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Effect of drought stress on the photosynthesis of *Acacia tortilis* subsp. *raddiana* at the young seedling stage

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Abstract

Water stress usually impairs photosynthesis and plant growth. *Acacia tortilis* subsp. *raddiana* is well adapted to dry environments. The aim of the present study was to determine the impact of a progressive decrease in soil water content on photosynthetic-related parameters at the young seedling stage. Drought-induced plant responses occurred according to two types of kinetics. Water potential, stomatal conductance, and transpiration rates were rapidly affected by a decrease in soil water content, while chlorophyll fluorescence-related parameters and chlorophyll concentrations decreased only when soil water content was lower than 40%. The maximal efficiency of PSII photochemistry in the dark-adapted state remained unaffected by the treatment, whatever the stress duration. *A. raddiana* accumulated high concentrations of soluble sugars in relation to a stress-induced early stimulation of sucrose-phosphate synthase activity, while stimulation of invertase and sucrose synthase led to fructose accumulation only at the end of the stress period. We suggested that sugar accumulation may be involved in osmotic adjustment and protection of stressed tissues. *A. raddiana* was thus able to protect its photosynthetic machinery under drought conditions and may be considered as a promising species for revegetation of dry areas.

Additional key words: gas exchange; growth parameters; stomata; sugar metabolism; water-use efficiency.

Introduction

Drylands have become the target of a significant loss of vegetation cover and biodiversity due to the increasing phenomenon of desertification. Changes in the pattern of land are also induced by human populations which contribute to the destruction of habitats, especially the Saharan pastures (Jaouadi *et al.* 2010). Such a vegetation disappearance is hastened by low and erratic rainfall and compromises livestock survival, which is of primary importance for autochthonous populations (Andersen and Krzywinski 2007). Biodiversity in harsh environments is, however, of paramount importance since it allows ecological system resilience by enhancing their ability to cope with numerous disturbances. Hence, dry land areas may allow us to identify valuable resources for the future since some species exhibit ecophysiological properties and genetic adaptations allowing them to sustain water shortage.

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Abbreviations: Chl – chlorophyll; DM – dry mass; *E* – instantaneous transpiration; ED – density of epidermal pavement cells; ETR – electron transfer rate; F_0 – the minimal fluorescence in the dark-adapted state; F_m – the maximal fluorescence in the dark-adapted state; FM – fresh mass; g_s – stomatal conductance; LA – leaf area; NPQ – nonphotochemical quenching; P_N – net photosynthesis; q_P – photochemical quenching; RWC – relative water content; SD – stomatal density; SI – stomatal index; SLA – specific leaf area; SPS – sucrose-phosphate-synthase; SuSy – sucrose synthase; SWC – soil water content; WUE – water-use efficiency; Ψw – shoot water potential; Ψs – shoot osmotic potential; Φ_{PSII} – actual PSII efficiency.
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Reforestation of desert areas requires the identification of drought-resistant woody plants that may constitute both shelter and food for mammals (Grouzis and Le Floc'h 2003). In this context, species belonging to the genus *Acacia* receive particular attention because of their positive impact on arid areas, where they improve seed trapping, as well as nutrients and moisture availability (Flores and Jurado 2003). Indeed, the genus *Acacia* (Tourn.) Miller (Fabaceae, subfamily Mimosideae, tribe Acacieae) has a wide distribution in the tropics, especially in arid and semiarid areas. These species of nitrogen-fixing plants are well adapted to the desert area, contributing to the setting of sand dunes and promoting the infiltration and redistribution of nutrients in the soil (Munzbergová and Ward 2002, Noumi *et al*. 2011).

Acacia tortilis (Forsk) Hayne ssp. *raddiana* (Savi) Brenan (hereafter designed as *A. raddiana*) was reported for the first time in Western Sahara by Cogne (1881, cited by Bensaid 1991) and is considered as one of the most xeric angiosperm trees (Bensaid 1991). It is an abundant and widespread leguminous woody species in the Algerian desert belt (Kennenni 1991, Bensaid 1988). It permits to create and to keep a microclimate and ecological niches favorable to the herbaceous strata and telluric microflora by its windbreak effect, its litter, its shadow, and its root exudations (Grego *et al.* 2003, Grouzis and Akpo 2003, Noureddine *et al.* 2010).

It is usually considered that drought resistance of *A. raddiana* is related to a deep root system allowing the adult plant to absorb water from deep horizons of the profile (Bensaid *et al.* 1996, Akinnifesi *et al.* 2004). In natural conditions, however, drought resistance at the young seedling stage is also crucial to allow the regeneration of the vegetation cover. At this early phenological stage, the root system is only poorly developed and consequently unable to explore soil at a sufficient depth to ensure the water supply. Hence, water stress resistance under such conditions is a direct function of physiological tolerance mechanisms in relation to the maintenance of photosynthetic process and stress-induced increase in the water-use efficiency allowing partial stomatal closure without detrimental effect on sugar synthesis (Warren *et al.* 2011). Water shortage may indeed impact stomatal behavior, photosynthetic pigment concentration, light phase of photosynthesis, CO_2 fixation, and enzyme activities involved in Calvin cycle, thus compromising sugar metabolism which directly conditions plant growth and survival (Delpérée *et al.* 2003, Vandoorne *et al.* 2012).

Following plant behavior during progressive water shortage allows to identify the sequence of stress-induced physiological modifications. This approach helps understand better the causes of growth inhibition and even death of stressed plants. In the closely related species, *Acacia arabica*, Lassouane *et al.* (2013) have recently demonstrated that a decrease in net photosynthesis induced by water stress was mainly due to stomatal limitations. However, this study did not quantify water stress impact on sugar metabolism. Moreover, to the best of our knowledge, no data are available in respect of *A. raddiana*, despite the ecological importance of this species. The present study was therefore undertaken in order to determine the impact of drought on photosynthesis of *A. raddiana* young seedlings exposed to a progressive drought stress during the early stages of their growth.

Material and methods

Plant materials and treatments: Seeds of *A. raddiana* were provided by Institut National de Recherche Forestière, Tamanrasset, South Algeria (22°47'13"N and 5°31'38"E). They were germinated on filter paper moistened with sterile demineralized water for three days in darkness at 29°C, then sown (one seed per pot) in square plastic pots (size 12×12 cm; 20 cm height) filled with loam (*Loam for Professionals*, *DCM N.V.*, Grobbendonk, Belgium). Seedling were sown under greenhouse conditions with a day/night temperature of 29/25°C, *ca.* 55% relative humidity, and 16 h photoperiod [mean radiation reached a maximum of 304 µmol(photon) m^{-2} s^{-1} at the top of the canopy]. Four lamps (*Philips HPIT 400 W*, *Philips Lighting S.A*., Brussels, Belgium) were used to supplement natural irradiation. After six weeks of culture, drought stress was imposed by withholding irrigation for 25 d. A control batch was maintained by irrigating the plants three times a week in order to maintain soil water content corresponding to 80% of the field capacity. Individual pots were randomly distributed within the greenhouse and their position was changed every week. All the leaves (except the first and the last one) were harvested and pooled on the 1st, 7th,12th, 17th, 22nd, and 25th day of drought (DD) and immediately transferred to liquid nitrogen for storage and subsequent biochemical analysis. Stem height and leaf number were recorded both in control and stressed plants for six plants per treatment. Stems, leaves, and roots were separated and weighed for fresh mass (FM) determination. Tissues were then incubated during 48 h in an oven at 70°C and weighed again to determine dry mass (DM) and calculate water content. The leaf area was estimated using a leaf area meter (*E-B95-AM350, Delta-T-Devices Ltd.*, Cambridge, UK). Specific leaf area (SLA) was calculated according to the formula SLA = leaf area/leaf DM.

Soil water content (SWC) and plant water status: Soil (30 g) was taken from the center of the pot at each sampling date. Soil wet mass (SWM) was determined both for control and stressed plants (five pots for each treatment) and oven dried at 105°C during 48 h for dry mass determination (SDM). Soil water content was calculated as: SWC [%] = (SWM – SDM) × 100/SDM.

The leaf water content of six plants for each treatment was measured after drying samples in an oven for 72 h at

70°C. The shoot water potential (Ψ_w) was measured at each treatment in the morning (between 10:30 and 11:30 h), using a Scholander pressure chamber (*PMS Instrument Co.*, Orlando, USA). For relative water content (RWC) determination, eight leaf discs (1 cm) were excised from three leaves and immediately weighed for FM determination. Discs were incubated in the dark at 4°C for 24 h, weighed again for turgescent mass (TM) determination and then oven dried at 105°C during 48 h and weighed again for DM assessment. Relative water content was calculated according to Barrs and Weatherley (1962) as: RWC = [(FM – DM)/(TM – DM)] × 100.

For osmotic potential (Ψs) determination, four leaves located at the middle portion of the stem were quickly collected from six plants per treatment, then placed in Eppendorf tubes perforated with four small holes and immediately frozen in liquid nitrogen. After being encased individually in another intact Eppendorf tube, they were allowed to thaw for 30 min and centrifuged at 15,000 × *g* for 15 min at 4°C. This step was repeated twice. The collected tissue sap was used for Ψs estimation. Osmolarity (C) was assessed with a vapor pressure osmometer (*5500*, *Wescor*, Logan, USA) and converted using the formula: Ψ_s [MPa] = −C [mOsmol kg^{-1}] × 2.58 × 10^{-3} according to the Van't Hoff equation (Zhu *et al.* 2001).

Stomatal density was estimated by counting the number of stomata per unit of leaf area. Clear nail polish was used to make prints of areas on the leaf abaxial surface. The number of stomata and epidermal cells were counted on four areas of 0.265 × 0.265 mm per leaf to determine stomatal and epidermal pavement cell density. Stomatal index (SI) was calculated using the following equation: SI = SD/ED, where SD is stomatal density and ED is the density of epidermal pavement cells.

Chlorophyll (Chl) fluorescence: Chl fluorescence-related parameters were measured in five plants per treatment by the *Fluorescence Monitoring System II* (*Hansatech Instruments*, Norfolk, UK) on the second and third fully unfolded leaf. All measurements (five per treatment) were performed on the middle part of the abaxial side of the leaf. Leaf portions were acclimated to darkness for 30 min. The minimal fluorescence level (F_0) was measured by measuring the modulated light (0.1 μmol m^{-2} s^{-1}). The maximal fluorescence level (F_m) with all PSII reaction centres closed was determined by a 0.8-s saturating pulse of 8,000 μmol(photon) m^{-2} s^{-1} in dark-adapted leaves. The leaf was then continuously illuminated with white actinic light of 1,200 μmol(photon) m^{-2} s^{-1} for 3 min. The steady-state fluorescence (F_s) was recorded and a second saturating pulse of 8,000 μmol(photon) m^{-2} s^{-1} was imposed to determine maximal fluorescence in the light-adapted state (F_m'). The actinic light was removed and the minimal fluorescence level in the light-adapted state (F_0') was determined by illuminating the leaf with a 3-s pulse of far-red light. Using both light and dark fluorescence parameters, the following parameters were calculated according to Maxwell and Johnson (2000): (*1*) maximal efficiency of PSII photochemistry in the dark-adapted state $F_v/F_m = (F_m - F_0)/F_m$; (*2*) photochemical quenching coefficient, $q_P = (F_m' - F_s)/(F_m' - F_0')$; (*3*) efficiency of excitation capture by open PSII reaction centres, $F_v'/F_m' = (F_m' - F_0')/F_m'$; (*4*) nonphotochemical quenching, $NPQ = (F_m - F_m')/F_m'$; and (*5*) actual PSII efficiency, $\Phi_{PSII} = (F_m' - F_s')/F_m$.

Photosynthtetic pigments: Chl *a*, Chl *b*, and total carotenoid (Car, xanthophylls, and β-carotene) concentrations were quantified on the whole leaves from five plants per treatment: *ca.* 100 mg of FM were ground in the dark in 10 ml of 80% (v/v) cold acetone and centrifuged at 5,000 × *g* for 10 min at 4°C. The absorbance of the sample was read at three different wavelengths (663.2, 646.8, and 470 nm) using a spectrophotometer (*UV-1800*, *Shimadzu*, Kyoto, Japan). The pigment concentrations were calculated according to Lichtenthaler (1987).

Gas exchange: Net photosynthesis (P_N) was recorded with an infrared gas analyzer (*LCA4 8.7*, *ADC Bioscientific*, Hoddesdon, Hertfordshire, UK) using a PLC Parkinson leaf cuvette on intact leaves for 1 min (20 records min^{-1}) with an air flow of 300 ml min^{-1}. Air taken in the greenhouse was sent to a chamber into which a leaf portion of 3.5 cm^2 was introduced. The P_N and instantaneous transpiration rate (E) were estimated on the second and third fully unfolded leaf. Five plants were measured for each treatment, and all measurements were performed around midday (between 12:30 and 14:30 h). The stomatal conductance (g_s) was measured using an *AP4* system (*Delta-T Devices*, Cambridge, UK) on the eighth leaf (acropetal numbering). Five plants were used for each treatment.

Total soluble sugars and starch concentration: Fresh leaves (*ca.* 300 mg of FM) collected from five plants per treatment were ground in liquid nitrogen, mixed with 7 ml of 70% ethanol (w/v) for 5 min on ice and centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was used for total soluble sugar quantification and identification of individual sugars, while the pellet was used for starch analysis. For total soluble sugars, 200 μl of the supernatant reacted with 1 ml of an anthrone solution (0.5 g anthrone, 250 ml of 95% H_2SO_4, and 12.5 ml of demineralized water); the absorbance was read at 625 nm (*DU 640*, *Beckman Coulter*, South Pasadena, USA) to quantify the total soluble sugars according to Yemm and Willis (1954). A calibration curve was established using glucose as the standard. The remaining supernatants were dried in a thermomixer under nitrogen flux (45 min at 50°C); dry residues were resuspended in 200 μl of milli-Q water and were filtered through 0.45 μm microfilters (*Minisart® RC4*, *Sartorius*, Goettingen, Germany) prior to analysis using a reversed-phase HPLC coupled to a refractive index detector (*RID-10A, Shimadzu,* The Netherlands). Samples (10 μl) were injected into a HPLC system (*Prominence*

System, Shimadzu, The Netherlands) equipped with a *Hypersil GOLD Amino* column (150 × 4.6 mm internal diameter, 3 μm particle size, *Thermo Scientific*, Hemel Hempstead, UK), a solvent delivery unit *LC-20AT*, a *SIL-HTc* autosampler, and a *RID-10A* refractive index detector (*Shimadzu,* The Netherlands). Samples were eluted at 28°C at a flow rate of 1 ml min^{-1} in an isocratic mobile phase which consisted of 83% acetonitrile and 17% pure water. Carbohydrates were quantified using seven-point calibration curves with custom-made external standard solutions, ranging from 5 to 100 mM and every ten injections, a check standard solution was used to confirm the calibration of the system.

For starch analysis, the pellets were treated with 5 ml of 65% perchloric acid overnight at 4°C and then centrifuged at 10,000 × *g* for 10 min according to McCready *et al.* (1950). The supernatant was collected and completed to 20 ml with demineralized water. The absorbance was read at 625 nm as described above.

Sugar-metabolizing enzymes: Sucrose-phosphate synthase (SPS, EC 2.4.1.14), sucrose-synthase (SuSy, EC 2.4.1.13), and invertase (acid and basic/neutral, EC 3.2.1.26) activities were determined in the leaves. For this purpose, 400 mg of fresh material was combined with 3.5 ml of extraction buffer (50 mM HEPES/KOH, pH 7.5, containing 100 mM KCl, 20 mM $MgCl_2$, 10 mM $NaHSO_3$, 2 mM EDTA, 1 mM phenylmethylsulphonylfluoride, 1 mM mercaptoethanol, and 0.1% Polyclar). The extract was then centrifuged at 10,000 × *g* for 10 min at 4°C. Two aliquots (1.5 ml each) were mixed with 6 ml of saturated ammonium sulphate. After 30 min at 0°C, the pellet was centrifuged at 10,000 × *g* for 10 min, washed twice with 80% (w/v) ammonium sulphate, and centrifuged as before. The obtained pellet was then resuspended in 3 ml of 50 mM HEPES/KOH buffer, pH 7.5, containing 100 mM KCl, 20 mM $MgCl_2$, and 2 mM EDTA. SPS activity was determined according to Huber *et al.* (1991). SuSy and invertase activities were assessed according to King *et al.* (1997), with slight modifications (Vandoorne *et al.* 2012) involving the use of a 3,5-dinitrosalicylic acid solution (45 mM 3,5-dinitrosalicylic acid, 10% NaOH, and 1 M potassium sodium tartrate) to stop the reaction after incubation. The protein concentration in the extract was estimated according to Bradford (1976) using bovine serum albumin as standard. and enzyme activities are expressed on a protein basis.

Statistical treatment: Experiment was repeated twice (June to October in 2011 and 2012) and results exhibited similar trends. Data presented hereafter are from one single experiment. The statistical analysis was performed using *STATISTICA* (*Version 6.0*, *Statsoft Inc.*). Data were analyzed using a one-way analysis of variance (*ANOVA*) at a significance level of $P<0.05$, $P<0.01$, and $P<0.001$ considering the presence or absence of stress as a factor. The statistical significance of the results was analyzed by the *Tukey*'s HSD test (Honestly Significant Difference).

Results

The soil water content remained at a constant value (78.5 ± 1.5%) throughout the experiment in controls, but it progressively decreased as a consequence of water withholding (Fig. 1). Differences between control and stressed treatments were significant already after 7 DD; SWC reached its minimal value (40.3 ± 4.1 %) at the end of the treatment.

The rate of leaf appearance was significantly affected by drought: the control plants produced a mean value of 14.4 leaves during the time course of the experiment, while stressed plants produced only 4.2 leaves (Fig. 2*A*). The shoot DM, the stem length, and the total leaf area were clearly lower in the stressed plants than in controls (Fig. 2*B–D*). The SLA, however, remained marginally affected, the difference between the control and stressed plants being significant only at the end of the treatment [337 ± 18 *vs.* 262 ± 14 cm^2 g^{-1}(DM) in controls and in the stressed plants, respectively].

The leaf RWC remained constant until the end of the experiment, but it drastically decreased at 22 and 25 DD (Fig. 3*A*). The shoot Ψ_w remained constant in well-watered plants but decreased already after 7 DD, reaching its lowest value at the end of the treatment (Fig. 3*B*). The leaf Ψ_s remained similar in the control and stressed plants until 12 DD and was slightly, but significantly lower in the stressed plants than in controls after 17 DD (Fig. 3*C*). Then, it drastically decreased until the end of the treatment.

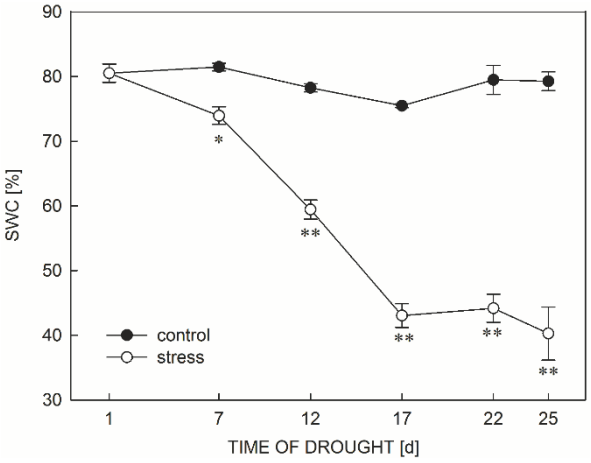

Fig. 1. Soil water content (SWC) in *Acacia raddiana* plants exposed to control (*closed circle*) and water stress (*open circle*) conditions. Mean ± SE ($n = 5$). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test.

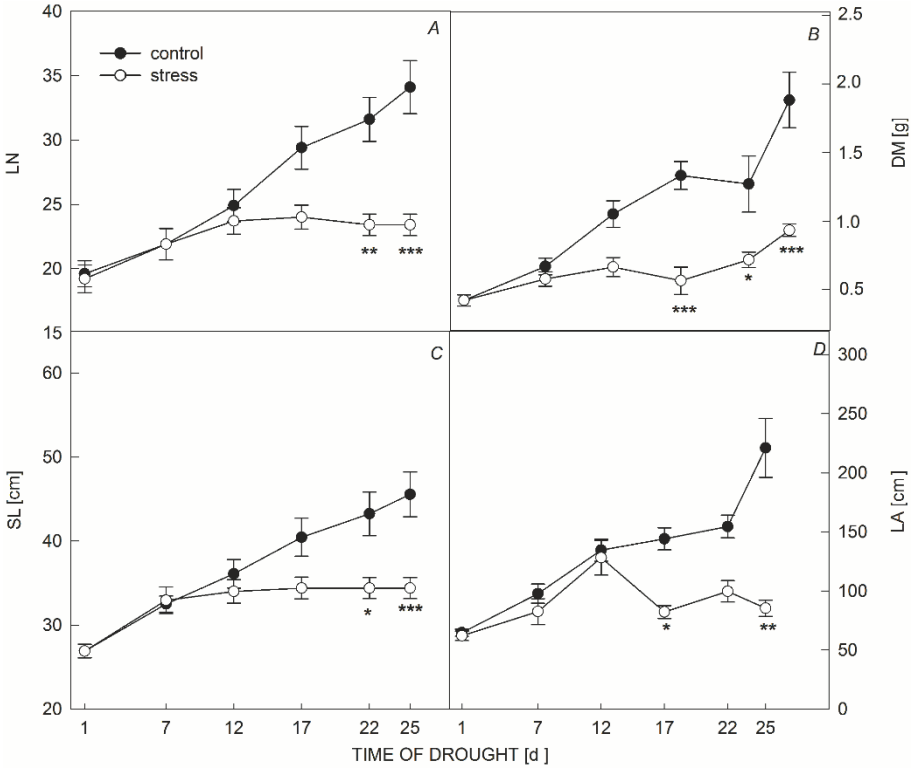

Fig. 2. Evolution of *Acacia raddiana* growth parameters under control (*closed circle*) and water stress (*open circle*) conditions: leaf number (LN) (*A*), dry mass (DM) (*B*), shoot length (SL) (*C*), and total leaf area (LA) (*D*). Mean ± SE (n = 6). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test.

P_N was lower in stressed plants than in controls (Fig. 4*A*). The lowest P_N value was recorded after 17 DD in the stressed plants. Then, it increased onwards, but remained significantly lower in the stressed than in control plants. The E was lower in the stressed plants than in controls already after 12 DD (Fig. 4*B*). Such a decrease in P_N and E values may be partly explained by a strong decrease in g_s starting already after 7 DD (Fig. 4*C*). It is, however, noteworthy that the instantaneous water-use efficiency (WUE) was higher in the stressed plants than in controls after 12, 22, and 25 DD (Fig. 4*D*).

Stomatal density was hardly affected by water stress (data not shown): an increase in stomatal density was, however, recorded for the youngest leaves of the stressed plants, which appeared after 17 DD (86.6 *vs*. 51.5 stomata mm^{-2} in the stressed plants and in controls, respectively). In those leaves, however, epidermal cells were also clearly smaller; therefore the stomatal index (number of stomata/number of epidermal cells) remained constant.

Total Chl and Car contents increased in the stressed plants at the end of the treatment, but Chl *a*/*b* ratio remained unaffected by drought (Fig. 5). Total protein concentration was higher in the stressed plants than in control after 17, 22, and 25 DD, thus demonstrating that protein synthesis and stability were not impaired by drought (Fig. 5*D*).

The maximal efficiency of PSII photochemistry in the dark-adapted state, F_v/F_m, remained unaffected by the treatment, whatever the stress duration (Table 1). The Φ_{PSII}, NPQ, and q_P remained similar in the control and stressed plants until 22 DD (Fig. 6*A*–*C*): at this time, both Φ_{PSII} and q_P were reduced by water stress, while NPQ increased at 25 DD. The electron transfer rate (ETR) decrease was significantly lower in the stressed plants compared with controls after 22 and 25 DD (Fig. 6*D*).

The total soluble sugar concentration increased and was significantly higher in the stressed plants than in control. Starch was also significantly higher in drought-treated plants after 7, 17, and 25 DD. Individual sugar quantification revealed an obvious increase in glucose concentration in the stressed plants compared with controls after 7 and 17 DD, while fructose significantly increased after 17 and 25 DD (Fig. 8). Fructose concentration was always higher than the glucose concentration. Sucrose concentration increased in response to drought; however, differences between the control and stressed plants were not significant at the end of the stress period.

Water stress had no significant impact on sugar-metabolizing enzyme activities after 1 DD (data not shown). As shown in Table 2, SPS activity increased with the age of the plant in controls but exhibited an opposite trend in the stressed plants. It is noteworthy that SPS activity was four times higher in the latter than in the former after only 7 DD. SuSy was two times higher in the stressed plants than in controls after 17 and 25 DD. Basic/neutral invertases were lower in the stressed plants than in the controls after 17 DD, while acid invertases were higher in the stressed plants, except at the end of the experiment.

Discussion

Acacia tortilis (subsp *raddiana*) is an important plant species in arid and semi-arid areas, where it may be used as a useful material for revegetation purposes. This plant is thus frequently exposed to a combination of abiotic stresses, such as drought, high temperatures, and high light intensities. Our experimental approach focused on the impact of water shortage at the seedling stage, as it may occur during natural regeneration below a dense canopy. Our data suggest that this plant species might cope with progressive soil dehydration and demonstrate that drought-induced physiological modifications occurred according to two types of kinetics. Indeed, some parameters (Ψ_w, g_s, total soluble sugars, and E) were quickly affected by the decrease in SWC, while others (Chl concentration and fluorescence, and Ψ_s to a lesser extent) were modified at the end of the experiment, when the water content in both soil and plant fell to very low values.

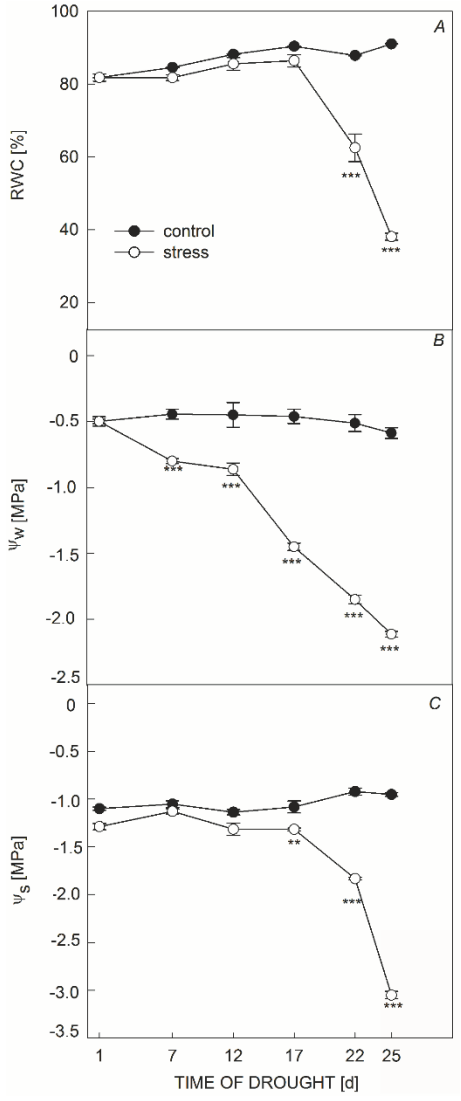

Fig. 3. Water status of *Acacia raddiana* under control (*closed circle*) and water stress (*open circle*) conditions: leaf relative water content (RWC) (*A*), water potential (Ψ_w) (*B*), and osmotic potential (Ψs) (*C*). Mean ± SE (n = 6). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test.

The increase in total soluble sugars occurred already after 7 DD, while at this time, SWC was reduced by less than 10% and growth was still significantly unaffected. Although osmotic adjustment may be a direct function of sugar accumulation (Chen *et al*. 2011) and has been reported as a major component in water stress resistance in *A. tortilis* (Otieno *et al.* 2005), sugar accumulation occurred before any decrease in Ψ_s under our experimental conditions. It did not result from starch consumption since starch also accumulated in the stressed plants. Such an increase might be related to a stimulation of SPS activity, leading to sucrose accumulation, which is known as one of the main sugars that accumulate in plants exposed to water deficit (Xu *et al*. 2007, Vandoorne *et al*. 2012). SPS stimulation was, however, transient in our drought-treated plants, while sucrose-cleaving enzymes (SuSy and invertases) were also stimulated on a longer term basis than SPS. These enzymes may contribute to fructose and glucose accumulation at a time when P_N started to decline, and accumulation of monosaccharides could contribute to the recorded decrease in Ψ_s starting at 17 DD concomitantly with SuSy stimulation. Sucrose indeed did not accumulate after 25 DD, while fructose still accumulated at a high concentration. The fact that glucose accumulation was not recorded at the end of the experiment may be, at least partly, explained by the stress-induced starch increase. Other *Acacia* species, such as *A. pycnantha*, were reported to accumulate galactose and arabinose (Vidanarachchi *et al.* 2009), but these sugars were detected only in very small amounts in *A. raddiana* (data not shown).

A decrease in water loss is of paramount importance for plants growing in soils with poor water availability. Hence, the recorded decrease in the leaf number and the lower LA may contribute to reduce water consumption. Otieno *et al*. (2005) reported that the reduction of LA occurring as a result of water stress in one-year old plants of *A. tortilis* may be explained by a reduced rate of leaf initiation. Our data corroborated this view and showed that this strategy might also occur in young plants, at the seedling stage. Xu and Zhou (2008) reported high flexibility in stomatal density in response to their water status. A lower stomatal density may improve drought resistance through the decrease in the transpiration rate (Barbieri *et al*. 2012, Orsini *et al*. 2012). Unexpectedly, the stomatal density expressed on the area basis increased rather than decreased in the expanding leaves of *A. raddiana* as a consequence of the reduced cell size. A limited impact of water stress on SLA also confirmed that modification in leaf morphology remained limited and supported the view of Novriyanti *et al*. (2012) that

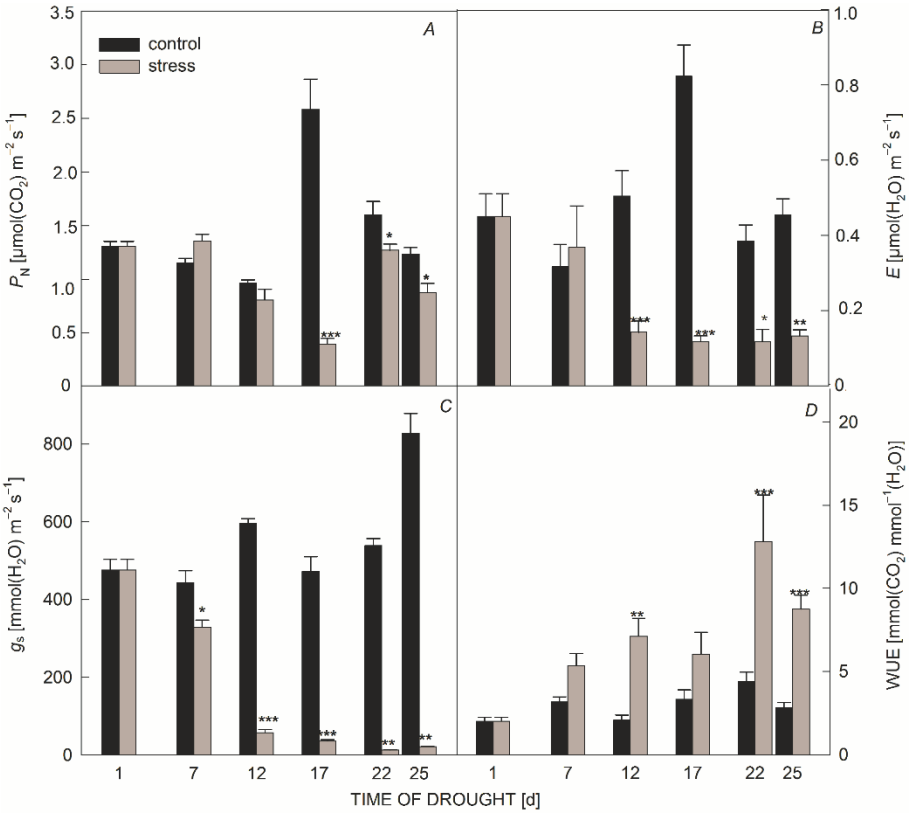

Fig. 4. Photosynthesis and gas exchange-related parameters of *Acacia raddiana* under control (*black*) and water stress (*grey*) conditions: net photosynthesis (P_N) (*A*), instantaneous transpiration (*E*) (*B*), stomatal conductance (g_s) (*C*), and instantaneous water-use efficiency (WUE) (*D*). Mean ± SE (n = 6). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test.

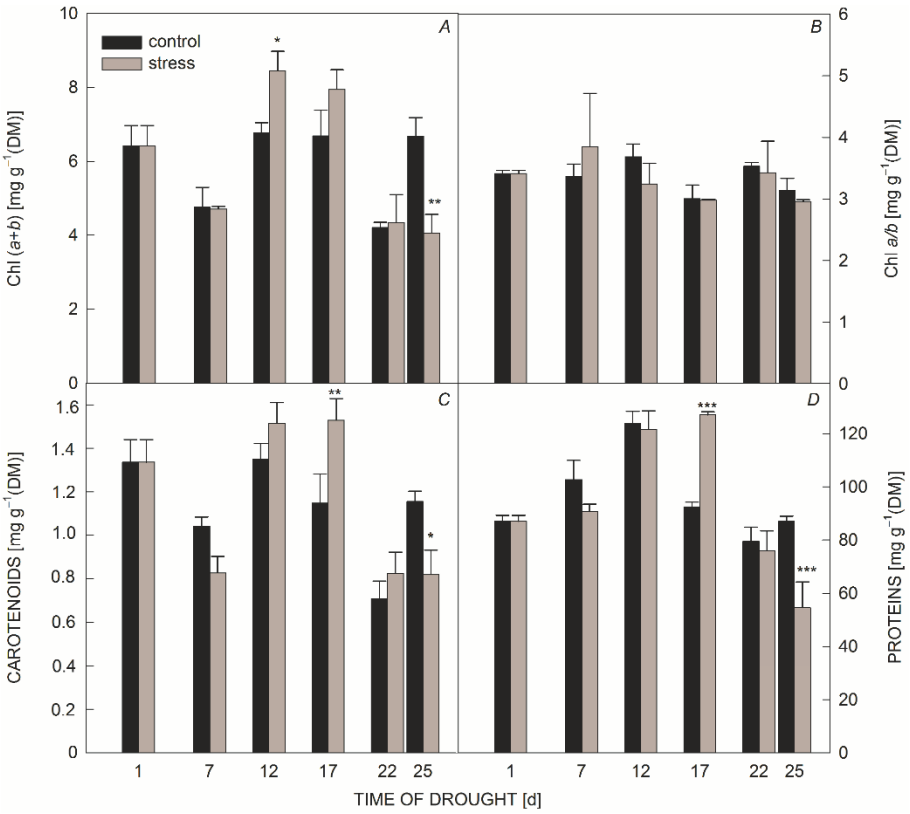

Fig. 5. Pigment and protein concentration in *Acacia raddiana* under control (*black*) and water stress (*grey*) conditions: total chlorophyll concentration (Chl *a* + *b*) (*A*), chlorophyll *a*/*b* ratio (Chl *a*/*b*) (*B*), total carotenoid concentration (*C*), and protein content (*D*). Mean ± SE (n = 5). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test. DM – dry mass.

Table 1. Maximal PSII efficiency (F_v/F_m) in *Acacia raddiana* plants exposed to well-watered (control) and drought (stress) conditions. Mean ± SE (n = 5). Significant difference with control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test.

Drought duration [d]	Control	Stress
1	0.85 ± 0.004	0.86 ± 0.004
7	0.84 ± 0.004	0.84 ± 0.008
12	0.83 ± 0.016	0.82 ± 0.026
17	0.86 ± 0.005	0.85 ± 0.013
22	0.83 ± 0.009	0.84 ±0.011
25	0.85 ± 0.004	0.84 ± 0.010

modification of leaf morphological properties was less efficient in acacias than in other xerophyte species such as eucalypts. In contrast to stomatal densities, the g_s was highly responsive to SWC decline and correlated to E values. High stomatal sensitivity helps to reduce water loss but also to avoid cavitation. Stomatal closure directly impacted P_N, but to a lesser extent than E (except at 17 DD). As a consequence, WUE steadily increased throughout the stress period. Carbon balance, however, depends not only on P_N but also on dark respiration, which was not estimated in this study. Gimeno *et al*. (2010) demonstrated that *Acacia* species regulate dark respiration homeostasis in such a way that carbon balance remains compatible with plant metabolism, even under periods of severe stress. The drought-induced growth inhibition could thus appear as an efficient strategy to reduce metabolite requirements during a stress period, when CO_2 assimilation is not guaranteed.

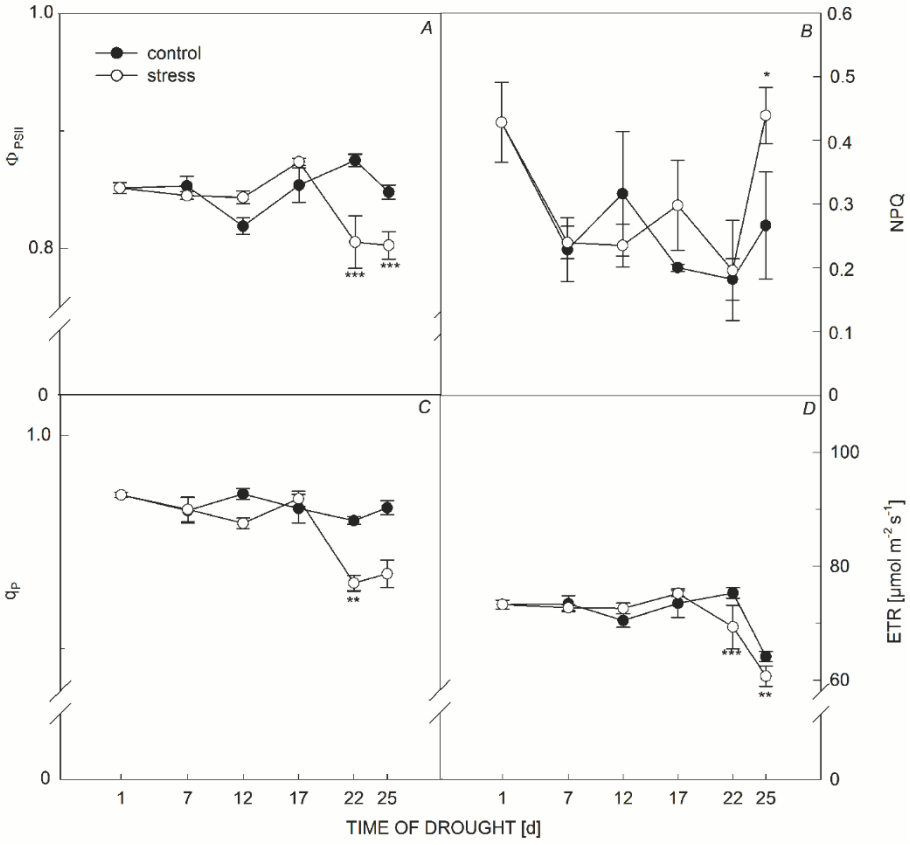

Fig. 6. Chlorophyll fluorescence-related parameters of *Acacia raddiana* under control (*closed circle*) and water stress (*open circle*) conditions: actual PSII efficiency (Φ_{PSII}) (*A*), nonphotochemical quenching (NPQ) (*B*), (*C*) photochemical quenching (q_P) (*C*), and electron transport rate (ETR) (*D*). Mean ± SE (n = 6). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test.

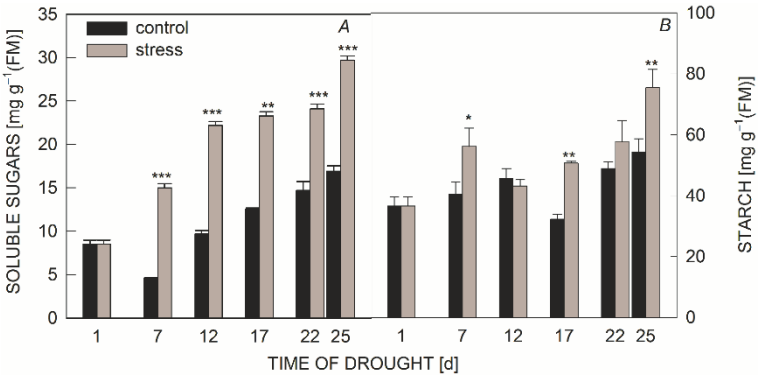

Fig. 7. Total soluble sugar (*A*) and starch (*B*) concentration in *Acacia raddiana* under control (*black*) and water stress (*grey*) conditions. Mean ± SE (n = 6). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test. FM – fresh mass.

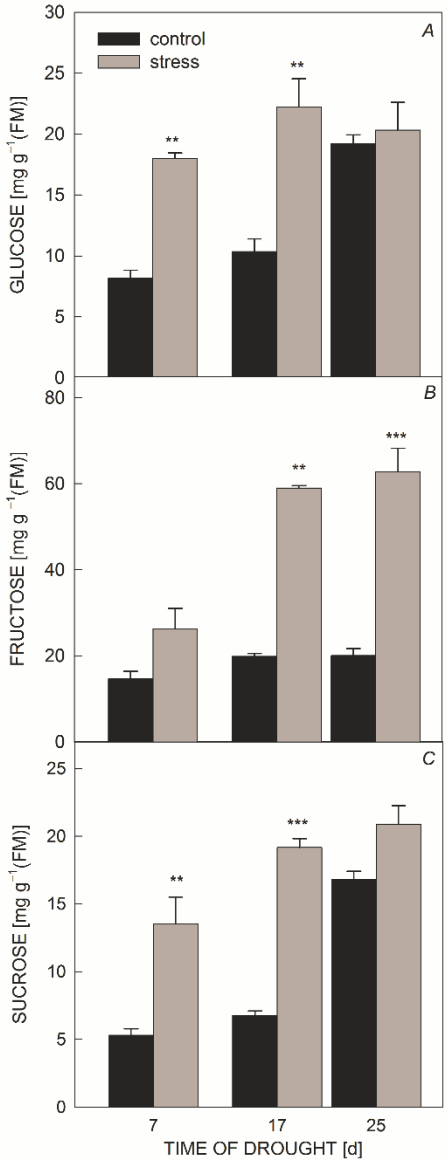

Fig. 8. Sucrose (*A*), glucose (*B*), and fructose (*C*) concentrations in *Acacia raddiana* under control (*black*) and water stress (*grey*) conditions. Data are given for 7, 17, and 25 d of treatment. Mean ± SE (n = 6). Significant difference from control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test. FM – fresh mass.

Acacia raddiana also displays an extraordinary capacity to protect the photosynthetic apparatus from deleterious impact of water stress. The PSII maximum quantum efficiency (F_v/F_m) remained unaffected, even under severe water deficit conditions. Photochemical quenching, which is a direct indicator of the proportion of open reaction centres, started to decline significantly only when RWC dropped to very low values; the reduction state of the Q_A pool thus increased only as a consequence of very low SWC. Since Φ_{PSII} is a direct product of q_P and the antenna conversion efficiency, the fact that Φ_{PSII} followed the same trend as q_P indirectly suggests that this efficiency was not drastically compromised by water stress. This might be related to the fact that Chl content was not reduced (and even increased in some cases) in response to water stress and that Chl *a*/*b* ratios exhibited a constant value. It has been postulated that low Chl *a* may be regarded as a protective adaptive mechanism preventing an excess of photon absorption (Ait Said *et al.* 2013). This may be the case under conditions of high light intensities, but it is not supposed to occur under our experimental conditions.

Nonphotochemical quenching includes thermal dissipation of excess absorbed photons. According to Yu and Ong (2002), it increased in *Acacia mangium* at high temperatures. As far as *A. raddiana* is concerned, NPQ did not represent a major parameter involved in PSII protection in seedlings exposed to soil water deficit under moderate temperature, since a significant increase was recorded only at 25 DD in highly dehydrated leaves. High NPQ capacity is often associated with a higher xantophyll cycle activity. Protecting Car content (which includes xantophylls in our case) increased after 12 DD, even if NPQ remained unaffected, and might thus protect thylakoid membranes from oxidative stress. Taken together, these observations confirmed the stability and plasticity of the photosynthetic machinery of *A. raddiana.* It explained the maintenance of ETR, which concerns the noncyclic transport of electron, except when Ψ_w values fell below –1.75 MPa. We hypothesize that photosynthesis of this species is mainly limited by stomatal rather than non-stomatal causes under drought conditions. The *Acacia* genus displays a wide range of morphological and physiological adaptations to environmental constraints (Otieno *et al.* 2005, Gimeno *et al.* 2010, Warren *et al.* 2011). The behavior of *A. raddiana* in response to water stress is, however, comparable to the behavior of *A. arabica*, which also shows an ability to maintain PSII efficiency, photochemical quenching, and Chl concentrations at very low soil water content (Lassouane *et al.* 2013).

It is noteworthy that leaf RWC dropped abruptly to very low values at the end of the experiment. This may suggest a sudden rupture of all metabolic strategies adopted by the plant to cope with transient water stress, when stress duration is too long and the plant capacity to cope with the constraint is overwhelmed. In such plants, sugar accumulation may be the consequence of a passive dehydration process rather than an active stimulation of sugar synthesis leading to accumulation. However, despite the very low water content, the plants still exhibited enzyme activities and net photosynthesis, suggesting that the metabolic activity was still maintained under these conditions. Rehydration experiments, should allow us to determine if those plants encountered a permanent wilting process or if they are still able to recover after the stress relief.

Taken together, our results demonstrated that *A. raddiana* was able to cope with a progressive decline of the soil water content at the seedling stage. It accumulated soluble sugars, reduced both water and osmotic potential,

Table 2. Leaf sucrose-phosphate synthase (SPS), sucrose synthase (SuSy), acid invertase (IA), and neutral invertase (IBN) activities in *Acacia raddiana* plants exposed to well-watered (control) and drought (stress) conditions. Mean ± SE (n = 5). Significant difference with control at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ by *Tukey*'s multiple test.

	Drought duration [d]	Control	Stress
SPS [nmol mg(protein)$^{-1}$ min^{-1}]	7	8,636 ± 95	3,227 ± 455***
	17	1,736 ± 216	1,434 ± 93
	25	2,357 ± 210	1,743 ± 225
SUSY [nmol mg(protein)$^{-1}$ min^{-1}]	7	261 ± 25	149 ± 11
	17	329 ± 47	929 ± 87***
	25	323 ± 58	715 ± 80***
IBN [nmol mg(protein)$^{-1}$ min^{-1}]	7	51 ± 6	142 ± 31.57*
	17	676 ± 22	332 ± 14***
	25	382 ± 44	283 ± 24*
IA [nmol mg(protein)$^{-1}$ min^{-1}]	7	52 ± 1	319 ± 38***
	17	752 ± 66	911 ± 52*
	25	349 ± 40	119 ± 5**

and exhibited a high stomatal sensitivity leading to a rapid stomatal closure under stress conditions. The photosynthetic apparatus appeared to be resistant to water stress as indicated by an increase in the carotenoid content and stability of chlorophyll-related fluorescence parameters. Hence, the drought-induced decrease in net photosynthesis occurred mainly due to stomatal limitations, but plants were able to adapt their growth in order to reduce assimilate consumption. This plant species appears to be a promising material for reforestation of arid areas.

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