Adjustment of photosynthetic carbon assimilation to higher growth irradiance in three-year-old seedlings of two Tunisian provenances of Cork Oak (*Quercus suber* L.)

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Three-year-old seedlings of two Tunisian provenances of cork oak (*Quercus suber* L.) differing in climatic conditions at their geographical origin were subjected to increasing light intensities. Gaâfour was the provenance from the driest site and Feija from the wettest site. Low-light adapted seedlings from both provenances were exposed to two light treatments: full sunlight (HL) and low light (LL, 15% sunlight) for 40 days. The CO_2-response curve of leaf net photosynthesis (A_n-C_i curve) established under saturated photon flux density was used to compare photosynthetic parameters between leaves subjected to continuous low light (LL leaves) and leaves transferred from low to high light (HL leaves). Transfer from low to high light significantly increased net photosynthesis (A_n) and dark respiration (Rd) in Gaâfour provenance but not in Feija. After transfer to high irradiance, specific leaf area (SLA) did not change in either provenance. This suggested that the increase in photosynthetic capacity on a leaf area basis in HL leaves of Gaâfour provenance was not due to increased leaf thickness. Only the seedlings from the Gaâfour provenance were able to acclimate to high light by increasing V_{cmax} and J_{max}.

Keywords: *Quercus Suber*, Photosynthesis, V_{cmax}, J_{max}, Stomatal Limitation

Introduction

Quercus suber L. is an evergreen oak growing across a wide range of environmental conditions in the Mediterranean Basin. Due to these diverse conditions, Mediterranean forest tree species are exposed to a range of selective pressures that may lead to phenotypic plasticity and local adaptation (Ramirez-Valiente et al. 2010). Light is one of the most heterogeneous factors affecting plant growth and development (Colin & Clive 2001) and acclimation of leaf photosynthesis to different light environments is a key factor that maximizes carbon gain (Evans & Poorter 2001, Katahata et al. 2007). Acclimation to fluctu-ations in light conditions plays a crucial role in determining the competitive ability of forest tree species (Grassi & Bagnaresi 2001). Acclimation to environmental stresses is achieved through structural, morphological and physiological adjustments at both leaf and whole-plant level (Pearcy & Sims 1994, Sims et al. 1998, Grassi & Bagnaresi 2001). The acclimation of plants to high light condition appeared in the ability to use high photosynthetic photon flux densities efficiently (Grassi & Bagnaresi 2001, Sun et al. 2016).

Plants can form sun and shade leaves in response to the variation in growth irradiance. Sun leaves have a higher photosyn-thetic capacity than shade leaves. Higher photosynthetic capacities in sun leaves correlate with a greater leaf thickness and is supported by a greater investment of nitrogen in photosynthetic enzymes (Boardman 1977, Evans & Seemann 1989, Price et al. 1998, Terashima et al. 2001, Evans & Poorter 2001, Oguchi et al. 2003, 2005, Pandey & Kushwaha 2005). Furthermore, strong correlations were reported between photosynthetic capacity and surface area of mesophyll cells (Pandey & Kush-waha 2005), between photosynthetic capacity and the chloroplast area facing the intercellular space (Oguchi et al. 2003) and between photosynthetic capacity and stomatal and mesophyll conductances to CO_2 diffusion (Hanba et al. 2002). Pandey & Kushwaha (2005) studied the relationship of leaf anatomy with photosynthetic acclimation in *Valeriana jatamansi* under full irradiance [FI, 1 600 μmol (PPFD) m^{-2} s^{-1}] and shade conditions [SC, 650 μmol (PPFD) m^{-2} s^{-1}]. FI plants had thicker leaves with lower specific leaf area (SLA), enabling to arrange all their chloroplasts along the mesophyll cell surface. Leaf thickness is determined by the irradiance during leaf development, and it changes little after leaf maturation (Sims & Pearcy 1992). Nevertheless, when leaves are subjected to higher irradiance after maturation, their photosynthetic capacity often increases. However, high photosynthetic capacity is not always associated with thicker leaves.

The aim of this study was to test whether the habitat of origin induced an intraspe-

cific variation in response to transfer of plants from low to high growth irradiance. To this purpose, seedlings of cork oak (*Quercus suber* L.) originating from two different natural habitats and representing a marked climate gradient were used. Changes in photosynthetic characteristics of mature leaves were examined when transferred from low to full sunlight condition. The photosynthetic capacity, the maximum carboxylation rate of Rubisco (Vc_{max}), the potential light-saturated electron transport rate (J_{max}) and the specific leaf area were determined for leaves grown under different light conditions.

Materials and methods

Seed origin and experimental design

Acorns of two *Quercus suber* populations originating from contrasting environments in the northwestern provinces of Tunisia were collected in October 2010. The first site, the National Park of Feija (36° 30′ 00″ N, 08° 20′ 00″ E), is located in the North of the Kroumirie Mountains and is characterized by a cold and humid climate. The altitude varies between 800 and 1500 m a.s.l. and the average annual rainfall is 1217 mm, with precipitation increasing with altitude and reaching 1800 mm year^{-1}. In January, the average temperature is 7 °C and can drop to 0 °C with snow which falls every year. In summer, the temperature rises notably and averages around 29 °C. This leads to a mean annual temperature of 14.3 °C. Cork oak trees are found in forest mosaics along with other tree species, including Zeen oaks (*Quercus faginea*), maritime pines (*Pinus pinaster*) and many shrub species.

The second site is located in Gaâfour (36° 32′ 190″ N, 09° 32′ 40″ E) in the southern hills and plains around the city of Siliana. It is characterized by a semi-arid climate (480 mm year^{-1}) with moderate winters and hot dry summers. Cork oak trees at this site are found in agroforestry systems containing olive trees. The altitude is 560 m a.s.l. and the mean annual temperature is 17 °C.

Immediately after collection, acorns were planted in a common greenhouse at the National Research Institute for Rural Engineering, Waters, and Forestry under low light conditions (LL, 15% of full sunlight). On October 1st, 2013, low-light adapted seedlings were growing in 5 litres pots containing a mixture of equal proportions of soil and compost. Seedlings were randomly assigned to one of two light treatments for 40 days: (i) high light (100% natural incident irradiance, HL); and (ii) low light (LL). In the LL treatments, light levels were obtained through the use of layers of neutral shade-cloth, while in the HL treatment seedlings were left uncovered. On a sunny day, photosynthetically active radiation (PAR), measured using a Li-190® device (Li-Cor Bioscience, Lincoln, NE, USA) ranged between 1630-1810 µmol m^{-2} s^{-1} and 215-315 µmol m^{-2} s^{-1} in the HL and LL treatments, respec-

tively. Irrigation to saturation was provided manually each day. Mean volumetric soil water contents of the pots containing seedlings in the two light treatments was monitored by a time domain refractometry (TDR, Trase system I, Soil moisture Equipment Corp., USA) and was approximately 25-30%. To ensure that all seedlings were exposed to a similar range of light throughout the experiment and minimize the possible effects of within-block light variability, each block containing twenty pots (ten for each provenance per light treatment) was periodically moved. All experiments were carried out using mature leaves that were fully expanded and developed prior to the light treatment.

CO₂ response curves

Photosynthetic traits were measured *in situ* on mature leaves of four to six different seedlings per treatment (one leaf per plant) during the late morning (08:00-11:00 h) and early afternoon (13:00 -16:00 h). At the beginning of the experiment, leaf gas exchange was measured in three low light-adapted seedlings from each provenance. After 40 days, four plants from Gaâfour and Feija provenances were transferred from low to high light and used to carry out gas exchange measurements. Three additional LL adapted Gaâfour and Feija seedlings were used to ensure that there was no change of photosynthesis parameters during the experiment for plants kept under LL. Photosynthetic carbon dioxide response curves were recorded using a portable gas-exchange system (LI-6400®, Li-Cor Inc., Lincoln, NE, USA) equipped with a 2 × 3 cm light-source chamber (6400-02B LED®, Li-Cor). Each leaf was adapted to dark for approximately 30 minutes in the measurement chamber. When the gas exchange reached a steady state, net CO₂ efflux was recorded as an estimate of dark respiration (Rd). At this point, incident PFD was set to 1600 µmol m^{-2} s^{-1} (saturating light). The leaf temperature was maintained at 25 °C and the humidity of the incoming air was kept at 50-60%. The external CO₂ partial pressure (C_a) variation consisted of 12 steps, starting by inducing photosynthesis at ambient CO₂ concentration of 400 µmol mol^{-1} until net photosynthesis (A_n) stabilized (A_n varied by less than ± 2%). This was important to ensure a steady-state activation of Rubisco (Long & Bernacchi 2003). The external CO₂ concentration (C_a) values were then decreased to 300, 200, 100 and 50 µmol CO₂ mol^{-1} respectively. Upon completion of the measurement at 50 µmol CO₂ mol^{-1}, C_a was increased back to 400 ppm to check whether the original A_n could be restored. If this was achieved, C_a was increased stepwise to 600, 700, 1000, 1200, 1500 and 2000 ppm. Leaves were allowed to equilibrate for at least 5 minutes at each step before data logging. At the end of each A-C_i curve, the leaf area and mass enclosed in the chamber was measured with a leaf area meter portable laser

(Model CI-202). The specific leaf area (SLA) was determined as the ratio of the leaf area to leaf dry mass of individual leaves. To estimate the CO₂-saturated rate of photosynthesis (A_{sat}), CO₂ response curves were fitted using a three components exponential function (Watling & Press 2000) according to the following equation (eqn. 1):

$$A_n = a\left(1 - e^{-bx}\right) + c$$

where A_n is leaf net photosynthetic rate and x is C_i. Using this equation, A_{sat} was calculated as (a+c). The CO₂ compensation point (Γ) was estimated from x-axis intercepts.

Estimation of V_{cmax}, J_{max} and relative stomatal limitation

Non-linear regression techniques, based on the equations of Farquhar et al. (1980) and later modified by Sharkey (1985) and Harley & Sharkey (1991), were used to estimate V_{cmax} (the maximum rate of carboxylation limited by Rubisco) and J_{max} (the maximum rate of carboxylation limited by electron transport). In some cases, carboxylation may also be limited by triose phosphate availability (Sharkey 1985, Harley & Sharkey 1991); however, this was not observed in any of the seedlings used in this study. V_{cmax} and J_{max} describe the upper limit to biochemical processes (amount, activity, and kinetics of Rubisco and regeneration of RuBP), both parameters are derived from different portions of the A_n-C_i curve. Points in the lower portion (at low C_i) of the curve are used for V_{cmax} and points in the upper portion (at high C_i) for J_{max}. The Michaelis-Menten constants for CO₂ and O₂ (K_c and K_o respectively) and the CO₂ compensation point in the absence of mitochondrial respiration (Γ^*), as well as their temperatures dependencies, were taken from Bernacchi et al. (2001).

The stomatal limitation was calculated according to Farquhar & Sharkey (1982) as follows (eqn. 2):

$$L_s = 1 - A/A_o$$

where A_o represents carbon assimilation under natural ambient CO₂ in the absence of stomatal limitation.

Statistical analysis

Gas exchange measurements were repeated 4 and 6 times for HL and LL seedlings respectively. Mean values and standard errors were calculated. One-way analysis of variance (ANOVA) followed by the *post-hoc* Duncan's test (α =0.05) were applied to test for differences among means of photosynthetic gas exchange parameters and specific leaf area.

Two-way analysis of variance was also conducted to test the interaction effect (provenance × light) on photosynthetic parameters. Pearson's correlation between the photosynthetic parameters and SLA

were also calculated. All ANOVA analyses were performed using the software package SigmaPlot® (Systat Software Inc., San Jose, CA, USA). Pearson's correlation analysis was done using XLSTAT v. 2014 (http://www.xlstat.com) after data standardization.

Results

Photosynthetic responses to transfer from low to high light

Gas exchange parameters between LL and HL leaves in Gaâfour and Feija seedlings at an ambient CO_2 concentration of 400 ppm were compared (Fig. 1). Stomatal conductance (g_s) and net carbon assimilation (A_n) measured under saturating light in LL leaves were found to be similar in both Feija and Gaâfour provenances.

In HL leaves from the Gaâfour provenance, a significant increase in A_n was observed when compared to LL leaves (P < 0.01). While the g_s in this environment also increased, it was not significant (P > 0.05). No notable variations in these parameters (A_n and g_s) were detected in Feija leaves under different light condition treatments. At HL, A_n and g_s were significantly higher in Gaâfour leaves than in Feija leaves (P = 0.027 and 0.003 for A_n and g_s, respectively).

It was expected that the variations of g_s may explain significant differences of A_n in HL leaves of the two provenances. However, g_s did not show such differences, since stomatal limitation to carbon assimilation (L_s) remained similar in both provenances at HL conditions. Similarly, the intercellular CO_2 concentration (C_i) did not change in the HL leaves of Gaâfour and Feija seedlings when compared with LL leaves. A significant contribution of g_s to the lower A_n in Feija leaves at HL is therefore not supported. Although g_s did not differ between light treatments in the same provenance, there was a highly significant treatment × provenance interaction effect on both g_s and A_n (P < 0.01 – Tab. 1).

There were no significant changes to the leaf dark respiration in the seedlings of Feija transferred from LL to HL in comparison to those maintained at LL (P > 0.05). Dark respiration was highest in HL leaves of Gaâfour provenance (Tab. 1) but did not show a significant treatment × provenance interaction effect (Tab. 1). No significant changes in specific leaf area were measured between leaves of seedlings maintained at LL and those transferred to a HL condition in either provenance (Fig. 1).
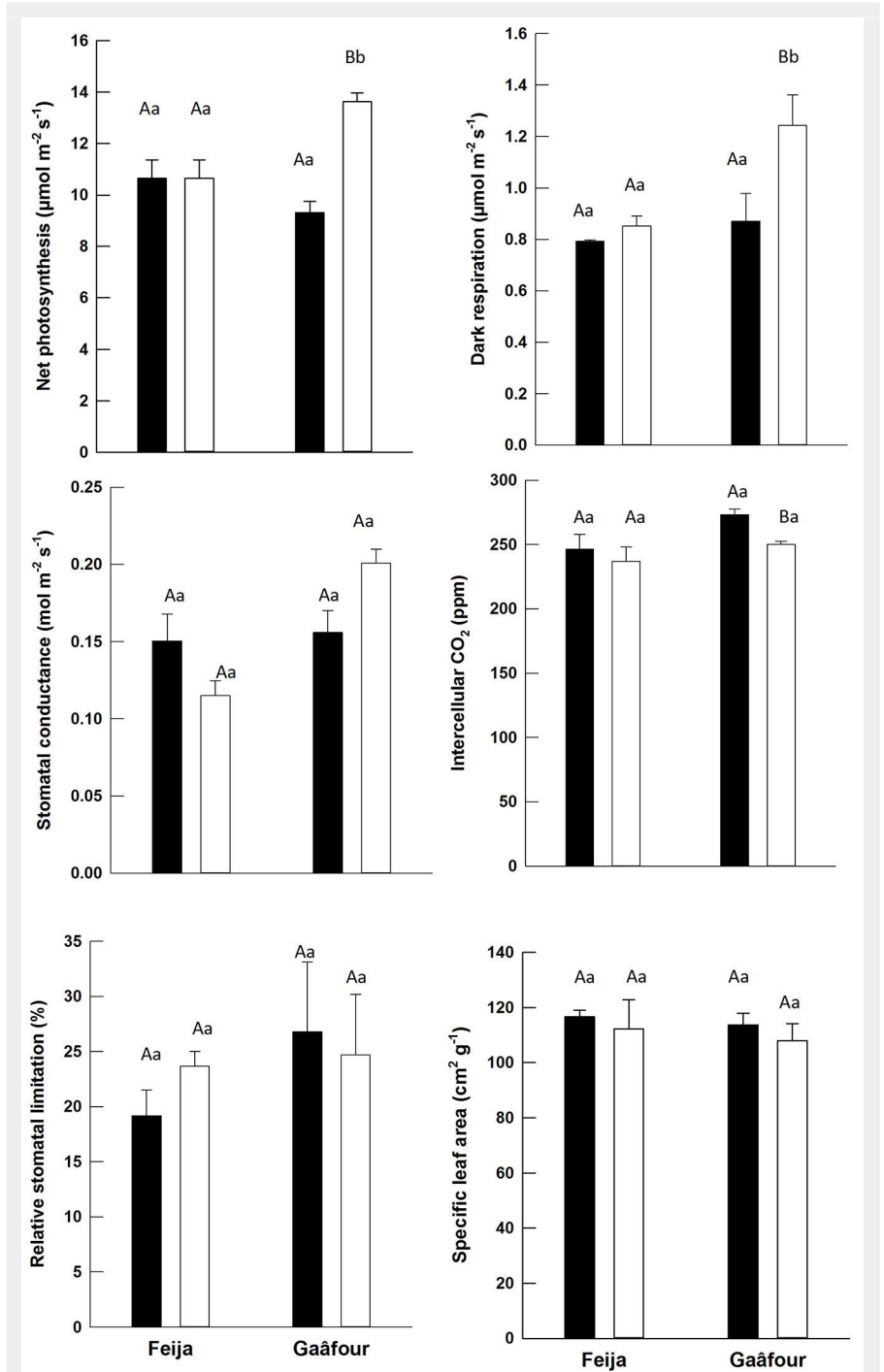

Fig. 1 - Leaf physiological traits of leaves maintained continuously in low light (black) or transferred from low to high light (white): net photosynthesis (A_n, μmol m^{-2} s^{-1}); dark respiration (Rd, μmol m^{-2} s^{-1}); stomatal conductance (g_s, mmol m^{-2} s^{-1}); intercellular CO_2 concentration (C_i, μmol mol^{-1}); relative stomatal limitation (L_s, %); and specific leaf area (SLA, cm^2 g^{-1}). Different uppercase letters indicate a significant difference between provenances in the same light environment, whereas different lowercase letters indicate significant difference between light environments in the same provenance (P ≤ 0.05).

Tab. 1 - Summary of two-way analyses of variance (*F*-values) for differences in gas exchange parameters between treatments and provenances. (ns): non-significant; (**): *P*< 0.01; (*): *P* <0.05.

Factors	A_n	Rd	g_s	C_i	L_s	Γ	V_{cmax}	J_{max}	A_{sat}
Provenance	2.36 [ns]	8.16*	12.44**	4.92*	0.8 [ns]	2.8 [ns]	32.52**	7.35*	11.68*
Sunlight	11.98**	6.9*	0.13 [ns]	3.33 [ns]	0.06 [ns]	2.27 [ns]	85.94**	1.77	0.15 [ns]
Provenance × sunlight	14.09**	3.61 [ns]	9.63**	0.57 [ns]	0.46 [ns]	1.37 [ns]	95.07**	2.35 [ns]	0.45 [ns]

Fig. 2 - Net photo-synthesis (A_n) as a function of intercellular CO_2 concentration (C_i) of leaves maintained continuously in low light (solid line) or transferred from low to high light (dotted line) in Feija and Gaâfour provenances. Inserts: Feija (closed symbols) and Gaâfour (open symbols).
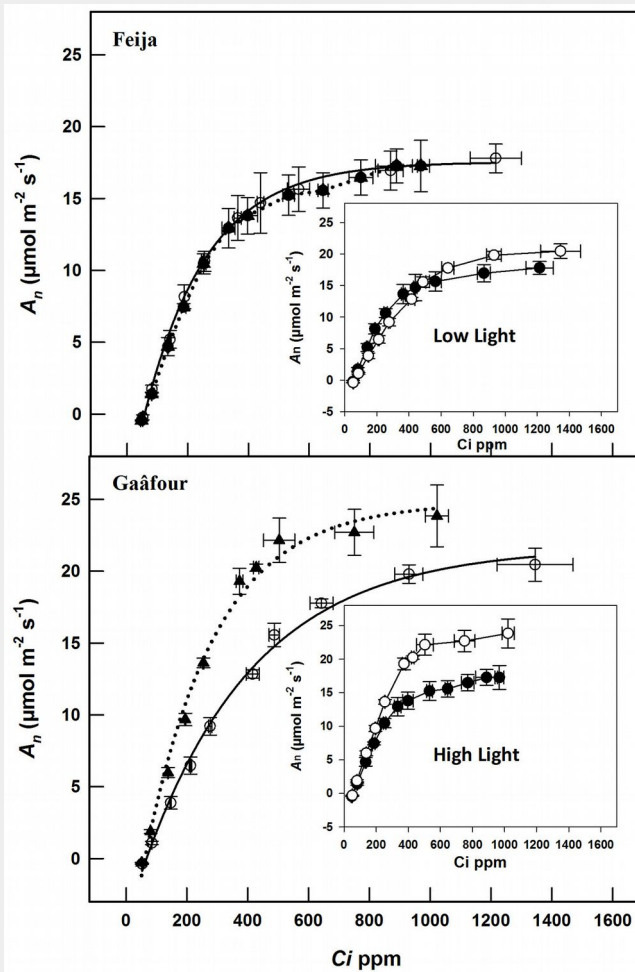
Photosynthesis in response to C_i

In order to characterize the photosynthetic activity in the leaves of Gaâfour and Feija provenances under the two light conditions, A_n was measured as a function of the intercellular CO_2 concentration (C_i). As shown in Fig. 2 (insert), A_n of LL leaves in Gaâfour provenance was similar to Feija provenance at low C_i but it was higher at high C_i. In the HL leaves of Gaâfour, A_n as a function of C_i increased in comparison to LL leaves. No differences were observed between Feija seedlings grown in either light condition.

Fig. 3 shows that the CO_2 compensation point (Γ) remained constant in leaves of both provenances when the leaves were transferred to full sunlight, despite a negligible decrease detected in Gaâfour leaves. Under both light regimes, leaves of Gaâfour seedlings had significantly higher CO_2-saturated photosynthesis rates (A_{sat}) than those of Feija ($P < 0.05$). The statistical analysis (two-way ANOVA), did not show any significant treatment × provenance interaction effect on either Γ or A_{sat} (Tab. 1).

Estimation of V_{cmax} and J_{max}

Stomatal constraints on photosynthesis were similar in the Feija and Gaâfour provenances, indicating that photosynthetic acclimation was curtailed at the biochemical level. The parameters describing the maximum rate of carboxylation by Rubisco (V_{cmax}) and the maximum rate of electron
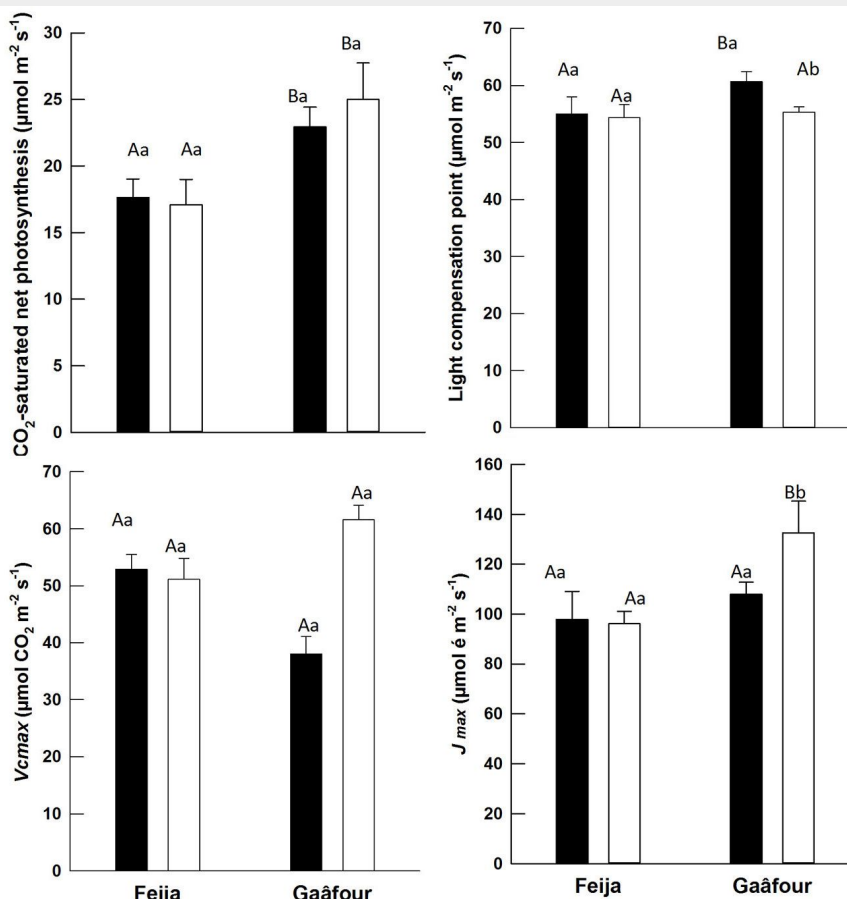

Fig. 3 - Photosynthetic parameters estimated from A_n/C_i curves of leaves maintained continuously in low light (black) or transferred from low to high light (white): the CO_2-saturated net photosynthesis (μmol CO_2 m^{-2} s^{-1}), the CO_2 compensation point Γ (ppm), the maximum ribulose 1.5- bisphosphate carboxylase/oxygenase (Rubisco) carboxylation V_{cmax} (μmol CO_2 m^{-2} s^{-1}) and the potential light-saturated electron transport rate J_{max} (μmol m^{-2} s^{-1}). Different uppercase letters indicate a significant difference between provenances in the same light environment, whereas different lowercase letters indicate significant difference between light environments in the same provenance ($P \leq 0.05$).

Tab. 2 - Correlations between photosynthetic gas exchange parameters. (*): $P \leq 0.05$; (**): $P \leq 0.01$.

Variables	A_n	Rd	g_s	C_i	L_s	Γ	V_{cmax}	J_{max}	A_{sat}	SLA
A_n	1	0.5895*	0.6205*	-0.1450	-0.0213	-0.4866	0.8207**	0.6425*	0.4425	-0.2974
Rd	-	1	0.6044*	0.1031	0.0754	-0.0648	0.7062*	0.6369*	0.3974	-0.4165
g_s	-	-	1	0.2751	-0.0041	-0.2484	0.5637	0.4559	0.4237	-0.3551
C_i	-	-	-	1	-0.0387	0.8235**	-0.2873	-0.0079	0.3514	0.2816
L_s	-	-	-	-	1	0.3311	0.0144	0.6661*	0.0988	0.0550
Γ	-	-	-	-	-	1	-0.3731	-0.0082	0.3580	0.3768
V_{cmax}	-	-	-	-	-	-	1	0.6889*	0.4536	-0.4946
J_{max}	-	-	-	-	-	-	-	1	0.7077*	-0.3083
A_{sat}	-	-	-	-	-	-	-	-	1	0.0186
SLA	-	-	-	-	-	-	-	-	-	1

transport (J_{max}) are shown in Fig. 3. In the LL leaves, V_{cmax} was higher in Feija than Gaâfour leaves ($P< 0.05$). When transferred to HL conditions, V_{cmax} increased significantly in the Gaâfour seedlings only. This increase of V_{cmax} in HL leaves of Gaâfour showed approximately a 60% enhancement compared to leaves that remained in LL conditions.

Although J_{max} in LL leaves were similar in Feija and Gaâfour provenances, Feija seedlings displayed significantly lower J_{max} after 40 days of growth under HL conditions. This suggests a lower capacity of electron transport in Feija leaves, leading to the limitation of RuBP regeneration. The statistical analysis showed a highly significant treatment × provenance interaction effect on V_{cmax}, but this interaction was non-significant on J_{max} (Tab. 1).

Discussion

We studied the effect of increasing light intensity on photosynthesis in cork oak (*Quercus suber* L.) seedlings of two provenances differing in climatic conditions at their geographical origin. Three-year-old cork oak seedlings of both provenances, grown in pots in a common greenhouse, were randomly assigned to one of two light treatments (HL, full sunlight and LL, 15% of sunlight).

The HL leaves of Gaâfour provenance proved to have a significantly higher photosynthetic capacity ($P < 0.05$) when compared to the Feija leaves. Different studies showed a possible intraspecific variability and phenotypic plasticity in Cork oak as an adaptation to contrasting regional climate conditions (Balaguer et al. 2001, Aranda et al. 2005, Staudt et al. 2008, Ramirez-Valiente et al. 2010, Matesanz & Valladares 2014, Gratani 2014). Investigating the influence of full-sun on seedling hardiness, Calzavara et al. (2015) suggested that the acclimation process can induce changes in physiological, anatomical, and morphological traits of plants, favoring their establishment after transplantation to the field. The results of the present study indicate that the lower A_n in the HL leaves of Feija were not related to the limited stomatal conductance. Furthermore, the higher A_n in Gaâfour leaves at full sunlight as compared to those of Feija, was consistently observed at all C_i values applied (Fig. 2). These findings do not exclude that diffusional limitations are important factors affecting photosynthesis. However, it does highlight that additional, non-diffusive limitations affect photosynthesis in Feija leaves compared to those of Gaâfour for seedlings transferred to HL condition. It is widely assumed that J_{max} and V_{cmax} parameters represent the major limitations to light-saturated photosynthesis (Long & Bernacchi 2003) and that the decrease in biochemical capacity (J_{max} and V_{cmax}) can limit A_n at high light, possibly by affecting nitrogen partitioning to Rubisco with increasing relative irradiance (Grassi & Bagnaresi 2001, Katahata et al. 2007, Fernàndez & Fleck 2016). Mechanistic photosynthetic models may be used to determine the impact of varying environmental conditions – including those predicted to be affected by climate change – on the biochemistry of photosynthesis and carbon acquisition at the leaf and plant levels (Patrick et al. 2009). The model by Farquhar et al. (1980) describes limitation processes in photosynthesis.

In this study V_{cmax} and J_{max} estimated from A_n/C_i curves were within the range observed for other Mediterranean species (Ghouil et al. 2003, Aranda et al. 2005, Niinements et al. 2006, Vaz et al. 2010). Correlations between the photosynthetic gas exchange parameters and SLA are shown in Tab. 2. The Rd, g_s, V_{cmax} and J_{max} were significantly and positively correlated with the A_n. The SLA and other gas exchange parameters were not significantly correlated with the A_n. This correlation analysis elucidates that V_{cmax} and J_{max} are the major contributors in the photosynthetic acclimation to HL conditions in cork oak.

The results from this study indicate that only Gaâfour seedlings were able to acclimate physiologically to a high light environment. This is in accordance with previous results indicating that the increase in V_{cmax} and J_{max} reflects a physiological plasticity at the cell levels caused by an increase in the photosynthetic capacity per unit of leaf tissue (Grassi & Bagnaresi 2001). The high photosynthetic capacity of HL leaves of Gaâfour was accompanied by a significant increase ($P < 0.05$) of the dark respiration.

A previous study showed that a large investment of nitrogen in photosynthetic enzymes (especially Rubisco) supports the increase of photosynthetic capacity in spinach leaves transferred from low to high growth irradiance (Terashima & Evans 1988). It also increases the respiration rate in wheat leaves (Makino & Osmond 1991). Oguchi et al. 2003 suggest that this increase in respiration rate in *Chenopodium album* is related to the maintenance processes of leaves, *e.g.*, protein turnover which consumes respiratory energy. The high dark respiration in HL leaves confirms the acclimation ability of Gaâfour seedlings to full sunlight, since variations in respiration have been widely proposed as a component of acclimation to photon availability (Pandey & Kushwaha 2005). Similar to A_n, the Rd was significantly and positively correlated to g_s, V_{cmax} and J_{max}.

A remarkable finding of the experiment was that the SLA did not differ among provenances or light treatments (Fig. 1), suggesting that leaf function can acclimate to changing light conditions despite no structural or morphological adjustments. A very common morphological response to high light is a lower SLA (or high leaf mass area), which is generally due to increased leaf thickness (Brooks et al. 1996). However, high photosynthetic capacity is not always accompanied by thicker leaves (Oguchi et al. 2003). Oguchi et al. (2003, 2005) explained the mechanism by showing that the transfer from low to high light increases the area of chloroplasts facing the intercellular space. The mesophyll cells of LL leaves had opened spaces along cell walls where chloroplasts were absent, which enabled the leaves to increase the photosynthetic maximum rate when they were exposed to high light. Leaf thickness determines an upper limit to the photosynthetic maximum rate of leaves subjected to a change from low to high light conditions. Shade leaves would only increase the photosynthetic maximum rate when they have open space to accommodate chloroplasts which elongate after light conditions improve, which possibly was not the case in Feija leaves. No significant correlation was observed between SLA and the gas exchange parameters (Tab. 2).

In conclusion, this finding corroborates previous results (Aranda et al. 2005, Ramírez-Valiente et al. 2014) showing a large

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provenance-level differentiation in cork oak with provenance from dry places exhibiting the higher tolerance.

List of abbreviations

A_{sat}: CO_2-saturated photosynthesis; HL: high light; LL: low light; PAR: photosynthetically active radiation; A_n: net photosynthesis; PPFD: photosynthetic photon flux density; LED: light emitting diode; C_a and C_i: external and intercellular CO_2 molar fractions; g_s: stomatal conductance; Γ: CO_2 compensation point; L_s: stomatal limitation; J_{max}: maximum electron transport rate; V_{cmax}: ribulose 1-5 biphosphate carboxylation.

References

Aranda I, Castro L, Pardos M, Gil L, Pardos JA (2005). Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak (*Quercus suber* L.) seedlings. Forest Ecology and Management 210: 117-129. - doi: 10.1016/j.foreco.2005.02.012

Balaguer L, Martinez-Ferri E, Pérez-Corona ME, Baquedano FJ, Castillo FJ, Manrique E (2001). Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. Functional Ecology 15: 124-135. - doi: 10.1046/j.1365-2435.2001.00505.x

Bernacchi CJ, Singsass EL, Pimentel C, Portis AR, Long SP (2001). Improved temperature response function for models of Rubisco-limited photosynthesis. Plant, Cell and Environment 24: 253-259. - doi: 10.1111/j.1365-3040.2001.00668.x

Boardman NK (1977). Comparative photosynthesis of sun and shaded plants. Annual Review of Plant Physiology 28: 355-77. - doi: 10.1146/annurev.pp.28.060177.002035

Brooks JR, Sprugel DG, Hinckley TM (1996). The effects of light acclimation during and after foliage expansion on photosynthesis of *Abies amabilis* foliage within the canopy. Oecologia 107: 21-32. - doi: 10.1007/BF00582231

Calzavara AK, Bianchini E, Mazzanatti T, Oliveira HC, Stolf-moreira R, Pimenta JA (2015). Morphology and ecophysiology of tree seedlings in semideciduous forest during high-light acclimation in nursery. Photosynthetica 53: 597-608. - doi: 10.1007/s11099-015-0151-0

Colin MO, Clive GJ (2001). Plants and resource mosaics: a functional model for predicting patterns of within-plant resource heterogeneity to consumers based on vascular architecture and local environmental variability. Oikos 94: 493-504. - doi: 10.1034/j.1600-0706.2001.940311.x

Evans JR, Poorter H (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant, Cell and Environment 24: 755-767. - doi: 10.1046/j.1365-3040.2001.00724.x

Evans JR, Seemann JR (1989). The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences, and control. In: "Photosynthesis" (Briggs WR ed). Liss, New York, USA, pp. 183-205.

Farquhar GD, Sharkey TD (1982). Stomatal conductance and photosynthesis. Annual Review of Plant Physiology 33: 317-345. - doi: 10.1146/annurev.pp.33.060182.001533

Farquhar GD, Von Caemmerer S, Berry JA (1980). A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149: 78-90. - doi: 10.1007/BF00386231

Fernàndez MJ, Fleck I (2016). Photosynthetic limitation of several representative subalpine species in the Catalan Pyrenees in summer. Plant Biology 18 (4): 638-648. - doi: 10.1111/plb.12439

Ghouil H, Montpied P, Epron D, Ksontini M, Hanchi B, Dreyer E (2003). Thermal optima of photosynthetic functions and thermostability of photochemistry in crok oak seedlings. Tree Physiology 23: 1031-1039. - doi: 10.1093/treephys/23.15.1031

Grassi G, Bagnaresi U (2001). Foliar morphological and physiological plasticity in *Picea abies* and *Abies alba* saplings along a natural light gradient. Tree Physiology 21: 959-967. - doi: 10.1093/treephys/21.12-13.959

Gratani L (2014). Plant phenotypic plasticity in response to environmental factors. Advanced in Botany 2014: 1-17. - doi: 10.1155/2014/208747

Hanba YT, Kogami H, Terashima I (2002). The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. Plant, Cell and Environment 25: 1021-1030. - doi: 10.1046/j.1365-3040.2002.00881.x

Harley PC, Sharkey TD (1991). An improved model of C3 photosynthesis at high CO_2: reversed O_2 sensitivity explained by lack of glycerate re-entry into the chloroplast. Photosynthesis Research 27: 169-178. [online] URL: http://link.springer.com/article/10.1007/BF00035838

Katahata SI, Naramoto M, Kakubari Y, Mukai Y (2007). Photosynthetic capacity and nitrogen partitioning in foliage of the evergreen shrub *Daphniphyllum humile* along a natural light gradient. Tree Physiology 27: 199-208. - doi: 10.1093/treephys/27.2.199

Long SP, Bernacchi CJ (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. Journal of Experimental Botany 54: 2393-2401. - doi: 10.1093/jxb/erg262

Makino A, Osmond B (1991). Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. Plant Physiology 96: 355-362. - doi: 10.1104/pp.96.2.355

Matesanz S, Valladares F (2014). Ecological and evolutionary responses of Mediterranean plants to global change. Environmental and Experimental Botany 103: 53-67. - doi: 10.1016/j.envexpbot.2013.09.004

Niinements U, Cescatti A, Rodeghiero M, Tosens T (2006). Complex adjustments of photosynthetic potentials and internal diffusion conductance to current and previous light availabilities and leaf age in Mediterranean evergreen species *Quercus ilex*. Plant, Cell and Environment 29: 1159-1178. - doi: 10.1111/j.1365-3040.2006.01499.x

Oguchi R, Hikosaka K, Hirose T (2003). Does the photosynthetic light-acclimation need change in leaf anatomy? Plant, Cell and Environment 26: 505-512. - doi: 10.1046/j.1365-3040.2003.00981.x

Oguchi R, Hikosaka K, Hirose T (2005). Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. Plant, Cell and Environment 28: 916-927. - doi: 10.1111/j.1365-3040.2005.01344.x

Pandey S, Kushwaha R (2005). Leaf anatomy and photosynthetic acclimation in *Valeriana jatamansi* L. grown under high and low irradiance. Photosynthetica 43: 85-90. - doi: 10.1007/s11099-005-5090-8

Patrick LD, Ogle K, Tissue DT (2009). A hierarchical Bayesian approach for estimation of photosynthetic parameters of C3 plants. Plant, Cell and Environment 32: 1695-1709. - doi: 10.1111/j.1365-3040.2009.02029.x

Pearcy RW, Sims AD (1994). Photosynthetic acclimation to changing light environments: scaling from the leaf to the whole plant. In: "Exploitation of Experimental Heterogeneity by Plants: Ecophysiological Process Above- and Below-ground" (Caldwell MM, Pearcy RW eds). Academic Press, San Diego, CA, USA, pp. 145-174. - doi: 10.1016/B978-0-12-155070-7.50010-X

Price GD, Von Caemmerer S, Evans JR, Siebke K, Anderson JM, Badger MR (1998). Photosynthesis is strongly reduced by antisense suppression of chloroplastic cytochrome bf complex in transgenic tobacco. Australian Journal of Plant Physiology 25 (4): 445. - doi: 10.1071/PP97164

Ramírez-Valiente JA, Valladares F, Sánchez-Gómez D, Delgado A, Aranda I (2014). Population variation and natural selection on leaf traits in cork oak throughout its distribution range. Acta Oecologica 58: 49-56. - doi: 10.1016/j.actao.2014.04.004

Ramirez-Valiente JA, Sanchez-Gómez D, Aranda I, Valladarres F (2010). phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. Tree Physiology 30: 618-627. - doi: 10.1093/treephys/tpq013

Sharkey TD (1985). Photosynthesis in intact leaves of C3 plants: physics, physiology and rate limitations. The Botanical Review 51: 53-105. - doi: 10.1007/BF02861058

Sims DA, Pearcy WP (1992). Response of leaf anatomy and photosynthetic capacity in *Alocasia macrorrhiza* (Araceae) to a transfer from low to high light. American Journal of Botany 79: 449-455. - doi: 10.2307/2445158

Sims DA, Seemann JR, Luo Y (1998). The significance of differences in mechanisms of photosynthetic acclimation to light, nitrogen and CO_2 for return on investment in leaves. Functional Ecology 12 (2): 185-194. - doi: 10.1046/j.1365-2435.1998.00194.x

Staudt M, Ennajah E, Mouillot F, Joffre R (2008). Do volatile organic compound emissions of

Tunisian cork oak populations originating from contrasting climatic conditions differ in their responses to summer drought? Canadian Journal of Forest Research 38: 2965-2975. - doi: 10.1139/X08-134

Sun Y, Zhu J, Sun OJ, Yan Q (2016). Photosynthetic and growth responses of *Pinus koraiensis* seedlings to canopy openness: implications for the restoration of mixed-broadleaved Korean pine forests. Environmental and Experimental Botany 129: 118-126. - doi: 10.1016/j.envexpbot.2016.02.005

Terashima I, Evans JR (1988). Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. Plant and Cell Physiology 29: 143-155. - doi: 10.1093/oxfordjournals.pcp.a077461

Terashima I, Miyazawa SI, Hanba YT (2001). Why are sun leaves thicker than shade leaves? Consideration based on analyses of CO_2 diffusion in the leaf. Journal of Plant Research 114 (1): 93-105. - doi: 10.1007/PL00013972

Vaz M, Pereira JS, Gazarini LC, David TS, David JS, Rodrigues A, Marocco J, Chaves MM (2010). Drought-induced photosynthetic inhibition and autumn recovery in two Mediterranean oak species (*Quercus ilex* and *Quercus suber*). Tree Physiology 30: 946-956. - doi: 10.1093/treephys/tpq044

Watling JR, Press MC (2000). Infection with the parasitic angiosperm *Striga hermonthica* influences the response of the C3 cereal *Oryza sativa* to elevated CO_2. Global Change Biology 6: 919-930. - doi: 10.1046/j.1365-2486.2000.00366.x