Acta Physiol Plant (2011) 33:1425–1433
DOI 10.1007/s11738-010-0677-5

ORIGINAL PAPER

Osmotic adjustment, water relations and growth attributes of the xero-halophyte *Reaumuria vermiculata* L. (Tamaricaceae) in response to salt stress

Mustapha Gorai · Mohamed Neffati

Received: 18 October 2010 / Revised: 18 November 2010 / Accepted: 7 December 2010 / Published online: 30 December 2010

Abstract *Reaumuria vermiculata* (L.), a perennial dwarf shrub in the family of Tamaricaceae, is a salt-secreting xero-halophyte found widely in arid areas of Tunisia. In the present study, physiological attributes of *R. vermiculata* were investigated under salt stress. Four-month-old plants were subjected to various salinity levels (0, 100, 200, 300, 400 or 600 mM NaCl) for 30 days under greenhouse conditions. Results showed that plants grew optimally when treated with standard nutrient solution without NaCl supply. However, increasing osmolality of nutrient solutions caused a significant reduction in biomass production and relative growth rate. This reduction was more pronounced in roots than in shoots. In addition, this species was able to maintain its shoot water content at 30% of the control even when subjected to the highest salt level, whereas root water content seemed to be unaffected by salt. Shoot water potential declined significantly as osmotic potential of watering solutions was lowered and the more negative values were reached at 600 mM NaCl (−3.4 MPa). Concentrations of Na^+ and Cl^- in the shoots of *R. vermiculata* were markedly increased with increasing osmolality of nutrient solutions, whereas concentration of K^+ was not affected by NaCl supply. Salt excretion is an efficient mechanism of Na^+ exclusion from the shoots of this species exhibiting high K^+/Na^+ selectivity ratio over a wide range of NaCl salinity. Proline accumulation in shoots was significantly increased with increase in salt level and may play a role in osmoregulation.

Keywords Osmoregulation · *Reaumuria vermiculata* · Relative growth rate · Salinity · Solute accumulation · Water relations

Communicated by R. Aroca.

M. Gorai (✉) · M. Neffati
Laboratoire d'Ecologie Pastorale, Institut des Régions Arides, 4119 Médenine, Tunisia
e-mail: gorai.mustpha@yahoo.fr

Introduction

Reaumuria vermiculata L. is a xero-halophytic perennial dwarf shrub distributed in many gypseous and saline areas in southern Tunisia (Gorai and Neffati 2007). It reaches 50–100 cm height and occurs along different bioclimatic stages with precipitations varying from 50 to 400 mm (Le Houérou 1993). *Reaumuria vermiculata* is highly tolerant to drought and salinity, and widely found in tropical and subtropical regions (Le Houérou 1993, 1995). This species, belonging to the Tamariaceae family, avoids salinity or excretes salts via the leaf surface by glandular structures that are similar to hydathodes (Le Houérou 1993) and possesses an environmental interest that consists in its capacity to desalinize saline soils by responsible salt glands. If plants were harvested at the end of the growing season, a significant reduction in soil salinity might be achieved (Gorai and Neffati 2007). Seeds are donned of silken hairs and flowering happens from February to June (Pottier-Alapetite 1979). In their natural habitats, seeds mature at the end of August and germination starts following rains in autumn and early winter, when temperatures were decreasing (Gorai and Neffati 2007). These authors indicated that germination was inhibited by either an increase or decrease in temperature from the most suitable temperature found (15°C) and increasing NaCl-salinity progressivily inhibited seed germination, which was less than 5% at 300 mM.

Environmental abiotic stress conditions, and especially drought and salinity, are currently the major factors responsible for the worldwide deterioration of plant cover and the erosion of soils (Boyer 1982; Owens 2001). The ability of plants to survive and maintain growth under saline conditions is known as salt tolerance. The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutritional imbalance, specific ion effect, hormonal imbalance and induction of oxidative stress, or a combination of these factors (Greenway and Munns 1980; Marschner 1995; Parida and Das 2005). Responses of species to salt stress depend on several interacting variables, including the magnitude (salt concentration and time of exposure) of the stress, plant genotype, plant developmental stage and cultural environment (Sultana et al. 1999; Jaleel et al. 2007). Salinity reduces the ability of plants to take up water, causing a reduction in growth along with a suite of metabolic changes (Munns 2002). A metabolic response to salt stress is the synthesis of compatible osmolytes (Ashraf and Foolad 2007). These mediate osmotic adjustment and therefore protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity (Zhu 2001; Mansour and Salama 2004).

The aim of the present study was to investigate the effects of salinity on growth, water relations and accumulation of organic and inorganic solutes in early seedlings stage of *R. vermiculata*.

Materials and methods

Plant material and culture conditions

Seeds of *R. vermiculata* were obtained from plants that were collected from a location near El Fjé, Medenine (33°30′N, 10°39′E; southeast Tunisia) in July 2007. This area is arid to semi-arid with a typical Mediterranean climate, characterized by irregular rainfall events and a harsh dry summer period (Gorai and Neffati 2007).

Seeds were cleaned and stored for 6 months in the seed bank of the Laboratoire d'Ecologie Pastorale at the Institut des Régions Arides (Médenine, Tunisia) in which relative humidity was set at 30% and temperature was maintained at 20°C. When experiments were carried out, seeds were surface sterilized with Na-hypochlorite. The pots were filled with sterilized mixture of sand and soil (1:2), three seeds per pot were planted and the soil irrigated with distilled water until germination. The maximum germination was observed after 15 days. At this stage, the seedlings were thinned to one per pot and irrigated with Hewitt nutrient solution (Hewitt 1966), containing macronutrients: 1.5 mM $MgSO_4$, 1.6 mM KH_2PO_4, 0.4 mM K_2HPO_4, 3 mM KNO_3, 2 mM NH_4NO_3 and 3.5 mM $Ca(NO_3)_2$. The medium also contained iron as complex EDTA–K–Fe (45 μM) (Jacobson 1951) and micronutrients as a mixture of salts: 8 μM $MnCl_2$; 0.7 μM $CuSO_4$ $5H_2O$; 0.76 μM $ZnSO_4$, $7H_2O$; 0.3 μM $Mo_7O_{24}(NH_4)_6$ $4H_2O$ and 46 μM H_3BO_3 (Arnon and Hoagland 1940). Plants were grown in a green-house as follows: 25 ± 1°C temperature, 50% day/75% night relative humidity and 16 h light/8 h dark regime.

Experimental design and NaCl treatments

Individual plants of homogeneous stage of development and size were selected. The experiment was arranged in a completely random design with six NaCl-salinity levels × six replicates. Plants were partitioned into six lots and irrigated with control nutrient solution lacking salt or the same nutrient solution supplemented with 100, 200, 300, 400 or 600 mM NaCl. The choice of salt concentrations was driven on previous studies, where the growth of *R. vermiculata* seedlings was completely arrested and all of them subsequently died at 800 mM NaCl (Gorai and Neffati 2007; Gorai unpublished data). To avoid osmotic shock, NaCl concentrations were increased stepwise in aliquots of 50 mM day^{-1}. An amount of 200 ml NaCl solution was supplied every 2 days, which equaled the amount that was flushed from the drained pots.

Growth measurements

Two harvests were made, at the beginning of treatment (4-month-old plants) and 30 days later. At the harvests, plants were divided into shoots and roots and their respective fresh mass (FM) was measured immediately. Dry mass (DM) of shoots was determined using a LCD-1 lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and that of roots was obtained after oven drying (60°C, 48 h). Lyophilized samples were used to determine chlorophyll contents, proline and soluble sugars on a dry-weight basis.

The relative growth rate (RGR) was determined as RGR ($g\ g^{-1}d^{-1}$) = $(\ln(W_2) - \ln(W_1))/(t_2 - t_1)$, where W is the dry matter at the beginning (W_1) and the end (W_2) of the 30-day treatment period, and ($t_2 - t_1$) is the duration of this period (Hunt 1990).

The sensitivity index (SI), i.e., the difference between dry matter production of salt-treated plants irrigated and the control, expressed in percent of the latter, was calculated as SI_{NaCl} (%) = $[(DM_{NaCl} - DM_{control})/DM_{control}] \times 100$. This parameter was more negative when the plant was more sensitive to NaCl (Saadallah et al. 2001).

Chlorophyll contents

Chlorophyll *a* and *b* were determined following the method described by Inskeep and Bloom (1985). The extract was analyzed for absorbance at 647 and 664.5 nm on a Jenway 6400 spectrophotometer (Jenway, London, UK). Chlorophyll content (mg g^{-1} DM) was calculated using the following equations: Chl $a = 12.70A_{664.5} - 2.79A_{647}$; Chl $b = 20.70A_{647} - 4.62A_{664.5}$; Total Chl $= 17.90A_{647} + 8.08A_{664.5}$, where *A*, absorbance in a 1 cm cuvette.

Water relations

The water content (WC) of shoots and roots was determined as WC (ml H_2O g^{-1} DM) = (FM − DM)/DM. Shoot water potential was measured using a pressure chamber (PMS Instruments Co., Corvallis, OR, USA) after 30 days of salt treatment, according to Scholander et al. (1965).

Ion contents

Ions were extracted from dried, milled plant material with nitric acid (HNO_3, 0.5%). Concentrations of Na^+ and K^+ were determined using an atomic absorption spectrophotometer (Schimazu AA 6800, Schimazu Crop, Kyoto, Japan), while Cl^- concentration was determined on the same extract with a chloride meter (Jenway PC LM3, London, UK).

K^+/Na^+ selectivity ratios

The selectivity ratios of K^+ over Na^+ ($S_{K/Na}$) for accumulation, uptake and transport were estimated as: $[K/Na]_{whole\ plant}/[K/Na]_{medium}$, $[K/Na]_{root}/[Ka/Na]_{medium}$ and $[K/Na]_{shoot}/[K/Na]_{root}$, respectively (Gorai et al. 2010a). K and Na represent, respectively, the quantity found in the whole plant (accumulation), roots (uptake) and shoots (transport).

Proline and soluble sugar contents

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

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

Tissue osmolarity due to K^+ plus Na^+ and associated anions

A conservative estimate of tissue osmolarity (*M*) due to K^+ plus Na^+, and associated anions, supposed univalent and soluble, were calculated as: $2 \times ([Na^+] + [K^+])/[H_2O]$, where brackets design ion (mmol g^{-1} DM) or water (ml g^{-1} DM) contents (Glenn and Brown 1998).

Vacuolar compartmentation

To appreciate the Na^+ compartmentalization degree in foliar tissues, we have correlated shoot water content with its Na^+ content according to Oertli's hypothesis (Flowers et al. 1991). When Na^+ is compartmentalized inside the cells, it was used for the osmotic adjustment and its vacuolar accumulation induced a supplement tissue hydration; however, if Na^+ is remained in the cell walls, it involved tissue dehydration.

Statistical analysis

Data were analysed using SPSS statistical package (SPSS 2002). Data were tested for normal distribution using the Shapiro–Wilk test, and heterogeneities of variance within treatments were tested using Levene's test. When necessary, log transformations were used to normalise distributions. A comparison of means was carried out using Duncan test at $P < 0.05$.

Results

Growth attributes

During NaCl-salinity exposure, salts were secreted from the salt glands in leaves and deposited as crystals on the upper surface of leaves of treated plants, whereas there was no deposition in controls. NaCl-salinity significantly reduced both shoot and root dry matter of *R. vermiculata* ($P < 0.01$). Plant growth was considerably reduced at the

highest salt concentrations (600 mM NaCl) either in shoots and roots, ca. 39 and 52% of the control value, respectively (Fig. 1a). Shoot fresh:dry weight ratio was significantly decreased with increase in salt level, but the highest salt level caused a sharp decline in this attribute. However, root fresh:dry weight ratio seemed to be unaffected by salt (Fig. 1b). The RGR followed a similar trend as that observed for dry matter production, and decreased with increasing salinity in both shoots and roots ($P < 0.01$). Root growth was more sensitive to NaCl than shoot growth (Fig. 1c). To appreciate the sensitivity of *R. vermiculata* to salinity, the SI was determined for shoots and roots (Fig. 1d). The roots presented with the more negative SI were considered as the highest sensitive tissue.

The chlorophyll content of *R. vermiculata* subjected at varying NaCl-enriched nutrient solutions is shown in Fig. 2. Salt stress significantly decreased chl *a*, chl *b* and Total chl contents ($P < 0.001$) with increase in salt level (Fig. 2a–c), whereas chl *a*/*b* ratio was significantly increased ($P < 0.001$) at higher NaCl concentrations (Fig. 2d).

Water relations

As shown in Fig. 3a, NaCl-salinity significantly affected shoot water content of *R. vermiculata* ($P < 0.001$). At 600 mM NaCl, shoot water content was drastically decreased (ca. 32% of the control value). In roots, water content was not affected by salt exposure and did not exceed 4 ml g^{-1} DM. There was a positive correlation between shoot WC and shoot DM, with $R^2 = 0.987$ (Fig. 3b). A one-way ANOVA indicated that treatments significantly affected water potential of salinized plant shoots relative to the control ones. At the highest NaCl concentration, shoot water potential dropped to −3.4 MPa ($P < 0.001$). There was a strong relationship between shoot water potential and the NaCl-enriched nutrient solutions, with $R^2 = 0.911$ (Fig. 3c).

Ions and organic solutes accumulation

Reaumuria vermiculata seedlings in the presence of NaCl-enriched nutrient solution accumulated higher amounts of Na^+ in the shoots ($P < 0.001$) as compared to that in roots ($P < 0.001$). Sodium concentration in the shoots ranged from 1.08 to 2.68 mol l^{-1} at 100 mM and 600 mM NaCl, respectively, whereas that in the roots varied from 0.18 to 0.36 mol l^{-1}, respectively (Fig. 4a). Chloride concentration in leaves displayed a similar pattern as that observed for Na^+, but at lower levels than sodium ($P < 0.001$). However, there were no significant differences in Cl^- concentration in roots with increasing NaCl supply (Fig. 4b). Increasing NaCl-salinity had no adverse effect on potassium concentration in shoots and high amounts were reached at the highest NaCl concentration ($P < 0.05$), whereas that in roots significantly decreased as compared to the controls ($P < 0.01$) (Fig. 4c).

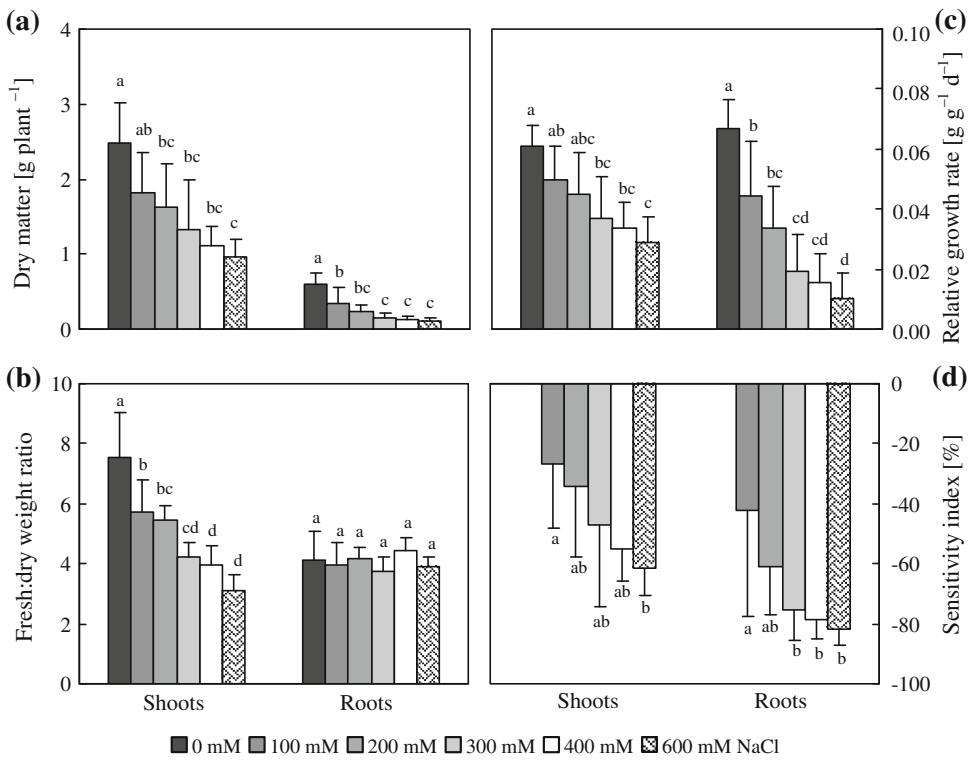

Fig. 1 Changes in tissue **a** dry mass (g plant^{-1}), **b** fresh:dry weight ratio, **c** relative growth rate (g g^{-1} day^{-1}), and **d** sensitivity index (%) of *Reaumuria vermiculata* when 4-month old plants were subjected for 30 days to salinity at varying NaCl-salinity concentrations (0–600 mM) in nutrient solution. Data represent mean ± 95% confidence limits, $n = 5$. *Different letters* indicate significant differences between treatments at $P < 0.05$ according to the Duncan test

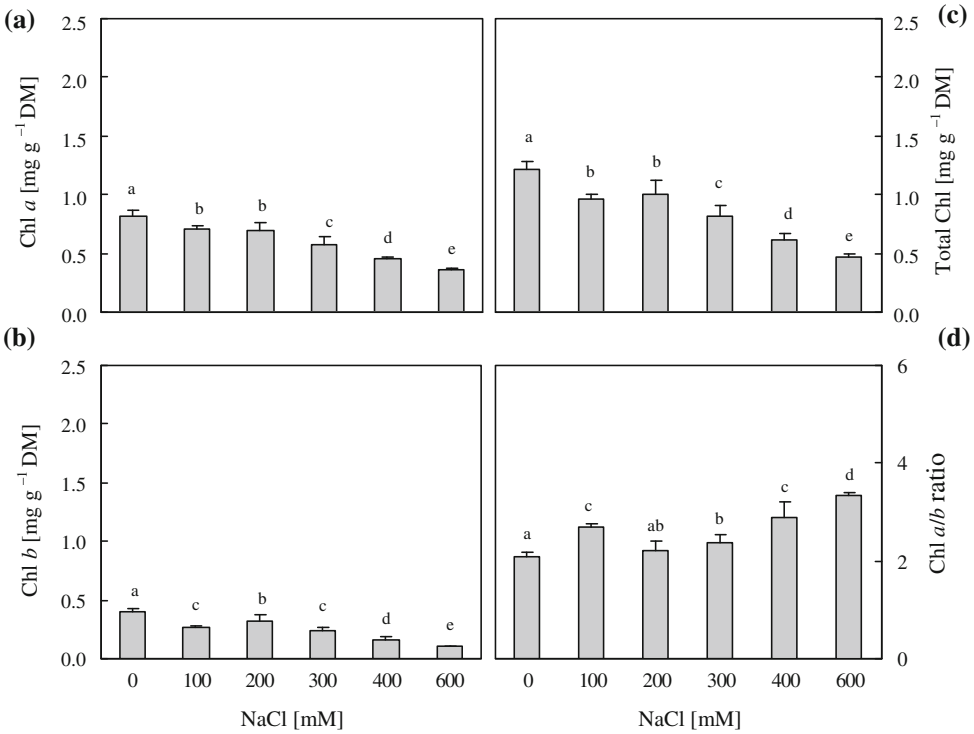

Fig. 2 Mean chlorophyll content (mg g^{-1} DM shoot tissue) of *Reaumuria vermiculata* when 4-month old plants were subjected for 30 days to salinity at varying NaCl concentrations (0–600 mM) in nutrient solution. Data represent mean ± 95% confidence limits, $n = 5$. *Different letters* indicate significant differences between treatments at $P < 0.05$ according to the Duncan test

Increasing NaCl concentration of nutrient solutions had a significant effect on proline concentration in shoots ($P < 0.001$), whereas that of soluble sugars seemed to be unaffected ($P > 0.05$) (Fig. 5a, b). At 600 mM NaCl, proline concentration represented ca. ninefold of the control value.

K^+/Na^+ selectivity ratios

To assess the ability of *R. vermiculata* for selective uptake of K^+ with increasing osmolarity of solutions, the $S_{K/Na}$ was calculated and given in Fig. 6. A one-way ANOVA of the $S_{K/Na}$ ratios revealed that NaCl-salinity significantly affected accumulation of K^+ in the whole plant ($P < 0.001$), uptake by roots ($P < 0.001$) and transport by shoots ($P < 0.001$). At all salinity levels, $S_{K/Na}$ for transport in shoots was lower than those of uptake and accumulation. At the highest NaCl concentrations, $S_{K/Na}$ for uptake, accumulation and transport represented ca. 4.5-, 3.0- and 1.5-fold of 100 mM NaCl-enriched nutrient solution, respectively.

Vacuolar compartmentation

Figure 7 shows the relationship between shoot Na^+ and water content in *R. vermiculata*. Shoot hydration was correlated with the accumulation of Na^+. Shoot water content fell as sodium concentration rose although not as much as in roots. Because the large accumulation of Na^+ ions resulted in tissue dehydration, it is likely that this cation was remained in the cell walls.

Tissue osmolarity due to K^+ plus Na^+ and associated anions

The osmolarity in shoots remained significantly higher than in the medium for all NaCl concentrations ($P < 0.001$); however, in roots only for NaCl concentrations lower than 300 mM NaCl. The concentration of soluble ions in shoots ranged from 0.77 to 5.80 M at 0 and 600 mM NaCl, respectively, whereas that in roots varied from 0.37 to 0.82 M.

Discussion

In the field, *R. vermiculata* naturally inhabits inland salines throughout the Tunisian desert and its wadies, where it is very common. A number of morphological characteristics associated with dehydration have been observed as adaptations under arid environment, including small leaf size and extremely thick cuticle (Gorai personnal observation). This study has demonstrated that growth of *R. vermiculata* seedlings was reduced by increasing NaCl concentrations in nutrient solution. Nevertheless, this species was able to survive at about sea-level concentrations of 600 mM NaCl. This corroborates previous studies on other halophytes, showing optimal growth in mediums

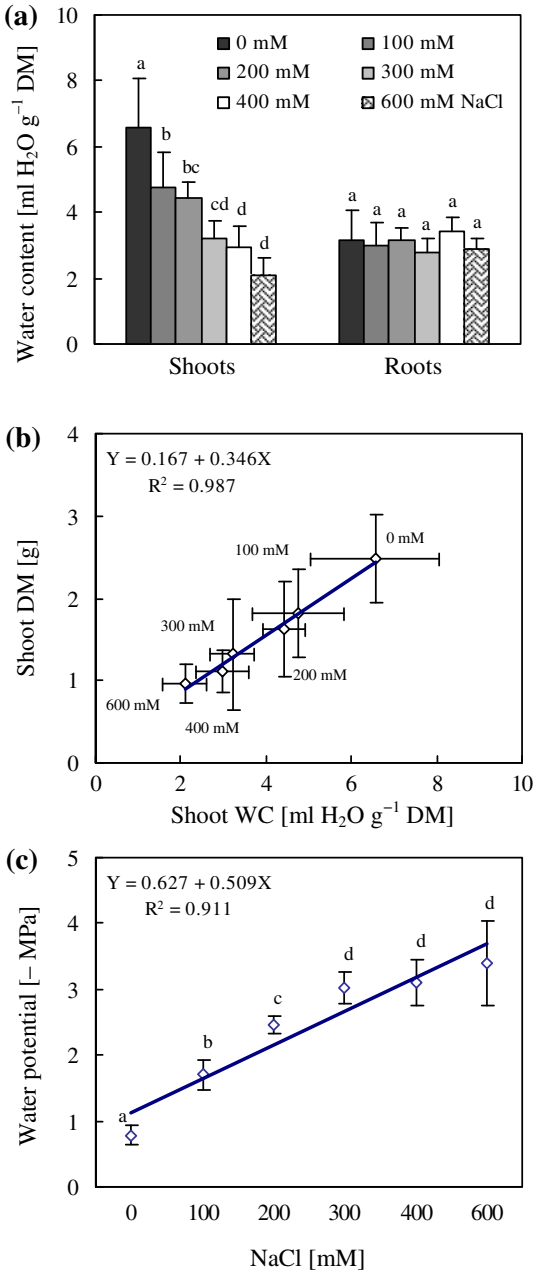

Fig. 3 Changes in **a** tissue water content (ml H_2O g^{-1} DM), and regression plots for **b** shoot dry matter (g $plant^{-1}$) and its water content (ml H_2O g^{-1} DM) and **c** shoot water potential (–MPa) at varying NaCl-salinity concentrations (0–600 mM) in nutrient solution of *Reaumuria vermiculata* when 4-month old plants were subjected for 30 days to salt stress. *Lines* describing the dependencies were obtained using a linear regression. Data represent mean ± 95% confidence limits, $n = 5$. *Different letters* indicate significant differences between treatments at $P < 0.05$ according to the Duncan test

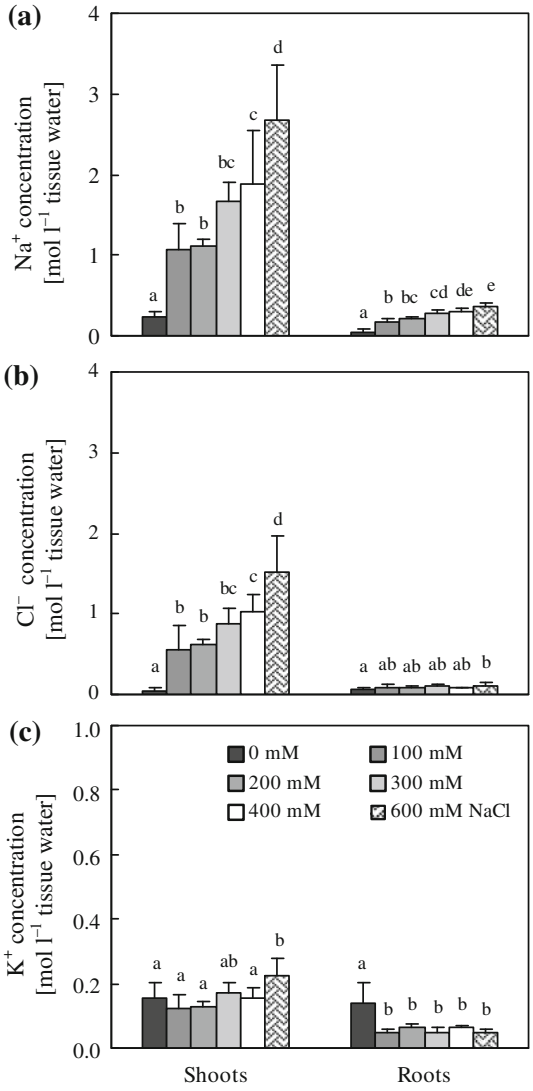

Fig. 4 Changes in Na^+ (**a**), Cl^- (**b**) and K^+ (**c**) concentrations (mmol l^{-1} tissue water) in shoots and roots of *Reaumuria vermiculata* when 4-month old plants were subjected for 30 days to salinity at varying NaCl-salinity concentrations (0–600 mM) in nutrient solution. Data represent mean ± 95% confidence limits, $n = 5$. *Different letters* indicate significant differences between treatments at $P < 0.05$ according to the Duncan test

lacking salts (Barhoumi et al. 2007; Naidoo et al. 2008). The loss of chlorophyll is often considered as a marker of a cellular component of salt stress (Singh and Dubey 1995). Thus, our data support the hypothesis that leaf cells of *R. vermiculata* were stressed when the plants were grown in a salty medium.

Many halophytes have salt glands that secreted excess stress-inducing ions that invade the plant and maintain internal ion concentration at lower level (Hogarth 1999; Tester and Davenport 2003; Naidoo et al. 2008). In *R. vermiculata* salts were secreted by salt glands present in leaves and deposited as crystals on the upper surface of leaves of NaCl-treated plants (Gorai personnal observation). As initially hypothesized by Oertli (1968) and later confirmed (Flowers et al. 1991), in the absence of efficient compartmentalization of Na^+ by leaf cells, their

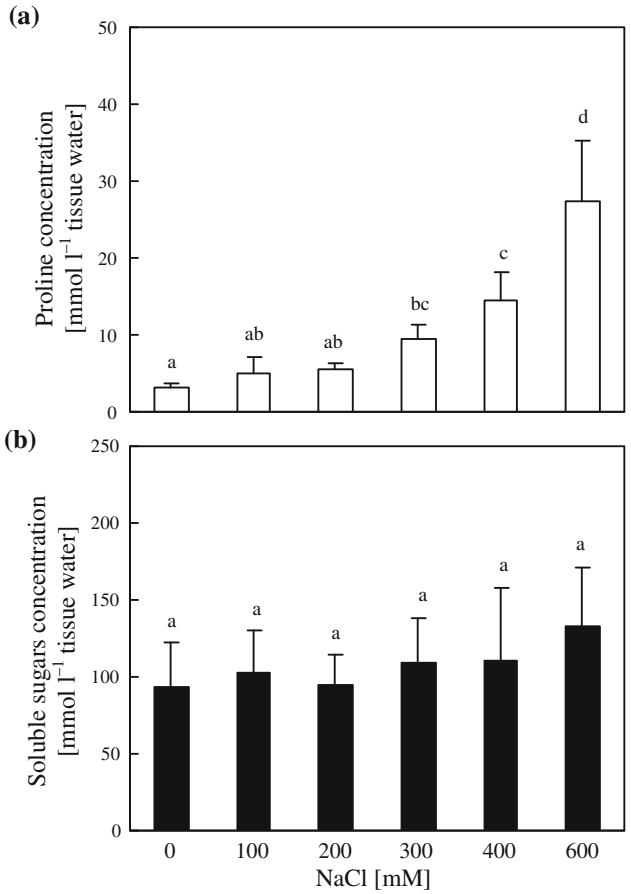

Fig. 5 Mean concentration (mmol l^{-1} tissue water) of proline (**a**) and soluble sugars (**b**) in shoots of *Reaumuria vermiculata* grown on nutrient solution supplied with different NaCl-salinity concentrations (0–600 mM). Data represent mean ± 95% confidence limits, $n = 5$. *Different letters* indicate significant differences between treatments at $P < 0.05$ according to the Duncan test

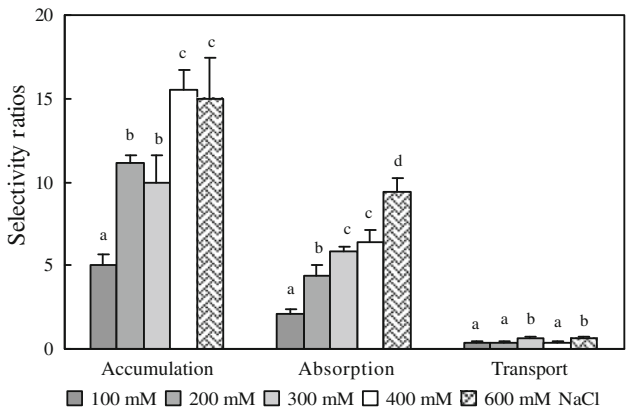

Fig. 6 Changes in the K/Na selectivity ratios ($S_{K/Na}$) for accumulation, uptake and transport in *Reaumuria vermiculata* when 4-month old plants were subjected for 30 days to salinity at varying NaCl concentrations (0 to 600 mM) in nutrient solution. Data represent mean ± 95% confidence limits, $n = 5$. *Different letters* indicate significant differences between treatments at $P < 0.05$ according to the Duncan test

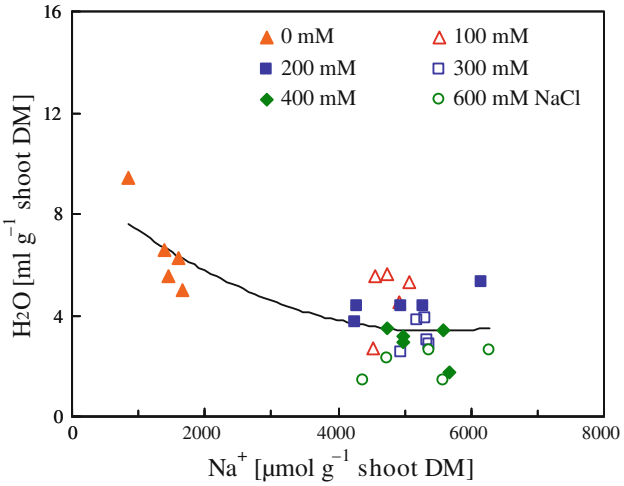

Fig. 7 Relationship between shoot water content (ml H_2O g^{-1} DM) and its sodium content (µmol Na^+ g^{-1} DM) of *Reaumuria vermiculata* when 4-month old plants were subjected for 30 days to salinity at varying NaCl concentrations (0–600 mM) in nutrient solution. A *line* describing the dependency was obtained using a polynomial regression, $R^2 = 0.52$. Values are from six treatments with five replicates ($n = 30$)

concentration in the leaf apoplast may reach excessive values, leading to leaf cell dehydration (Munns and Passioura 1984) and membrane disruption (Speer and Kaiser 1991). This was also true in the present study, as shown by the negative relationship between Na^+ tissue concentration and the shoot hydration (Fig. 7). It is possible that a proportion of Na^+ ions which accumulated within the shoots of *R. vermiculata* remained in the cell walls, bringing about an efflux of water from the protoplast to the apoplast and the subsequent loss of this water by transpiration. It was found by Ramadan (1998) that salt glands of *R. hirtella* secrete a variety of ions, but NaCl is the most abundant salt excreted. Sodium and chloride, which were the predominating ions in the soil solution of the root environment, constituting ca. 89% of salts secreted. This author estimated that more than 67% of the absorbed NaCl was secreted during the day, and the rest is accumulated by the plant leaves.

Water relations in plants are affected by salinity (Hasegawa et al. 2000). The inaptitude of plants to hydrate their tissues appropriately on saline soils causes a physiological drought. Several research reports that plants grown under salt stress manifest acclimation to succeed in their establishment, by lowering both water and osmotic potentials in the leaves (Sultana et al. 1999; Koyro 2006). Osmotic adjustment by net accumulation of solutes in cells in response to a fall in the water potential of their environment can in part offset this deterioration of growth conditions. In *R. vermiculata*, shoot water potential reaching the lowest values (down to −3.4 MPa) at the highest salt level (600 mM NaCl). A conservative estimate

of the concentration of osmotically active (soluble) ions in the tissues was obtained assuming that these ions comprised K^+ and Na^+ plus the anions associated with them (assumed to be univalent) (Glenn and Brown 1998). The data of Fig. 8 show that the concentration of soluble ions in shoots reached high values with 5.8 M at the highest NaCl concentration (600 mM). In *Thellungiella halophila*, a halophytic species, M'rah et al. (2006) showed that the concentration of these ions reached only 1.3 M when treated with NaCl concentration of 200 mM. Proline is a compatible solute that accumulates in response to osmotic stress, and the accumulation of this osmolyte represents an important adaptive response to salt and drought stress (Ashraf and Harris 2004; Parida and Das 2005). In our study, *R. vermiculata* subjected to salt stress accumulated ninefold more proline than controls. By studying the metabolic acclimation of *R. soongorica* during water loss, Liu et al. (2007) showed that elevated levels of proline may assist in osmotic adjustment during desiccation.

Many studies on halophytes and some tolerant glycophytes plants showed that a high $S_{K/Na}$ for transport is a salt tolerance criterion (Gorham et al. 1990; Shachtman et al. 1991; Wolf et al. 1991; Yeo 1998; Debez et al. 2004; Gorai et al. 2010b). *Reaumuria Vermiculata* appears to be able to maintain a high $S_{K/Na}$ for accumulation in the whole plant and the uptake by roots; however, the transport in shoots reached lower values when salinity increases in the medium. The ability of this species to maintain a substantial growth rate under saline conditions is directly related to an efficient $S_{K/Na}$. Zhu (2001) reported that the capacity of plants to counteract salinity stress strongly depends on the status of their potassium nutrition. Maintenance of a high K^+ cytosolic concentration with increasing salinity apparently occurs via highly selective pathways at the root–soil interface and is an important determinant of salt tolerance (Maathuis and Amtmann 1999; Rodriguez-Navarro 2000). Ion selectivity also occurs in salt glands. For example, in *R. hirtella* grown under natural saline conditions, Ramadan (1998) found that the secretion mechanism has a high specifcity for Na^+ and Cl^- and a low specifcity for secretion of K^+, $Ca^{2+,}$ Mg^{2+} and $SO_4{}^{2-}$, and $PO_4{}^{3-}$ and $NO_3{}^-$ seems to be unavailable for secretion, possibly because of their rapid incorporation and utilization in the metabolism. *Sporobolus spicatus* was found to secrete 93% NaCl by weight of salts secreted by plants from four different sites while K^+, $Ca^{2+,}$ Mg^{2+} and $SO_4{}^{2-}$ constituted only 5% of salts (Ramadan 2001).

In conclusion, this study shows that *R. vermiculata* grown in the presence of NaCl-salinity appears to be able to survive up to 600 mM and could accumulate large amounts of ions in its shoots without damage. This accumulation leads to (1) the tissue dehydration, and (2) the chlorophyll loss. The more negative values of shoot water potential were achieved by the accumulation of osmotically active solutes. Further investigations are necessary to understand the strategies for adaptation of *Reaumuria* grown under saline environments.

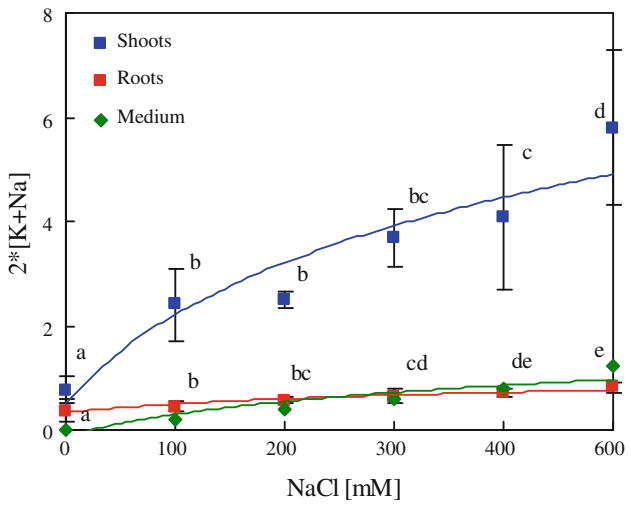

Fig. 8 Regression plots for tissue osmolarity of *Reaumuria vermiculata* when 4-month old plants were subjected for 30 days to salinity at varying NaCl concentrations (0–600 mM) in nutrient solution. *Lines* describing the dependencies were obtained using a logarithmic regression. Data represent mean ± 95% confidence limits, $n = 5$. *Different letters* indicate significant differences between treatments at $P < 0.05$ according to the Duncan test

References

Arnon DI, Hoagland DR (1940) Crop production in artificial solutions and in soils with special reference to factors affecting yields and absorption of inorganic nutrient. Soil Sci 50:463–484

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3–16

Barhoumi Z, Djebali W, Smaoui A, Chaïbi W, Abdelly C (2007) Contribution of NaCl excretion to salt resistance of *Aeluropus littoralis* (Willd) Parl. J Plant Physiol 164:842–850

Bates S, Waldren RP, Teare ID (1973) Rapid determination of the free proline in water stress studies. Plant Soil 39:205–208

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Debez A, Ben Hamed K, Grignon C, Abdelly C (2004) Salinity effects on germination, growth and seed production of the halophyte *Cakile maritima*. Plant Soil 262:179–189

Flowers TJ, Hajibagheri MA, Yeo AR (1991) Ion accumulation in the cell walls of rice plants growing under saline condition: evidence for the Oertli hypothesis. Plant Cell Environ 14:319–325

Glenn EP, Brown JJ (1998) Effects of soil salt levels on the growth and water use efficiency of *Atriplex canescens* (Chenopodiaceae) varieties in drying soil. Am J Bot 85:10–16

Gorai M, Neffati M (2007) Germination responses of *Reaumuria vermiculata* to salinity and temperature. Ann Appl Biol 151:53–59

Gorai M, Ennajeh M, Khemira H, Neffati M (2010a) Influence of NaCl-salinity on growth, photosynthesis, water relations and solute accumulation in *Phragmites australis*. Acta Physiol Plant. doi:10.1007/s11738-010-0628-1

Gorai M, Ennajeh M, Khemira H, Neffati M (2010b) Combined effect of NaCl-salinity and hypoxia on growth, photosynthesis, water relations and solute accumulation in *Phragmites australis* plants. Flora 205:462–470

Gorham J, Bristol A, Yopung EM, Wyn Jones RG, Kashour G (1990) Salt tolerance in the Triticeae: K/Na discrimination in barley. J Exp Bot 41:1095–1101

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Ann Rev Plant Physiol 31:149–190

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Ann Rev Plant Physiol Plant Mol Biol 51:463–499

Hewitt EJ (1966) Sand and water culture methods used in the study of plant nutrition. Commonw Bureau Horticult Tech Com 22:431–446

Hogarth PJ (1999) The Biology of Mangroves. Oxford University Press, NewYork

Hunt R (1990) Basic growth analysis. Plant growth analysis for beginners. Unwin Hyman, London

Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll *a* and *b* in *N*,*N*-dimethylformamide and 80% acetone. Plant Physiol 77:483–485

Jacobson L (1951) Maintenance of iron supply in nutrient solutions by a single addition of ferric-potassium-ethylene-diamine-tetracetate. Plant Physiol 26:411–413

Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, Panneerselvam R (2007) Alterations in germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. S Afr J Bot 73:190–195

Koyro HW (2006) Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). Environ Exp Bot 56:136–146

Le Houérou HN (1993) Salt tolerant plants for the arid regions of the Mediterranean isoclimatic zone. In: Lieth H, Al-Masoom A (eds) Towards the rational use of high salinity tolerant plants. Kluwer, Dordrecht, pp 403–422

Le Houérou HN (1995) Forage halophytes in the Mediterranean basin. In: Choukr-Allah R, Malcom CV, Hamdy A (eds) Halophytes and biosaline agriculture. Marcel Dekker, New York, pp 115–135

Liu YB, Wang G, Liu J, Zhao X, Tan HJ, Li XR (2007) Anatomical, morphological and metabolic acclimation in the resurrection plant *Reaumuria soongorica* during dehydration and rehydration. J Arid Environ 70:183–194

M'rah S, Ouerghi Z, Berthomieu C, Havaux M, Jungas C, Hajji M, Grignon C, Lachaâl M (2006) Effects of NaCl on the growth, ion accumulation and photosynthetic parameters of *Thellungiella halophila*. J Plant Physiol 163:1022–1031

Maathuis FJM, Amtmann A (1999) K^+ Nutrition toxicity: the basis of cellular K^+/Na^+ ratios. Ann Bot 84:112–133

Mansour MMF, Salama KHA (2004) Cellular basis of salinity tolerance in plants. Environ Exp Bot 52:113–122

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250

Munns R, Passioura JB (1984) Hydraulic resistance of plants. III. Effects of NaCl in barley and lupin. Aust J Plant Physiol 11:351–359

Naidoo G, Somaru R, Achar P (2008) Morphological and physiological responses of the halophyte, *Odyssea paucinervis* (Staph) (Poaceae), to salinity. Flora 203:437–447

Oertli JJ (1968) Extra cellular salt accumulation, a possible mechanism of salt injury in plants. Agrochimica 12:461–469

Owens S (2001) Salt of the earth. Genetic engineering may help to reclaim agricultural land lost due to salinisation. EMBO Rep 2:877–879

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotox Environ Safe 60:324–349

Pottier-Alapetite G (1979) Flore de la Tunisie. Angiosperme-Dicotylédones. Vol. I: Apétales-Dialypétales. Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture, Tunis

Ramadan T (1998) Ecophysiology of salt excretion in the xerohalophyte *Reaumuria hirtella*. New Phytol 139:273–281

Ramadan T (2001) Dynamics of salt secretion by *Sporobolus spicatus* (Vahl) Kunth from sites of differing salinity. Ann Bot 87:259–266

Robyt JF, White BJ (1987) Biochemical techniques—theory and practice. Books/Cole Publishing Company, Monterey, pp 267–275

Rodriguez-Navarro A (2000) Potassium transport in fungi and plants. Biochem Biophys Acta 1469:1–30

Saadallah K, Drevon JJ, Abdelly C (2001) Nodulation et croissance nodulaire chez le haricot (*Phaseolus vulgaris*) sous contrainte saline. Agronomie 21:627–634

Scholander PF, Hammel HT, Bradstreet ED, Henningsen EA (1965) Sap pressure in vascular plants. Science 148:339–346

Shachtman D, Munns R, White Cross MI (1991) Variation in sodium exclusion and salt tolerance in *Triticum tauschii*. Crop Sci 31:992–997

Singh AK, Dubey RS (1995) Changes in chlorophyll a and b contents and activities of photosystems I and II in rice seedlings induced by NaCl. Photosynthetica 31:489–499

Speer M, Kaiser WM (1991) Ion relations of symplastic and apoplastic space in leaves from *Spinacia oloraceae* L. and *Pisum sativum* L. under salinity. Plant Physiol 97:990–997

SPSS (2002) SPSS 11.5 for Windows Update. SPSS Inc, Chicago

Sultana N, Ikeda T, Itoh R (1999) Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environ Exp Bot 42:211–220

Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 91:503–527

Wolf O, Munns R, Tounet ML, Jeschke WD (1991) The role of the stem in the partitioning of Na^+ and K^+ in salt-treated barley. J Exp Bot 42:697–704

Yeo AR (1998) Molecular biology of salt tolerance in the context of whole-plant physiology. J Exp Bot 49:915–929

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6:66–71