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Full Length Research Paper

Physiological and biochemical traits of different water and light intensities on cork oak (*Quercus suber* L.) seedlings

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The present study was conducted to assess the impacts of different water and light intensities on the physiological and the biochemical aspects of cork oak (*Quercus suber* L.) seedlings from acorns collected from Tabarka (Northern Tunisia). The experiments were divided into two treatments: Water stress (well-watered: 25 to 35%, moderate drought stress: 15 to 20%, and severe drought stress: 7 to 10%) and light intensity stress (full sunlight: 100% of full sunlight, 1,200 µmol m^{-2} s^{-1}, moderate sunlight: 65 to 70% of full sunlight, 800 µmol m^{-2} s^{-1}, low sunlight: 20 to 25% of full sunlight, 300 µmol m^{-2} s^{-1}, and extremely low sunlight: 3 to 4% of full sunlight, 40 µmol m^{-2} s^{-1}). Proline accumulation was higher in the severe drought stress and extremely low sunlight compared with other water and light intensity levels. Total nitrogen concentration (T-N) on a dry mass basis was significantly higher in seedlings grown in the low sunlight and the extremely low sunlight than in the full sunlight and the moderate sunlight. The photosynthetic rate (P_N), the stomatal conductance (*G*s), and the transpiration rate (*T*r) of the seedlings of *Q. suber* showed similar tendencies both in the three drought water levels and in the four light intensity levels during treatment periods, while the water use efficiency (WUE) was not significantly different among each treatment. P_N for the full sunlight was lower at a maximum value (≈ 8.2 µmol m^{-2} s^{-1}) compared with the moderate sunlight. On the other hand, the maximum photosynthesis was higher for the moderate sunlight seedlings when compared to the full sunlight seedlings, although the leaf from the full sunlight was light saturated at near 1000 µmol m^{-2} s^{-1} compared with the moderate sunlight. In all treatments, both maximum Rubisco activity and electron transport capacity expressed from the *A*/*C*i response curves (V_{cmax} and J_{max}) increased with well-watered (WW) and the full sunlight (FL). Under water treatments, the slopes and explained variances for severe drought stress were considerably lower than those under WW and moderate drought stress (MDS) at 60 and 90 days of treatment. In conclusion, the different water and light intensity levels affected the physiological and the biochemical parameters of *Q. suber*. The lower assimilation rate was associated with lower stomatal conductance, the nitrogen allocation to photosynthetic functions, maximal Rubisco activity (V_{cmax}) and electron transport rate (J_{max}).

Key words: Water, light intensity, relative water content, proline content, total nitrogen content, chlorophyll content, photosynthetic rate, maximum Rubisco activity, maximum electron transport capacity, *Quercus suber* L.

INTRODUCTION

A sclerophyllous tree, cork oak (*Quercus suber* L.), distributes within the habitat type of a quite narrow geographical range as compared with the other Mediterranean-climate evergreen oak species such as *Quercus coccifera* including *Quercus calliprinos* (holly oak) and *Quercus ilex* (holm oak) (Lumaret et al., 2005). Cork oak woodland (444 hectares, 70% of land utilization) is a major factor in Tunisia due to forestland with small treeless areas (Campos et al., 2008).

During the past two decades, the substantial decline phenomena of *Quercus* spp. forests have often been

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observed over wide-area of central Europe and sub-Mediterranean basin (Barbero et al., 1990; Führer 1998; Thomas et al., 2002). Under Mediterranean climate conditions, summer drought is the one of the most important environmental restriction factors (Larcher, 2000).

Light interception by canopy-shade is the most important ecological filter limiting understory plant establishment in closed-canopy forest. Thus, plants exhibit many morphological and physiological adaptations to cope with these environmental stresses (Aranda et al., 2005; Quero et al., 2006). Oak species also possess various effective morphological and physiological adaptations such as smaller leaf size, thicker leaf thickness, thicker cuticle thickness, greater stomatal density and decreased stomatal size that improve drought resistance, decrease photochemical damage, and contribute to minimal degree of photosynthesis under drought stress (Abrams, 1990).

A relative water content (RWC) estimating the degree of water stress caused by drought can be physiologically quantified by either the leaf water potential or the relative leaf area (Hunt et al., 1987; Mayak et al., 2004). The effects of proline, an osmoprotectant, which is known to be accumulated under osmotic stress such as salinity and water stress, have been suggested to osmotic compensation by an increase in osmotically active solutes per cell (Lansac et al., 1994). Physiological and biochemical study of plant species grown under several environmental stresses such as water-deficit and shaded understory is one of the most frequently discussed topics in the ecosystem-management and restoration. The physio-biochemical studies are also important to establish the basic data about cork oak decline to regeneration capacity investigation in dry and hot weather during summer for *Q. suber* seedlings grown under Mediterranean-climate zone. A systematic study by the influences of abiotic stresses such as the increasing shade and drought on leaf morphological and physiological properties in relation to the leaf and whole-plant growth and productivity has been performed in the previous study of cork oak (Aranda et al., 2005; Valladares et al., 2005; Quero et al., 2006) but no such knowledge of the biochemical and ultrastructural properties exhibits for cork oak. A careful consideration of the eco-physiological specialty of oak species (*Quercus* spp.) on the effects of various water-deficit levels and irradiances is the first important step in order to gain a better understanding of the oak decline phenomenon (Führer, 1998). Photosynthetic capacity measured as leaf maximum carboxylation rate (V_{cmax}) and leaf maximum electron transport rate (J_{max}) is a key factor in *in vivo* estimate of biochemical parameters of gas exchange models applied under different experimental conditions (Wang et al., 2008).

Therefore, in Mediterranean area, a physiological and biochemical model to describe the decline symptoms of cork oak is important to assess the fundamental phases of the photosynthetic parameters in both the exogenous abiotic and the endogenous physiological factors. The biochemical and physiological mechanisms regulating this interaction are not completely understood and this limits our capacity to predict the response of vegetation to future environmental changes.

The main objective of this chapter was to assess the biochemical and physiological aspects in response to different water and light intensity on *Q. suber* seedlings.

MATERIALS AND METHODS

Experimental description and plant material

The study was carried out on cork oak (*Q. suber* L.) seedlings growing in two nursery stands located in the greenhouse at the University Campus (University of Seoul, Seoul). *Q. suber* seedlings were prepared with acorns collected from Ain Snoussi, Northern Tunisia.

Seedlings, four-months after germination, were planted in 3-L plastic pots in April 2008 and placed in greenhouse of University of Seoul. The 3-L pots is filled with an artificial soil (Perlite+Vermiculite+Peatmoss, 1:1:1 [v/v/v]) with soil pH 5, and electrical conductivity (EC) 15.4 µS cm^{-1}. The experimental design in the greenhouse was performed with a completely randomized design of 18 replicates per treatment with three irrigations and four light regimes for five months.

Seedlings were subjected to three irrigation regimes: Well-watered (WW, 25 to 35%), moderate drought stress (MDS, 15 to 20%), and severe drought stress (SDS, 7 to 10%) regime. Soil moisture was recorded three times a week by time domain reflectometry (TDR, Trase System I, Soil Moisture Equipment Corp. USA). Seedlings under four light regimes with 18 replicates were randomly divided according to the different layers of shade cloth screening. The light intensity (the photosynthetic photon flux density, PPFD) of full sunlight was measured on a sunny day with a quantum sensor (type SKP 215, Skye Instruments, Powys, UK). The average photosynthetic photon flux density (PPFD, *Q*) under each light intensity regime during a sunny day was: FL, full sunlight (100% of full sunlight, 1,200 µmol $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$); ML, moderate sunlight (65 to 70% of full sunlight, 800 µmol $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$); LL, low sunlight (20 to 25% of full sunlight, 300 µmol $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$); ELL, extremely low sunlight (3 to 4% of full sunlight, 40 µmol $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$). Seedlings were grown in a randomized block design with metal frames to produce three irrigation and four light intensity regimes.

Physiological traits

Leaf water status (*RWC, relative water content*)

Relative water content (RWC) was a useful measure of the physiological water status estimating the degree of water stress caused by drought (Mayak et al., 2004). Sampling was done at pre-dawn, and only mature leaves were utilized for measurement. The different leaf samples were weighed (0.5 g) to determine fresh weight. Turgid weight of the samples was determined by the tissues in flasks containing tri-distilled water and maintaining them in darkness at 4°C (to minimize respiration losses) until they reached a constant weight. Full turgor was typically reached after 48 h. Dry weight was obtained after placing the samples in a hot air oven at 75°C for 48 h. Five to six replicates were used for each treatment. Relative water content (RWC) was calculated using the formula of Weatherley (1950) as follows:

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

Proline determination

Free proline content was determined as described by the procedures of Bates et al. (1973) and Tamayo and Bonjoch (2001). Leaves collected for determination of the proline were similarly prepared with physiological age collected for total nitrogen. Each 0.5 g sample of frozen tissue (leaves) was homogenized in a mortar with 5 ml of 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was centrifuged with 15,000 g at 4°C for 10 min. The supernatant (2 ml) of brain homogenates after centrifugation was reacted by incubating with 2 ml of acid-ninhydrin (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid, and adding 20 ml 6 M phosphoric acid: Acid ninhydrin was kept stable only for 24 h at 4°C) and 2 ml of glacial acetic acid for 1 h at 100°C, and the reaction was terminated in a ice bath. The reaction mixture was extracted with 4 ml toluene (mixed vigorously on a vortex mixer for 15 to 20 s). One milliliter of the chromophore-containing toluene was pipetted for spectrophotometric measure at room temperature. Its optical density was measured using a UV-Vis spectrophotometer (Optizen 2120 UV, Mecasys, Korea) at 520 nm.

As previously described, the proline standard curve was established using a standard curve of L-proline solutions derived from 0.0, 1.9, 7.8, 15.62, 31.25, 62.5 and 125 μg ml^{-1} concentration.

Total nitrogen

Two to three leaves per five seedlings of each treatment, including the leaves used for gas exchange measurements, were immediately harvested for determining total nitrogen content and pigment content.

Three mature leaves were randomly sampled just above the point. Leaves were packed in bags and immediately carried to the laboratory. After measurement of leaf area (excluding the petiole) and leaf fresh mass, leaves were dried at 75°C for 48 h for leaf dry mass estimation. These same leaves finely ground with a mill (1.5 mm mesh) and used for the determination of total nitrogen content by the Kjeldahl method (using $H_2SO_4/HClO_4$ as a digestion accelerator) using a Foss Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). Total nitrogen content was expressed to percentage dry weight of plant material (Baker and Thompson, 1992; Horneck and Miller, 1998).

Pigment analysis (*Chlorophyll contents and total carotenoids*)

Leaf discs were taken for the quantitative determination of a whole pigment (Chl_a, chlorophyll a; Chl_b, chlorophyll b; Chl_T, total chlorophyll; Car_T, total carotenoids). Leaf samples of 0.1 g discs were extracted using 8 ml with 80% acetone solution at 4°C for 7 days.

The ratio of Chl_a and Chl_b (Chl_a/Chl_b ratio) is known to indicate the quantitative function of the pigment apparatus and light adaptation of the photosynthetic apparatus (Lichtenthaler et al., 1981).

The amounts of Chl_a, Chl_b, Chl_T, and Car_T, total carotenoids were determined as the method of Lichtenthaler (1987) using a UV-Vis spectrophotometer (Optizen 2120 UV, Mecasys, Korea) by reading the absorbance at wavelengths of 663, 647 and 470 nm, respectively. The equations of Chl and Car content per fresh mass were calculated by Lichtenthaler and Buschmann (2001):

Acetone with 20% (v/v) water:

$$Chl_a = 12.25 \times A_{663} - 2.79 \times A_{647}$$

$$Chl_b = 21.50 \times A_{647} - 5.10 \times A_{663}$$

$$Chl_T = 7.15 \times A_{663} - 18.71 \times A_{647}$$

$$Car_T = 1000 \times A_{470} - 1.82 \times Chl_a - 85.02 \times Chl_b$$

where A_x is the absorbance of the extract solution in a 1 cm path length cuvette at wavelength x. Units for all of the aforementioned equations are μg g^{-1}.

Photosynthetic water use efficiency (*PWUE*)

Instantaneous photosynthetic water use efficiency (PWUE, ratio of photosynthesis: transpiration rates on unit leaf area basis) and intrinsic transpiration efficiency (TE) were calculated using the following formula (Cernusak et al., 2007; Condon et al., 2002; Pou et al., 2008):

$$\text{PWUE } (\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}) = A\ (\mu\text{mol m}^{-2}\text{ s}^{-1}) \,/\, Tr\ (\text{mmol H}_2\text{O m}^{-2}\text{ s}^{-1})$$

where *A* is the leaf net CO_2 assimilation rate (μmol m^{-2} s^{-1}) and *T*r is the transpiration rate (mmol H_2O m^{-2} s^{-1}).

The leaf net CO_2 assimilation rate (*A*) and transpiration rate (*T*r) were measured with an open gas-exchange system at an ambient CO_2 concentration of 400 μmol mol^{-1}, 50 to 60% relative humidity, 25°C, and a photosynthetic photon flux density (PPFD, *Q*) of 1500 μmol m^{-2} s^{-1} using infrared gas analyzer a Li-Cor 6400 photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA).

Gas exchange measurements

The calculations of the leaf gas exchange measurements were performed as described by Farquhar et al. (1980). The leaf gas exchange measurements (that is, the leaf net CO_2 assimilation rate, *A*; the stomatal conductance, *G*s; the transpiration rate, *T*r; the internal CO_2 concentration, *C*i) were conducted under cloudless sky in the greenhouse conditions using a LI-6400 Portable Photosynthetic System (Li-Cor Inc., Lincoln, NE, USA), measured at an ambient CO_2 concentration of 400 μmol mol^{-1}, 50 to 60% relative humidity, 25°C, and a photosynthetic photon flux density (PPFD, *Q*) of 1500 μmol m^{-2} s^{-1}, and taken in four consecutive seasons in seedlings grown under three water and four shade levels in April to September, 2008.

Measurements of photosynthetic and physical parameters were defined from approximately 08:00 to 11:00 h in the morning on the third or fifth fully expanded leaf from apex with cloudless days to minimize water loss according to midday stomatal closure (preliminary measurements indicated no evidence of midday stomatal closure) (Aleric and Kirkman, 2005).

Biochemical traits

Light response curves (*the A/Q curves*)

Two sets of photosynthetic measurements, *A*/*C*i and *A*/*Q* curves, were conducted according to the model of leaf photosynthesis proposed by Feng et al. (2007): the response of leaf photosynthetic CO_2 uptake per unit leaf area (*A*, μmol m^{-2} s^{-1}) to intercellular CO_2 concentration (*C*i, μmol mol^{-1}), and to photosynthetic photon flux density (PPFD: *Q*, μmol m^{-2} s^{-1}) as measured in gas exchange systems, respectively.

The *A*/*C*i curves were measured at 0, 30, 40, 60, 80, 100, 120, 150, 200, 300, 400 and 600 μmol mol^{-1} CO_2 under a *Q* of 2,000 μmol m^{-2} s^{-1}, in the reference chamber with a Li-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA). The *A*/*Q* curves were generated at a *Q* of 0, 30, 50, 100, 150, 300, 500, 800, 1100, 1500 and 2000 μmol m^{-2} s^{-1} and with constant CO_2 concentration 400 μmol mol^{-1} in the cuvette. P_N, *T*r and *C*i were recorded when the sample leaf was balanced for 200 s under each CO_2 step.

P'_{max}, light- and CO_2- saturated photosynthetic rate in μmol m^{-2} s^{-1}, was determined after 500 s under 2,000 μmol m^{-2} s^{-1} (*Q*) and 600 μmol mol^{-1} (CO_2). Maximum carboxylation rate was calculated from *A*/*C*i curves by nonlinear regression methods based on the model of Farquhar et al. (1980).

Measurement of A-Ci curve and estimation of derived parameters, V_{cmax} and J_{max}

The rate of maximum Rubisco carboxylation (V_{cmax}, μmol m^{-2} s^{-1}) was derived by estimates related to the initial slope of the *A*/*C*i curve.

The *A*/*C*i curve was obtained by extrapolating the linear fitted model using the linear equation ($A = k\,C\text{i} + \text{i}$) within 50 to 200 μmol mol^{-1} *C*i, where *k*, the initial slope of the *A*/*C*i curve, may be described as CE (carboxylation efficiency), and –i/*k* was equal to Γ* (CO_2 compensation point in μmol mol^{-1}) in the absence of the mitochondrial respiration.

The parameters derived from the *A*/*C*i curves such as V_{cmax} and R_d (the rate of dark respiration in μmol m^{-2} s^{-1}) were calculated from the following equations (Farquhar and Sharkey, 1982; Feng et al., 2007):

$$V_{cmax} = \{k \times [C\text{i} + K_c \times (1 + O / K_o)]^2 / [\Gamma^* + K_c \times (1 + O / K_o)]\}$$

$$R_d = \{V_{cmax} \times (C\text{i} - \Gamma^*) / [C\text{i} + K_c \times (1 + O / K_c)] - (k \times C\text{i} + \text{i})\}$$

where K_c and K_o were 404.9 and 278.4 mmol mol^{-1} at 25°C, respectively (Bernacchi et al., 2001), and *O* was 210 mmol mol^{-1}. J_{max}, the rate of maximum electron transport (μmol m^{-2} s^{-1}) related to the plateau of the *A*/*C*i curve, was calculated using the following equation (Loustau et al., 1999):

$$J_{max} = \{[4 \times (P'_{max} + R_d) \times (C\text{i} + 2 \times \Gamma^*)] / (C\text{i} - \Gamma^*)\}$$

Statistical analysis

The effects of water and light intensity stress on the different morphological and anatomical parameters of the seedlings were analysed separately for each extraction by a multifactor analysis of variance (ANOVA) using the version 9.2 of the SAS statistical software package (Systat 9.2, Systat Software Inc., Richmond, USA). The test for differences among arithmetic treatment means were performed by the least significant difference (LSD) multiple range test method. The significance levels were set at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Physiological traits

Leaf water status (*RWC, relative water content*)

A relative water content (RWC) estimating the degree of water stress caused by drought can be physiologically quantified by either the leaf water potential or the relative leaf area (Mayak et al., 2004; Hunt et al., 1987).

Relative water content (RWC) was calculated according to the formula below: RWC = [(FW, fresh weight - DW, dry weight) / (TW, turgid weight - DW, dry weight)] × 100. The leaf water status, as measured by RWC, varied significantly among water treatments (Figure 1A), but there were no significant differences among shade treatments (Figure 1B).

In water treatments (Figure 1A), a reduction in RWC of *Q. suber* seedlings occurred compared with the well-watered (WW) treatment. However, the shade treatments showed similarly patterns of RWC and the lowest values were observed during the low sunlight (LL) treatment. Full sunlight (FL) treatment had a better RWC maintenance than other shade treatments (ML, LL, and ELL). The relative water content (RWC) is a good indicator, as a useful measure of the physiological water status of seedlings, resulting in a water stress situation reflected by a reduction in RWC (Mayak et al., 2004).

Rhizopoulou et al. (1991) reported that the minimum values of RWC were observed during the entire summer drought period. The indices of leaf water status showed that severe drought stress (SDS) had lower RWC, indicating higher water loss, compared with the other treatments.

When stomata are tightly closed in leaves exposed to direct sunlight for most of the day, the contribution of transpiration against water loss may be important in Mediterranean-climate species during the hot and dry summer (Bacelar et al., 2004). Jorba et al. (1984) reported that a reduction in RWC induced a reduction in photosynthesis of potted olive trees. Therefore, the low RWC of SDS in *Q. suber* probably induced the inhibition of photosynthesis during the hot-dry summer.

Proline determination

Plants activate relatively various metabolic pathways. They also have defense systems to exist in response to different environmental stresses such as drought and shade. The accumulation of the major compatible osmoprotectant solutes frequently is induced in plants and microorganisms by different stresses such as water stress and salinity stress (Tamayo and Bonjoch, 2001). In the present study, free proline accumulation was remarkable in *Q. suber* seedlings in response to different water and light intensity (Figure 2). The severe drought stress (SDS) treatment caused a significant increase in leaf proline concentration in *Q. suber* seedlings (Figure 2A).

In well-watered (WW) conditions, proline concentration showed similar values both in June (60 days after treatment) and in July (90 days). Proline level showed the same value (≈ 3.0 μmol g^{-1} fresh wt) in seedlings grown under WW and moderate drought stress (MDS) (Figure 2A). After a period of 90 days with water stress, the

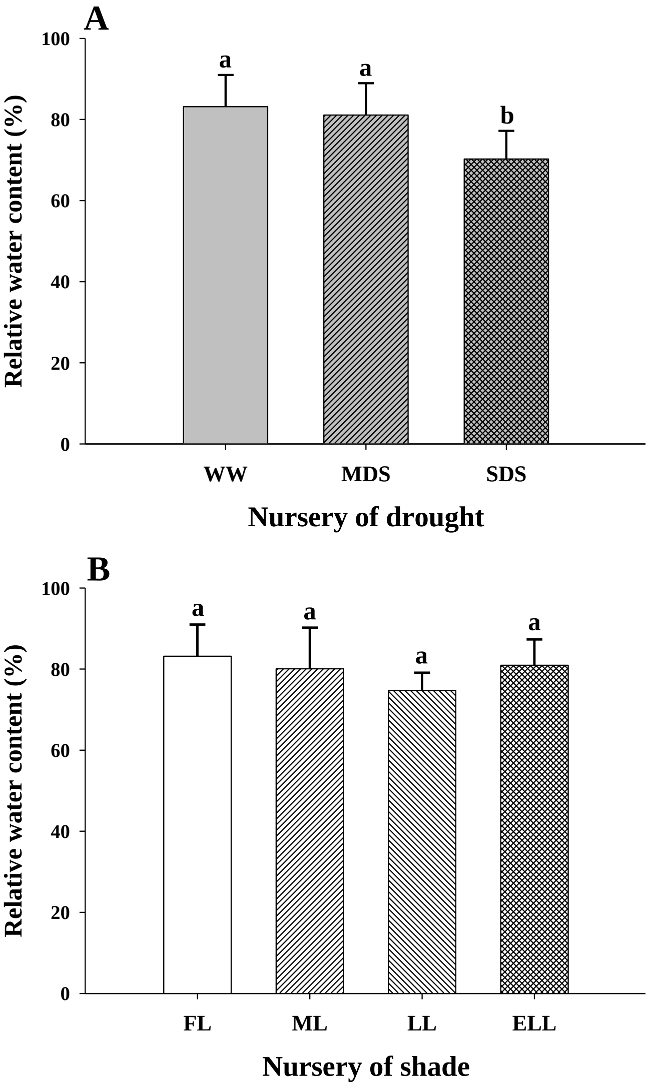

Figure 1. Relative water content (RWC) of the leaves of *Q. suber* seedlings subjected to different levels of water (A) and light intensity (B) at the end of the experiment. Vertical bars indicate ± SD of mean (n = 5): The same letter are not significantly different at $P \leq 0.05$ according to the least significant difference (LSD) multiple range test. WW, well-watered; MDS, moderate drought stress; SDS, severe drought stress; FL, full sunlight; ML, moderate sunlight; LL, low sunlight; ELL, extremely low sunlight.

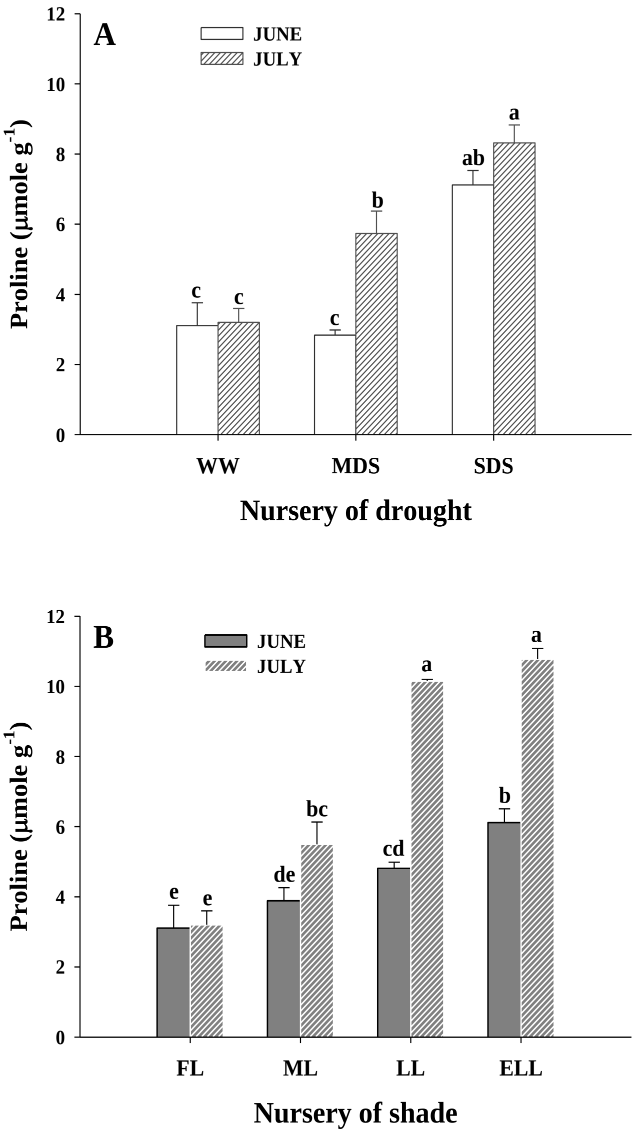

Figure 2. Proline concentration (µmol g^{-1} fresh wt.) of the leaves of *Q. suber* seedlings subjected to different levels of water (A) and light intensity (B) at the end of the experiment. Vertical bars indicate ± SE of mean (n = 3): The same letter are not significantly different at $P \leq 0.05$ according to the least significant difference (LSD) multiple range test. WW, well-watered; MDS, moderate drought stress; SDS, severe drought stress; FL, full sunlight; ML, moderate sunlight; LL, low sunlight; ELL, extremely low sunlight.

concentration of proline in severe drought stress (SDS) seedlings increased up to 8.3 µmol g^{-1} of leaf fresh weight compared to 5.7 µmol g^{-1} of leaf fresh weight observed in moderate drought stress (MDS) seedlings (Figure 2A).

The severe water stress induces irregularly numerous metabolic processes in plants. A tremendous free proline accumulation (up to 100 times the normal) is one of the most dramatic stress features; it has been used as a single parameter to measure physiological desiccation (Bates, 1973).

The role of the accumulation of compatible solutes such as free proline named ‘osmoprotectants’ in drought-stressed plants helps maintain turgor, facilitates physiological and biochemical processes with preservation of enzyme structure and activity, acts as scavenger of reactive oxygen species (ROS) and other free radicals, serve as a carbon and nitrogen storage compound. Furthermore, after water deficit ceases, degradation of accumulated free proline produces energy usable for the restoration of cell functions (Tamayo and Bonjoch, 2001).

However, Barker et al. (1993) described no correlation between proline accumulation and drought stress resistance, arguments being based on the physiological role of proline accumulation was uncertain because even dramatic increases in leaf proline concentration were insufficient to influence osmotic potential, suggested a little contribution to the osmotic adjustment in response to drought stress compared with other osmolytes such as the neutral sugars.

Proline accumulation by the water and the light intensity stress was observed both under the severe drought stress (SDS) treatment and under the extremely low sunlight (ELL) treatment (Figure 2). The previous authors have suggested that the proline concentration increase under drought stress (Oufir et al., 2009) and shade stress (Rhizopoulou et al., 1991) in Mediterranean *Quercus* species. The severe drought stress (SDS) treatment showed leaf proline concentration with a 2.6-fold greater compared with that of well-watered seedlings (Figure 2A). Similarly, in shade-induced treatments, ELL conditions indicated the larger contents than proline accumulation of seedlings grown under the full sunlight (FL) treatments (Figure 2B). The proline accumulation with a positive correlation between the increase of drought stress and the increase of proline was observed in Maize (Ibarra-Caballero et al., 1988).

This correlation indicated by the water status measured as RWC (that is, the positive correlation between the decreased RWC values and the increased proline content). Increased proline concentration directly related to drought stress resistance by osmotic adjustment in SDS treatment. During a period of drought stress may be affected a compensatory mechanism for a better seedling survival in the accumulation of the proline, as a reservoir of nitrogen and carbon sources which might help maintain physiological mechanism processes under drought stress environment (Tamayo and Bonjoch, 2001).

Proline accumulation was higher in ELL treatment compared with other shade treatments (Figure 2B). However, the accumulation of high proline under SDS and ELL treatment could not contribute a better recovery of these seedlings, suggesting that proline was not the major solute contribution to turgor maintenance (Figure 2).

The effect of osmoprotectant, proline, which is known to accumulate under osmotic stress such as salinity and water stress, has been suggested to osmotic compensation by an increase in osmotically active solutes per cell (Lansac et al., 1994). Tamayo and Bonjoch (2001) have considered that the proline accumulation may be only a pathological symptom of stress rather than an adaptive response. Nevertheless, the proline accumulation is a general response to the environmental stresses such as water and shade stresses, it contributes also as an important harbinger of chlorophyll and total nitrogen synthesis, and is able to contribute directly to the preservation of water in plants (Rhizopoulou et al., 1991).

Total nitrogen

Nitrogen contents of leaves are one of the most important components that determine the major physiological processes such as photosynthesis and respiration in higher plants (Makino and Osmond, 1991).

The total nitrogen concentration (T-N) on a dry mass basis was significantly higher in seedlings grown in the low sunlight (LL) and the extremely low sunlight (ELL) treatment than in the full sunlight (FL) and the moderate sunlight (ML) treatment (Figure 3B). Although leaf total nitrogen (T-N) changed significantly in shade treatments and did not show a significant change in water treatments (Figure 3A). The increase of total nitrogen with shade treatment was a consequence of the increment in the specific leaf area (SLA) with increasing shade (Figure 3B) (Aranda et al., 2007).

The previous results also have reported that the total nitrogen content showed the decreasing patterns on a leaf area basis (N_{area}) and was found the opposite trend in a leaf mass basis (N_{mass}) (Aranda et al., 2005; Jimenez et al., 2009). The total nitrogen variables showed strong interactions of light and water effects in the leaves of oak seedlings. Leaves of *Q. suber* seedlings revealed difference less than other *Quercus* species in specific leaf area (SLA) and in nitrogen under drought stress. The effects of shade on physiological variables were higher than the effects of drought (Quero et al., 2006).

In many species, the growth under the lower light increased greatly the partition of the nitrogen into the chlorophyll and the thylakoids (Evans, 1989; Evans and Poorter, 2001). Plants also could induce the adaptive mechanisms to their light environment at several integration levels such as the fraction of biomass, the investment of nitrogen. At the cellular level, the final level for acclimation is the re-allocation of nitrogen between the various pools in relation to the photosynthesis apparatus.

The total nitrogen concentrations per unit leaf mass were constant among the three water treatments, but seedlings grown under the extremely low sunlight (ELL) treatment partitioned a larger fraction of leaf nitrogen into light harvesting.

The most important features of leaves grown under high-light than those under low-light were:
(1) Less chlorophyll per unit nitrogen;
(2) A higher chlorophyll *a*:*b* ratio;
(3) An increased electron transport capacity per unit chlorophyll;
(4) A slightly greater ratio of electron transport capacity to Rubisco activity (Evans, 1989; Evans and Poorter, 2001).

The photosynthetic capacity of leaves was related to the nitrogen content primarily because the proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen (Evans, 1989; Evans and Poorter, 2001). However, irradiance had little effect on foliar nitrogen concentration (Niinemets et al., 1998).

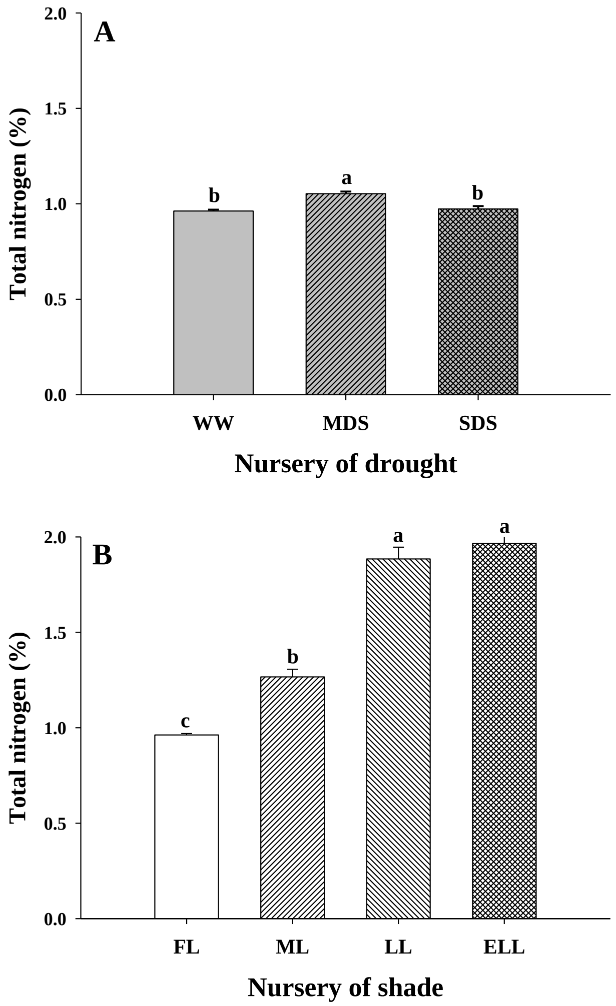

Figure 3. Total nitrogen contents of the leaves of *Q. suber* seedlings subjected to different levels of water (A) and light intensity (B) at the end of the experiment. Vertical bars indicate ± SD of mean (n = 3): The same letter are not significantly different at $P \leq 0.05$ according to the least significant difference (LSD) multiple range test. WW, well-watered; MDS, moderate drought stress; SDS, severe drought stress; FL, full sunlight; ML, moderate sunlight; LL, low sunlight; ELL, extremely low sunlight.

As a result, the higher total nitrogen contents of the extremely low sunlight (ELL) treatment might be able to induce the higher chlorophyll contents. However, the accumulation of high total nitrogen contents under the extremely low sunlight (ELL) treatment could not contribute to the recovery of the photosynthetic ability.

Pigment analysis (*Chls contents, Total carotenoids*)

The adaptative ability of plant in relation to water and light during growth may be associated with specific changes in the morphological, physiological, and biochemical characteristics of chloroplasts. The ratio of Chl$_a$ and Chl$_b$ (Chl$_a$/Chl$_b$ ratio) is known as indicator of the the energetic activity of of light harvesting complex II (LHC II) system in the pigment apparatus and light adaptation of the photosynthetic apparatus (Lichtenthaler et al., 1981).

As shown in Tables 1 and 2, chlorophyll contents of the seedlings of *Q. suber* showed statistically little differences among the three drought levels and the ratio chlorophyll a/b (Chl$_a$/Chl$_b$) and cartenoids contents (Car$_T$) was not significantly different among each treatment (Table 1). Chl$_b$ was contained only within the pigment antenna system, while Chl$_a$ existed in the reaction centers of PS I and II and in the pigment antenna (Lichtenthaler et al., 1981).

Chlorophyll contents (Chl$_a$, Chl$_b$, and Chl$_T$) of the extremely low sunlight (ELL) and the low sunlight (LL) seedlings was significantly greater compared with those of the full sunlight (FL) and the moderate sunlight (ML).

On the other hand, the extremely low sunlight (ELL) chloroplasts possessed the lower values for the ratio chlorophyll a/b (Chl$_a$/Chl$_b$) as compared to the full sunlight (FL) and the moderate sunlight leaves (ML), which exhibit higher values in the ratio of Chl$_a$/Chl$_b$ (Table 2).

The higher level for Chl$_a$/Chl$_b$ in sun-type chloroplasts indicated a lower level of the light-harvesting Chl$_a$/Chl$_b$ protein (LHCP) per a PSI chlorophyll a-protein complexs (CPI) than in shade-type chloroplasts. The lower value of the light harvesting Chl$_a$/Chl$_b$ protein of sun-type chloroplasts was related with smaller gran stacks and the lower stacking degree of thylakoids (Lichtenthaler et al., 1981). Therefore, as compared to the full sunlight (FL) leave, the lower value of Chl$_a$/Chl$_b$ for shade-type chloroplasts (LL and ELL) may be associated with the broader grana and a higher stacking degree.

The other authors reported that the photosynthetic apparatus of sun-type chloroplasts grown under sun light intensities contained the lower chlorophyll content per chloroplast, higher values for the ratio of chlorophyll a/b (Chl$_a$/Chl$_b$) (Young and Smith, 1980; Meier and Lichtenthaler, 1981; Lichtenthaler et al., 1981; Yang, 2007; Gregoriou et al., 2007).

In addition, sun-type leaves of *Q. suber* may possess a higher capacity of the photosynthetic apparatus, indicating the higher photosynthetic rate and the bio-chemical capacity (Tables 1 and 2), which was confirmed by ultrastructural aspects (Figure 6).

Gas exchange measurements

The changes induced by drought and shade stress in the photosynthetic rate (P_N), the stomatal conductance (Gs), the transpiration rater (Tr), and the water use efficiency (WUE) relationships are shown in Figure 4. Photo-synthetic parameters (P_N, Gs, and Tr) of both drought (Figures 4A, C and E) and shade stress (Figures 4B, D and F) seedlings varied with the treatment period (Figure 4). The photosynthetic rate, the stomatal conductance, and the transpiration rate of the seedlings of *Q. suber*

Table 1. Results of the mean values (± S.D.) for chlorophyll contents and biochemical traits, according to several water stresses for *Quercus suber* in June and July.

Month of Measurement	Parameters	Drought stress			
		WW^z	MDS	SDS	LDS
Chlorophyll contents					
June	$\mathrm{Chl}_a{}^y$	15.5 ± 0.7 ab^x	13.7 ± 0.7 b	17.9 ± 0.1 a	2.6840
	Chl_b	7.0 ± 0.2ab	6.3 ± 0.4 b	7.9 ± 0.0 a	1.1602
	Chl_T	22.6 ± 0.9ab	20.0 ± 1.2 b	25.9 ± 0.1 a	3.7758
	$\mathrm{Chl}_a/\mathrm{Chl}_b$	2.2 ± 0.1	2.2 ± 0.0	2.3 ± 0.0	
	Car_T	4.3 ± 0.3	3.7 ± 0.1	4.6 ± 0.1	
July	Chl_a	12.6 ± 0.8 ab	11.1 ± 0.8 b	15.5 ± 0.4 a	3.1482
	Chl_b	3.9 ± 0.4 ab	2.9 ± 0.3 b	4.9 ± 0.2 a	1.4457
	Chl_T	16.5 ± 1.2 ab	14.0 ± 1.1 b	20.4 ± 0.6 a	4.5747
	$\mathrm{Chl}_a/\mathrm{Chl}_b$	3.3 ± 0.2	3.8 ± 0.1	3.2 ± 0.0	
	Car_T	4.5 ± 0.3	4.1 ± 0.2	5.0 ± 0.1	
Biochemical traits					
June	P_N	11.5 ± 0.2 a	10.6 ± 0.1 b	6.0 ± 0.1 c	0.4708
	V_cmax	95.0 ± 3.5 a	86.1 ± 0.3 b	53.6 ± 0.4 c	5.9466
	J_max	92.4 ± 2.8 a	75.6 ± 0.4 b	39.4 ± 0.4 c	4.7528
July	P_N	10.7 ± 0.3 a	7.1 ± 0.2 b	4.7 ± 0.5 c	1.0804
	V_cmax	67.0 ± 0.5 a	68.7 ± 0.7 a	23.0 ± 2.4 b	5.1267
	J_max	102.5 ± 0.5 a	90.6 ± 0.7 b	38.0 ± 1.0 c	2.5290

^{z} W, well-watered; MDS, moderate drought stress; SDS, severe drought stress. ^{y} Chl_a, chlorophyll a; Chl_b lorophyll b; Chl_T, total chlorophyll; $\mathrm{Chl}_a/\mathrm{Chl}_b$, the ratio of Chl_a and Chl_b; Car_T, total carotenoids; P_N , photosynthetic rate; V_cmax, the maximum rate of carboxylation; J_max, the maximum rate of the electron transport. ^{X} Means (n=5) in a row followed by the same letter are not significantly different at $P \leq 0.05$ according to the least significant difference (LSD) multiple range test.

showed similar tendencies both under the three drought levels (Figures 4A, C and E) and under the four shade levels (Figures 4B, D and F) during treatment periods, while water use efficiency (WUE) was not significantly different among each treatment (Figures 4G and H). Figure 4 demonstrates that seedlings of well-watered (WW) treatment had a higher P_N and Tr compared with moderate drought stress (MDS) and severe drought stress (SDS) during the treatment period of 150 days (Figures 4A and E).

The trend in WUE induced by the drought stress was approximately similar (Figure 4G). Tree species grown under environmental stress such as in high temperature and in strong sunlight required the need to maintain the high photosynthetic water use efficiency (PWUE; P_N/Tr) (Mulkey et al., 1996). Separately, induced drought stress indicated that P_N (Figure 4A) and Tr (Figure 4E) were peaked after time intervals of 60 and 90 days, respectively. Water use efficiency (WUE, ratio between these traits) was observed a similar tendency toward a gradual decrease (the peak at 30 days) among treatments during the treatment period of 150 days (Figure 4G).

Q. suber seedlings grown under the extremely low sunlight (ELL) treatment increased their SLA (Figure 3), but they had necessarily a lower physiological performance, in terms of net photosynthetic rate and transpiration rate when subjected to the extremely low sunlight (ELL) treatment (Figures 4B and F).

In contrast, under similar water-provided conditions, the moderate sunlight (ML) seedlings were able to achieve higher photosynthetic rate when compared to the full sunlight (FL) treatment during the treatment period of 150 days (Figure 4B). In addition, the low sunlight (LL) and the extremely low sunlight (ELL), as well as full sunlight (FL) treatment, showed a gradual decrease in P_N after 60 days. In transpiration rate (Tr), shade-induced treatments (ML, LL, and ELL) were a similar tendency with the exception of the full sunlight (FL) treatment. WUE induced by the four shade treatments also found a similar tendency (Figure 4H).

As a result, both the severe drought stress (SDS) and the extremely low sunlight (ELL) types had fairly rapid decreases in photosynthesis during treatment periods. These differences are similar to values reported interactions of drought and shade effects on leaves of the *Q. pyrenaica* (Quero et al., 2006).

Under the severe drought stress (SDS), WUE improved at 120-day of the summer dry season than other treatments. The water-use efficiency can be defined as the ratio of the instantaneous rates of photosynthesis (P_N) and transpiration (Tr) at the stomata (Cernusak et al., 2007). Therefore, water-use efficiency (WUE), the ratio of carbon gain during CO_2 assimilation (A, μmol m^{-2} s^{-1}) to water loss during transpiration (Tr, mmol m^{-2} s^{-1}), were of major importance to the survival, productivity and fitness

Table 2. Results of the mean values (± S.D.) for chlorophyll contents and biochemical traits, according to several light stresses for *Quercus suber* in June and July.

Month of Measurement	Parameters	Shade stress				
		FL^z	ML	LL	ELL	LDS
Chlorophyll contents						
June	Chl$_a$^{y}	15.5 ± 0.7 c^x	26.1 ± 0.0 b	33.3 ± 0.4 a	32.9 ± 0.7 a	2.0814
	Chl$_b$	7.0 ± 0.2 c	11.2 ± 0.2 b	17.9 ± 0.6 a	16.6 ± 0.5 a	1.5879
	Chl$_T$	22.6 ± 0.9 b	37.4 ± 0.2 b	51.3 ± 1.0 a	47.7 ± 1.2 a	3.4651
	Chl$_a$/Chl$_b$	2.2 ± 0.1 a	2.3 ± 0.1 a	1.9 ± 0.0 b	1.9 ± 0.0 b	0.1675
	Car$_T$	4.3 ± 0.3 c	5.8 ± 0.0 b	6.4 ± 0.0 ab	7.1 ± 0.1 a	0.7004
July	Chl$_a$	12.6 ± 0.8 c	23.5 ± 1.3 b	30.9 ± 1.3 a	30.3 ± 1.0 a	4.3753
	Chl$_b$	3.9 ± 0.4 b	8.4 ± 0.6 b	16.9 ± 2.1 a	14.7 ± 1.5 a	5.3083
	Chl$_T$	16.5 ± 1.2 c	31.8 ± 1.9 b	47.7 ± 3.4 a	44.9 ± 2.5 a	9.3876
	Chl$_a$/Chl$_b$	3.3 ± 0.2 a	2.8 ± 0.0 a	1.9 ± 0.2 b	2.0 ± 0.1 b	0.5395
	Car$_T$	4.5 ± 0.3 c	6.4 ± 0.3 b	6.9 ± 0.2 ab	7.7 ± 0.2 a	0.9902
Biochemical traits						
June	P_N	11.5 ± 0.2 b	12.2 ± 0.2 a	11.8 ± 0.4 a	4.5 ± 0.1 b	0.9433
	V_{cmax}	95.0 ± 3.5 a	73.6 ± 0.4 b	44.2 ± 0.2 c	9.8 ± 0.2 d	5.0925
	J_{max}	92.4 ± 2.8 a	87.9 ± 0.5 a	72.9 ± 0.2 b	45.7 ± 3.6 c	6.6139
July	P_N	10.7 ± 0.3 b	13.1 ± 0.2 a	6.9 ± 0.3 c	2.0 ± 0.2 d	1.5453
	V_{cmax}	67.0 ± 0.5 b	75.3 ± 0.2 a	62.1 ± 4.8 b	27.5 ± 1.2 c	8.1304
	J_{max}	102.5 ± 0.5 a	99.5 ± 0.1 a	88.7 ± 3.8 b	46.8 ± 0.3 c	6.1999

^{z} FL, full sunlight; ML, moderate sunlight; LL, low sunlight; ELL, extremely low sunlight. ^{y} Chl$_a$, chlorophyll a; Chl$_b$, chlorophyll b; Chl$_T$, total chlorophyll; Chl$_a$/Chl$_b$, the ratio of Chl$_a$ and Chl$_b$; Car$_T$, total carotenoids; P_N, photosynthetic rate; V_{cmax}, the maximum rate of carboxylation; J_{max}, the maximum rate of the electron transport. ^{X} Means (n=5) in a row followed by the same letter are not significantly different at $P \leq 0.05$ according to the least significant difference (LSD) multiple range test..

of individual plants (Ni and Pallardy, 1991).

Moreover, under water-limiting drought environments, growth and yield productivity in plants may be increased by improving WUE (biomass to water loss ratio) (Ebdon and Kopp, 2004) and may be essential to avoid drought stress by enhancing WUE (Deng et al., 2006).

However, the higher value of WUE in severe drought stress (SDS) treatment was interpreted as due to lower transpiration rate by stomata closure as summer drought progress. Therefore, when seedlings were imposed under severe drought stress conditions, a reduction in the biochemical capacity for carbon assimilation and utilization might occur with restriction in gaseous diffusion (Yordanov et al., 2000).

In addition, to allow the uptake of CO_2, the stomata permit the passage of water vapor from the interior of the leaf into the surrounding air. In order to take up CO_2, the plant necessarily gives water off, and when trying to reduce water loss, the influx of CO_2 is equally reduced (Larcher, 2003).

Although stomata closure produced higher drought in severe drought stress (SDS) leaves (Figure 4) within each treatment, these leaves maintain lower transpiration for a given leaf-to-air humidity difference (Figure 4E). High photosynthetic water use efficiency (PWUE) was similar to those reported for other species grown during dry-season conditions (Mulkey et al., 1992).

The result might be correlated with lower transpiration rate. Therefore, in such leaves, assimilation may not be greatly affected even when stomata are partially closed (Figures 4 and G). Also, stomata closure decreases the intercellular CO_2 concentration (Ci), which in turn alters photosynthetic mechanisms.

These photosynthetic mechanisms also may be independently influenced by water stress; therefore, it was very difficult to determine the exact sequence of events. Nevertheless, stomata closed with mild water stress, and this closure increased resistance to CO_2 diffusion into the leaf and water diffusion out of the leaf (Dickson and Tomlinson, 1996). When plants were placed in the gradually increased water-deficit conditions, the leaf transpiration rate and stomata conductance usually decreased faster than the carbon assimilation, leading to increased water use efficiency, WUE (Chaves and Oliveira, 2004).

The pattern of the variation in P_N and Tr induced by the different water stress treatments was similar for all treatments, but seedlings grown under the shade treatments were similar only in P_N. An interesting difference in the response of well-watered (WW), moderate drought stress (MDS), and severe drought stress (SDS) towards prolonged drought stress was observed: After 150 days of

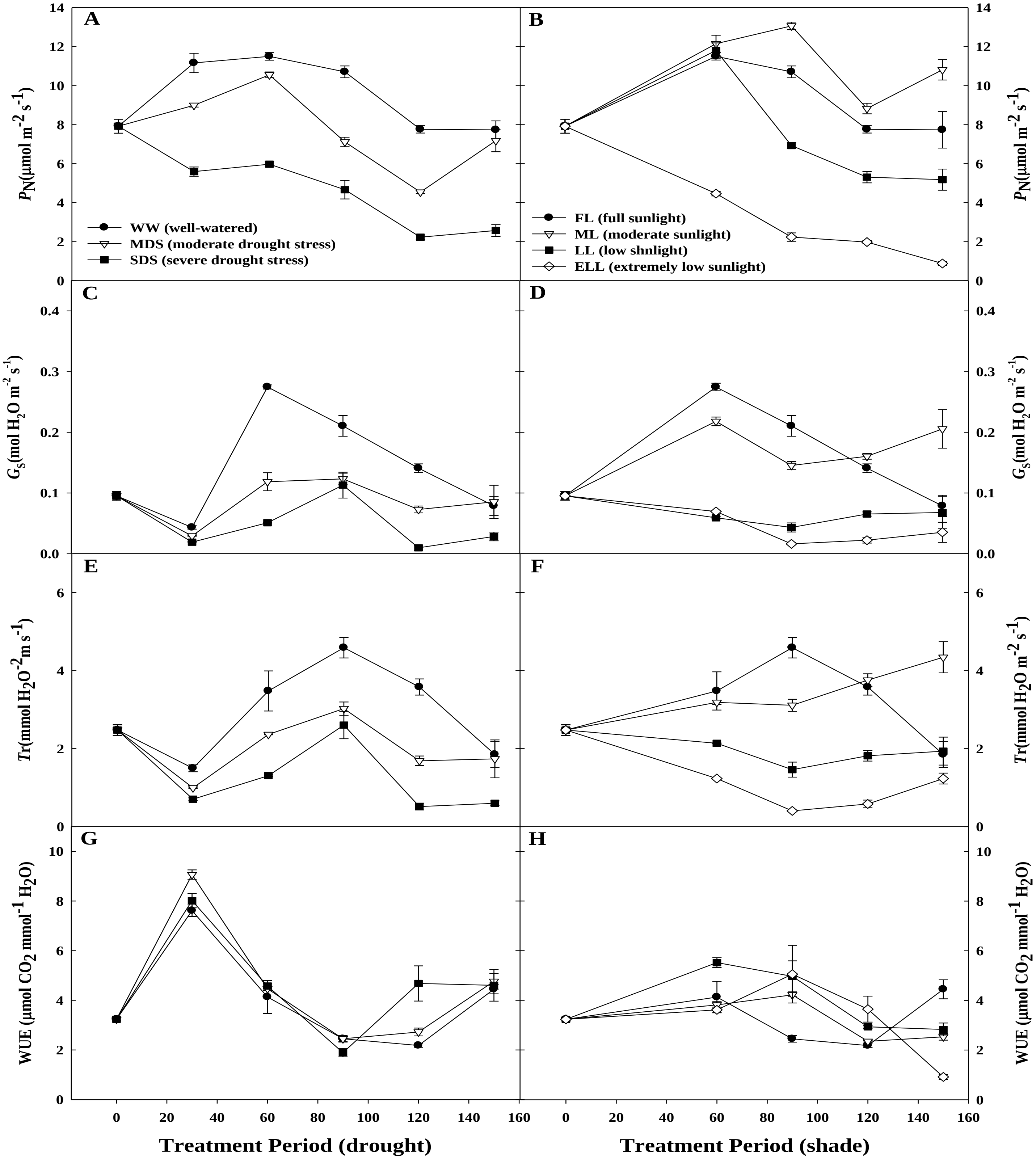

Figure 4. Response of the P_N (A, B), Gs (C, D), *Tr* (E, F), and WUE (G, H) induced by the leaves of *Q. suber* seedlings in response to water (A, C, E, and G) and light intensity (B, D, F, and H) during the period of the experimental treatment. Values represent mean S.E. (*n* = 5). In each Figure, seedlings under water treatments (WW, well-watered treatment [●]; MDS, moderate drought stress [▽]; and SDS, severe drought stress [■]) are distinguished from seedlings subjected to shade treatments (FL, full sunlignt [●]; ML, moderate sunlignt [▽]; LL, low sunlight [■]; ELL, extremely low sunlight [◇]).

drought stress, CO_2 assimilation parameters (P_N and *T*r) in moderate drought stress (MDS) seedlingsultimately indicated up to similar values of photosynthesis compared to well-watered (WW).

In contrast, severe drought stress (SDS) caused nearly complete inhibition of the photosynthesis and the transpiration. Similar results have been reported previously that the photosynthesis of *Q. rubra* rapidly decreased as water stress increases and often drop to zero under severe water stress (Weber and Gates, 1990).

Biochemical traits

Light response curves (*the A/Q curves*)

The reduction in the availability of water and light intensity stress affected physiological performances in the leaves of oak seedlings. Differences in daily courses of the photosynthetic rate (*A*) to the photosynthetic photon flux density (PPFD, *Q*) are presented for water and light intensity stresses of *Q. suber* in Figures 5 and 6.

Photosynthesis for well-watered (WW) seedlings approached a maximum value of ≈ 8.1 µmol m^{-2} s^{-1} for sunlit periods at midday. When *Q. suber* seedlings were in severe drought stress (SDS) treatment, P_N averaged about one-third (≈ 2.5 µmol m^{-2} s^{-1}) of the maximum values recorded during WW treatment. Under shaded treatments, P_N for the moderate sunlight (ML) treatment seedlings showed a maximum value of ≈ 9.5 µmol m^{-2} s^{-1}, and displayed about 4.5-fold increase in the maximum values for the extremely low sunlight (ELL) treatment. P_N for the ELL treatment showed a maximum of only 2.1 µmol m^{-2} s^{-1}. Moreover, P_N showed a lower maximum value (≈ 8.2 µmol m^{-2} s^{-1}) under the full sunlight (FL) treatment as compared with that under ML treatment. The light intensity at which photosynthesis became saturated was greater in seedlings grown under WW treatment compared with those grown under SDS treatment (Figure 6A). The well-watered (WW) seedlings became light saturated at about 1000 µmol m^{-2} s^{-1} with a maximum P_N of ≈ 7.6 µmol m^{-2} s^{-1}. The severe drought stress (SDS) seedlings revealed the saturated light at 800 µmol m^{-2} s^{-1}, and obtained a maximum P_N of ≈ 2.04 µmol m^{-2} s^{-1}. Under different light intensity treatments, the moderate sunlight (ML) seedlings became light saturated about 800 µmol m^{-2} s^{-1} with a maximum P_N of ≈ 8.3 µmol m^{-2} s^{-1}. The extremely low sunlight (ELL) seedlings became lowest light saturated at about one-quarter the photosynthetic photon flux density necessary for moderate sunlight (ML) seedlings (200 µmol m^{-2} s^{-1}), and indicated a maximum P_N of only ≈ 1.15 µmol m^{-2} s^{-1}.

Both the full sunlight (FL) and the moderate sunlight (ML) seedlings had similar photosynthetic rates at quantum fluxes < ≈ 500 µmol m^{-2} s^{-1} and similar light compensation points of near 40 µmol m^{-2} s^{-1} (Figure 6A). Considerable differences in the in photosynthetic rate (P_N), transpiration rate (*T*r), and water use efficiency (WUE) relations, as well as leaf structure (morphological and anatomical traits), existed between drought and shade stress treatments of *Q. suber*. The patterns of photosynthesis in response to the photosynthetic photon flux density (PPFD, *Q*) under experimental conditions were very similar to the pattern reported for several other species from field as well as the glasshouse studies(Quero et al., 2006).

Leaves from well-watered seedlings displayed photosynthetically saturated light at nearly 1.25 times the photosynthetic photon flux density that was necessary for light saturation in severe drought stress (SDS) seedlings and had maximum values of photosynthesis that were approximately four times greater (Figure 5A).

On the other hand, the maximum photosynthesis was higher for the moderate sunlight (ML) seedlings when compared to the full sunlight (FL) seedlings, although the leaf from the full sunlight (FL) treatment was light saturated at near 1,000 µmol m^{-2} s^{-1} compared with the moderate sunlight (ML, 800 µmol m^{-2} s^{-1}) treatment (Figure 6A). In Mediterranean climates, the abundance, distribution, and composition of the understory seedlings in the forest were more consistently affected by changes in soil moisture and other resources, especially light during the dry summer months. The success of long-term advanced regeneration in the Mediterranean region depends on the capacity of seedlings to survive prolonged drought and deep shade (Aranda et al., 2007).

The relative range of the moderate sunlight (ML) for optimal photosynthesis for leaves from among shade treatments may reflect an adaptation in *Q. suber* to fluctuating light which was common in the understory (Young and Smith, 1980; Mendes et al., 2001; Terashima et al., 2006; Gregoriou et al., 2007). In South Portugal, *Q. suber* dominated the western and moister areas whereas *Q. rotundifolia* occupied the drier eastern inland areas. This geographical distribution seemed to reflect different tolerances to drought between species (David et al., 2004). Also, the maximum photosynthetic rate during the moderate sunlight (ML) conditions of *Q. suber* (≈ 9.5 µmol m^{-2} s^{-1}) were somewhat similar compared with *Q. suber* grown under 60% full sunlight, higher than that reported for seedlings under full sunlight (Hernández et al., 2009).

These relatively high photosynthetic rates may represent an adaptation in *Q. suber* which enables utilization of the greater photosynthetic quantum flux density that occurs in more open locations (that is, the moderate sunlight) within the understory.

Measurement of A-Ci curve and estimation of derived parameters, V_{cmax} and J_{max}

A mechanistic and biochemical model in relation to leaf photosynthesis proposed by Farquhar et al. (1980) as

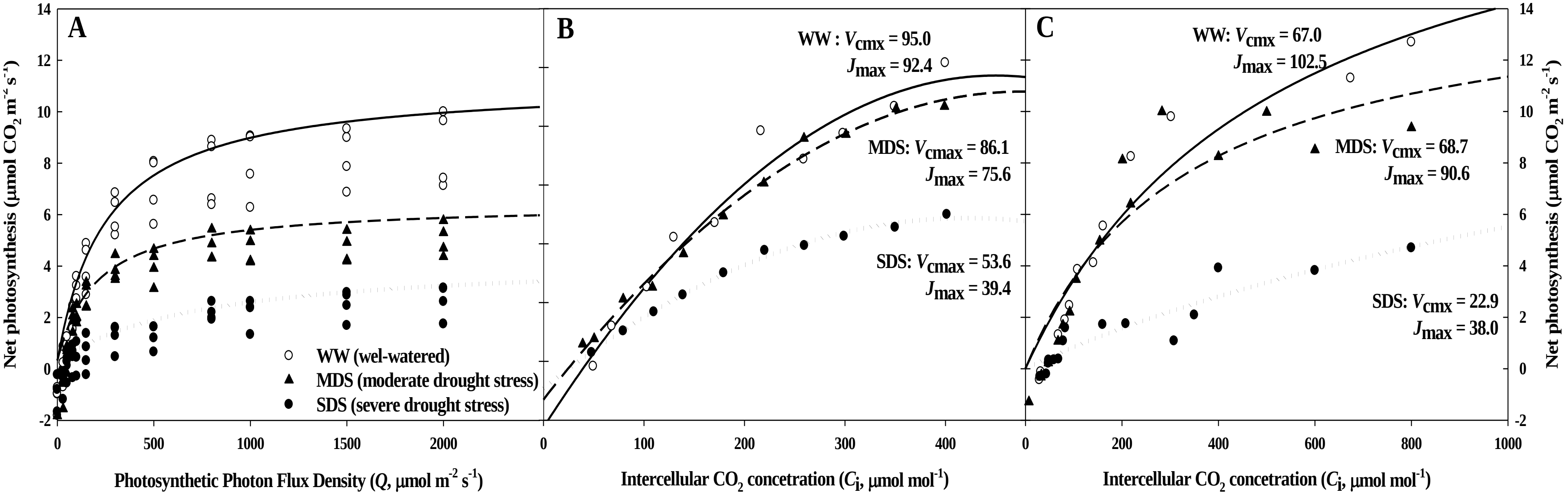

Figure 5. Simulated and measured responses of the net photosynthetic rate (*A,* μmol CO_2 m^{-2} s^{-1}) to the photosynthetic photon flux density (*Q*, μmol m^{-2} s^{-1}), the *A*/*Q* curves (A) after 90 days and the relationship between leaf photosynthesis (*A*) and intercellular CO_2 concentration (*C*i, μmol mol^{-1}), the *A*/*C*i curves after 60 days (B) and 90 days (C) of three water treatments for *Q. suber* seedlings. WW, Well-watered [○], MDS, moderate drought stress [▲], and SDS, severe drought stress [●].

modified by Sharkey (1985) was analyzed according to the *A*/*C*i response curves. Recently, more research has been focused on investigating whether the relationship between biochemical and physiological traits in leaves (e.g., photosynthetic rate, V_{cmax}, and J_{max}) have effects on growth rate similar to those of their analogues in leaves (e.g., specific leaf area, photosynthetic rate).

Physiological and biochemical responses associated with carbon fixation can be conveniently divided into stomatal and non-stomatal (that is, biochemical) responses of photosynthesis under water stress. Stomatal control in response to water stress is probably the most important step in this process. The maximum Rubisco activity (V_{cmax}) and electron transport capacity (J_{max}) were the key parameters determining photosynthetic capacity (Abrams, 1990; Dickson and Tomlinson, 1996; Juarez-Lopez et al., 2008).

In all treatments, both maximum Rubisco activity and electron transport capacity expressed from the *A*/*C*i response curves (V_{cmax} and J_{max}) increased with well-watered and the full sunlight treatment (Figures 5 and 6 and Tables 1 and 2). Under water treatments, the slopes and explained variances for severe drought stress (SDS) were considerably lower than for well-watered (WW) treatment and moderate drought stress (MDS) at 60 and 90 days of treatments (Figures 5B and C).

As a result, the curves obtained from water treatments of *Q. suber* leaves demonstrated that well-watered (WW) treatment had the highest discriminative curves on the response except for *A*/*C*i, and severe drought stress (SDS) was much more severe than the effect of moderate drought stress (MDS). The *A*/*C*i response curves of shade treatments demonstrated that moderate sunlight (ML) treatment had larger curves compared with the full sunlight (FL) treatment, and the extremely low sunlight (ELL) treatment suppressed the response nearly completely (Figures 5 and 6).

The rate of maximum Rubisco carboxylation (V_{cmax}, μmol m^{-2} s^{-1}) and the rate of maximum photosynthetic electron transport (J_{max}, μmol m^{-2} s^{-1}) in response to separately induced drought and shade stress treatments after 60 and 90 days of treatment are illustrated by the *A*/*C*i response curves (Figures 5 and 6). Both 60 and 90 days of

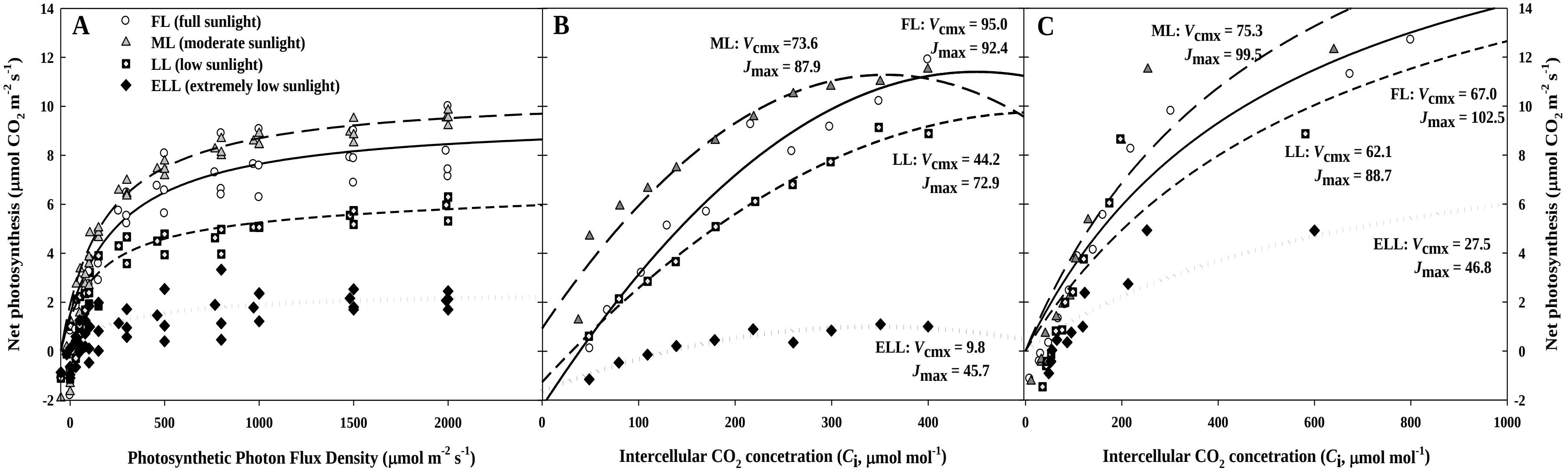

Figure 6. Simulated and measured responses of the net photosynthetic rate (*A*, μmol CO_2 m^{-2} s^{-1}) to the photosynthetic photon flux density (*Q*, μmol m^{-2} s^{-1}), the *A*/*Q* curves (A) after 90 days and the relationship between leaf photosynthesis (*A*) and intercellular CO_2 concentration (*C*i, μmol mol^{-1}), the *A*/*C*i curves after 60 days (B) and 90 days (C) of three water treatments for *Q. suber* seedlings. FL, full sunlight [○]; ML, moderate sunlight [▲]; LL, low sunlight [◘]; ELL, extremely low sunlight [♦\.

treatment revealed the similar *A*/*C*i responsecurves in V_{cmax} and J_{max} among each treatment (Figures 5 and 6). Seedlings of well-watered (WW) treatment tended to have greater V_{cmax} (≈ 95.0 μmol m^{-2} s^{-1}) and J_{max} (≈ 92.4 μmol m^{-2} s^{-1}) compared to the other two water stress treatments both 60 and 90 days (Figures 5B and C). Moreover, for moderate drought stress (MDS) and severe drought stress (SDS) treatment, V_{cmax} showed only about 90 and 56%, respectively, whereas J_{max} had the values of only about 82 and 43%, respectively, than those of the well-watered (WW) treatment.

On the other hand, under shade treatments, the full sunlight (FL) seedlings tended to have larger V_{cmax} and J_{max} than more shade-induced seedlings, although the moderate sunlight (ML) treatment showed greater photosynthetic rate compared with the full sunlight (FL) treatment. For optimum allocation of resources, Rubisco and electron transport should co-restrict photosynthesis such that no excess capacities remain (Niinemets et al., 1998).

Changes in leaf photosynthesis in response to variations in the intercellular CO_2 concentration (*C*i, μmol mol^{-1}) under experimental conditions were similar to those reported for several other species grown under the growth chamber study (Heerden van and Krüger, 2002). Hollinger (1992) suggested that there was a strong relationship between the leaf nitrogen content and the fitted maximum rates of carboxylation. For example, *Q. lobata* and *Q. agrifolia* increased V_{cmax} with increasing nitrogen contents, and had also a higher the rate of maximum Rubisco carboxylation (V_{cmax}) in leaves of the deciduous oak *Q. lobata* than in the co-occuning evergreen oak *Q. agrifolia*. In contrast, as in the previous study, a lower leaf nitrogen concentration of seedlings was associated with lower maximal Rubisco activity and electron transport rate.

A lower assimilation rate was associated with lower stomata conductance, nitrogen allocation to photo-synthetic functions, maximal Rubisco activity (V_{cmax}) and electron transport rate (J_{max}) (Abrams, 1990; Dickson and Tomlinson, 1996; Juarez-Lopez et al., 2008).

Conclusions

This study examined the physiological and biochemical aspects to examine in response to water and light intensity on *Q. suber*. Specifically, it focused on processes affecting drought and shade on *Q. suber*.

Seedlings of *Q. suber* grown under the water stress lead to the decreased photosynthesis due to thedecreased biochemical parameters such as V_{cmax} and J_{max}. Under the shaded environment, *Q. suber* seedlings was observed generally to have slower growth, lower photosynthetic rate, and transpiration rate, although they possess relatively higher chlorophyll content, proline accumulation, and total nitrogen content compared with their severe drought stress. Specially, seedlings grown under the extremely low sunlight possess decreased photosynthesis due to decreased V_{cmax} and J_{max}. Although shade-tolerant species are generally manifested as an enhanced phenotypic adaptation in the morphological alterations in comparison with physiological alterations, this study had shown a higher adjustment in water stress than in light intensity (shade) stress.

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