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REGULAR PAPER

Effect of sodium chloride on the response of the halophyte species *Sesuvium portulacastrum* grown in mannitol-induced water stress

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Abstract *Sesuvium portulacastrum* is a halophytic species well adapted to salinity and drought. In order to evaluate the physiological impact of salt on water deficit-induced stress response, we cultivated seedlings for 12 days, in the presence or absence of 100 mmol l^{-1} NaCl, on a nutrient solution containing either 0 mmol l^{-1} or 25 mmol l^{-1} mannitol. Mannitol-induced water stress reduced growth, increased the root/shoot ratio, and led to a significant decrease in water potential and leaf relative water content, whereas leaf Na^+ and K^+ concentrations remained unchanged. The addition of 100 mmol l^{-1} NaCl to 25 mmol l^{-1} mannitol-containing medium mitigated the deleterious impact of water stress on growth of *S. portulacastrum*, improved the relative water content, induced a significant decrease in leaf water potential and, concomitantly, resulted in enhancement of overall plant photosynthetic activity (i.e. CO_2 net assimilation rate, stomatal conductance). Presence of NaCl in the culture medium, together with mannitol, significantly increased the level of Na^+ and proline in the leaves, but it had no effect on leaf soluble sugar content. These findings suggest that the ability of NaCl to improve plant performance under mannitol-induced water stress may be due to its effect on osmotic adjustment through Na^+ and proline accumulation, which is coupled with an improvement in photosynthetic activity. A striking recovery in relative water content and growth of the seedlings was also recorded in the presence of NaCl on release of the water stress induced by mannitol.

Keywords Halophyte · Osmotic adjustment · Photosynthesis · Proline · Sodium · Water stress

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Introduction

In semi-arid regions, with a progressive reduction of the vegetation cover, desertification coupled with rapid soil erosion is becoming a serious problem (Martínez et al. 2005). Various plant associations, including psammophytic species (plants native to sandy soils), often thrive well in the dry regions. Some of the fast growing species belonging to genera such as *Sesuvium*, *Batis* and *Mesembryanthemum* (Menzel and Lieth 1999) have the ability to cover barren soil in an incredibly short time. *S. portulacastrum*, in particular, exhibits a great potential as a soil cover and landscaping plant. This species produces attractive branches with pink-purplish and occasionally white flowers (Pasternak and Nerd 1995). Recent results demonstrate that *S. portulacastrum* accumulates large amounts of Cd^{2+} in its shoots, suggesting the possibility of its potential use in the phyto-remediation of saline soils polluted by cadmium (Ghnaya et al. 2005). Additionally, this plant has medicinal value (Burits et al. 2001), and the secondary metabolites obtained from this species have been shown to have a great

potential as a substitute for some synthetic raw materials in the food, perfumery, cosmetic and pharmaceutical industries (Lis-Balchin and Deans 1997). The essential oil from the leaves of *S. portulacastrum* exhibits notable antibacterial activity against both gram-positive and gram-negative bacteria and displays significant antifungal and antioxidant activity (Magwa et al. 2006).

Successful introduction of *S. portulacastrum* in arid salty ecosystems depends largely on its capacity to tolerate the specific environmental constraints, such as salinity, drought and nutritional disturbances. In previous work we showed that *S. portulacastrum* is able to express high growth potential, even under severe salinity (Messedi et al. 2003, 2005). In fact, salt-induced growth stimulation in *S. portulacastrum* may be maintained in the presence of 800 mmol l^{-1} NaCl if a part of the root system is protected from the effect of salt (Messedi et al. 2004). As with salinity, even when subjected to long-term water deficit, *S. portulacastrum* is known to retain its growth potential and nutrient acquisition systems and exhibits a dramatic accumulation of proline (Slama et al. 2006). Surprisingly, only a few studies have been conducted to evaluate the combined effects of water and salt stresses on plants (Glenn and Brown 1998). In halophytes, Na^+ often assumes a positive function in response to water stress, as evidenced by a specific increase in Na^+ absorption when the plants are subjected to drought on non-saline substrate (Martínez et al. 2003, 2005). According to Glenn and Brown (1998), tolerance to water deficit and salt stress in *Atriplex canescens* is linked through a common mechanism of Na^+ uptake, which is directly used for osmotic adjustment. Martínez et al. (2003, 2004, 2005) estimated that, despite significant stimulation of Na^+ absorption by the plant during stress, the direct contribution of this element to osmotic adjustment is negligible from a quantitative point of view. However, the beneficial impact of Na^+ in a stressed plant has been shown to be related to an increase in zeaxanthin (Qiu et al. 2003), glycine betaine (Subbarao et al. 2001) and soluble sugar (Martínez et al. 2005) levels.

The objective of the present study was to determine whether the salt can modify the response of *S. portulacastrum* seedlings to water stress and to monitor some physiological reactions involved in this response. For this purpose, the plants were subjected to water stress by mannitol in the presence or absence of NaCl, and an analysis was made of the parameters related to water status, photosynthetic gas exchanges, ion nutrition and organic solute accumulation. The ability of plants to recover on release of water stress induced by mannitol was also assessed.

Materials and methods

Plant material and multiplication

S. portulacastrum, a dicotyledonous halophyte belonging to the family Aizoaceae, is a perennial species native to sandy soils. The plants used in this study were obtained by multiplication of the selected plants by cuttings. The mother plants were cultivated at our experimental station near the sea shore, 35 km north-east of Tunis (10°10′ E, 36°48′ N; mean annual rainfall and temperature were 19.4°C and 456 mm, respectively), in outdoor containers filled with a mixture of sandy soil and organic matter, and irrigated with tap water. Three-centimetre-long stems having one node and two opposite leaves were cut from mother plants, disinfected for 5 min in saturated calcium hypochlorite solution, rinsed thoroughly with distilled water, and rooted in a nutrient solution (Hewitt 1966) prepared with deionised water. One hundred and fifty rooted cuttings were then put individually into 750 ml Rivera plastic pots. The plants were grown in a greenhouse at 30 ± 5°C and 16 ± 2°C, with a relative humidity of 60 ± 5% and 90 ± 5% during the day and night, respectively. The light regime was 14 h light:10 h dark per day. Shoots and roots were harvested for analysis in the morning after 12 days.

Osmotic treatments

Plants were divided in two groups: the first group was maintained on basal nutrient solution without salt, while the second one was grown in the same basal nutrient solution that was supplemented with NaCl (100 mmol l^{-1}). One week later, plants from each treatment were divided into two further groups and grown either in the presence or absence of mannitol (25 mmol l^{-1}) for 12 days. After 6 days, one lot of plants growing in mannitol was transferred to a mannitol-free medium. Thus, the following treatments were used: (1) basal nutrient solution at 0 mmol l^{-1} mannitol containing 0 mmol l^{-1} or 100 mmol l^{-1} NaCl; (2) basal medium containing 25 mmol l^{-1} mannitol with 0 mmol l^{-1} or 100 mmol l^{-1} NaCl; (3) basal medium containing 0 mmol l^{-1} mannitol with 0 mmol l^{-1} or 100 mmol l^{-1} NaCl, but the plants had first been grown in 25 mmol l^{-1} mannitol for 6 days.

The nutrient solution in the pots was constantly bubbled with pressurised air to stir the solution and to prevent anoxia of the plants' root systems.

Growth, water relationship measurements and leaf water potential

We determined fresh weight (FW) and dry weight (DW) of shoots and roots of each plant after counting the number of leaves. Relative water content (RWC) was measured in the second- or third-youngest fully expanded leaves harvested in the morning, using the following equation:

$$\mathrm{RWC}(\%) = 100 \times (\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW})$$

(Schonfeld et al. 1988).

FW was determined within 2 h after harvest. Turgid weight (TW) was obtained after the leaves had been soaked in distilled water in test tubes for 12 h at room temperature (approximately 20°C), under low light conditions in the laboratory. After being soaked, the leaves were quickly and carefully blotted dry with tissue paper and used for determining the TW. DW was obtained after the leaf or shoot samples had been oven dried at 60°C for 48 h. The leaf water potential was evaluated immediately after sampling, by the pressure chamber method.

Gas exchange

Net CO_2 assimilation and stomatal conductance were recorded with a portable photosynthesis system (LCA4). Measurement conditions were as follows: 1300 μmol $mm^{-2}s^{-1}$ photosynthetically active radiation (PAR), 375 μmol mol^{-1} ambient CO_2 concentration and 28 ± 2°C leaf temperature. The vapour pressure deficit (VPD = 1.3 kPa) was calculated from saturation vapour pressure and relative humidity (Buck 1981). Measurements were carried out in the morning between 10 o'clock and 12 o'clock (six replicates per treatment). Data were automatically collected every minute after the photosynthesis rate had stabilised.

Cation assay and determination of proline and soluble sugars

Na^+ and K^+ were assayed by flame emission spectrophotometry after nitric acid extraction (HNO_3, 0.5%) of the finely ground dry matter. Free proline was quantified spectrophotometrically using the method of Bates et al. (1973), while the soluble sugars were determined by the anthrone reagent method according to Yemm and Willis (1954).

Statistical analysis

Analysis of variance (ANOVA), using the AV1W MSUSTAT program with orthogonal contrasts and mean comparison procedures, was performed to detect differences between treatments. Mean separation procedures were carried out using the multiple range tests with Fisher's least significant difference (LSD) ($P < 0.05$).

Results

NaCl effects on growth of mannitol-treated plants

In comparison with the control (0 mmol l^{-1} NaCl, 0 mmol l^{-1} mannitol), incorporation of salt (100 mmol l^{-1} NaCl, 0 mmol l^{-1} mannitol) in the nutrient medium increased the biomass production of the whole plant by 20% (Fig. 1). In contrast, in the plants growing in the presence of mannitol without salt, a significant reduction of growth (40%) was observed. However, an addition of 100 mmol l^{-1} NaCl in the mannitol containing nutrient solution mitigated the deleterious effect of mannitol on growth. In fact, the DW was twice as high in plants grown in mannitol and salt in combination as in those grown in mannitol alone. Transfer of plants grown in mannitol for 6 days to a mannitol-free medium did not elicit much effect

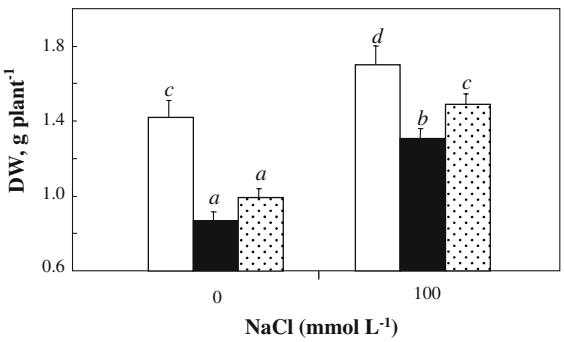

Fig. 1 Dry matter production in *Sesuvium portulacastrum*. Plants were grown for 12 days in nutrient solution containing 0 mmol l^{-1} (*open columns*) or 25 mmol l^{-1} mannitol (*closed columns*) in the absence or in the presence of 100 mmol l^{-1} NaCl. After growth in mannitol for 6 days, plants were transferred to a mannitol-free medium in the presence or in the absence of 100 mmol l^{-1} NaCl (*dotted columns*). The *y*-axis does not start at zero. Values are the means of six replicates, *vertical bars* are SEs. Values sharing a common letter are not significantly different at $P < 0.05$

on the recovery of plant growth. However, in the presence of 100 mmol l^{-1} NaCl, the growth recovery level was found to be quite significant after the release of water stress induced by mannitol.

Generally, the roots were less affected by water stress induced by mannitol than the shoots (Fig. 2). Thus, the root/shoot ratio was significantly higher in plants reared in mannitol in the absence or presence of 100 mmol l^{-1} NaCl than that observed in plants reared in the absence of mannitol.

Water relationships

As shown in Fig. 3a, leaf RWC was significantly increased by the addition of 100 mmol l^{-1} NaCl. However, RWC decreased significantly under water stress induced by mannitol, especially in the salt-free medium. At the end of the recovery period, leaf RWC was partially restored.

In the absence of mannitol, NaCl significantly decreased leaf water potential (ψ_w) (Fig. 3b). In mannitol and NaCl, in combination, ψ_w showed even greater reduction. However, at the end of the recovery period, ψ_w increased significantly in the presence of NaCl.

Gas exchange

The highest values of CO_2 assimilation and stomatal conductance were observed in the presence of 100 mmol l^{-1} NaCl (Table 1). The exposure of plants to water stress reduced the values of these parameters in the absence, as well as in the presence, of NaCl.

Sodium and potassium concentration

Regardless of the presence of mannitol and NaCl in the nutrient solution, Na^+ concentration was always higher in leaves than in roots: leaves accumulated up to four-times more salt than the roots accumulated (data not shown). The transfer of salt-treated plants to mannitol increased leaf Na^+ content (Fig. 4a). When salt was present in the medium, recovery after water stress relief led to a significant decrease of Na^+ concentration, reaching values similar to those observed in plants treated with 100 mmol l^{-1} NaCl alone.

As observed for Na^+, K^+ content was also found to be relatively more abundant in the leaves than in the roots (data not shown). The presence of salt in the nutrient solution significantly reduced (30%) leaf K^+ concentration (Fig. 4b). In contrast, exposure of plants to mannitol in absence of salt had no effect on the concentration of K^+ in the leaves. However, mannitol induced a significant increase in the level of K^+ in the presence of 100 mmol l^{-1} NaCl.

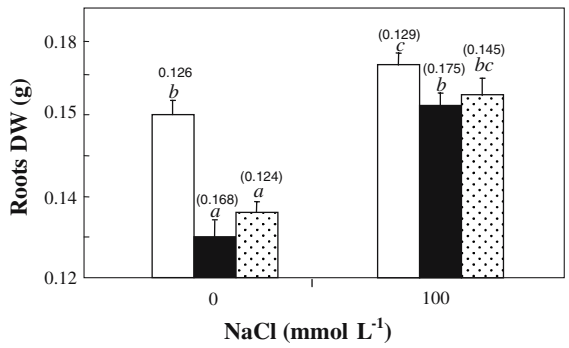

Fig. 2 Root dry matter production in *Sesuvium portulacastrum*. Plants were grown for 12 days in nutrient solution containing 0 mmol l^{-1} (*open columns*) or 25 mmol l^{-1} mannitol (*closed columns*) in the absence or presence of 100 mmol l^{-1} NaCl. After growth in mannitol for 6 days, plants were transferred to a mannitol-free medium in the presence or absence of 100 mmol l^{-1} NaCl (*dotted columns*). Values mentioned on the error bars correspond to root/shoot DW ratio of each treatment. The *y*-axis does not start at zero. Values are the means of six replicates, *vertical bars* are SEs. Values sharing a common letter are not significantly different at $P < 0.05$

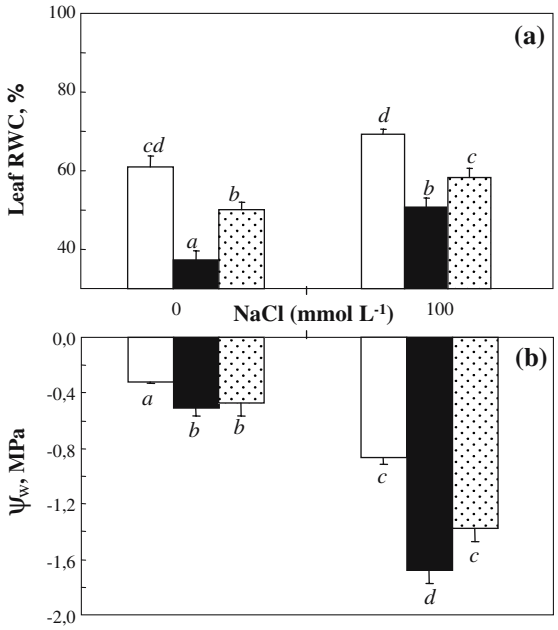

Fig. 3 Changes in leaf RWC (%) (**a**) and leaf water potential (ψw, MPa) (**b**) in *Sesuvium portulacastrum*. Plants were grown for 12 days in nutrient solution containing 0 mmol l^{-1} (*open columns*) or 25 mmol l^{-1} mannitol (*closed columns*) in the absence or presence of 100 mmol l^{-1} NaCl. After growth in mannitol for 6 days, plants were transferred to a mannitol-free medium in the presence or absence of 100 mmol l^{-1} NaCl (*dotted columns*). The *y*-axis does not start at zero. Values are the means of six replicates, *vertical bars* are SEs. Values sharing a common letter are not significantly different at $P < 0.05$

Table 1 CO_2 net assimilation rate (μmol $m^{-2}s^{-1}$) and stomatal conductance (*gs* mol $m^{-2}s^{-2}$) in leaves of *S. portulacastrum*. Plants were grown for 12 days in nutrient solution containing 0 mmol l^{-1} or 25 mmol l^{-1} mannitol in the absence or presence of 100 mmol l^{-1} NaCl. After growth in mannitol for 6 days, plants were transferred to a mannitol-free medium in the presence or absence of 100 mmol l^{-1} NaCl (*Re*). Values are the means of six replicates

Mannitol (mM)	NaCl (mM)					
	0			100		
	0	25	0 Re	0	25	0 Re
A (μmol CO_2 $m^{-2}s^{-1}$)	22 ± 1.5	8 ± 1.9	10 ± 0.5	29 ± 3.2	18 ± 1.2	21 ± 1.3
gs (mol $m^{-2}s^{-1}$)	0.3 ± 0.02	0.07 ± 0.01	0.09 ± 0.01	0.42 ± 0.02	0.2 ± 0.02	0.37 ± 0.03

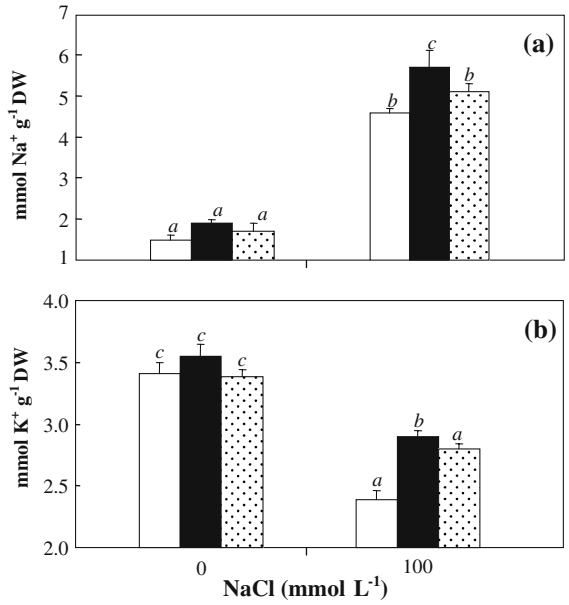

Fig. 4 Changes in leaf Na^+ (**a**) and K^+ (**b**) concentrations in *Sesuvium portulacastrum*. Plants were grown for 12 days in nutrient solution containing 0 mmol l^{-1} (*open columns*) or 25 mmol l^{-1} (*closed columns*) mannitol in the absence or presence of 100 mmol l^{-1} NaCl. After growth in mannitol for 6 days, plants were transferred to a mannitol-free medium in the presence or absence of 100 mmol l^{-1} NaCl (*dotted columns*). The *y*-axis does not start at zero. Values are the means of six replicates, *vertical bars* are SEs. Values sharing a common letter are not significantly different at $P < 0.05$

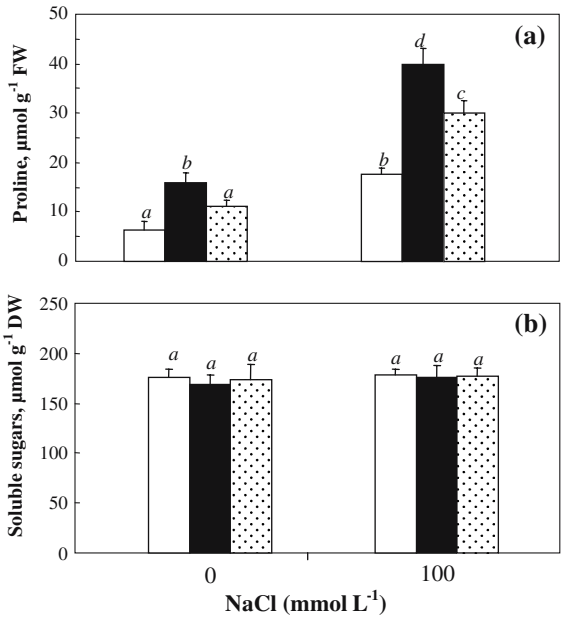

Fig. 5 Changes in concentrations of proline (**a**) and soluble sugars (**b**) in leaves of *Sesuvium portulacastrum*. Plants were grown for 12 days in nutrient solution containing 0 mmol l^{-1} (*open columns*) or 25 mmol l^{-1} (*closed columns*) mannitol in the absence or presence of 100 mmol l^{-1} NaCl. After growth in mannitol for 6 days, plants were transferred to a mannitol-free medium in the presence or absence of 100 mmol l^{-1} NaCl (*dotted columns*). Values are the means of six replicates, *vertical bars* are SEs. Values sharing a common letter are not significantly different at $P < 0.05$

Accumulation of proline and soluble sugars

In the presence of NaCl a significant increase was recorded in the level of proline (Fig. 5a). Plants grown in the presence of 100 mmol l^{-1} NaCl accumulated approximately three-times more proline than did the controls. As in salt, exposure of plants to mannitol also resulted in an increased proline concentration in the leaves. Addition of NaCl to the mannitol-containing nutrient solution resulted in a further increase in proline content, reaching the highest value of all the treatments. The level of proline accumulation at the end of the recovery period decreased significantly either in the presence or in the absence of salt. Irrespective of the treatments, the leaf soluble sugar content (Fig. 5b) remained, by and large, unchanged.

Discussion

Plant growth and photosynthetic capacity

In *S. portulacastrum*, water stress induced by 25 mmol l^{-1} mannitol strongly reduced growth, whereas

the plants grown in 100 mmol l^{-1} NaCl showed the highest levels of dry matter production and tissue hydration. This observation defies explanation on the basis of an osmotic effect only, which, in effect, should be much smaller with 25 mmol l^{-1} mannitol than with 100 mmol l^{-1} NaCl. According to Hohl and Schopter (1991), mannitol may penetrate the free space of the wall and induce plasmolysis resulting in growth reduction. Thus, some secondary effects of mannitol treatment, unrelated to water stress induction, cannot be excluded.

Sesuvium portulacastrum requires NaCl to express its growth potential. In a previous work (Messedi et al. 2003), we observed that the growth and tissue hydration in *S. portulacastrum* were maximal in the presence of 100–400 mmol l^{-1} NaCl. Additionally, using a split root system (Messedi et al. 2004), we demonstrated that even at high salinity levels (800 mmol l^{-1} NaCl), the growth of *S. portulacastrum* is limited by the restriction imposed by NaCl on N uptake rather than ionic and/or osmotic stresses.

Water stress strongly restricted root and shoot growth, despite the fact that the roots were relatively more tolerant. Consequently, plants grown in mannitol in the presence or in the absence of salt exhibited a higher root/shoot DW ratio than did plants growing in a mannitol-free medium. This may be related to the preferential allocation of dry matter to roots (Kage et al. 2004) and may facilitate adaptation to drought (Yin et al. 2005). Previously, we had observed a similar effect in *S. portulacastrum* during long-term water stress lasting 70 days induced by irrigation with 25% of field capacity (Slama et al. 2006).

A close relationship was found between CO_2 net assimilation rate and leaf relative water content (Fig. 6a), stomatal conductance (Fig. 6b) and dry matter production (Fig. 6c), suggesting that the severe reduction of growth in the presence of mannitol alone was strongly related to the reduction in leaf gas exchange properties, which are known to be strongly dependent on water status. Since *S. portulacastrum* is an obligate halophyte, salt (100 mmol l^{-1} NaCl) may prove to be optimal for its photosynthetic capacity. Our results also indicate that the accumulation of salt in the leaves of *S. portulacastrum* may be compatible with a high photosynthetic rate. This conclusion finds credence in the fact that the highest leaf sodium concentration was recorded in plants grown simultaneously in salt and mannitol or in salt alone (480 mmol l^{-1} and 382 mmol l^{-1} water tissue, respectively), and these plants exhibited the highest values of CO_2 assimilation and stomatal conductance. In accordance with these findings, Debez et al. (2006) showed

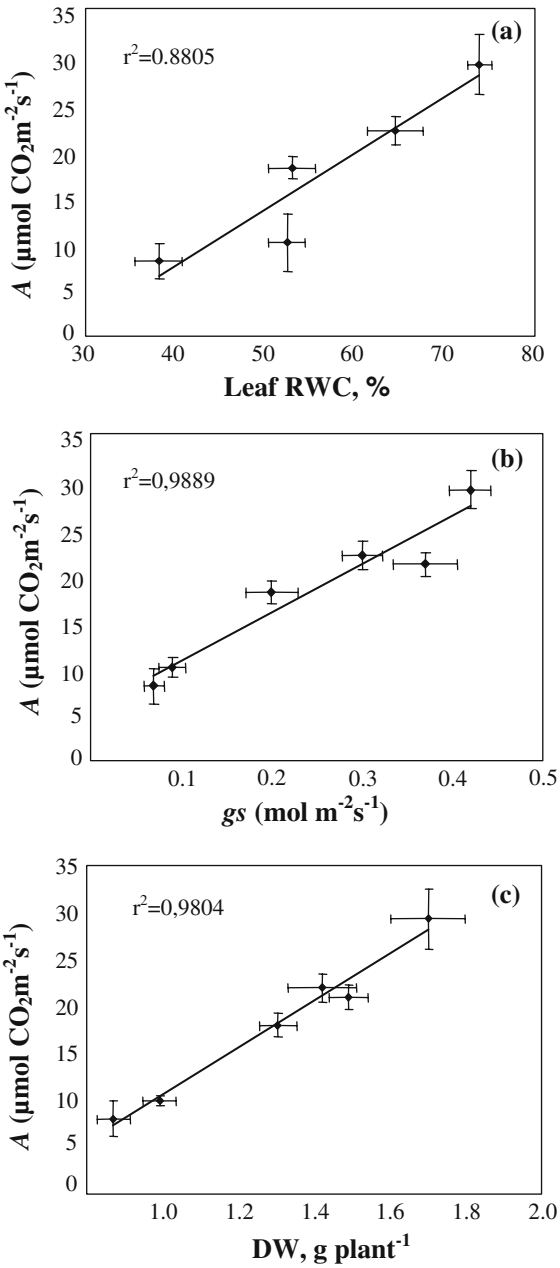

Fig. 6 Relationship between CO_2 net assimilation rate (µmol m^{-2} s^{-1}) and leaf relative water content (%) (**a**), stomatal conductance (*gs* mol $m^{-2}s^{-2}$) (**b**) and dry matter production (g $plant^{-1}$) (**c**) in *Sesuvium portulacastrum*. Values are the means of six replicates, *vertical bars* are SEs

that the leaf sodium concentration up to 200 mmol l^{-1} water tissue were also found to be positively correlated with net CO_2 assimilation in the halophyte *Cakile maritima*. It may be mentioned here that the nature of salt influence on photosynthesis partly depends on the ion properties of the stomata, i.e. their ability to use Na^+ instead of K^+ to regulate guard cell turgor (Robinson et al. 1997; Kerstiens et al. 2002).

The improvement of photosynthetic parameters in plants subjected to salt and mannitol together may also be explained on the basis of a positive impact of Na^+ on photosynthesis in plants (Murata et al. 1992). Sodium may also play an important role in the maintenance of the granal stacking, which provides a suitable site for energy transfer between photosystem II (PSII) and photosystem I (PSI) (Qiu et al. 2003).

Osmotic adjustment

Contribution of Na^+ to osmotic adjustment

The addition of 100 mmol l^{-1} NaCl to the mannitol-containing medium resulted in increased leaf Na^+ concentration. This observation is in accordance with an improvement of tissue hydration and is in conformity with the previously observed salt-accumulating character of *S. portulacastrum* and its capacity to sequester Na^+ in the vacuoles for osmotic adjustment (Messedi et al. 2004). In halophytes, the involvement of Na^+ in osmotic adjustment has been amply highlighted, if it is assumed that it is mainly present in the vacuoles and that this compartment occupies approximately 90% of the total cell volume. Martínez et al. (2005) reported that, for *A. halimus*, the contribution of Na^+ to the total osmotic adjustment did not exceed 15% in leaves. However, our data indicate that the contribution of Na^+ to the total osmotic adjustment in *S. portulacastrum* varied from approximately 8% in plants subjected to water stress induced by mannitol to 47% in plants grown simultaneously in the presence of salt (100 mmol l^{-1} NaCl) and mannitol. This apparent difference between *S. portulacastrum* and *A. halimus* could be explained by the fact that, in the latter species, a considerable part of the Na^+ absorbed may be accumulated in trichomes that cover the leaf surface (Mozafar and Goodin 1970).

Contribution of proline to osmotic adjustment

In addition to sodium, several organic compounds may also contribute to osmotic adjustment. These compounds are considered to accumulate in the cytosol and organelles. Members of the Aizoaceae family are thought to accumulate high amounts of proline in response to salinity or drought (Messedi et al. 2004; Slama et al. 2006). Our results showed that, when mannitol was added to the nutrient solution, the concentration of proline in the leaves increased significantly. A strong increase in proline concentration was also observed in the presence of 100 mmol l^{-1} NaCl. In addition, a high positive correlation was established between proline and Na^+ concentration in leaves (Fig. 7), suggesting the positive role of Na^+ in proline accumulation. Martínez et al. (2005) demonstrated that, in *A. halimus*, proline was also accumulated mainly in response to NaCl. In red beet, on the other hand, Na^+ stimulated glycine betaine synthesis (Subbarao et al. 2001). Earlier, it had been demonstrated (Huber 1974) that salt induced inhibition of pyrroline-5-carboxylate dehydrogenase, an enzyme involved in proline degradation, and enhanced pyrroline-5-carboxylate reductase, which is involved in proline synthesis. In addition, it has also been demonstrated that the level of Δ^1-pyrroline-5-carboxylate synthetase mRNA increased in salt-stressed moth bean roots (Hu et al. 1992).

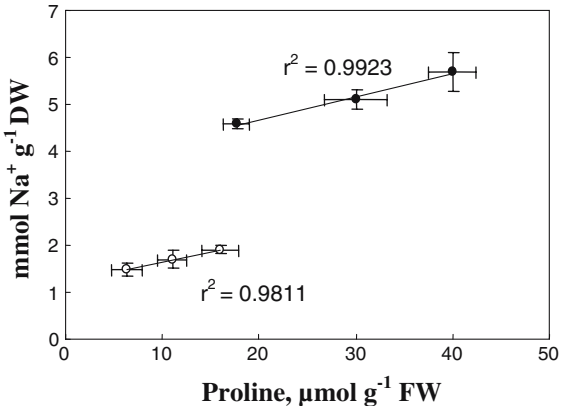

Fig. 7 Relationship between proline and Na^+ concentration in leaves of *Sesuvium portulacastrum*. Plants were grown for 12 days in nutrient solution containing 0 mmol l^{-1} (*open circles*) or 100 mmol l^{-1} NaCl (*closed circles*), in the absence or presence of 25 mmol l^{-1} mannirol and 6 days after stress release. Values are the means of six replicates, *vertical bars* are SEs

Our results point out that proline is involved in intracellular osmotic adjustment. Indeed, the improvement of water status in plants subjected to water stress in the presence of salt (100 mmol l^{-1} NaCl) was found to be correlated with the highest proline content in the shoots. Plants grown in salt also exhibited the lowest leaf water potential. It had been reported earlier that the lowering of osmotic potential by osmolyte accumulation in response to stress improves the capacity of the cells to maintain their turgor pressure at low water potential. This appears to be essential for physiological processes such as photosynthesis, enzyme activity and cell expansion (Tyree and Jarvis 1982; Claussen 2005). In *S. portulacastrum*, the contribution of proline to the total osmotic adjustment increased from 3.3% in plants subjected to water-deficit stress to 6.1% in plants exposed to mannitol and NaCl in combination. The contribution of the proline to the osmotic adjustment becomes quite significant by the fact that this compatible osmolyte is concentrated

mostly in the cytosol and the chloroplasts (Büssis and Heineke 1998; Aubert et al. 1999). The concentration of cytosolic proline in *S. portulacastrum*, evaluated with the supposition that cytoplasm volume represents 5% of total cell volume (Flowers and Yeo 1986), reaches more than 50% of the vacuolar ionic concentration, estimated as twice the sum of the concentration of sodium and potassium ions. Thus, it appears plausible that in *S. portulacastrum* the osmotic balance between vacuole and cytoplasm may be primarily achieved through the accumulation of proline.

In addition to the implication of the involvement of proline in osmotic adjustment, the highest level of growth rate expressed by plants grown simultaneously in the presence of salt and mannitol can be linked to the other roles that have been credited to proline. It has been reported that this compatible osmolyte protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, and may serve as an energy and nitrogen source (Matysik et al. 2002; Shetty 2004; Kishor et al. 2005).

In conclusion, our results indicate that it is possible that salt improves the ability of *S. portulacastrum* plants to cope with mannitol-induced water stress by improving the functions of parameters related to photosynthesis and by inducing enhanced accumulation of Na^+ and proline. The latter two compounds appear to be the main contributors to osmotic adjustment in *S. portulacastrum*.

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