Journal of Plant Nutrition, 28: 1365–1374, 2005

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1081/PLN-200067462

Taylor & Francis
Taylor & Francis Group

Salt Stimulation and Tolerance in an Intertidal Stem-Succulent Halophyte

M. Ajmal Khan, Irwin A. Ungar, and Allan M. Showalter

Department of Environmental and Plant Biology, Molecular and Cell Biology Program, Ohio University, Athens, Ohio, USA

ABSTRACT

Salt tolerance of *Arthrocnemum macrostachyum* (Moric.) C. Koch (Chenopodiaceae), a stem-succulent halophyte most commonly found in the intertidal regions of the provinces of Sind and Balochistan, Pakistan, was investigated. Plants were grown for 125 d at six sodium chloride (NaCl) concentrations from 0 to 1000 mM to determine the effects of salinity on ion accumulation, plant water status, and biomass. Shoot biomass was greatest at 200 to 400 mM NaCl, but it was inhibited at salinities of 600 mM NaCl or higher. Tissue water content (g g^{-1}dry mass) of shoots under 200 to 600 mM NaCl treatments was higher than under the control nutrient solution, equal to the control at 800 mM NaCl, but significantly lower at 1000 mM NaCl than under all other treatments, indicating an increase in shoot succulence at salinity levels up to that of seawater. Ash content increased with added salt, but was about 60% of plant dry mass under all salinity treatments. The Na^+ and Cl^- concentrations of shoots were significantly higher under 1000 mM NaCl than under the control treatment. These results indicate that *A. macrostachyum* is salt tolerant and capable of accumulating large quantities of Na^+ and Cl^- when treated with from 200 to 1000 mM NaCl.

Keywords: *Arthrocnemum macrostachyum*, biomass, halophyte, salt stimulation, tolerance

INTRODUCTION

Maritime salt marshes exist in many parts of the world and comprise vegetated land areas bordering the sea that are subject to periodic inundation by tidal action (Ahmad and Malik, 2002). Since there is a gradual change in elevation

Received 29 December 2003; accepted 9 August 2004.

Address correspondence to Dr. M. Ajmal Khan, Department of Botany, University of Karachi, Karachi 75270, Pakistan. E-mail: ajmal@halophyte.org

and micro-environmental conditions between seaward and the landward portion of a marsh, there is also often a change in vegetation, reflecting elevation or hydroperiod gradients. In the sub-tropical salt marshes of Pakistan, lower elevations that are regularly inundated by seawater are less saline (500–650 mM NaCl) than higher elevations that are infrequently flushed by tidal action but may attain soil-water salinity levels as high as 1000 mM NaCl (Gul and Khan, 1998). Above-ground biomass production is lowest in the seaward zone and highest in the intermediate zone of the marsh, ranging from 972 to 1420 g m^{-2} $yr^{-}1$. In this study *Arthrocnemum macrostachyum* was grown over a range of salinities to determine under what conditions biomass production is stimulated or inhibited.

Some coastal species, such as *Tetragonia trigyna* and *T. tetragonoides*, show the greatest biomass production in control nutrient solution but are able to tolerate salinities of up to 300 mM NaCl (Watkins et al., 1988; Wilson et al., 2000). Other stem-succulent halophytes are salt-stimulated and show increased biomass production, including *Salicornia* sp. (170 mM NaCl) (Ungar, 1978), *Sarcocornia natalensis* (300 mM NaCl) (Naidoo and Rughunanan, 1990) *Salicornia bigellovii* (200 mM NaCl) (Ayala and O'Leary, 1995), and *Halosarcia perganulata* (340 mM NaCl) (Short and Colmer, 1999). *Suaeda fruticosa*, which grows in association with *A. macrostachyum*, is reported to show its greatest biomass production at 400–600 mM NaCl, with little mortality up to 1000 mM NaCl (Khan et al., 2000).

Succulent halophytes, which require salinity for their optimal growth, may accumulate Na^+ and Cl^- ions in their tissues to achieve osmoregulation (Neumann, 1997; Subbarao et al., 2002; Wang et al., 2002). Winter et al., (1976) reported that two halophytes from the coastal habitats of Israel tended to have approximately equivalent concentrations of Na^+ and Cl^- when expressed as mmol kg^{-1} fresh weight: *A. macrostachyum* (455 mmol Na^+ and 490 mmol Cl^-), and *Halimione portulacoides* (385 mmol Na^+ and 453 mmol Cl^-). Khan et al. (2000) showed that the stem-succulent perennial *Haloxylon stocksii* had a shoot-ion content that ranged from 215 to 488 mmol Cl^-kg^{-1} dry mass and 278 to 528 mmol Na^+kg^{-1} dry mass during the growing season. This study sought to be determine if *A. macrostachyum* accumulates ions in its shoots, a process which may serve as a mechanism for osmoregulation.

Arthrocnemum macrostachyum (Moric.) C. Koch (Chenopodiaceae) is a perennial, stem-succulent halophyte that grows in salt marshes of the Mediterranean region, North and East Africa, and East and South Asia (Lieth et al., 1999). It is found in the intertidal zone of the Arabian Sea at Karachi, Pakistan, where seawater salt concentrations range from 600 to 700 mM NaCl (Gul and Khan, 1998). It has a small, shrub-like habit and is found in pure patches from the margin of the mangrove zone dominated by *Avicennia marina* to the mean high-tide line of coastal salt marshes of Karachi, Pakistan; occasionally, it is also found with other species such as *Limonium stocksii*, *Urochondra setulosa, Aeluropus lagopoides*,*and Suaeda fruticosa* (Gul

and Khan, 1998). Populations of *A. macrostachyum* are subjected to spatial and temporal variation in soil salinity because of the drought and flooding periods they are exposed to under field conditions. Very little information is available on the salt tolerance of this species, which is used as forage and whose seeds are a potential source of high-quality edible oil. The present investigation was designed to test the following questions: (1) How tolerant is *Arthrocnemum macrostachyum* to soil salinity and is its growth salt stimulated and (2) Does an increase in NaCl concentration of the medium cause an accumulation of $Na^+$and Cl^-in shoots of *Arthrocnemum macrostachyum*?

MATERIALS AND METHODS

Seeds of *Arthrocnemum macrostachyum* were collected during the autumn of 1994 from salt flats located on the Karachi University Campus, Pakistan, and stored at 4°C. Seeds were surface-sterilized using the fungicide Phygon (whose active ingredient is dichlone: 2,3-dichloro-1,4 napthoquinone) and germinated in spring 1995.

Plants were grown in a growth chamber at a thermoperiod of 25°C/35°C (night/day) and a photoperiod of 12 h (300 μmol m^{-2} s^{-1}, 400–700 nm at plant level). Ten replicates containing one plant in each pot were grown in 10 cm-diameter pots at 0, 200, 400, 600, 800, and 1000 mM sodium chloride (NaCl) in sand culture. A half-strength Hoagland and Arnon No. 2 nutrient solution was used to supply the macronutrients and micronutrients (Moore, 1960). Seedling growth in sand culture was begun with half-strength Hoagland and Arnon No. 2 nutrient solution for 30 d. Subsequently, salinity concentrations were increased gradually by 200 mM NaCl at 2 d intervals to reach all of the experimental salinity levels after 10 d. Pots were placed in trays and sub-irrigated; the water level was adjusted daily with tap water to correct for evaporation. Salt solutions containing half-strength Hoagland and Arnon No. 2 nutrient solution were completely replaced once a week to maintain the salinity levels in pots. A plant from each of 10 pots per treatment was harvested at 125 d after the salt-concentration treatments were established. Fresh and dry mass of plant shoots and roots were measured for 10 plants in each treatment. Roots were rinsed in distilled water to remove sand from their surface. Dry mass was determined after drying for 48 h in a forced-draft oven at 60°C.

The same 10 dried plants from each NaCl treatment were ashed in a muffle furnace at 500°C for 12 h to determine their ash content and ash-free dry mass. Ash was treated with 5 ml of 20% concentrated sulfuric acid for 24 h and then diluted to 100 mL with distilled water. Chloride ion (Cl) content was measured with a Beckman specific-ion electrode. Cation content of plant organs was determined with a Perkin Elmer model 360 atomic absorption spectrophotometer. Flame-emission spectrophotometry was used to determine sodium (Na^+) and

potassium (K^+) and atomic absorption spectrophotometry for calcium (Ca^{2+}) and magnesium (Mg^{2+}) content of plant-organ extractions.

Data for fresh and dry mass and ionic content were analyzed by one-way ANOVA to determine whether significant differences were present among means. A post-hoc Bonferroni multiple-range test was carried out to determine whether significant ($P < 0.05$) differences occurred between individual treatments (SPSS, 1999).

RESULTS

Both shoot fresh mass ($F = 20.023$, $P < 0.001$) and root fresh mass ($F = 8.89$, $P < 0.001$) were affected significantly by salinity (Figure 1a). Final trial fresh mass of shoots increased in salinity treatments at 200 and 400 mM NaCl, but further increases in salinity yielded much reduced biomass at 800 and 1000 mM NaCl. Root fresh mass was not salt stimulated or inhibited at 200 and 400 mM NaCl, but at higher salinities it was lower than the controls.

Salinity also affected significantly both shoot dry mass ($F = 15.81$, $P < 0.001$) and root dry mass ($F = 11.69$, $P < 0.001$). Shoot dry weight reached its peak at 200 and 400 mM NaCl, achieving about double the biomass of the non-saline controls (Figure 1b). Root dry mass was promoted at 200 mM NaCl and declined at salinities above 400 mM NaCl (Figure 1b).

The fresh mass:dry mass ratio of shoots remained constant up to and including 800 mM NaCl and then decreased ($F = 6.67$, $P < 0.05$) at 1000 mM NaCl (Figure 1c). The root fresh mass:dry mass ratio increased substantially ($F = 24.62$, $P < 0.0001$) at the lower salinities, reaching from about 4 in non-saline treatments (controls) to more than three times this level at 400 mM NaCl (Figure 1c), but declined to the control levels at 1000 mM NaCl.

Ash content of *A. macrostachyum* shoots was 36% of oven dry mass in the non-saline medium and increased significantly, to 58%, in 200 mM NaCl ($F = 20.14$, $P < 0.01$) (Figure 1d). There was no further increase in ash content with a further increase in salinity. Ash content of the root increased at 400 mM NaCl, but did not differ significantly at higher salinities. Root ash content was about 50% less than in the shoot for all treatments (Figure 1d). Percentage of ash-free dry mass (organic weight) of shoots declined at 200 mM NaCl with no change at higher salinities ($F = 8.64$, $P < 0.05$) (Figure 1e), while percentage ash-free dry mass of roots did not change significantly among treatments ($F = 1.64$, $p > 0.05$) (Figure 1e).

Tissue water content (g g^{-1} dry mass) was higher than the control in shoots at 200 to 600 mM NaCl, but was lower than the control at 1000 mM NaCl. Root-tissue water content increased under 200 to 400 mM NaCl, but declined at the highest salinity (Figure 1f).

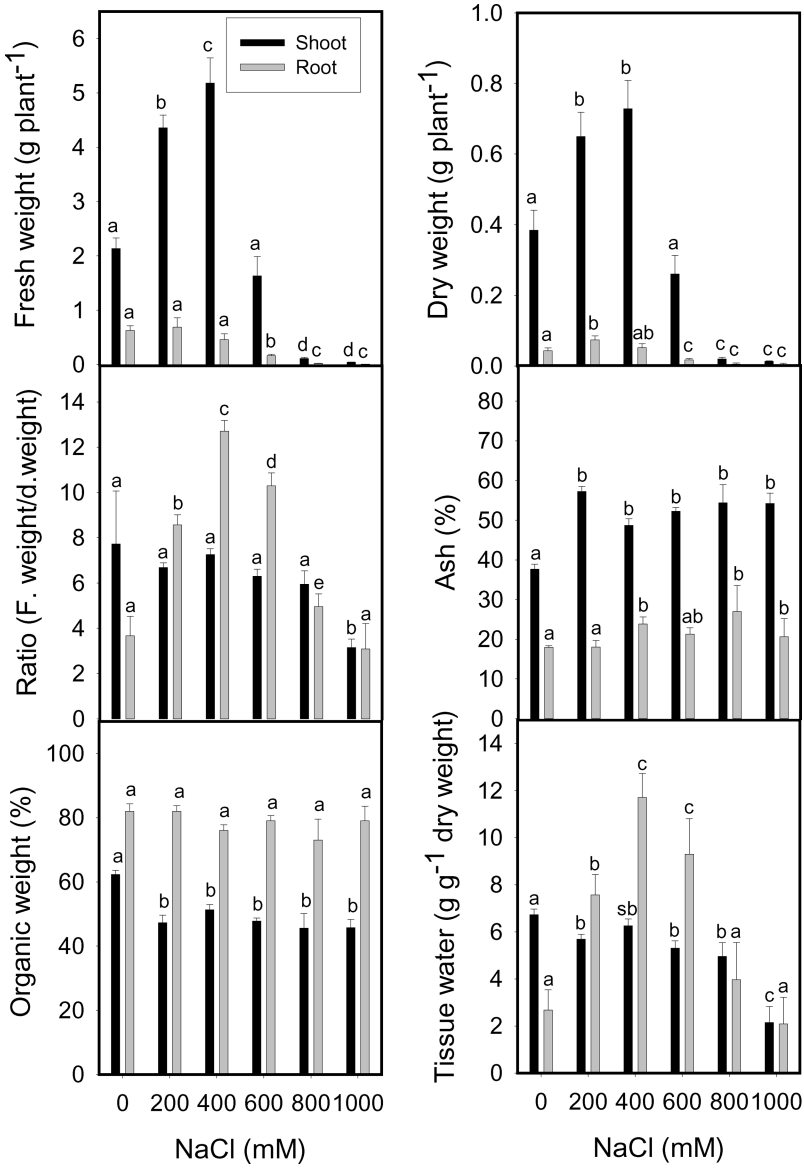

Figure 1. Effect of NaCl on (a) fresh mass, (b) dry mass, (c) fresh mass:dry mass ratio, (d) ash content based on dry mass, (e) ash-free dry mass, and (f) tissue water content of *Arthrocnemum macrostachyum* shoots and roots. Bars represent mean ± standard error. Different letters above bars represent a significant difference ($P < 0.05$) between treatments.

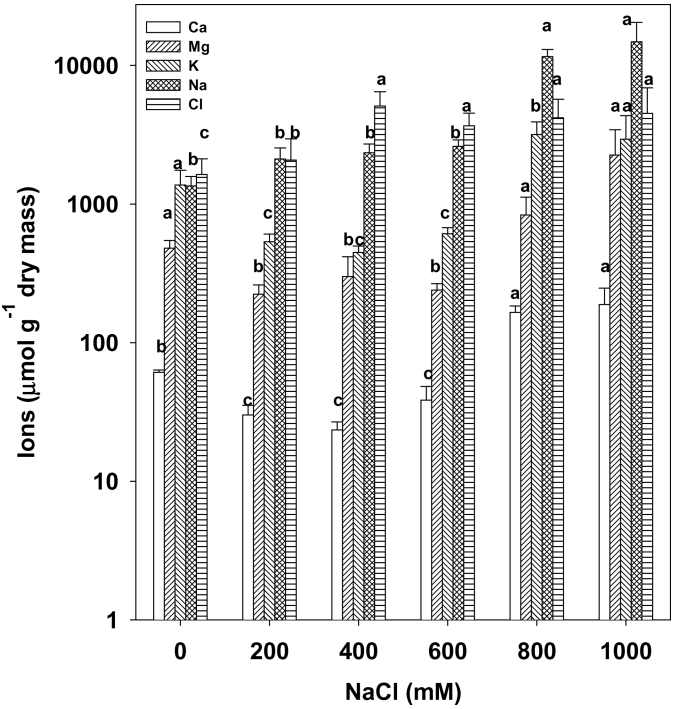

Figure 2. Effect of NaCl on ion content of *Arthrocnemum macrostachyum* shoots. Bars represent mean ± standard error. Different letters above bars represent a significant difference ($P < 0.05$) between treatments for a specific ion.

Concentrations of Ca^{2+} ($F = 33.21$, $P < 0.001$), Mg^{2+} ($F = 6.51$, $P < 0.0001$), and K^+ ($F = 23.55$, $P < 0.001$) in shoots were affected significantly by NaCl treatments (Figure 2). They were lower than the controls in up to 600 mM NaCl. The concentration of Na^+ ($F = 33.56$, $P < 0.001$) in shoots increased significantly with increases in salinity at 800 and 1000 mM NaCl, while Cl^- concentration increased at 400 mM NaCl and higher ($F = 39.62$, $P < 0.0001$). The K:Na ratio was affected significantly ($F = 45.23$, $P < 0.001$) by salt treatments, decreasing in plants under the controls to that achieved for plants in the 200 mM NaCl treatment, then staying about the same under the 400 to 1000 mM NaCl treatments (Figure 3).

DISCUSSION

Similar to other stem and leaf-succulent members of the Chenopodiaceae, *A. macrostachyum* is a halophyte that is salt stimulated in up to 400 mM NaCl and has a high degree of salt tolerance. Shoot biomass production of

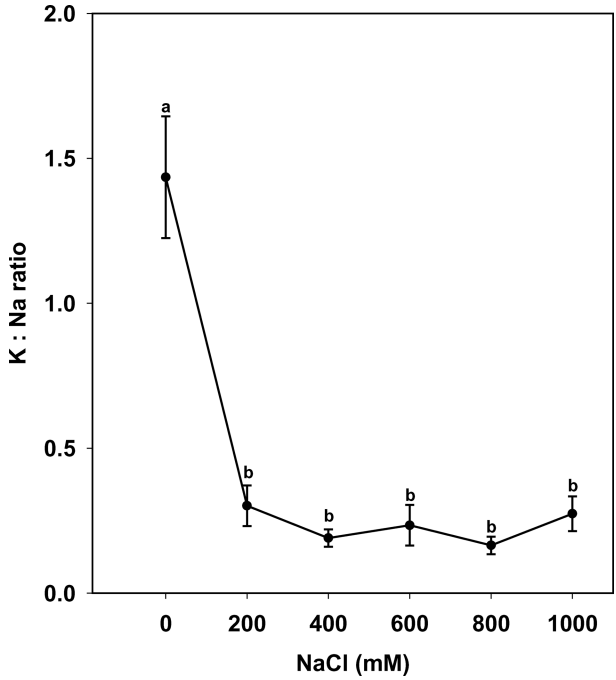

Figure 3. Effect of NaCl on K:Na ratio of *Arthrocnemum macrostachyum* shoots. Points represent mean ± standard error. Different letters above points represent a significant difference ($P < 0.05$) between treatments.

A. macrostachyum was stimulated for plants grown in from 200 to 400 mM NaCl, but was reduced at higher salinities. Earlier work on *Sarcocornia natalensis*, another stem-succulent coastal halophyte in the Chenopodiaceae from coastal marshes in South Africa showed that its shoot dry mass was greatest for plants grown at 300 mM NaCl and that exposure to 500 mM NaCl for seven months resulted in only a moderate decrease in shoot mass (Naidoo and Rughunanan, 1990). Similar results were reported for *Allenrolfea occidentalis* (Gul et al., 2000) and *Salsola chaudhryi* (Al-Khateeb, 2002). It is likely that the causes of reduction in growth at both suboptimal and supraoptimal salinity differ, indicated by the higher rate of photosynthesis at suboptimal salinity (Ayala and O'Leary, 1995). Further study of the response of high-salinity-tolerant halophytes to suboptimal salinity is needed.

Succulence is an adaptive strategy that contributes to the regulation of internal ion concentrations in many halophyte species in the family Chenopodiaceae (Sen et al., 2002). Exposure to high concentrations of NaCl increased succulence in *Sarcocornia natalensis* (Naidoo and Rughunanan, 1990), *Allenrolfea occidentalis* (Gul et al., 2000), and *Halosarcia pergranulata* (Short and

Colmer, 1999). The present work showed that tissue water (g g^{-1} dry mass) content in shoots was significantly ($P < 0.05$) higher under the 200 to 600 mM NaCl treatments than under the control, indicating an increase in succulence with an increase in salinity. However, at 1000 mM NaCl, the shoot tissue water content was significantly ($P < 0.05$) lower than under the control treatment.

Ash content of *A. macrostachyum* plants increased from 35% under the controls to about 60% under 200 mM NaCl and did not differ significantly with increases in salinity up to 1000 mM NaCl. High ash content from ion accumulation is considered to be of major adaptive significance in the Chenopodiaceae for osmotic adjustment (Sen et al., 2002; Ungar, 1991). In *Suaeda maritima*, a succulent-leaved coastal halophyte, inorganic ions constituted 27% of the dry mass in plants grown in non-saline controls and 45% in those treated with 340 mM NaCl (Yeo and Flowers, 1980). In *Disphyma australe*, inorganic ions constituted 55% of the total dry mass (Neals and Sharkey, 1981), while in *Suaeda fruticosa* (Ungar, 1978) and *Halosarcia pergranulata* the ash contact was 60% and 50% for each species, respectively (Short and Colmer, 1999). Ash content in *A. macrostachyum* is at the upper range compared with that in these other species.

Concentrations of Na^+ and Cl^- in succulent shoots of *A. macrostachyum* were regulated by ion accumulation, because an increase in the external NaCl from 0 to 1000 mM resulted in a significant increase in internal Na^+ and Cl^- content at higher salinities. The K^+:Na^+ ratio in shoots declined at 200 mM NaCl from the control value and remained lower than the control at salinities from 400 to 1000 mM NaCl. A low K^+:Na^+ ratio in salt treatments has been reported previously in other halophytes (Ayala and O'Leary, 1995; Naidoo and Rughunanan, 1990; Subbarao et al., 2002). *Arthrocnemum macrostachyum* reached its greatest biomass at 200 to 400 mM NaCl and had reduced biomass at higher salinities. This salinity is lower than that of seawater of the Arabian Sea near Karachi, which ranges between 600 to 700 mM NaCl, but represents salinity levels at Manora channel near Sandspit, Karachi, after monsoon rains in June, when dry mass production is high (Moore, 1960). These data indicate that *Arthrocnemum macrostachyum* is a salt accumulator, based on its ability to accumulate high concentrations of Na^+ and Cl^- and the 50%–60% ash content in shoots of plants treated with from 200 to 1000 mM NaCl. This is an important adaptation that permits *A. macrostachyum* to grow in intertidal habitats where soil salinity levels may range seasonally from 150 to 1000 mM NaCl (Moore, 1960).

CONCLUSIONS

Arthrocnemun macrostachyum is a highly salt tolerant, stem-succulent halophyte, whose growth was stimulated at 200–400 mM NaCl. It is interesting to

note that the leaf succulence also reached its peak at the same concentration, indicating some relationship between succulence and optimal growth. *Arthrocnemum macrostachyum* is perhaps tolerant of a low K:Na ratio because of solute compartmentation at the cellular level. Further studies on water relations, compatible organic solutes, and solute compartmentation are required to enhance the understanding of salinity tolerance in *A. macrostachyum.*

ACKNOWLEDGMENTS

We thank the CIES for providing a Fulbright Scholar research grant and Ohio University for a postdoctoral fellowship to M. Ajmal Khan. This research was supported in part by National Science Foundation Research Grant INT-97-30882.

REFERENCES

Ahmad, R., and K. A. Malik. 2002. *Prospect for saline agriculture*, Task for Vegetation Science 37. Dordrecht, Netherlands: Kluwer Academic Publishers.

Al-Khateeb, S. A. 2002. Differential response of *Seidltzia rosemarinus* L. and *Salsola chaudaryi* L. to NaCl induced salinity under eastern Saudi Arabian conditions. *Pakistan Journal of Agricultural Research* 17: 81–87.

Ayala, F., and J. W. O'Leary. 1995. Growth and physiology of *Salicornia bigellovii* Torr. at suboptimal salinity. *International Journal of Plant Science* 156: 197–205.

Gul, B., and M. A. Khan. 1998. Population characteristics of the coastal halophyte *Arthrocnemum macrostachyum. Pakistan Journal of Botany* 30: 189–197.

Gul, B., D. J. Weber, and K. A. Khan. 2000. Effect of salinity and planting density on the physiological responses of *Allenrolfea occidentalis*. *Great Basin Naturalist* 60: 188–197.

Khan, M. A., I. A. Ungar, and A. M. Showalter. 2000. The effect of salinity on the growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forssk. *Journal of Arid Environment* 45: 73–84.

Lieth, H., M. Moschenko, M. Lohmann, H.-W. Koyro, and A. Hamdt. 1999. *Halophyte uses in different climates* I. Leiden, Germany: Backhuys Publishers.

Moore, R. H. 1960. *Laboratory guide for elementary plant physiology*. Minneapolis: Burgess Publishing.

Naidoo, G. R., and R. Rughunanan. 1990. Salt tolerance in the succulent halophyte, *Sarcocornia natalensis*. *Journal of Experimental Botany* 41: 497–502.

Neals, T. F., and P. J. Sharkey. 1981. Effect of salinity on growth and on mineral and organic constituents of the halophyte *Disphyma australe* (Soland.) J.M. Black. *Australian Journal of Plant Physiology* 8: 165–179.

Neumann, P. 1997. Salinity resistance and plant growth revisited. *Plant Cell and Environment* 20: 1193–1198.

Sen, D. N., P. K. Kasera, and S. Mohammad. 2002. Biology and physiology of saline plants. In *Handbook of plant and crop physiology*, ed. M. Pessarakli, 563–581. New York: Marcel Dekker.

Short, D. C., and T. D. Colmer. 1999. Salt tolerance in the halophyte *Halosarcia pergranulata* subsp. *pergranulata. Annals of Botany* 83: 207–213.

Subbarao, G. V., G. W. Stutte, R. M. Wheeler, and W. L. Berry. 2002. Sodium: A functional nutrient in plant. In *Handbook of plant and crop physiology*, ed. M. Pessarakli, 363–384. New York: Marcel Dekker.

SPSS. 1999. *SPSS: SPSS Base 9.0 User Guide.* Chicago, Illinois: SPSS.

Ungar, I. A. 1978. The effects of salinity and hormonal treatments on growth and ion uptake of *Salicornia europaea. Bulletin de la Societe Botanik Francaise* 3–4: 95–104.

Ungar, I. A. 1991. *Ecophysiology of vascular halophytes*. Boca Raton, FL: CRC Press.

Wang, D., J. A. Poss, T. J. Donovan, M. C. Shannon, and S. M. Lesch. 2002. Biophysical properties and biomass production of elephant grass under saline conditions. *Journal of Arid Environment* 52: 447–456.

Watkins, C. B., J. M. A. Brown, and F. I. Dromgoole. 1988. Salt tolerance of the coastal plant, *Tetragonia trigyna* Banks et Sol. Ex Hook. (climbing New Zealand spinach). *New Zealand Journal of Botany* 26: 153–162.

Wilson, C., S. M. Lesch, and C. M. Grieve. 2000. Growth stage modulates salinity tolerance of New Zealand spinach (*Tetragonia tetragonoides*, Pall.) and Red Orach (*Atriplex hortensis* L.). *Annals of Botany* 85: 501–509.

Winter, K., J. Troughton, M. Evenari, A. Laüchli, and U. Lüttge. 1976. Mineral ion composition and occurrence of CAM-like diurnal malate fluctuation in plants of coastal and desert habitats of Israel and the Sinai. *Oecologia* 25: 125–136.

Yeo, A. R., and T. J. Flowers. 1980. Salt tolerance in the halophyte *Suaeda maritima* L. Dum.: Evaluation of the effect of salinity upon growth. *Journal of Experimental Botany* 1: 71–1183.