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Biomass production, photosynthesis, and leaf water relations of *Spartina alterniflora* under moderate water stress

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Abstract The perennial smooth cordgrass, *Spartina alterniflora*, has been successfully introduced in salty ecosystems for revegetation or agricultural use. However, it remains unclear whether it can be introduced in arid ecosystems. The aim of this study was to investigate the physiological response of this species to water deficiency in a climate-controlled greenhouse. The experiment consisted of two levels of irrigation modes, 100 and 50% field capacities (FC). Although growth, photosynthesis, and stomatal conductance of plants with 50% FC were reduced at 90 days from the start of the experiment, all of the plants survived. The water-stressed plants exhibited osmotic adjustment and an increase in the maximum elastic modulus that is assumed to be effective to enhance the driving force for water extraction from the soil with small leaf water loss. An increase in the water use efficiency was also found in the water-stressed plants, which could contribute to the maintenance of leaf water status under drought conditions. It can be concluded that *S. alterniflora* has the capacity to maintain leaf water status and thus survive in arid environment.

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Introduction

Salinity and water shortage are the major constraints affecting fodder production and yield stability in most arid and semiarid regions. The climate is mainly characterized by low rainfall, high temperatures in summer and low temperatures in winter. This, together with scarcity and low quality of the water resources, prevents a quick recovery of the soil plant covering (Save et al. 1999). Because of these restrictions, the use of salt- and drought-tolerant species for revegetation and preservation of soils with little plant covers is one possible alternative (Morales et al. 1998). However, some halophytes can tolerate salt stress, but not drought, and some xerophytes can tolerate drought, but not salt stress (Kefu et al. 2003).

Spartina alterniflora Loisel is a rhizomatous perennial, native to the Atlantic and Gulf cost of North America (Bradley and Morris 1991). It is recognized as one of the most important costal grass species and commonly dominates costal salt marshes (Pomeroy and Wiegert 1981). It has also been introduced along the Pacific coast of North America, and in many other countries, because this species can be used as fodder for fowl, goats, and pigs (Zheng and Xu 1994; Zheng and Zhang 1995; Lin and Li 1999), as a source of bioactive material (Hu and Qin 1998), and for sewage treatment (Liu and Tian 2002) and the production of green fertilizer (Wang et al. 1996).

Successful introduction of *S. alterniflora* in arid salty ecosystems of Tunisia depends largely on its capacity to tolerate the specific environmental constraints, such as salinity and drought stress. Recently, Vasquez et al. (2006)

showed that *S. alterniflora* could tolerate a wide range of salinity stresses. However, limited and sporadic reports were made about drought tolerance of *S. alterniflora* (Brown et al. 2006; Brown and Pezeshki 2007).

Drought tolerance of plants species in arid areas may be partially explained by the involvement of complex functional and structural adaptations, among which are growth regulation, osmotic adjustment, and changes in the elastic properties of leaf tissues (Serrano et al. 2005; Stoyanov 2005; Martínez et al. 2007). Osmotic adjustment is achieved by synthesizing and accumulating organic solutes. These soluble compounds, which include soluble carbohydrates, glycine betaine, polyols, and amino acids (Colmer et al. 1996; Dichio et al. 2003), protect plants from osmotic stress by cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Bohnert and Jensen 1996; Ashraf and Foolad 2007). Thus, salt tolerance of *S. alterniflora* was associated with high accumulation of Na^+, soluble carbohydrate, glycine betaine, and proline (Colmer et al. 1996).

Some data indicate that plants subjected to dehydration may avoid reduced water potential and maintain turgor by reduction of their cell volume via shrinkage associated with elastic adjustment of their cell walls (Martínez et al. 2007). However, inelastic cell walls, although precluding turgor maintenance at low water contents, do have several advantages over elastic cell wall. Rigid cell walls may help maintain lower water potential at any given volume than do elastic ones. This can result in an increase in the gradient in water potential between the soil and the plant, thereby promoting more effective water uptake from drying soils and/or accelerating recovery after re-watering (Patakas et al. 2002). Nevertheless, the effects of drought stress on the cell wall elasticity are not clear (Mustard and Renault 2004).

The aim of this study was to examine whether *S. alterniflora* has the capacity to maintain leaf turgor and photosynthetic activity and to realize positive growth under drought conditions.

Materials and methods

Plant material and multiplication

Spartina alterniflora, a monocotyledoneous halophyte of the family *Poaceae*, is a perennial species native to salty marginal lands. The mother plants from its native range (USA) were cultivated in outdoor containers filled with a mixture of sandy soil and organic matter, and irrigated with tap water in an experimental station near the sea shore, 35 km northeast of Tunis (10°10′E, 36°48′N; mean annual rainfall and temperature were 19.4°C and 456 mm,

respectively), at an altitude of 10 m. *S. alterniflora* cuttings (0.25 m height) were taken and washed before transplanting them individually into 4-l blow-molded containers (one plant plug per container) filled with limono-sandy soil containing 0.25, 0.95, 0.65, and 0.05 meq/100 g of dry soil of Na^+, K^+, Ca^{2+}, and Cl^-, respectively, and 0.24 and 0.45 g/kg of dry soil of P_2O_5 and total N, respectively. The pH and the electrical conductivity of the aqueous extract (1/10) were 6.65 and 0.05 mmhos cm^{-1}, respectively. The field capacity (FC) of soil, measured according to the technique of Bouyoucos (1983), was 11.5%.

Drought treatment

Two irrigation modes were introduced after 30 day of transplanting: 100 and 50% FC. The latter was selected to represent water deficit stress on the basis of a preliminary experiment carried out on *S. alterniflora* watered with 100, 50, and 25% FC, showing that only up to a moderate water deficit stress (50%) a significant osmotic adjustment took place. After 1 month of ample watering (100% FC), an initial harvest was achieved. Plants were divided in two plots that were rewatered to weight every 2 days with tap water at 100% FC (control plants) for the first one and at 50% FC (drought treatment) for the second one. Evaporation from the soil surface was prevented by enclosing all pots in plastic bags sealed at the base of the rhizome of each cutting.

In addition, ten pots without plants were used to monitor evaporative water loss from the soil surface throughout each watering regime. For all treatments, tap water was enriched with diluted nutrient solution (Hewitt 1966). Independently of the procedure for watering (100 or 50% FC), plants received the same quantity of nutrients and were grown in ten randomised rows under mono-specific conditions (natural light, an average temperature of 25/18°C day/night and a relative humidity of 65/90%). Each row contained two individuals with different water availability, so that there were ten samples per treatment.

Shoots and roots for ten plants were harvested monthly for 90 days. Harvesting was done at 9:00 am.

Growth and electrolyte leakage

Fresh and dry weights of shoots and roots of each plant were determined after counting the leaf number. The fresh weight (FW) was determined 1 h after harvest; during this time, samples were wrapped in wet paper and enclosed in a plastic bag. The dry weight (DW) was obtained after leaf or shoot samples were dried to constant weight at 60°C.

Electrolyte leakage was measured as described by Dionisio-Sese and Tobita (1998). Fresh leaves (200 mg) were

cut into pieces of 5-mm length and placed in test tubes containing 10 ml distilled deionised water. The tubes were incubated in a water bath at 32°C for 2 h, and the initial electrical conductivity of the medium (EC1) was measured. The samples were then autoclaved at 121°C for 20 min to release all electrolytes and finally cooled to 25°C for the measurement of the electrical conductivity (EC2). The electrolyte leakage (EL) was calculated by using the formula:

$$EL = (EC1/EC2) \times 100.$$

Measurements of leaf water potential, photosynthesis, transpiration, and stomatal conductance

Leaf water potential (LWP) was measured on fully expanded mature leaves ($n = 10$ per treatment) at the end of the drought treatment (90 days), using the pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA) according to Scholander et al. (1965). Diurnal changes in stomata conductance, photosynthesis, and transpiration were also measured at the end of the drought treatment, using a portable gas-exchange system (Li-Cor 6200, Li-Cor Lincoln, NE). The ratio of net photosynthetic rate to stomatal conductance was taken as an estimate of intrinsic water use efficiency (Mediavilla et al. 2002). Measurements were made on fully expanded mature leaves ($n = 10$ per treatment).

Pressure–volume curves

The various components of water potential, osmotic adjustment, and the elastic modulus of tissues were calculated using P–V curves analysis on 7–10 completely expanded leaves per treatment. Leaves were collected predawn at 05:00 am, wrapped in damp paper, and enclosed in a plastic bag. The tissues were rehydrated by immersing them in distilled water in a beaker sealed with parafilm. Beakers were then stored in complete darkness at 2–4°C. Full rehydration was, on average, achieved in 24–48 h. Water potential at full turgor (Ψ_s^{100}) of leaves was close to zero (−0.01 MPa). P–V curves were determined using the Scholander pressure-chamber technique (Scholander et al. 1965). The flow of nitrogen was set to give a pressure rise of approximately 0.005 MPa s^{-1}.

To obtain the first points on the P–V curve, the leaves were subjected to stepwise increases of 0.2 MPa. At pressures above 2.5 MPa, stepwise increases of 0.5 MPa were applied to obtain the remaining points on the curve up to pressures of 5.0 MPa.

At each step, the quantity of xylem sap expressed from the cut surface was determined. The dry weight of the leaves was measured afterwards. The P–V curves of each leaf were obtained by expressing the relationship between the RWC values and the reciprocals of the water potentials measured ($-1/\Psi$). Osmotic potential at full turgor (Ψ_s^{100}), which equals the cell turgor at full hydration, was estimated via linear regression of data in the straight-line region of the P–V curves. Osmotic potential at zero turgor (Ψ_s^0) was derived from the X and Ψ coordinates, respectively, of the first point in the straight-line region of the P–V curves (Patakas and Notsakis 1999). The degree of total osmotic adjustment (OA_t) was defined as the difference in Ψ_s^{100} between the control ($\Psi_{s_c}^{100}$) and the stressed plants ($\Psi_{s_s}^0$) (Martínez et al. 2004).

The cell wall elasticity (CWE) of leaf tissue was estimated through the determination of the elasticity module (ε) value. This parameter was determined after Patakas and Notsakis (1999) using the expression:

$$\varepsilon = \left(\Psi_s^{100} - \Psi_s^0\right)(1 - AWC)/(1 - RWC_0)$$

where AWC is the apoplasmic water content and RWC_0 is the relative water content at zero turgor.

Inorganic ion assay

Nitrate contents were determined in aqueous extracts using 0.1–0.2 g dry powder in 10 ml distilled water (Soltani et al. 1992). Cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (phosphorus, Pi and Cl^-) were extracted by adding 30 ml nitric acid 0.1 N to 30 mg of plant dry powder. Cations were assayed by flame emission photometry, chloride by coulometry with a Haake–Buchler chloridometer, and inorganic phosphorus (Pi) photometrically by the method of Fleury and Leclerc (1943) modified by Soltani et al. (1992). The concentration of organic acids or carboxylates (C–A) was determined as the difference between the sum of inorganic cations (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}; total = C) and the sum of inorganic anions ($H_2 \cdot PO_4^{2-}$, NO_3^-, Cl^-, SO_4^{2-}; total = A) (Soltani et al. 1992).

Analysis of tissue carbon and nitrogen

Homogenized dry material of green leaves was analysed for carbon and nitrogen content (% dry weight) using a CNS analyser (Macro analyser Vario Max, Elementar Analysensysteme manufacturer, Hanau, Germany).

Analysis of soluble carbohydrates and proline content

Free proline and soluble sugars in 1 g fresh weights of leaf samples were determined spectrophotometrically according to Bates et al. (1973) and Staub (1963), respectively.

Contribution of solutes to osmotic adjustment

Concentrations of soluble sugars, proline, and inorganic ions were calculated for symplastic water volume at full turgor, according to the different fractions in control (100% FC) and stressed leaves (50% FC), estimated by the pressure–volume technique (Patakas et al. 2002). These concentrations were used to estimate the contribution of each solute to total osmotic adjustment, assuming that 40 μmol g^{-1} of symplastic water corresponds to 0.1 MPa (Patakas et al. 2002).

The contribution of each solute (s) to the total osmotic adjustment (OA_t) was calculated using the formula:

$$OA_{(s)} \text{ in } \% = \left[\frac{\left([s]_{stressed} - [s]_{control} \right) \times 0.1 \times 100}{40} \right] \Big/ OA_t$$

Statistical analysis

Two-way analysis of variance (ANOVA) was performed for all data sets with treatment and row considered as the main factors. When the ANOVA was significant at $P < 0.05$, Duncan's multiple range test was used for means comparisons. All data were analyzed by a SAS statistical package.

Results

Plant growth

Water deficit significantly decreased the plant growth only at the final harvest (Fig. 1a). Dry matter accumulation of drought-treated plants was 60% of the control ones; both leaf area and number of living leaves decreased by 36 and 42%, respectively, as compared to control plants (Fig. 1b, c). None of the drought-treated plants died during the treatment period (90 days).

Photosynthesis, transpiration, stomatal conductance, and water use efficiency

Figure 3 shows the effects of drought treatment on the rates of photosynthesis and transpiration and stomatal conductance of fully expanded mature leaves in *S. alterniflora*. Diurnal photosynthetic (Pn) and transpiration rates (Tr) were significantly lower in stressed plants than in control plants (Fig. 3a, b). In accordance with this result, diurnal changes in stomatal conductance was also significantly lowered in dehydrated plants than in control plants and followed the same pattern as Pn and Tr in both treatments (Fig. 3c). Stomatal conductance of stressed plants exhibited the lowest value when water potential reached a minimum
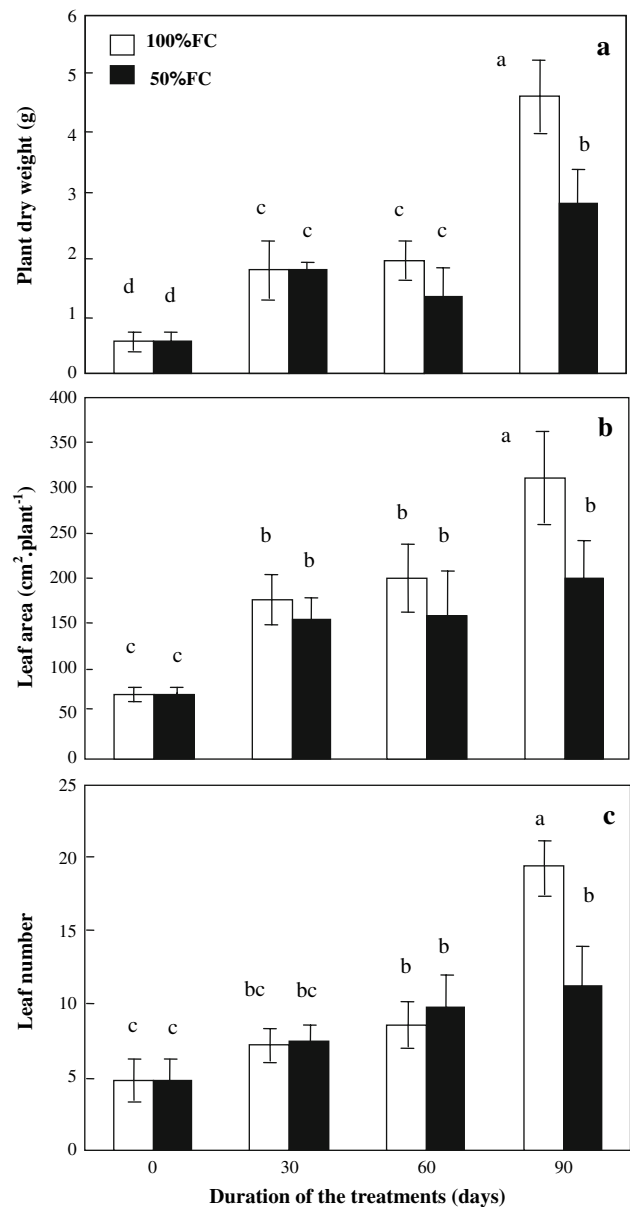
Fig. 1 Whole-plant dry weight (**a**), leaf area (**b**), and the number of leaves per plant (**c**) of *S. alterniflora* watered with 100 and 50% field capacities (*FC*). *Boxes* represent means and error bars represent ±SE of the means (*n* = 10). Values with different letters are significantly different at *P* = 0.05 level (Duncan's multiple range test)

value of 2.5 MPa at midday (Fig. 2). Water use efficiency remained almost constant in control plants and significantly increased in stressed plants, particularly at midday.

Plant water relations and electrolyte leakage

Water relation parameters for both control and water-stressed plants estimated from the corrected P–V curves are listed in Table 1. Both osmotic potential at full turgor (Ψ_s^{100}) and at turgor loss point (Ψ_s^{0}) decreased dramatically in water-

stressed plants. However, the percentage of apoplasmic water content (AWC) increased significantly in water-deficient, as compared with well-watered plants (Table 1). The leaf bulk modulus of elasticity, calculated at potentials near full turgor, was higher in water-deficient than in well-watered plants. Membrane damage, assessed by solute leakage of well-watered and droughted plants, was significantly higher in water-deficient plants (Table 1).

Leaf carbohydrate, proline, nitrogen, carbon, and inorganic ion content

Water stress induced a significant increase in proline and soluble carbohydrate content in leaves of *S. alterniflora*
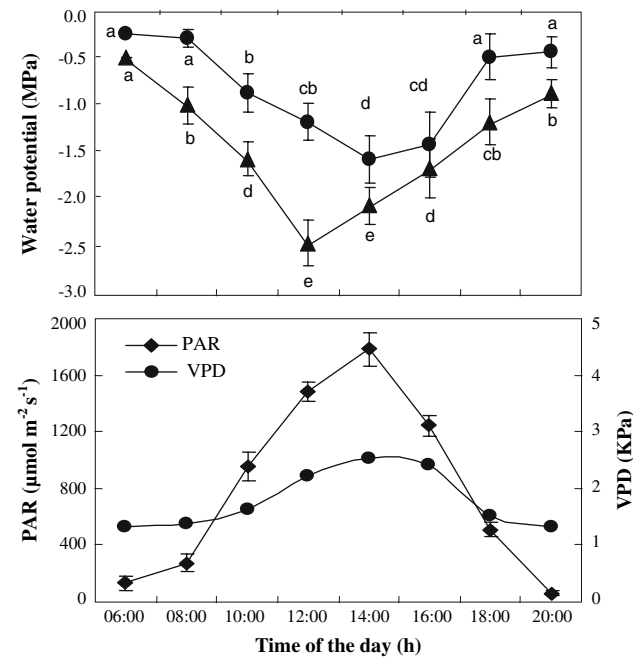
Fig. 2 Diurnal changes in leaf water potential in stressed and control plants and diurnal courses of environmental factors [photosynthetically active radiation (PAR) and air vapour pressure deficit] at the end of the treatment cycle (90 days). The vapour pressure deficit (VPD) was calculated from the saturation vapour pressure and relative humidity. *Bars* indicate the standard error of the mean of ten replicates. Values with different letters are significantly different at $P = 0.05$

(Table 2). Organic acid concentration, estimated by the difference between the sum of inorganic cations (C) and the sum of inorganic anions (A) on a unit symplastic water volume basis, was 1.3-fold higher in water-stressed than control plants (58 and 72.8). Water stress did not affect the C content of *S. alterniflora*. Therefore, the 1.3-fold increase in organic acid concentration seemed to be caused by the lack of inorganic anions uptake. Although the concentrations of total soluble carbohydrates were doubled and of proline were quadrupled, the carbon content in leaves of *S. alterniflora* remained almost constant under water deficit stress (Tables 2, 3). However the leaf N content increased significantly in conformance with NO_3^- (Table 2) and proline (Table 2) concentration.

Contribution of solutes to osmotic adjustment

The involvement of sugars and proline at the osmotic potential at full turgor (Ψ_s^{100}) increased from 4.5 to 0.3% in well-watered plants to 7.1 and 1% in water-deficient plants, respectively (Table 4). Inorganic ion concentrations accounted for 49.9 and 60% of the Ψ_s^{100} in stressed and well-watered plants (Table 4). Osmotic adjustment was probably due to the accumulation of organic compounds.

Discussion

Biomass accumulation

Growth, photosynthesis, and stomatal conductance were reduced in plants with 50% FC at 90 days from the start of the experiment. However, water use efficiency was significantly increased in the water-stressed plants (Figs. 1, 3), in agreement with the results of Brown and Pezeshki (2007). These authors could show that drought lead, in addition to reduced growth, to a decline of the photosynthetic activity of *S. alterniflora* due to a decrease of the stomatal conductance, an inhibition of chloroplast activity, and a breakdown of the chlorophyll content. The consistent decrease in gs measured in *S. alterniflora* at the end of the

Table 1 Water relation parameters as derived from pressure–volume analysis and percentage injury in control and drought-treated plants during dehydration

	Ψ_s^{100} (MPa)	Ψ_s^0 (MPa)	AWC (%)	ε_{max} (MPa)	EL	RWC_0
Control	-2.63 ± 0.31b	-3.24 ± 0.29b	22.9 ± 5.97b	2.9 ± 0.34b	12.96 ± 0.31b	84 ± 3.55b
Drought	-3.02 ± 0.43a	-3.68 ± 0.62a	38.3 ± 5.17a	4.9 ± 0.71a	26.11 ± 0.25a	89.6 ± 2.83a

Osmotic potential at full turgor (Ψ_s^{100}), osmotic potential at turgor loss point (Ψ_s^0), apoplastic water content (AWC), electrolyte leakage (EL), relative water content at turgor loss point (RWC_0), and bulk modulus of elasticity at full hydration (ε_{max})

Data presented as mean $\pm$ SE ($n = 10$ for water potential parameters, 3 for electrolyte leakage). Different letters within the same column indicate significant differences between treatments at $P < 0.05$

Table 2 Solute concentration in leaves of control and drought-treated plants expressed on the basis of symplastic water content at full turgor

Solute concentration (μmol g^{-1} symplastic water)	Control	Drought
K$^+$	144.4 ± 15a	134.5 ± 14a
Ca^{2+}	67.8 ± 12a	85.9 ± 14b
Mg^{2+}	27.1 ± 11a	21.9 ± 10a
Na$^+$	105.4 ± 17a	95.6 ± 15a
H$_2$PO$_4^-$	90.1 ± 19a	77 ± 18a
NO$_3^-$	10.8 ± 6a	30 ± 7b
SO$_4^{2-}$	62 ± 6a	50.4 ± 3a
Cl$^-$	123.8 ± 18a	107.7 ± 24a
Total soluble carbohydrates	47.16 ± 3a	86.7 ± 14b
Total inorganic ions	631.4 ± 19a	603.1 ± 25a
Total organic acids	58 ± 7a	72.8 ± 9b
Proline	2.9 ± 0.3a	11.7 ± 2b

Values represent the mean ± SE of five replicates per treatment. Different letters within the same line indicate significant differences between treatments at $P < 0.05$

Table 3 Carbon and nitrogen content in leaves of control and drought-treated plants expressed on the basis of dry weight

CN data S. alterniflora in % of dry weight	Control	Drought
C	39.60 ± 0.17a	39.48 ± 0.15a
N	2.05 ± 0.08a	2.43 ± 0.02b

Values represent the mean ± SE of five replicates per treatment. Different letters within the same column indicate significant differences between treatments at $P < 0.05$

Table 4 Contribution of total soluble carbohydrate, total inorganic ions, and proline to osmotic potential at full turgor (Ψ_s^{100}) and osmotic adjustment at full turgor (Ψ_s^{100}) in control and drought-treated plants

	Contribution to Ψ_s^{100} (%)		Contribution to osmotic adjustment (%)
	Control	Drought	
Total soluble carbohydrate	4.5	7.1	25.4
Total inorganic ions	60.0	49.9	−18.1
Proline	0.3	1.0	5.6

water-deficit treatment suggested an efficient stomatal control of transpiration by this species.

Water relations

The analysis of P–V curve data lead to the suggestion that *S. alterniflora* responded to water stress with an active

osmotic adjustment in leaves. At the end of the drought treatment, water-stressed plants exhibited significantly lower leaf osmotic potential at full turgor (Ψ_s^{100}) and at turgor loss point (Ψ_s^0) than the control plants. Besides the reduction of the osmotic potential, water stress also induced a decrease in leaf tissue elasticity. Such an elastic adjustment is in agreement with previous findings on plants subjected to drought (Nardini et al. 1999; Stoyanov 2005). The higher ε_{max} of the leaves, i.e., the higher rigidity of the living cell walls (Table 1), seems to enhance the driving force for water extraction from the soil with smaller water loss when transpirational demand is high (Fig. 2).

Water-stressed *S. alterniflora* plants showed significantly higher values of apoplasmic water fraction compared to well water plants (Table 1). This change can contribute to passive concentration of solutes and thus to turgor maintenance in stressed plants (Patakas and Noitsakis 1999).
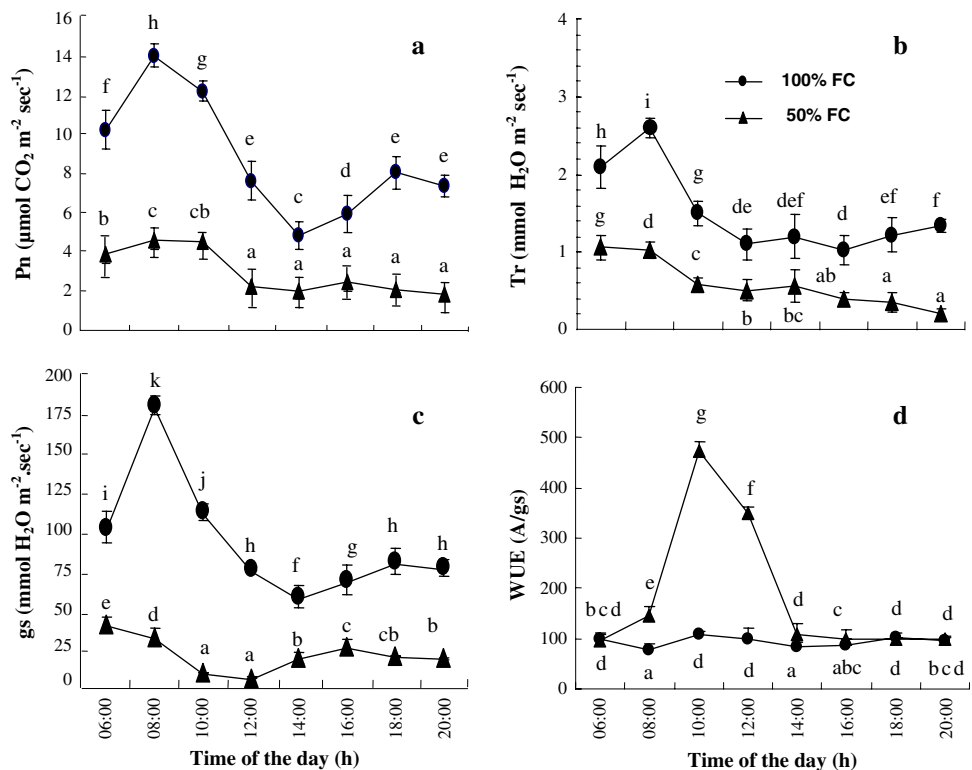
Osmotic adjustment

The significantly lower values of Ψ_s^{100} in stressed *S. alterniflora* plants (Table 1) suggest an increase in leaf solute concentration. Proline and soluble carbohydrate contents in *S. alterniflora* were raised by water deficit stress, indicating their contribution to osmotic adjustment. Assuming that 40 μmol g^{-1} of symplastic water corresponds to 0.1 MPa (Patakas et al. 2002), the concentration of soluble carbohydrate, proline, and inorganic ions was used to estimate the contribution of each solute to osmotic adjustment. Thus, soluble carbohydrate in *S. alterniflora* represented 25.4% of the osmotic adjustment observed (Tables 1, 2, 4). Similar results were obtained by Koyro and Huchzermeyer (2004a) in *S. alterniflora* in response to salt stress.

The concentration of the free amino acid proline was also fourfold higher at water stress conditions. However, even after the imposition of stress, the proline pool was insufficient to account for a significant contribution to the total osmotic adjustment. Comparatively to soluble sugars, its contribution to the osmotic adjustment is only 5.6% (Tables 1, 2, 4). Sánchez et al. (2004) reported that, in response to drought or salinity, proline is accumulated mainly in the cytoplasm (symplast) and might play a more complex role in conferring osmotic resistance than simply contributing to osmotic adjustment.

The concentrations of inorganic ions (Table 2) did not increase under water deficiency. It seems that inorganic ions are not significantly involved in the osmotic adjustment in drought-treated S*partina* (Brown et al. 2006). However, the proportion of organic acids, estimated by the difference between inorganic cations and

Fig. 3 Diurnal changes in photosynthetic rate (**a**), transpiration rate (**b**), stomatal conductance (**c**), and water use efficiency (**d**) in stressed and control plants at the end of the treatment cycle (90 days). See Fig. 2 for diurnal changes in photosynthetically active radiation (*PAR*) and air vapour pressure deficit. *Bars* indicate the standard error of the mean of ten replicates. Values with different letters are significantly different at $P = 0.05$

anions (C–A), was 1.3-fold higher in stressed compared to control plants, suggesting a notable contribution of these organic compounds at the osmotic adjustment. The energy stored in osmotically active organic compounds diverts most of the energy otherwise available for growth. However, it is necessary to confirm this hypothesis by direct measurement of organic acids. Since only a part of the decrease in osmotic potential can be explained by soluble sugars, with a lesser degree of proline and probably organic acids, we assume also that other solutes, such as glycine betaine, asparagine, and dimethylsulph-niopropionate (DMSH), may also play an important role in the osmotic adjustment of *S. alterniflora* (Cavalieri 1983; Colmer et al. 1996).

Conclusion

Despite reductions in growth, *S. alterniflora* was capable of surviving moderate water stress. The survival of this species was related to its ability to adjust osmotic potential, to enhance rigidity, and to maintain some capacity for photosynthesis. Furthermore, the high water use efficiency under water stress significantly contributed to maintain leaf water status of this species. Our results indicate that *S. alterniflora* has a promising potential for revegetation or fodder production in arid environments.

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