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The Influence of Water-Deficit Stress on Growth, Water Relations and Solute Accumulation in Wild Jujube (*Ziziphus lotus*)

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Abstract

Wild jujube, *Ziziphus lotus*, is a multipurpose xerophytic shrub of the Rhamnaceae family widely distributed in arid and semi-arid regions of Tunisia, where it occupies most soil types. The fruit is the edible part of the plant by local population. The reintroduction of this shrub requires the control of its multiplication in response to water shortage. This study aims to evaluate growth and water relations of wild jujube seedlings under water deficit stress. After multiplication and growth under well-watered conditions, water deficit stress was imposed to seedlings by controlled deficit irrigation to 40 and 70% of field capacity (FC) for 15, 30 and 45 days. Soil of control plants was maintained at 100% FC throughout the experiments. Best growth was recorded for control plants, while water deficit successively reduced dry matter production and leaf number per plant. In addition, relative water content of leaves and branch water potential decreased significantly under severe drought stress. Plants subjected to 40% FC, accumulated respectively, 1.5 and 15-fold more soluble sugars and proline in leaves than controls. There was a strong negative relationship identified between leaf proline concentration and branch water potential with $R^2 = 0.85$, reflecting the importance of this amino acid ability for osmotic adjustment in *Z. lotus*.

Keywords: Drought, Growth, Osmotic adjustment, Proline, Soluble sugars, Water potential, *Ziziphus lotus*.

INTRODUCTION

Drought stress is considered to be the main environmental factor limiting plant growth and yield of many agronomic and horticultural crops, especially in semi-arid areas (Boyer, 1982). In Mediterranean-type ecosystems, seasonal water shortage is the main factor constraining survival and growth of plants (Di Castri *et al.,* 1981). Accordingly, agronomic or horticultural plants adapted to survive under these conditions are resistant to recurrent summer drought and therefore offer an excellent model to study their life strategies (Von Willert *et al.,* 1990; Munné-Bosch *et al.,* 2009). Soil depth and texture are considered the most important edaphic properties that influence the moisture regime in arid environments with episodic rainfall (Grigg *et al.,* 2008). To date, a great deal of effort has been focused on physiological process underlying plant responses to drought stress (Van Hees, 1997).

Mediterranean shrubs are excellent models to study plant responses to drought since they are generally very resistant and well adapted to decreased soil water availability during the summer. Several native species are potentially interesting under aspects of dune stabilization and extension of plant cover, including some *Ziziphus* species. The ability of some species of the genus *Ziziphus* to withstand drought has been attributed to a combination of avoidance and tolerance mechanisms, including osmotic adjustment and sensitive stomatal closure (Arndt *et al.,* 2001; Pareek, 2001).

The genus *Ziziphus* (Rhamnaceae) includes evergreen or deciduous trees or shrubs which are usually armed with unequal stipular spines. In Tunisia, *Ziziphus* is represented by 3 species: *Z. spina-christi* (L.) Willd, *Z. vulgaris* Lam. and *Z. lotus* (L.) Lam. (Maraghni *et al.,* 2010). The latter species, known as "Sedra", is indigenous to Tunisia. The edible fruit called a nabk is a subglobose dark yellow drupe (c. 1–1.5 cm in diameter) at maturity (Maraghni *et al.,* 2010). It is has a wide ecological and geographical distribution and grows under a variety of environmental conditions (Maraghni *et al.,* 2010" Gorai *et al.,* 2010).

Ziziphus lotus differs from the other two species by its deciduous shrubby habit with intricately branched stems and smaller flowers and fruits (Jafri, 1977). It is a shrub that reaches 2–5 m and is found in depressions with deep sandy soil. Mounds composed of wind-borne sediment that accumulated around *Z. lotus* thorn scrub have long been reported from the Tunisian steppe regions (Tengberg and Chen, 1998). *Ziziphus lotus* is dormant from October through March and flowers in May and June and produce fruits in August (Gorai *et al.,* 2010). It is browsed by livestock and its leaves are valuable animal forage and fodder under open grazing conditions.

As dried powder leaves and fruit were typically used as emollient in the treatment of boils (Le Floc'h, 1983). Recently, the anti-inflammatory, analgesic and anti-spasmodic activities of this plant were demonstrated in rodents (Borgi *et al.,* 2008; Borgi and Chouchane, 2009). These characteristics make *Z. lotus* a valuable multipurpose shrub for semi-arid to arid ecological areas. To our knowledge, no study has, as yet, been carried out on the effects of water deficit stress on this arid fruit plant. Therefore, the present investigation was undertaken to characterize the potential of cultivating *Z. lotus* under conditions of low water availability.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Mature fruits were collected in September 2007 from plants in natural *Z. lotus* populations growing at Samaâlyate (33°17'N, 10°55'E; Ben Gardane, Southeast Tunisia) where annual mean precipitation is around 186 mm and annual mean temperature is 19.4°C with a minimum temperature 3.9°C in January and a maximum temperature 35.9°C in August. Fruits were cleaned and stored for six months in the seed bank of the Laboratoire d'Ecologie Pastorale at the Institut des Régions Arides, Médenine (20°C, 30% RH).

After removing pulps from fruits, endocarps were cracked using a manual peeler. The pots filled with a mixture of sand and soil (1:2, by volume), three seeds were planted per pot (20 cm in diameter) and watered to field capacity (FC) to facilitate germination. The maximum germination was observed after 15 days. At this stage, the seedlings were thinned to one per pot. Plants were grown in a growth chamber under the following conditions: 25±1°C temperature, 40% day and 75% night relative humidity and 16 h light/8 h dark regime with 250 μmol m^{-2} s^{-1} photosynthetic flux density (PFD).

Individual plants of uniform stage of development and size were selected. The experiment was arranged in a completely randomized design (CRD) with three levels of water deficit stress (100, 70 and 40% FC) × four replicates. After 3 months of ample irrigation (100% FC), the water deficit stress was applied and plants were harvested after 15, 30 and 45 days. Regular weightings of pots and plants (every 2 days) enabled to restore the moisture of soil with nutrient solution (Hewitt, 1966) at 40, 70 and 100% FC. Fresh mass (FM) and dry mass (DW) of shoots and roots of each plant were determined after counting all leaves. Leaves were dried using a LDC-1 lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) to determine proline and soluble sugar concentrations and dry mass of roots was obtained after oven drying (60°C, 48 h).

Water Relations

Leaf relative water content (RWC) was estimated by recording the turgid mass (TM) of fresh leaf samples by keeping them in distilled water overnight under low light conditions, followed by oven dry for 48 h at 60°C for dry mass measurement. Leaf RWC was calculated as RWC (%) = (FM–DM)/(TM–DM) × 100. The branch water potential (Ψ_w) was measured using a pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA) according to Scholander *et al.,* (1965).

Determination of Organic Solutes

Free proline was assayed spectrophotometrically by the ninhydrin method (Bates *et al.,* 1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 14,000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene, and absorbance was read at 520 nm using L-proline as standard. The leaf proline concentration was expressed on dry weight basis.

Soluble sugars were quantified following the phenolsulfuric acid method (Robyt and White, 1987). 100 mg dry weight of shoots was extracted in 80% (v/v) methanol heated to 70°C in a water bath. The extract was then centrifuged at 5,000 × g for 10 min. The supernatant was used for the estimation of soluble sugars concentrations. The reaction mixture consisted of 1 ml 5% phenol and 5 ml 98% sulphuric acid. Once the extract had cooled, its absorbance was determined at 490 nm using D-glucose as standard.

Statistical Analysis

Statistical analyses, including test for homogeneity of variance, were performed using SPSS for Windows, version 11.5. The experimental data were analyzed with a one-way ANOVA to determine if significant differences were present among means. A Tukey test was used to determine the significant ($P < 0.05$) differences among treatments.

RESULTS

Growth Analysis

Water deficit significantly reduced whole plant biomass accumulation after 30 days of treatment. At the end of experimental period, the dry matter production of plants was 48 and 35%

of the controls when treated with 70 and 40% FC, respectively (Fig. 1A). The plants receiving a water regime of 100% FC have the highest number of leaves. In 45-day water-stressed plants, this parameter decreased significantly by 56 and 82% as compared to controls, respectively, at 70 and 40% FC (Fig.1B). Water-deficit stress increased dry matter allocation to the roots (data not shown). On the other hand, the root to shoot DW ratio increased significantly under severe stress treatment from 45 days (Fig. 2). This ratio allows evaluating the effect of water-deficit stress on the dry mass allocation of the plants. A reduction of the aerial biomass production to allow limited its water losses by the phenomenon of transpiration and to guarantee the turgescence of its cells.

Water Relations

The changes in the leaf RWC along with increase in water-deficit stress are presented in Fig 3A. At the end of treatment, the depressive effect of water deficit was more pronounced when watering regime intensified. Water deficit at 40% FC decreased the leaf RWC by 20% as compared to controls after 45 days of treatment (Fig. 3A). The Ψ_w was significantly lower in plants subjected to water-deficit stress than in controls (Fig. 3B). Water deficit at 40% FC decreased Ψ_w reaching the most negative values. With the same watering regime, the Ψ_w reached –2.1 MPa at the end of treatment.

Organic Solute Accumulation

At the end of experimental period, proline concentration was significantly increased in response to drought stress (Fig. 4A). The lowest concentration was recorded in control plants (3.06 μmol g^{-1} DM) and plants subjected to 70 and 40% FC accumulated, respectively, 5 and 15-fold more proline than the control.

Leaf-soluble sugar concentrations were significantly increased at 70 and 40% FC and represented, respectively ca. 132 and 151% of the controls (Fig. 4B). Linear regression analysis was used to determine the relationships between leaf proline concentration and branch water potential of plants subjected for 45 days to different drought stress. There was a strong negative relationship between these parameters, with a coefficient of determination R^2 = 0.85 (Fig. 5).

DISCUSSION

The drought stress is a very important limiting factor during early seedling growth and establishment (Jaleel *et al.,* 2009). It affects both elongation and expansion growth (Shao *et al.,* 2008). Results from this study indicate that water-deficit stress reduced the growth of *Z. lotus* by restricting leaf formation. Furthermore, the effect of drought stress indicating that shoot growth is more sensitive to water availability than root growth (Ashraf and Foolad, 2007). Our data are consistent with findings reported on *Z. mauritiana* (Clifford *et al.,* 1998) and *Z. rotundifolia* (Arndt *et al.,* 2001). Both *Ziziphus* species have developed various mechanisms to cope with restricted water supply. Osmotic adjustment may occur concomitant with an increased root growth, followed by leaf loss and ultimately drought-enforced dormancy (Arndt *et al.,* 2001; Clifford *et al.,* 1998). In particular, *Z. rotundifolia* has a high degree of plasticity in response to water deficits. It appears that the extensive root systems and readiness to shed leaves under severe drought constitute the main mechanism of success of *Ziziphus* species in extremely hot and arid environments (Sankhla, 1998; Jones, 1999). According to Gorai *et al.*, (2010), wild jujube is a typical phreatophyte by maintaining its vegetative growth throughout summer months and behaves as arido-active species (Evenari *et al.,* 1982). Gorai *et al.,* (2010) reported that this deep-rooted shrub is able to obtain water from lower soil horizons, and possibly from a free water table. This conforms to the report that rooting depth of *Z. lotus* in Morroco can reach about 60 m (Le Houérou, 1972).

Measurement of plant water potential has gained widespread acceptance as a useful approach to quantify plant and soil water status (Pallardy *et al.,* 1991). Water losses result in the decline of turgor and, this both results in a decrease in leaf water potential (Boyer, 1982). Maintaining leaf RWC under lowering leaf Ψ_w in drought stress condition is short term adaptation exhibited by *Z. mauritiana* to improve the ability of plant to extract moisture from a progressively drying soil profile (Clifford *et al.,* 2002),. In the present study the decline in Ψ_w can be related to drought tolerance and the water storage in the plant. Similar results were found in two species of the same genus, *Z. mauritiana* (Clifford *et al.,* 1998) and *Z. rotundifolia* (Arndt *et al.,* 2001). During the hot and dry summer, the midday water potential of wild jujube reached values almost near –4 MPa and the diurnal amplitude ($\Delta\Psi$) was more pronounced during the dry season than that of wetting months, revealing a high biological activity of this species (Gorai *et al.,* 2010).

Different abiotic stress factors may induce osmotic stress, oxidative stress and protein denaturation in plants, which lead to similar cellular adaptive responses such as accumulation of compatible solutes, induction of stress proteins, and acceleration of reactive oxygen species scavenging systems (Zhu, 2002). The accumulation of compatible solutes such as sugars, proline or glycine betaine in plants benefit stressed cells by protecting and stabilizing macromolecules and structures from damage induced by stress conditions (Papageorgiou and Murata, 1995; Bohnert and Jensen, 1996;). Such solute accumulation in response to drought stress is quite well documented and is an important part of osmotic adjustment (Martínez *et al.,* 2004; Ennajeh *et al.,* 2006). In our study plants subjected to severe water-deficit stress during 45 days, accumulate higher concentrations of proline (15-fold) and soluble sugars (1.5-fold) than controls. Proline accumulation under moisture stress was previously reported in *Ziziphus* species; besides, this amino acid plays an important role as a compatible solute in *Z. mauritiana* (Clifford *et al.,* 1998; Arndt *et al.,* 2000) and *Z. rotundifolia* (Arndt *et al.,* 2001). Previous studies on *Ziziphus* species have shown that the intensity of drought stress is an important factor in the expression of drought tolerance or avoidance with osmotic adjustment (Clifford *et al.,* 1998; Arndt *et al.,* 2001). Mucilages and glucans in the leaves of *Z. mauritiana* and *Z. rotundifolia* are reported to function as hydraulic capacitors and remobilizers of solutes for osmotic adjustment (Clifford *et al.,* 2002), thus enabling more effective water uptake and assimilate redistribution into roots and stems.

According to the analysis of morphological and physiological traits, and biomass allocation pattern under drought stress conditions, it is concluded that wild jujube plants were able to tolerate conditions of low water availability. Further, osmotic adjustment in leaves is an important mechanism enabling plants to cope with extreme drought.

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Figures

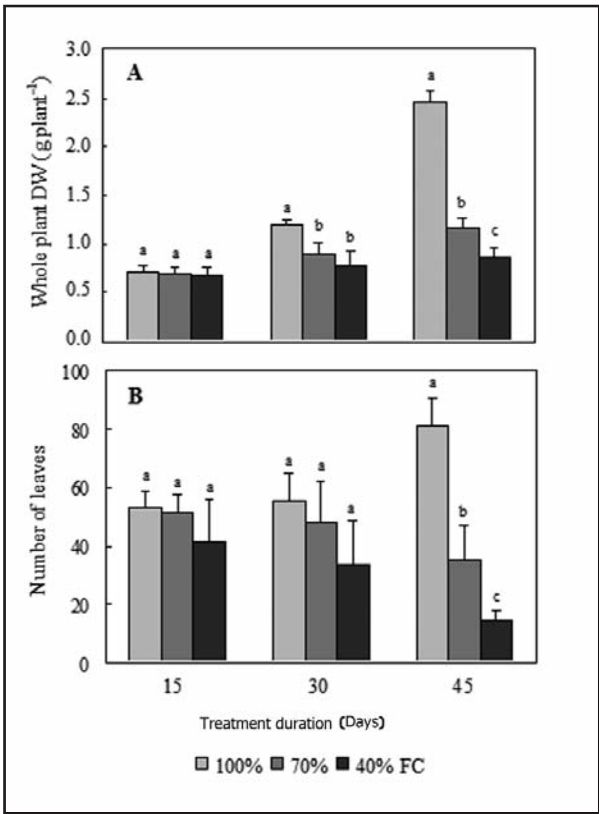

Fig. 1. Water-deficit stress effect on (A) whole plant dry mass (DW, g plant^{-1}) and (B) leaf number of *Ziziphus lotus* when 3-month old plants were subjected for 15, 30 and 45 days to three water regimes (100, 70, and 40% FC). Data represent mean ± 95% confidence limits, n = 4. Different letters indicate significant differences between treatments (P < 0.05).

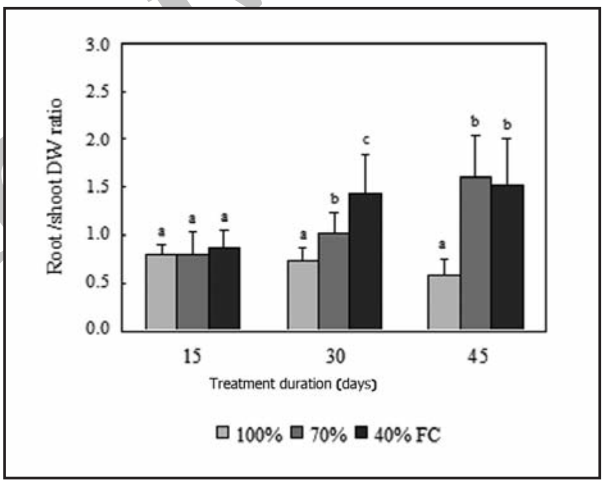

Fig. 2. Changes in the root/shoot dry mass ratio of *Ziziphus lotus* when 3-month old plants were subjected for 15, 30 and 45 days to three water regimes. Data represent mean ± 95% confidence limits, n = 4. Different letters indicate significant differences between treatments (P < 0.05).

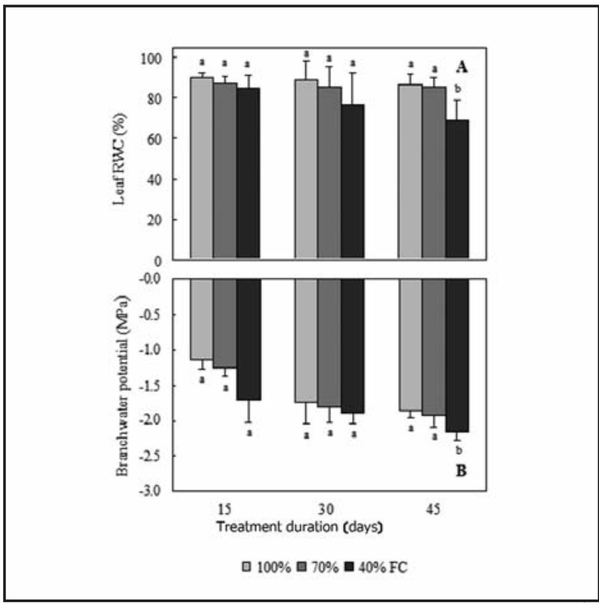

Fig. 3. Changes in (A) leaf relative water content (RWC, %) and (B) branch water potential (Ψ_W, MPa) of *Ziziphus lotus* when 3-month old plants were subjected for 15, 30 and 45 days to three water regimes. Data represent mean ± 95% confidence limits, n = 4. Different letters indicate significant differences between treatments (P < 0.05).

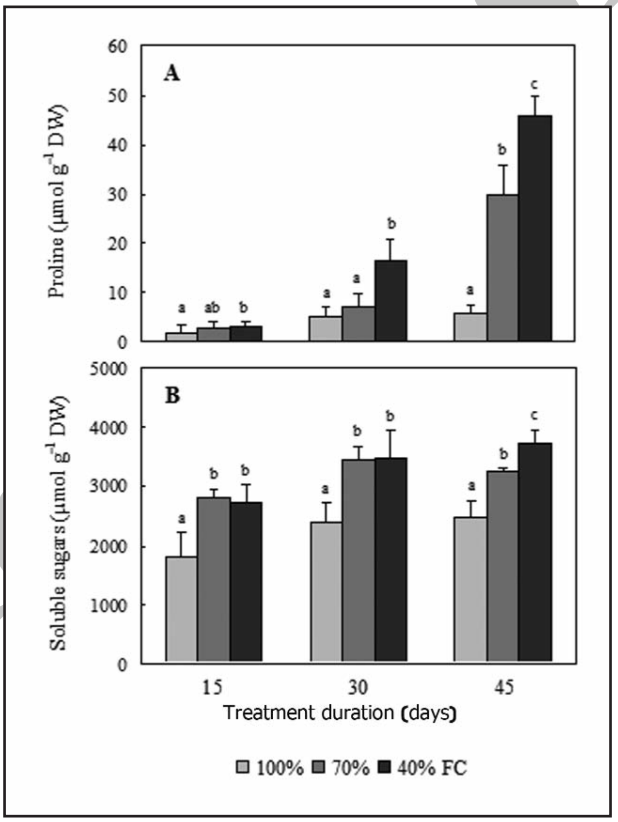

Fig. 4. Mean concentrations (µmol g^{-1} DW) of (A) proline and (B) soluble sugars in leaves of *Ziziphus lotus* when 3-month old plants were subjected for 15, 30 and 45 days to three water regimes. Data represent mean ± 95% confidence limits, n = 4. Different letters indicate significant differences between treatments (P < 0.05).

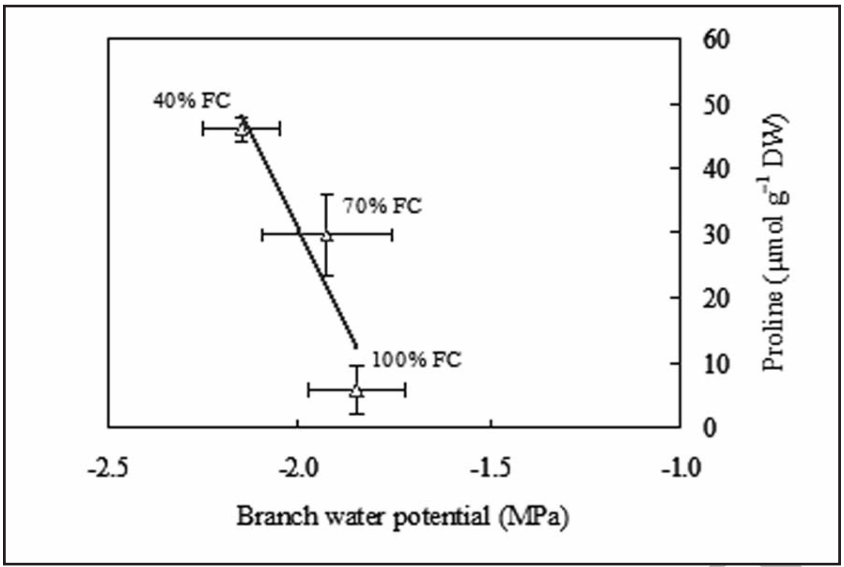

Fig. 5. Relationship between leaf proline concentration and branch water potential of *Ziziphus lotus* when 3-month old plants were subjected for 45 days to three water regimes. Data represent mean ± 95% confidence limits, n = 4. The line describing the dependency was obtained using linear regression, $R^2 = 0.85$.