570)	0.40 (**0.0003**)	0.59 (< **0.0001**)	
ε_{max}	–0.37 (**0.0011**)	–0.30 (**0.0090**)	–0.56 (< **0.0001**)	0.27 (**0.0195**)	–0.39 (**0.0007**)

Table III. Linear regression analysis, using the equations $\Psi\pi_0 = a + b.\Psi_{pd}$ or $\Psi\pi_{100} = a' + b'.\Psi_{pd}$. *F* statistic and summary results for the fit of the functions are shown. (1) functions are fitted to data from each light treatment independently; (2) a unique fit is made for all the data; (3) data from the four light treatments are grouped in high light (HL = HL1 + HL2) and low light (LL = LL1 + LL2) environments, and two new functions are fitted to them.

Light groups	*n*	$\Psi\pi_0$ vs. Ψ_{pd}		$\Psi\pi_{100}$ vs. Ψ_{pd}	
		F	*P > F*	*F*	*P > F*
(1) vs. (2)	75	5.823	6.300E–5	4.063	0.0016
(1) vs. (3)	75	0.500	0.736	0.613	0.655

	$\Psi\pi_0$ vs. Ψ_{pd}			$\Psi\pi_{100}$ vs. Ψ_{pd}		
	Parameter estimates		r^2	Parameter estimates		r^2
	a	b		a'	b'	
HL	1.09	–2.07	0.40	0.94	–1.48	0.37
LL	1.33	–1.48	0.35	0.92	–1.19	0.28

showed a higher soluble sugar concentration than seedlings grown under low irradiances. Similar results have been reported by Niinemets [36] in three temperate woody species and by Johnson et al. [24] in *Fagus sylvatica*, but contrast to those presented by Meletiou-Christou et al. [30] in four Mediterranean evergreen sclerophylls, which showed no substantial differences on soluble sugars when comparing sun and shade leaves. Changes on water availability through the growing season did not affect either soluble sugar concentration in two-year-old cork oak seedlings grown outdoors in Portugal, although mean values are higher than ours (between 1.0 and 1.7 mg glucose·cm^{-2}), probably related to seedling age [12]. The higher soluble sugar concentrations in seedlings grown under higher irradiances provoked a decrease of the osmotic potential at full turgor ($\Psi\pi_{100}$) and are at least partly responsible for osmotic adjustment [31].

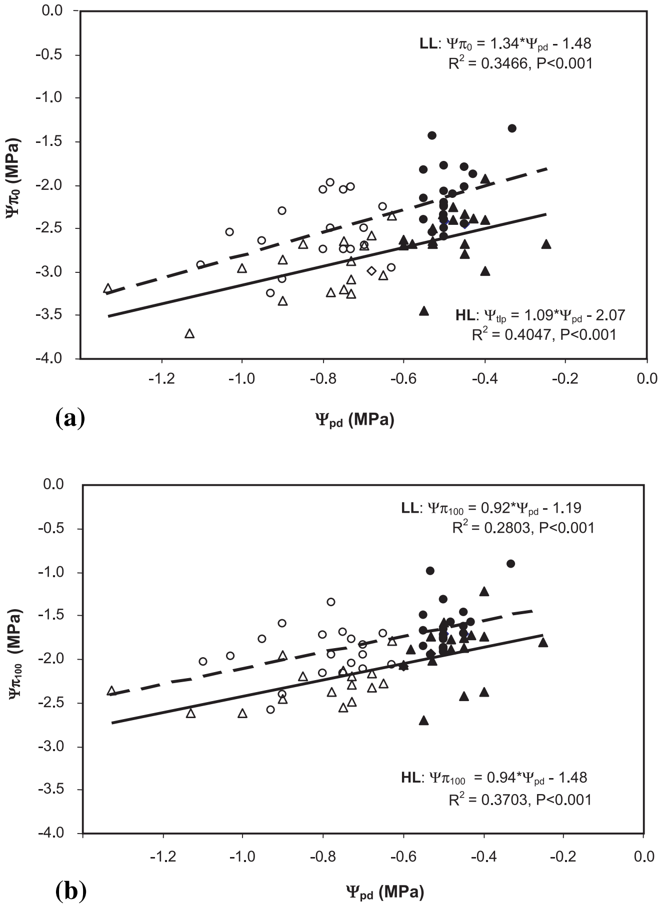


Figure 5. **(a)** Osmotic potential at turgor-loss point ($\Psi\pi_0$) and **(b)** at full turgor ($\Psi\pi_{100}$) plotted against predawn water potential (Ψ_{pd}). Data are derived from seedlings watered to field capacity, W+ (solid symbols) or subjected to a moderate water stress, W–(open symbols), and grown under high light, HL (triangles) or low light conditions, LL (circles). Also shown are fitted curves for pooled data of seedlings grown under high light (——) or low light conditions (-----).

As the response to light environment was the same under both water levels, with the highest values under the high light levels (HL1 and HL2) and the lowest under the low light levels (LL1 and LL2), seedlings could be arranged in two groups according to their response to the light environment: low light level (LL = LL1 + LL2 < 16% of full sun light) and high light level (HL = HL1 + HL2 > 40% of full sun light). Similar groups were defined when regressions between $\Psi\pi_{100}$ vs. Ψ_{pd} and $\Psi\pi_0$ vs. Ψ_{pd} were made (Fig. 5). Leaves grown under high light typically developed lower osmotic potential relative to shaded leaves [4, 8]. The lower $\Psi\pi_{100}$ and $\Psi\pi_0$ irrespective of Ψ_{pd} as irradiance increased has been related to lower daily leaf water potential, due to a more droughty environment [37]. Although the relationship between $\Psi\pi_{100}$ vs. Ψ_{pd} with high coefficients of determination ($r^2 > 0.7$) has been reported [3, 19], the lower coefficients in our study ($r^2 > 0.28$) can result from the moderate water stress applied.

In oak species, drought commonly results in decreases in $\Psi\pi_{100}$ of about 0.6 MPa or less [2, 3, 6, 13, 14]. Decreases in osmotic potential near to 0.5 MPa under moderate drought conditions and differences in osmotic adjustment between seedlings grown under low light (LL1 and LL2) and high light (HL1 and HL2) environments of 40% demonstrated an osmotic adjustment capacity in accordance to water conditions in our study. In any case, and under both light environments, cork oak readily displayed osmotic adjustment with little change in Ψ_{pd}, similarly to results reported in chestnut oak in an upland oak forest during a dry year [49]. Moreover, our results suggested a more limited osmotic adjustment under low light conditions (0.28 MPa) than under high light conditions (0.47 MPa), as reported by Augé et al. [8] with *Rosa* seedlings. Osmotic adjustment in seedlings grown under the high light environment (HL1) was also accompanied by an elastic adjustment of 3.6 MPa. The increase in osmotic adjustment capacity with light went in parallel with an increase in photosynthetic capacity (data not shown). Previous studies demonstrated the higher ability to osmotic adjustment in plants growing under high light environments [15, 31, 40]. The values of osmotic adjustment for seedlings grown under low light conditions are similar to those obtained in xeric North American oak species [3]. Values of osmotic adjustment under high light conditions are similar to those reported by Collet and Guehl [13] on sessile oak after subjecting seedlings to a slow rate of water deficit increase.

The decrease in osmotic potential at turgor loss point and at full turgor in moderate stressed seedlings was accompanied by an increase in ε_{max}. This is a characteristic of plants adapted to tolerate water stress [20, 43]. This increase in ε_{max} will result in a more rapid loss of turgor for a given loss in tissue water content and hence may appear to be a disadvantage during drought [17]. However, an increase in tissue rigidity may confer physiological and ecological advantages [8]. A lower cell wall elasticity would allow leaf water potential to drop rapidly and substantially as soon as the leaves began to loose water [34, 38] and a rapid recovery after a decrease in soil water content, which is shown as an efficient mechanism to overcome water stress [14]. It has been suggested that a higher ε_{max} value increases water absorption in a drying soil by increasing the soil-plant water potential gradient [18]; although both lower and higher elasticity have been suggested as promoting turgor maintenance.

Results of this study suggest the development of different physiological mechanisms to withstand environmental conditions in sun and shade-grown seedlings. Osmotic and elastic adjustments are found to help cork oak seedlings to maintain turgor during moderate water stress and under high light environment (> 40% of full sun). In contrast, in seedlings grown under moderate water stress and low light environment (< 16% of full sun), their main strategy in coping with stress was osmotic adjustment; but its lower ability for such adjustment suggests a lower efficiency in developing physiological mechanisms for drought tolerance, under low light conditions.

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