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Coordinated changes in photosynthetic machinery performance and water relations of the xerophytic shrub *Ziziphus lotus* (L.) Lam. (Rhamnaceae) following soil drying

M. MARAGHNI*, M. GORAI*,**,+, K. STEPPE***, M. NEFFATI*, and M.C. VAN LABEKE****

*Laboratoire des Ecosystèmes Pastoraux et Valorisation des Plantes Spontanées et des Micro-organismes Associés, Institut des Régions Arides, Université de Gabès, 4119 Médenine, Tunisia**

*Unité de Valorisation des Biomolécules Actives, Institut Supérieur de Biologie Appliquée de Médenine, Université de Gabès, 4119 Médenine, Tunisia***

*Laboratory of Plant Ecology, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium****

*Department of Plant Production, Faculty of Bioscience Engineering Ghent University, Coupure links 653, 9000 Gent, Belgium*****

Abstract

Aim of this study was to investigate the effect of water shortage in wild jujube plants, *Ziziphus lotus* (L.) Lam, and how it is related to its ecological success. All leaf anatomical traits were significantly affected following soil drying. Stressed plants displayed more negative stem water potential (ψ_w) and osmotic potential values of *ca*. −3.5 and −4.5 MPa, respectively, after 30 d. The relative water content declined although it still maintained high values (≥ 75%). The net photosynthetic rate, stomatal conductance, and transpiration rate were significantly inhibited as ψ_w decreased. However, the intrinsic water-use efficiency increased as water deficit was intensified; the difference became significant only after 30 d. As a consequence, the effective quantum yield of PSII photochemistry and the photochemical quenching coefficient significantly decreased although the maximal quantum yield of PSII photochemistry ratio remained statisticaly unchanged. Plants could maintain their water status sufficiently by increasing proline 1.7-fold and sucrose 1.8-fold in their leaves, respectively. The largest accumulation of both solutes may avoid photodamages at cellular level and play a critical role in maintaining osmotic adjustment.

Additional key words: chlorophyll fluorescence; drought; gas exchange; osmotic adjustment; water relations..

Introduction

Drought is the most complex and devastating abiotic stress on a global scale and its frequency is expected to increase as a consequence of climate changes (Ceccarelli 2010). Water availability is an important factor affecting photosynthesis, growth, and survival of plants, mainly in arid and semiarid regions, such as the Mediterranean basin. In recent years, many studies have focused on characterizing the adaptive strategies of plants to water stress (Kreuzwieser and Rennenberg 2014, Gorai *et al.* 2015). Plants express various responses to drought stress and develop a wide range of physiological and morphological strategies that allow them to cope with drought stress (Torrecillas *et al.* 1999, Augé *et al.* 2003). Chlorophyll (Chl) fluorescence constitutes an important selection criterion to verify plant tolerance to abiotic stresses (Maxwell and Johnson 2000, Baker and Rosenqvist 2004, Song *et al*. 2009, Zhang *et al*. 2010). Under water stress conditions, regulation of sunlight is necessary to balance the absorption and utilization of light energy, thereby minimizing the potential for photooxidative damage (Müller *et al.* 2001). The photoprotective mechanism competes with photochemistry for the absorbed energy, which in turn leads to decreasing quantum yield of PSII (Genty *et al.* 1989). Moreover, water shortage decreases the rate of plant CO_2 assimilation as a consequence of the reduced stomatal conductance (g_s) and/or by direct damage to carbon metabolism (Aroca *et al.* 2012, Nikinmaa *et al.* 2013). A metabolic response to drought stress is the synthesis of compatible organic solutes, such as soluble sugars, sugar alcohols, proline, and betaines (Serraj and Sinclair 2002, Gorai *et al.* 2015).

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+Corresponding author; phone: (+216)75633919, fax: (+216)75633918, e-mail: gorai.mustapha@yahoo.fr

Abbreviations: Chl – chlorophyll; DM – dry mass; *E* – transpiration rate; F_0 – minimum fluorescence; F_m – maximum fluorescence; F_v/F_m – maximum quantum efficiency of PSII ($F_v = F_m - F_0$); FM – fresh mass; g_s – stomatal conductance; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; RWC – relative water content; WUE_i – intrinsic water-use efficiency ($= P_N/g_s$); Φ_{PSII} – effective quantum yield of PSII photochemistry; ψ_w – water potential; ψ_π – osmotic potential.

These solutes reduce the oxidative damage caused by free radicals, especially in response to severe drought stress (Attipali *et al.* 2004).

Mediterranean shrubs represent excellent models for studying plant responses to drought, which are generally tolerant and well adapted to decreased soil water availability during the seasonal drought. *Ziziphus* species developed a series of adaptation mechanisms allowing them to tolerate severe conditions. They prevent transpiration and oxidative damage in tissues by strong stomatal control (Choudhary *et al.* 1996, Clifford *et al.* 1998, Arndt *et al.* 2001, Maraghni *et al.* 2014). *Ziziphus lotus* is endemic to the Mediterranean basin, while in Tunisia it has a wide ecological and geographical distribution and grows under a variety of environmental conditions. Wild jujube is dormant from October through March and mature plants flower in May, their fruits ripen in August, and their leaves begin to fall in early September (Gorai *et al.* 2010). In arid ecosystems, this multipurpose shrub contributes to biodiversity conservation. Wild jujube has as great economic and ecological interest: it stabilizes sand dunes and has feed, fuel, and medicinal uses (Le Floc'h 1983, Gorai *et al.* 2010, Maraghni *et al.* 2010). The jujube fruits are formed of red exocarp, edible fleshy mesocarp and stony endocarp with 1–2 seeds (Maraghni *et al.* 2010). The drupe can be consumed fresh, dried or processed (jams, loaf, cakes, jelly, *etc*.).

The aim of this study was to investigate the photosynthetic performance in drought-treated *Ziziphus lotus* plants, both in terms of CO_2 assimilation, as measured by leaf gas exchange, and of the functionality of the photosynthetic apparatus, as assessed by Chl *a* fluorescence measurements. Additionally, coordinated changes between photosynthesis and water relations were designed to increase understanding of preliminary alterations of physiological characteristics for this species**.**

Material and methods

Plant material, growth conditions, and experimental design: The experiments were carried out in a glasshouse at the Faculty of Bioscience Engineering, Ghent University, Belgium (51°02'N, 03°42'E). Seeds were provided by the seed bank of the Laboratoire des écosystèmes pastoraux et valorisation des plantes spontanées et des microorganismes associés at the Institut des Régions Arides, Médenine, Tunisia.

After surface sterilization, seeds were sown in alveolar plates filled with loam and placed at controlled conditions (Maraghni *et al.* 2010). After two weeks the seedlings were selected according to their uniformity and transplanted in plastic bags (14 cm in diameter and 60 cm in depth) attached by metal brackets and filled with a mixture of soil (loam soil) and sand (2:1; v/v). They were watered (drip irrigation) with water-soluble fertilizer of 10–10–10 ($N–P_2O_5–K_2O$) (*Scotts-Sierra Horticultural Products Co.*, Marysville, OH, USA). The pots were placed in a glasshouse with natural sunlight and the mean average day and night time temperatures were 30 and 18°C, respectively. After eight months of culture, the seedlings were subjected to two irrigation treatments; (*1*) irrigation to field capacity (control) and (*2*) withholding water during 14 and 30 d. They were randomly allocated to four blocks and each block consisted of 12 plants (4 plants for each treatment) giving a total of 16 plants per treatement. At the beginning of the experiment, all bags were saturated with water and allowed to drain freely by the drainage holes.

Plant water relations: The water status was evaluated by measuring relative water content (RWC) on fully expanded leaves according to Morgan (1984): RWC = [(FM – DM)/(TM – DM) × 100], where FM, DM, and TM are the fresh, dry, and turgid masses, respectively. Stem water potential (ψ_w) was measured using a Scholander pressure chamber (*PMS Instruments Co.*, Corvallis, R, USA) and osmotic potential (ψ_π) was determined according to Callister *et al.* (2006). About 20 mg of leaf DM were extracted in 1 ml of distilled water. The homogenate was placed in a water bath at 100°C for 1 h and centrifuged at 10,000 × *g* for 10 min at 4°C. A 100-µl aliquot of the extract was used to determine osmolarity with a micro-osmometer (*Fiske® Model 210, Fiske® Associates*).

Photosynthetic gas exchange and Chl fluorescence: The net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (*E*) of fully expanded leaves were determined between 9:00 h and noon using a *LI-COR 6400* portable photosynthesis system (*LI-COR,* Lincoln, NE, US*A*). Leaf temperature was kept constant at 25°C, relative air humidity in the cuvette at 60%, the CO_2 concentration in the chamber was 400 µmol mol^{-1}, and a light intensity of 1,500 µmol(photon) m^{-2} s^{-1}. Intrinsic water-use efficiency (WUE_i) was calculated from the ratio P_N/g_s.

Chl *a* fluorescence in dark- and light-adapted leaves was measured with a portable fluorometer (*PAM-2500,* Walz, Effeltrich, Germany). After 30 min of dark adaptation, the maximal quantum yield of PSII photochemistry (F_v/F_m) was calculated as [(F_m – F_0)/F_m], where F_m [induced by a short pulse (0.6 s) of saturating light of 3,450 µmol(photon) m^{-2} s^{-1}] and F_0 were the maximal and minimal fluorescence (Genty *et al.* 1989). After 4 min of illumination with continuous red, nonsaturating actinic light [447 µmol(photon) m^{-2} s^{-1}] and saturating pulses every 25 s, maximum (F_m') and steady-state (F_s) fluorescence signals were measured in light-adapted leaves. Then, the actinic light was turned off and a far-red pulse was applied to obtain the minimal fluorescence after the PSI excitation (F_0'). The effective quantum yield of PSII photochemistry (Φ_{PSII}) was calculated as [(F_m' – F_s)/F_m'] and q_P was calculated as [(F_m' – F_s)/(F_m' – F_0')] (Van Kooten and Snel 1990). Values were obtained for four leaves (one leaf per plant) and per treatment.

Leaf anatomical measurements: Newly formed leaves on the sun-exposed side of seedlings were selected for anatomical observations. In the laboratory, the selected leaves were disinfected in 5% sodium hypochlorite followed by three rinses with distilled water. The sections were taken from the middle of the leaves to avoid differential thickness along the leaf. Samples were fixed in mixture of (FAA) formalin: acetic acid: alcohol (ethanol). The fixed samples were dehydrated through an alcohol series (50, 60, 70, 85, and 100%) and embedded in paraffin during 24 h at 40°C. The sections were cut using a microtome and they were then stained with aqueous safranin and Fast Green to distinguish the different cells. After successive steps in the alcohol series and xylene (5–10 min, per solution) each section was mounted on Canada balsam. Finally, three sections per leaf were selected and used to determine the thickness (µm) of the palisade and spongy mesophyll and the upper and lower epidermis using an *Olympus IX81* inverted fluorescence microscope equipped with an *X-Cite Series 120Q* UV lamp and an *Olympus XM10* camera, which was connected to a *Nikon A1r* laser scanning microscope equipped with *Axiovision* software (*LiMiD*).

Determination of organic solutes: Free proline content was quantified as described by Bates *et al.* (1973). Leaf samples (0.5 g) were homogenized in 3% (w/v) sulphosalicylic acid and homogenate was filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, resulting mixture was heated at 100°C for 1 h in water bath. Reaction was then stopped in ice. The mixture was extracted with toluene, and the absorbance was read at 520 nm (*Nanoquant Infinite M200, TECAN Austria GmbH,* Austria). Proline concentration was determined using a calibration curve and expressed as µmol(proline) g^{-1}(FM).

Sugars from leaves were extracted with 80% ethanol at 70°C, and centrifuged at 5,000 × *g* for 10 min. Glucose, fructose, and sucrose were analysed using high-pH anion-exchange chromatography with pulsed amperometric detection (*Waters*; *CarboPac MA1* column with companion guard column, eluent: 50 mM NaOH, 22°C).

Statistical analysis: Means were compared using the least significant difference (LSD) test ($p \leq 0.05$). Statistical analyses were performed using *SPSS v.17.0*.

Results

Leaf anatomical traits: All leaf anatomical traits were significantly affected following soil drying (Table 1). Epidermis from both sides of *Z. lotus* leaves was thicker in stressed plants compared to well-irrigated plants as water deficit intensified. The epidermis thickness was more pronounced for the upper side compared to the lower one. Leaf mesophyll had different thickness layers and palisade mesophyll was thicker than spongy mesophyll in well-watered plants. As water deficit intensified, the palisade mesophyll thickness decreased reaching similar thickness of spongy mesophyll after 30 d. Both mesophyll tissues evolve in an inverse trend as a response to water shortage, where the thickness declined by 22% for palisade tissue, and increased by 7% for spongy tissue after withholding water for 30 d.

Stem water relations: ψ_w was related to relative water content (RWC), indicating that its decrease during the drought stress period was associated with a decrease in RWC (Fig. 1*A*). Withholding watering throughout the experimental period significantly affected RWC ($F_{2,6}$ = 10.63, $P<0.05$), which was decreased by 3.8 and 9.5% compared with control plants after 14-d and 30-d of the drought stress treatment, respectively. Stem ψ_w and ψ_π decreased significantly with on-going drought stress ($F_{2,6}$ = 75.55 and 19.74, respectively; $P<0.05$). The largest reduction in ψ_w occurred in drought-stressed stems on day 30, when it dropped to −3.5 MPa. A similar pattern was observed in ψ_π but there was an additional decline to −4.2 MPa. There was a significant positive correlation between ψ_w and ψ_π ($r = 0.88$; $P<0.01$). Stem turgor values were derived from plots of ψ_w and ψ_π recorded during the experiment (Fig. 1*B*). The proximity of points to the zero line showed that variations in turgor potential (ψ_p) were the smallest for well-irrigated plants and largest for drought-stressed plants after 14 and 30 d, respectively, which is explained by accumulation of organic solutes to cope with water shortage.

Gas exchange and Chl *a* fluorescence: Drought stress induced a significant decline in net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (*E*) ($F_{2,6}$ =35.36, 48.42, and 13.00, respectively; $P<0.01$; Fig. 2*A*–*C*). The values of P_N of well-watered plants reached 13.5 µmol(CO_2) m^{-2} s^{-1}; however, withholding water decreased P_N of stressed plants which became significant after 14 d reaching a value of around 8.5 µmol(CO_2) m^{-2} s^{-1} (Fig. 2*A*). There was additional decline in P_N after 30 d which was inhibited by 59% compared with control plants. In parallel, *E* reached the highest value [8.5 mmol(H_2O) m^{-2} s^{-1}] in control plants, and was inhibited by 31% after withholding water for 14 d, and by 59% after 30 d (Fig. 2*B*). As compared to controls, g_s of drought-treated plants decreased by 44 and 66% after 14 d and 30 d of withholding water, respectively (Fig. 2*C*). The P_N/g_s ratio which is an index of leaf intrinsic water-use efficiency (WUE_i) increased by prolonging water deficit.

Table 1. Leaf anatomical traits in drought-treated *Ziziphus lotus*. Means ± SE ($n = 4$).

Parameter size [µm]	Control	Drought 14-d	 30-d	*p*-value
Upper epidermis	23.88 ± 1.10	27.54 ± 0.30	27.86 ± 0.03	0.010
Lower epidermis	16.30 ± 0.48	19.68 ± 0.20	19.16 ±1.04	0.025
Palisade mesophyll	58.36 ± 1.56	57.45 ± 0.00	45.57 ± 0.00	0.000
Spongy mesophyll	42.80 ± 0.86	43.44 ± 0.00	45.67 ± 0.01	0.016

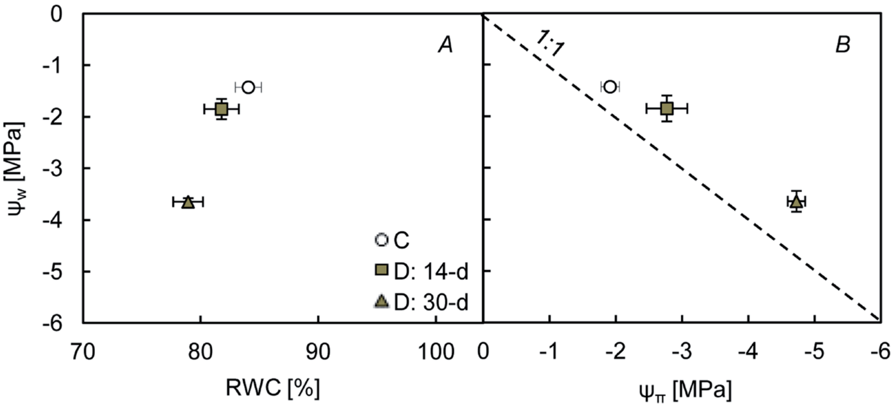

Fig. 1. Relationship between water potential (ψ_w) and relative water content (RWC) (*A*) and between ψ_w and ψ_π (osmotic potential) (*B*) for *Ziziphus lotus* in control and drought-treated plants by withholding watering throughout the experimental period. C – control plants (○), D – 14- and 30-d drought period (■ and ▲, respectively). Data represent mean ± SE, $n = 4$. The 1:1 line indicates $\psi_w = \psi_\pi$ and hence turgor potential = 0.

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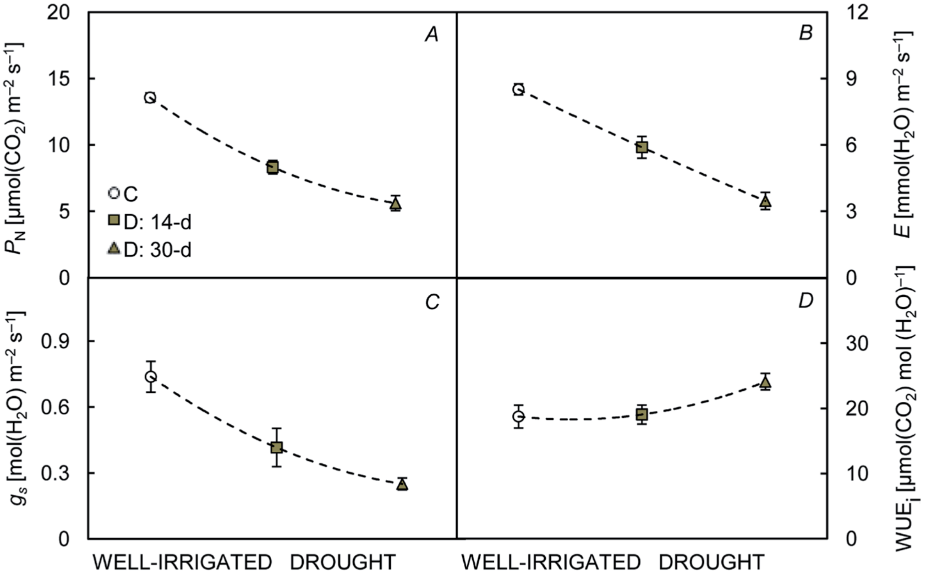

Fig. 2. Changes in (*A*) potosynthetic assimilation rate (P_N), (*B*) transpiration rate (*E*), (*C*) stomatal conductance (g_s), and (*D*) intrinsic water-use efficiency (WUE_i) of *Ziziphus lotus* in control and drought- treated plants by withholding watering throughout the experimental period. Lines describing the dependencies were obtained using a polynomial regression. C – control plants (○), D – 14- and 30-d drought period (■ and ▲, respectively). Values represent means ± SE, $n = 4$.

Stressed plants displayed higher WUE_i values than that in control plants only after 30 d, where the differences become significant (Fig. 2*D*).

Water-deficit stress significantly affected the effective quantum yield of PSII photochemistry (Φ_{PSII}) and photochemical quenching coefficient (q_P) ($F_{2,6}$ = 80.20 and 17.61, respectively; $P<0.01$) (Fig. 3*B*,*D*). Well-watered plants displayed higher Φ_{PSII} than stressed plants, which was markedely declined by 17% after withholding water for 14 d, and to 53% after 30 d. Similarly, q_P decreased gradually with on-going drought stress and the largest reduction in q_P occurred in stressed leaves on day 30, when it dropped by 60% compared with control ones. In contrast, maximal quantum yield of PSII photochemistry (F_v/F_m) and nonphotochemical quenching coefficient (q_N) did not differ significantly (Fig. 3*A*,*C*). The values of F_v/F_m ranged predominantly between 0.75 and 0.77. The q_N had no consistent pattern of increase or decrease reaching a value of 0.6.

Accumulation of organic solutes: Sucrose significantly accumulated in leaves as water deficit stress intensified representing *ca.* 121 and 183% of the control value after withholding water for 14 d and 30 d, respectively ($F_{2,6}$ = 16.28, $P<0.01$; Fig. 4*A*). Similarly, accumulation of proline was significantly induced by 12% after withholding water for 14 d, and to 73% after 30 d ($F_{2,6}$ = 59.7, $P<0.001$; Fig. 2*B*). In contrast, glucose and fructose did not change significantly by prolonging drought stress (Fig. 4*A*).

Discussion

Results from this study indicate that drought-stressed plants displayed less negative ψ_w as water deficit intensified and allowed their leaves to carry out photosynthesis. The additional decrease in ψ_π resulted in accumulation of compatible solutes such as sucrose and proline, indicating their contribution to osmotic adjustment. Futhermore, plants shifted their leaf anatomical behaviour by moving from mesic (well-watered) to xeric conditions (drought-stressed). They exhibited a significant increase in the thickness of epidermis and spongy parenchyma under

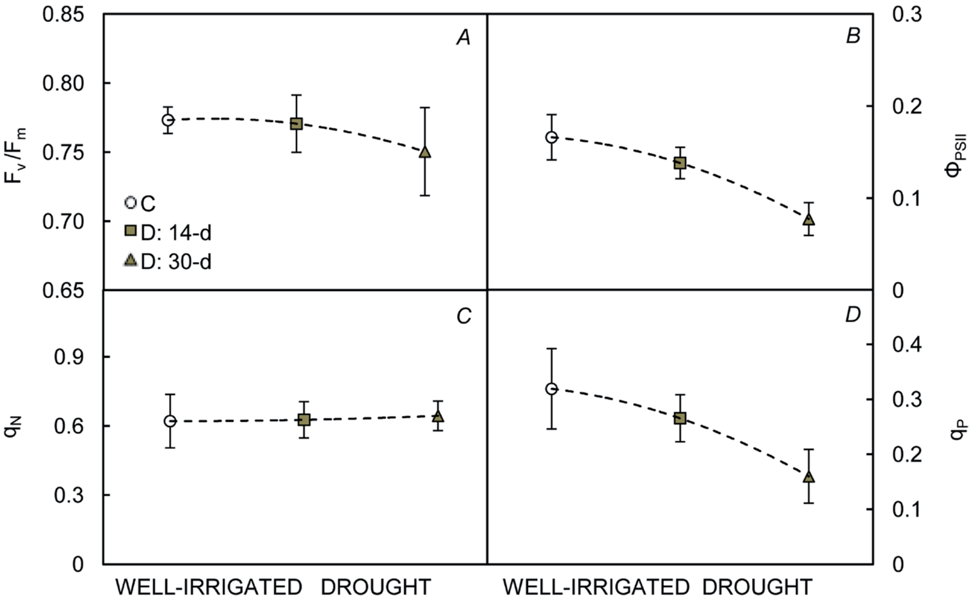

Fig. 3. Changes in (*A*) maximal quantum yield of PSII photochemistry (F_v/F_m), (*B*) effective quantum yield of PSII photochemistry (Φ_{PSII}), (*C*) nonphotochemical quenching coefficient (q_N), and (*D*) photochemical quenching coefficient (q_P) of *Ziziphus lotus* in control and drought-treated plants by withholding watering throughout the experimental period. Lines describing the dependencies were obtained using a polynomial regression. C – control plants (○), D – 14- and 30-d drought period (■ and ▲, respectively). Values represent means ± SE, *n* = 4.

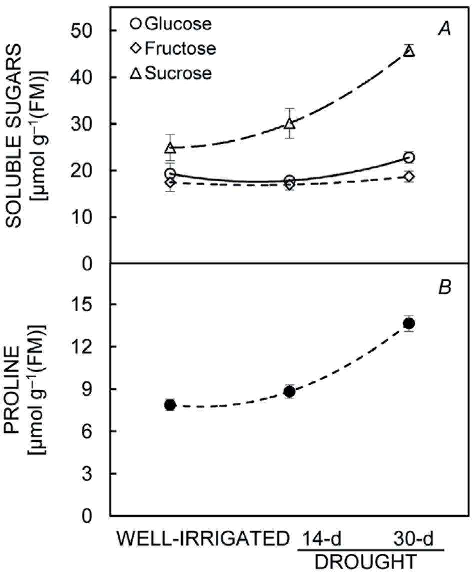

Fig. 4. Contents of (*A*) soluble sugars (glucose, fructose, and sucrose) and (*B*) proline in leaves of *Ziziphus lotus* in control and drought-treated plants by withholding watering throughout the experimental period. Lines describing the dependencies were obtained using a polynomial regression. Values represent means ± SE, *n* = 4.

water deficit stress, whereas palisade parenchyma was slightly reduced. The increase in the number of epidermal cells per leaf surface unit reflects a better control of water loss through cuticular transpiration (Bosabalidis and Kofidis 2002). This leaf anatomical behavior may enhance survival and growth under deficit water by improving water relations and providing higher protection for the internal cells (Bacelar *et al.* 2004). ψ_w and ψ_π were linearly related to RWC, which declined although it still maintained high values. This supports findings on other species of the same genus including *Z. mauritiana* (Clifford *et al.* 1998), *Z. rotundifolia* (Arndt *et al.* 2001), and *Z. mauritiana* (Kulkarni *et al.* 2010). It was shown (Lawlor and Cornic 2002) that photosynthetic rate of leaves in higher plants is known to decrease as the RWC and leaf ψ_w decrease. Maintaining leaf RWC under lowering ψ_w is an important adaptation exhibited by *Z. lotus* to cope with extreme drought conditions. Similarly, Kala *et al.* (2008) reported higher tolerance ability of *Z. nummularia* after 28 d of drought stress, which is associated with maintenance of better leaf RWC as compared to *Z. rotundifolia*. The relationship between RWC and ψ_w has often been used to quantify the dehydration tolerance: tissues, which maintain a high RWC as ψ_w decreases are more tolerant to dehydration. In the present study, leaves of *Z. lotus* decreased their RWC values from 84 to 79% and did not drop below 60%, which is a typical characteristic of drought-tolerant species (Kawaguchi *et al.* 2004, Gorantla *et al.* 2007, Perera *et al.* 2008, Gorai *et al.* 2015).

Lowering leaf gas exchange (H_2O and CO_2) is a main physiological response for *Z. lotus* to adapt to water deficit stress. Similar results have also been documented for some *Ziziphus* species such as *Z. mauritiana* (Clifford *et al.* 1998, Kulkarni *et al.* 2010) and *Z. rotundifolia* (Arndt *et al.* 2001). The inhibition of carbon assimilation in *Z. lotus* leaves by withholding watering is associated with a decline in stomatal conductance by involving stomatal closure. This pattern in leaf gas exchange is due to the cessation of plant growth associated with reduced availability of photoassimilates to apical sink region.

Xerophytic plants reduce stomatal conductance as soil water potential decreases and vapour pressure deficit of

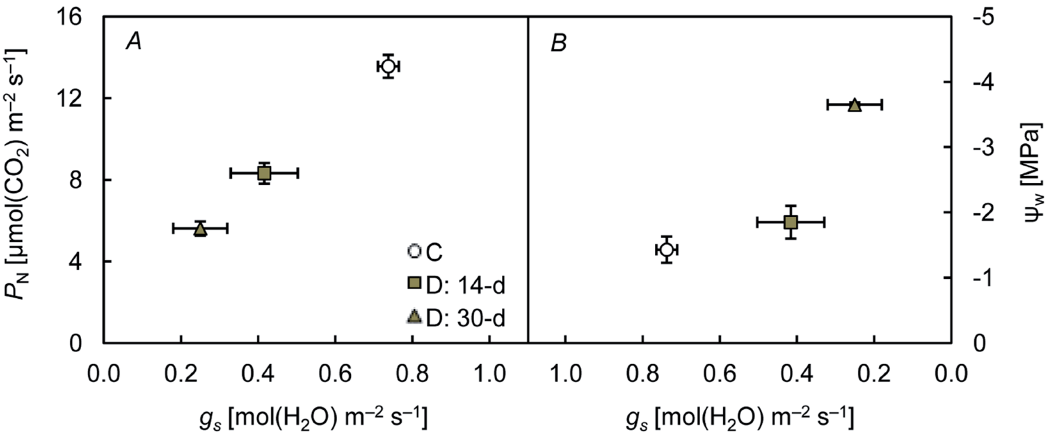

Fig. 5. Relationship between P_N and g_s (*A*), and between ψ_w and g_s (*B*) for *Ziziphus lotus* in control and drought-treated plants by withholding watering throughout the experimental period. C – control plants (○), D – 14- and 30-d drought period (■ and ▲, respectively). Values represent means ± SE, *n* = 4.

the air drops, thereby maintaining a relatively constant ψ_w (Chapotin *et al.* 2006). The relationship of stomatal conductance with ψ_w depends on the genotype (Tardieu and Simmonneau 1998), the environmental conditions during osmotic stress (Sharkey 1990), and the intensity of osmotic stress (Flexas *et al.* 1999). *Z. lotus* displayed sensitive stomatal responses, which result from lowering stem ψ_w ($r = 0.88$, $P<0.01$) (Fig. 5*B*). The decrease in *E* along with the lower stem ψ_w and ψ_π suggesting that g_s was effective in regulating the water balance of this species during drought stress. The relationship between P_N and g_s have received increasing recent attention due to the trade-off between water loss and carbon gain experienced by plants during extreme, climate change-type drought (McDowell *et al.* 2008). Our data show that both parameters are involved in decreasing gradually with on-going drought stress in a manner that improves water-use efficiency (Fig. 5*A*), indicating an optimisation of carbon uptake *vs.* water loss (Raven 2002). This positive correlation ($r = 0.84$, $P<0.01$) revealed that g_s plays a strong control over photosynthetic assimilation and the diffusional limitations restricting the supply of CO$_2$ to metabolism of *Z. lotus* plants. Moreover, photochemical processes are affected not only by photoinhibition and photo-oxidation but stomatal factors are also involved. According to Chaves (1991), this close relationship between P_N and g_s is also a common feature of drought-adapted species. The present data is in agreement with various studies (Arndt *et al.* 2001, Rouhi *et al.* 2007, De Smedt *et al.* 2012). The maintaining of higher leaf RWC of *Z. lotus* plants during water deficit stress was probably the result of their better water-use efficiency through controlled *E* under drought stress to avoid excess water loss.

Chl fluorescence data show that F_v/F_m did not change significantly with prolonging drought, indicating that photosynthetic efficiency was stable and was not affected when P_N declined. This behavior confirms that F_v/F_m ratio change only when drought stress becomes more severe and it is not a sensitive indicator of drought stress. Results from this study demonstrate that withholding watering significantly decreased Φ_{PSII} and q_P. The same finding has been reported on *Olea europaea* (Boussadia *et al.* 2008) and *Vigna unguiculata* (Souza *et al.* 2004). Changes in q_N in response to drought were relatively small, thus allowing maintenance of electron transport through photosystems at considerable rates. The decrease in photosynthetic capacity during drought stress was accompanied by reduction in Φ_{PSII}. This decline could be considered as a down-regulation of PSII that reflect the protective or regulatory mechanism to avoid photodamage of photosynthetic apparatus (Demming-Adams *et al.* 1996). The q_P represent the fraction of open PSII reaction centres (Genty *et al.* 1989) and can contribute to protect the photosynthetic apparatus by transferring electrons to O$_2$ under drought stress (Ort and Baker 2002). There was a strong positive correlaltion identified between Φ_{PSII} and q_P ($r = 0.93$, $P<0.001$). This finding may indicate that a q_P is associated with a deactivation of PSII excitation.

A very common response to drought is an increased production of compatible organic solutes, such as soluble sugars, sugar alcohols, proline, and betaines (Serraj and Sinclair 2002). Proline and carbohydrates are the two most important organic solutes that are accumulated in higher plants under drought conditions (Changhai *et al.* 2010). The current data show increased leaf concentrations of sucrose which seems to play a key role in the integration of plant growth and appear to be part of a wider mechanism for balancing carbon acquisition and allocation within and between organs (Farrar *et al.* 2000). During drought stress the decrease of carbon assimilation caused by low photosynthetic rates may contribute to increasing carbohydrate concentrations in leaves (Arndt *et al.* 2001). Furthermore, proline concentration markedely increased in leaves of stressed plant compared to well irrigated as water deficit intensified. According to Clifford *et al.* (1998), leaf proline accumulation seems to be a necessary and important mechanism to regulate redox potentials and functions as a hydroxyl radical scavenger. In response to osmotic stress induced by PEG-6000, *Z. lotus* seedlings increase the organic solute accumulation, such as proline and soluble sugars, in leaves and the activity of antioxidant enzymes, such as catalase and ascorbate peroxidase, in roots

(Maraghni *et al.* 2014). It was found (Gorai *et al.* 2015) that proline accumulation was significantly correlated to low ψ_w suggesting the involvement of this solute in osmotic adjustment. Similar trends of increase in proline and carbohydrates in leaves of some *Ziziphus* species during drought stress were also reported (Choudhary *et al.* 1996, Clifford *et al.* 1998, Arndt *et al.* 2001, Maraghni *et al.* 2011, 2014).

Overall, results of this study provide insight of the different mechanisms developed by *Z. lotus* in order to deal with water shortage. This xerophytic shrub was able to overcome drought stress by reducing its photosynthetic performance and water relations, and finally modifying its leaf anatomical tissues. Consequently, an osmotic adjustment was developed by this shrub as a tolerance mechanism to withstand drought stress.

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