Influence of salinity on Na^+ and K^+ accumulation, and gas exchange in *Avicennia germinans*

N. SUÁREZ* and E. MEDINA

Laboratorio de Ecofisiología Vegetal, Instituto Venezolano de Investigaciones Científicas, Aptdo. 21827, Caracas 1020A, Venezuela

Abstract

We analysed plant growth, ion accumulation, leaf water relations, and gas exchange of *Avicennia germinans* (L.) L. subjected to a long-term, controlled salinity gradient from 0 to 55 ‰. Growth and leaf area were affected by salinity higher than 10 ‰. As salinity increased, the predawn leaf water potential (Ψ_w) and leaf osmotic potential (Ψ_s) decreased. Leaf Ψ_w was at least –0.32 MPa lower than the Ψ_w of solution. Na^+ and K^+ ions explained about 78 % of decrease in Ψ_s. K^+ tissue water concentration decreased by more than 60 % in all salinity treatments as compared with those grown at 0 ‰. Inversely, Na^+ concentration in tissue water increased with nutrient solution salinity. The maximum net photosynthetic rate (P_N) and stomatal conductance (g_s) decreased by 68 and 82 %, respectively, as salinity increased from 0 to 55 ‰; the intercellular CO_2 concentration (C_i) followed the same trend. The P_N as a function of C_i showed that both the initial linear slope and upper plateau of the P_N *vs*. C_i curve were markedly affected by high salinity (40 and 55 ‰).

Additional key words: growth; leaf water relations; net photosynthetic rate; potassium; stomatal conductance.

Introduction

Salinity tolerance in mangrove species has been associated with a large investment in root biomass and high water use efficiency (Andrews and Muller 1985, Ball 1988, Clough and Sim 1989, Ball and Passioura 1995). Both these plant characteristics may represent a high carbon cost, reducing growth rate in the long term (Ball 1988, Clough and Sim 1989, Ball and Passioura 1995). Plants utilizing seawater require ion storage within the leaf cells and ion uptake regulation by the roots (Flowers *et al*. 1977, Clough *et al.* 1982, Rada *et al.* 1989). Mangroves are able to maintain their osmotic potential below the substratum water potential, necessary to maintain water uptake, while restraining the excess ion accumulation (Rada *et al.* 1989, Suárez *et al.* 1998, Suárez and Sobrado 2000). Changes in leaf water relations associated with ion accumulation within photosynthetically active tissue have considerable implications for the photosynthetic activity (Ball and Farquhar 1984, Seemann and Critchley 1985, Sobrado 1999); the decrease of photosynthetic rates with increasing salinity may be related to both stomatal closure, minimizing water loss, and to non-stomatal limitations associated with internal total ion accumulation (Ball and Farquhar 1984, Seemann and Critchley 1985, Sobrado 1999). Decreasing photosynthetic rates at high salinity may be due to K^+ deficiency rather than to the accumulation of ions in leaves up to toxic levels (Ball and Farquhar 1984, Ball *et al*. 1987).

Few studies have been carried out on mangrove species grown for long periods at constant salt concentrations above that of seawater. We analysed plant growth, ion accumulation, leaf water relations, and gas exchange of *Avicennia germinans*, a mangrove tree species with high salinity tolerance.

Received 11 April 2005, *accepted* 16 September 2005.
*Corresponding author; fax: +58 212 9063064 or 9063046, e-mail: natsuare@usb.ve
Present address: Departamento de Biología de Organismos, Universidad Simón Bolívar, Aptdo. 89000, Caracas 1080A, Venezuela.
Abbreviations: C_i – intercellular CO_2 concentration; d.m. − dry mass; g_s – stomatal conductance; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; R_D – respiration in darkness; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; V_{cmax} – maximum carboxylation rate; Φ_c – apparent quantum efficiency of CO_2 assimilation; Γ – CO_2 compensation point; Ψ_p – pressure potential; Ψ_s – leaf osmotic potential; Ψ_{sol} − solution osmotic potential; Ψ_w – leaf water potential at predawn.
Acknowledgements: To CONICIT for providing NS with a PhD fellowship. Financial support for this work was provided by IVIC and the International Institute of Tropical Forestry (USDA-Forest Service). To G. Aguiar for assistance with ion analyses. I am grateful to the editor, anonymous referees, and Prof. M.A. Sobrado for helpful suggestions to improve this manuscript.

Materials and methods

Plants: *Avicennia germinans* (L.) L. propagules were collected in August 1998 in Cumaná, Venezuela (10º27'N, 64º10'W). They were planted in pots filled with washed sand, and separated into 5 groups (20 plants per group). After 18 weeks, when cotyledons fell, raw marine salt (98 % NaCl) was added to the nutrient solution (40 % Hoagland solution) to obtain 5 treatments of increasing salinity. Marine salt was preferred to pure NaCl as the source of salinity as it simulates the effect of salinity under natural conditions. Salinity in nutrient solutions was measured with a refractometer and was increased at 1 ‰ per day until reaching salinities of 10, 25, 40, and 55 ‰. Plants grown at 0 ‰ salinity were used as controls. Twenty plants for each salinity treatment were grown in a greenhouse for 23 months under natural irradiance with a 12 h photoperiod. The saline solutions were constantly recycled through 0.2 m^3 containers by means of pumps and salt concentration was adjusted every 5 d. The maximum photosynthetic photon flux density (PPFD) was 1 550±370 μmol m^{-2} s^{-1}, air temperature averaged 30.5±3.4 ºC during the day and 16.3±2.0 ºC during the night, and the relative air humidity ranged between 50 and 78 % during the day.

Measurements: At seven (t_1) and twenty-three (t_2) months from the beginning of treatments, four to five plants per treatment were sampled. Each plant was separated into roots, stems, and leaves, and fresh mass and leaf area were determined. All samples were rinsed with distilled water, blotted with tissue paper, and dried in a ventilated oven for 72 h at 70 ºC to determine dry mass (d.m.). Plants growing at 55 ‰ salinity suffered from high mortality and after 23 months (at t_2) only two plants remained alive and were used for measurements. Dried samples were grounded and digested (1 h at 200 ºC) in a binary acid solution (80 % sulphuric acid and 20 % perchloric acid). Na^+ and K^+ concentrations were determined in digests by means of an atomic absorption spectrophotometer model *AA6* (*Varian Techtron*, Walnut Creek, CA, USA). K^+/Na^+ selectivity ratio ($S_{K,Na}$) of leaves was calculated according to Pitman (1965):

$$S_{K,Na} = (K^+_{leaf}/Na^+_{leaf})/(K^+_{solution}/Na^+_{solution})$$

At the end of the experiment the predawn water potential (Ψ_w) was determined (five plants per treatment) using a pressure chamber model *3000* (*Soilmoisture Equipment Corp.*, Santa Barbara, CA, USA). Thereafter, leaves were rinsed with distilled water and dried with tissue paper to eliminate surface salt. Leaf blades were placed in a syringe and frozen in liquid nitrogen. The frozen samples were thawed (1/2 h) at room temperature before sap extraction to determine leaf osmolality with a vapour pressure osmometer model *5500* (*Wescor*, Logan, UT, USA). Leaf osmotic potential (Ψ_s) and solution osmotic potential (Ψ_{sol}) were determined simultaneously.

Gas exchange was measured 8 and 11 months after the beginning of treatments on 15–30 fully expanded leaves from seven to ten plants per treatment, using an open system model *6400* (*Licor*, Lincoln, NE, USA). Chamber temperature was kept at 27 ºC and vapour pressure deficit ranged from 29 to 13 kPa; CO_2 concentration was 380 μmol mol^{-1}. The photosynthetic (P_N) response to irradiance was measured at quantum flux densities decreasing from 2 000 to 0 μmol m^{-2} s^{-1}. P_N was measured in a CO_2 range from 50 to 800 μmol mol^{-1}. Gas exchange parameters and intercellular CO_2 concentration of the leaf (C_i) were calculated as described by Caemmerer and Farquhar (1981).

Statistical analysis: The statistical significance of gas exchange, biomass, leaf water relations, and Na^+ and K^+ accumulation means among treatments were tested using one-way ANOVA (Sokal and Rohlf 1969). Least significant difference and Dunnet's T3 tests were performed *a posteriori* when homogeneous or non-homogeneous variance, respectively, was found in the data. A significance value of $p \leq 0.05$ was considered.

Results

Plant growth: Plant biomass and total leaf area of plants grown at salinities ≥10 ‰ were lower than those of control plants (Fig. 1). After 23 months from the beginning of treatments, total plant biomass decreased by 51, 87, and 95 % at salinities of 25, 40, and 55 ‰, respectively. Roots were always the larger compartment accounting for 36 to 52 % of the total plant mass (Fig. 1*A*); the root/shoot ratio was not modified by salinity and ranged between 0.6 and 1.1. Increases in stem and root biomass were observed in treatments up to 40 ‰. Plants did not grow at 55 ‰ and showed a 95 % mortality at the end of the experiment. Total leaf area was reduced in a pattern similar to that of total dry mass (Fig. 1*B*).

Leaf water relations and Na^+ and K^+ concentration: With increasing salinity, Ψ_w and Ψ_s decreased steeply. Ψ_w decreased from –0.41 at 0 ‰ to –3.28 MPa at 40 ‰, and Ψ_s showed the same trend (Table 1). In all cases, Ψ_s was lower than Ψ_{sol} and differences between plant and solution water potential ranged between –0.32 and –1.12 MPa (Table 1). Ψ_p remained positive in all salinity treatments; the lowest values were measured in plants cultivated at 40 ‰ (Table 1).

In all treatments K^+ concentration in tissue water was

higher than in the nutrient solution (Fig. 2*A*-*C*). K^+ concentration per unit of tissue water was significantly higher in control plants as compared to plants grown under varying salinity. Salinity treatments decreased K^+ concentration by more than 60 % (Fig. 2*A*–*C*). Inversely, Na^+ concentration in tissue water increased with the salinity of the nutrient solution, but the increase was not linear and showed substantial differences among tissues (Fig. 2*B*–*D*). Control plants accumulated Na^+ above the level of the nutrient solution, and this accumulation was markedly stimulated by the 10 and 25 ‰ treatments. In harvests t_1 and t_2, roots and leaves maintained similar ion concentrations, while a strong increase in Na^+ concentration in stems was observed at t_2 (Fig. 2*D*). The K^+/Na^+ ratio in leaves decreased with salinity and the calculated selectivity ratio ($S_{K,Na}$) indicated a preferential uptake of K^+ over Na^+ (Table 1).

Gas exchange: P_N and g_s decreased by 68 and 82 %, respectively, as salinity increased from 0 to 55 ‰ (Table 2), while C_i decreased from 244±20 at 0 ‰ to 188±39 μmol mol^{-1} at 55 ‰, as indicated by the C_i/C_a ratio (Table 2). The relation between maximum P_N and g_s, for all salinity treatments showed a significant positive correlation ($r^2 = 0.89$, $p \leq 0.05$; Table 2) indicating that intrinsic water use efficiency increases with salinity in correspondence with the lower C_i/C_a ratios. P_N as a function of PPFD did not show significant differences in Φ_c among treatments but tended to decrease with salinity (Table 2, Fig. 3*A*). Photon-saturated P_N differed significantly between plants grown at 0–25 ‰ and at 40–55 ‰ salinities (Fig. 3*A*). The CO_2-compensation concentration (Γ) showed a slight tendency to increase from 55 (0–25 ‰) up to 68 μmol m^{-2} s^{-1} (40–55 ‰) whereas R_D was significantly higher at the intermediate salinities (Table 2). P_N as a function of C_i showed that both the initial slope and upper plateau of the P_N *vs*. C_i curve were markedly affected at high salinity (40 and 55 ‰; Fig. 3*B*). The calculated maximum carboxylation rate (Vc_{max}, initial slope of the P_N *vs*. C_i curve) did not show significant differences in plants

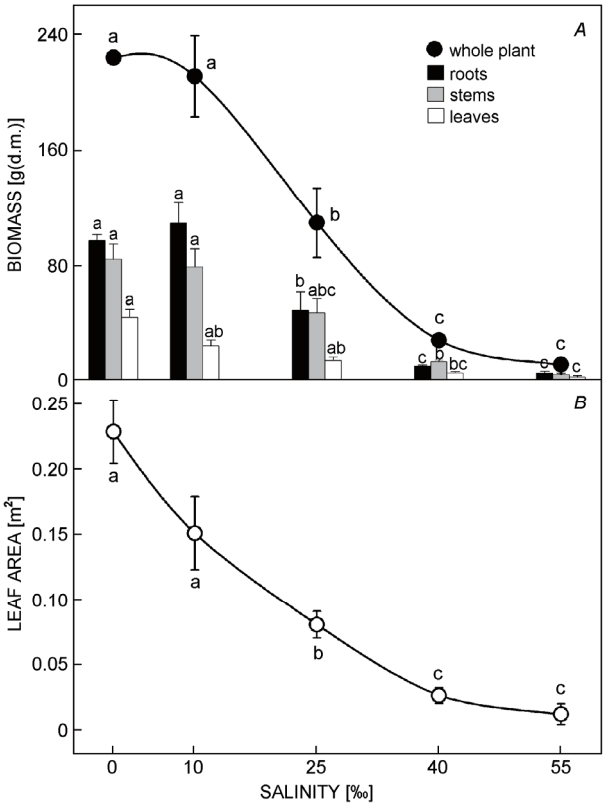

Fig. 1. Total plant biomass and biomass of roots, stems, and leaves (*A*), and total leaf area per plant (*B*) of *A. germinans* grown at 0, 10, 25, 40, and 55 ‰ salinity. The measurements were carried out at t_2 (23 months after the beginning of salt treatment). *Vertical lines* correspond to the standard error of the average; n = 4–5. *Different letters* indicate significant differences at a $p \leq 0.05$, between different salinity treatments.

Table 1. Predawn leaf water potential (Ψ_w), leaf osmotic potential (Ψ_s), pressure potential (Ψ_p), difference between plant and solution water potential ($\Delta\Psi_{plant\text{-}sol}$) measured at t_2 (month 23 after beginning of salinity treatment), Na^+ and K^+ concentration ratio in nutrient solution, Na^+ and K^+ contents in leaves, and selectivity ratios ($S_{K,Na}$) at the two harvest times (t_1 after 7 months and t_2 after 23 months) in leaves of *A. germinans* plants grown at salinities of 0, 10, 25, 40, and 55 ‰. Means ± SD, n = 4–5. At 55 ‰ for t_2 n = 2. *Different letters for the same harvest and for each variable* indicate statistical difference at $p \leq 0.05$.

		Salinity [‰] 0	10	25	40	55
Ψ_w [MPa]		–0.41 ± 0.09 a	–1.75 ± 0.09 b	–2.16 ± 0.18 c	–3.28 ± 0.50 d	-
Ψ_s [MPa]		–2.14 ± 0.27 a	–2.56 ± 0.06 b	–3.08 ± 0.17 c	–3.77 ± 0.35 d	-
Ψ_p [MPa]		1.73 ± 0.30 a	0.81 ± 0.08 b	0.92 ± 0.27 b	0.50 ± 0.42 b	-
$\Delta\Psi_{plant\text{-}sol}$		–0.38 ± 0.09 a	–1.12 ± 0.09 b	–0.52 ± 0.18 a	–0.32 ± 0.50 a	-
$K^+/Na^+_{solution}$		1.520	0.027	0.018	0.023	0.010
	Harvest					
K^+/Na^+_{leaf}	t_1	3.41 ± 1.62 a	0.21 ± 0.03 b	0.25 ± 0.06 b	0.20 ± 0.05 b	0.17 ± 0.05 b
	t_2	4.82 ± 2.65 a	0.20 ± 0.05 b	0.17 ± 0.04 b	0.16 ± 0.03 b	0.17
$S_{K,Na}$	t_1	2.24 ± 1.07a	7.83 ± 1.17 b	13.58 ± 3.05 b	8.56 ± 2.07 b	16.44 ± 4.66 b
	t_2	3.17 ± 1.75 a	7.28 ± 1.83 b	9.59 ± 1.97 b	6.87 ± 1.34 b	16.74

grown at 0–25 ‰ but decreased by 25 and 51 % in plants grown at 40 and 55 ‰ salinities, respectively (Table 2). Finally, in plants grown in the presence of salt, maximum P_N and calculated Vc_{max} were negatively correlated with leaf Na^+ concentration ($r^2 = 0.81$, $p \leq 0.05$).

Discussion

A marked decrease in plant growth and leaf area was observed under treatments with salinity higher than 10 ‰. Several mangrove tree species reach optimum growth at salinity between 2 and 10 ‰ (Downton 1982, Clough 1984, Ball 1988, Burchett *et al*. 1989, Suárez and Medina 2005). Reduction of plant growth at high salinity may be a consequence of limited plant capacity to increase leaf area (Suárez and Medina 2005). In this study the root/shoot ratio was not significantly modified by salinity and averaged 0.82±0.28, while in previous studies with mangrove seedlings this ratio tended to increase with salinity and was not higher than 0.5 (Downton 1982, Ball 1988). High root/shoot ratios may be related with higher water use efficiency, which contributes to maintain low rates of ion uptake and an adequate water balance (Ball 1988).

In *A. germinans,* the absence of salt did not inhibit plant growth. This result contrasts with reports on other mangrove species (Downton 1982, Clough 1984, Burchett *et al.* 1989, Werner and Stelzer 1990). However, Sobrado (1999) found that plants growing without added NaCl were healthy and did not show leaf tissue damage

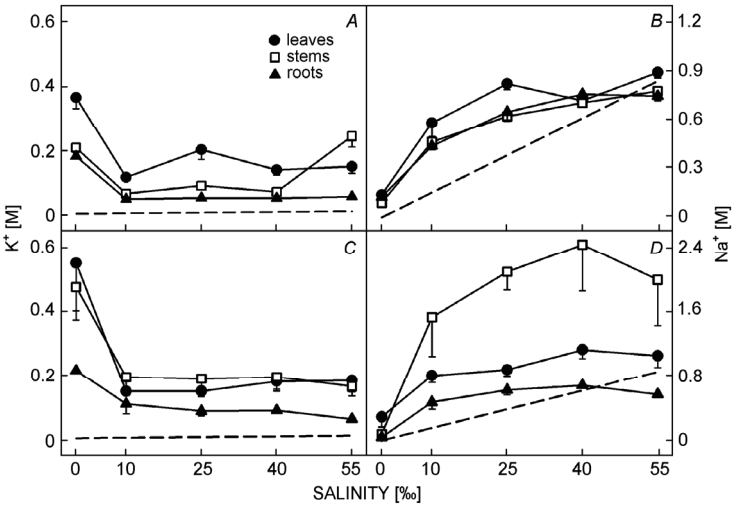

Fig. 2. K^+ and Na^+ concentrations per unit of tissue water in leaves, stems, and roots of *A. germinans* grown at 0, 10, 25, 40, and 55 ‰ salinity. *A* and *B*: harvests at t_1, 7 months after the beginning of treatments. *C* and *D*: harvests at t_2, 23 months after the beginning of treatments. *Dashed lines* show nutrient solution contents. *Vertical lines* correspond to the standard error of the average, $n = 4–5$.

Table 2. Maximum net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular to ambient CO_2 concentration ratio (C_i/C_a), P_N/g_s ratio, apparent quantum efficiency of CO_2 assimilation (Φ_c), respiration rate in the dark (R_D), maximum carboxylation rate (Vc_{max}), and CO_2 compensation concentration (Γ) in fully expanded leaves of *A. germinans* plants subjected to different salinity concentration treatments. The measurements were carried out between 8 and 11 months after beginning of treatments, at ambient CO_2 concentration of 380 μmol mol^{-1} and irradiance of 2 000 μmol m^{-2} s^{-1}. Means ± SD, n = 5−10 for Φ_c, R_d, V_{cmax}; n = 17–25 for maximum P_N, g_s, and C_i/C_a. *Different letters in the same row* indicate that the mean values for the variable were statistically different at $p \leq 0.05$.

	Salinity [‰] 0	10	25	40	55
P_N [μmol m^{-2} s^{-1}]	14.9 ± 2.6 a	14.8 ± 3.1 a	13.8 ± 3.0 a	7.9 ± 3.4 b	4.8 ± 1.7 c
g_s [μmol m^{-2} s^{-1}]	0.266 ± 0.096 a	0.332 ± 0.103 a	0.236 ± 0.099 a	0.112 ± 0.063 b	0.049 ± 0.022 c
C_i/C_a [μmol mol^{-1}]	0.641 ± 0.052 a	0.693 ± 0.040 b	0.617 ± 0.064 a	0.597 ± 0.091 a	0.496 ± 0.102 c
P_N/g_s [mol(CO_2) mol^{-1}(H_2O)]	0.061 ± 0.015 a	0.047 ± 0.010 b	0.065 ± 0.017 ac	0.078 ± 0.022 c	0.107 ± 0.025 d
Φ_c [μmol μmol^{-1}]	0.070 ± 0.08 a	0.062 ± 0.07 a	0.061 ± 0.02 a	0.058 ± 0.013 a	0.058 ± 0.006 a
R_d [μmol m^{-2} s^{-1}]	–1.47 ± 0.21 a	–1.48 ± 0.16 a	–2.22 ± 0.24 b	–1.77 ± 0.16 c	–1.44 ± 0.022 a
V_{cmax} [μmol μmol^{-1}]	69.95 ± 8.51 a	64.57 ± 14.10 a	67.56 ± 6.08 a	48.49 ± 12.44 b	33.04 ± 9.98 c
Γ [μmol m^{-2} s^{-1}]	51.6 ± 6.4 a	57.5 ± 5.0 a	56.6 ± 3.7 a	67.3 ± 12.5 b	67.7 ± 3.1 b

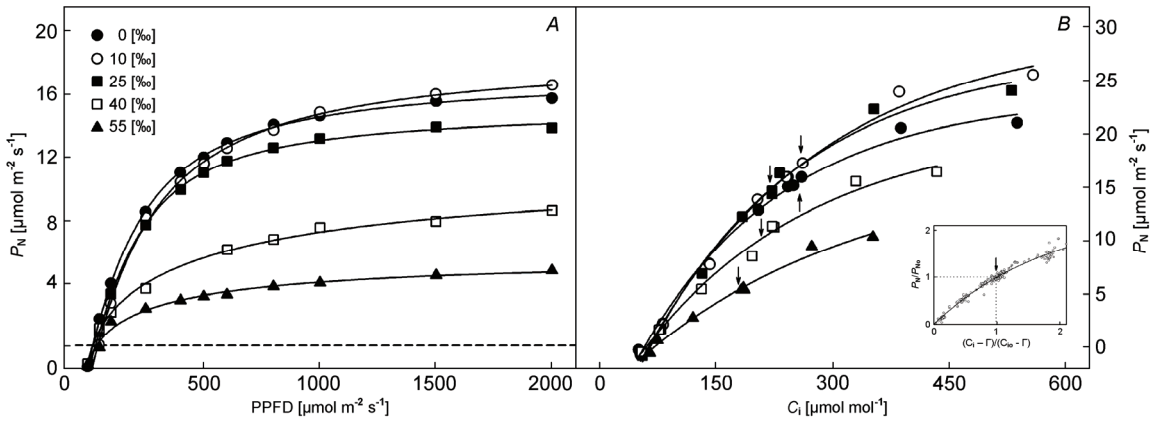

Fig. 3. Net photosynthetic rate (P_N) as a function of *A*: photosynthetic photon flux density (PPFD) and *B*: intercellular CO_2 concentration (C_i) in leaves of *A. germinans* cultivated at 0, 10, 25, 40, and 55 ‰ salinity. The measurements were carried out at ambient CO_2 concentration of 380 μmol mol^{-1} in *A*, and at irradiation of 2 000 μmol m^{-2} s^{-1} in *B*. Each curve shown represents 7–10 curves per salinity treatment. Lines were fitted by non-rectangular hyperbolae in *A* and by non-linear regression in *B*, following Caemmerer and Farquhar (1981). The fittings were statistically different at $p \leq 0.05$. The arrows indicate the operational C_i for each curve. *The insert* shows the normalization of the P_N *vs*. C_i curves for the salinity treatments considered, to underline the position of operational points (calculation following Ball and Farquhar 1984).

suggesting leaf dysfunction. Thus, *A. germinans* took up effectively other ions from the nutrient solution, developing a negative leaf sap osmotic potential (Ψ_s).In fact, Ψ_s at 0 ‰ was –2.14±0.27 MPa (Table 1). A high K^+ uptake and probably the accumulation of sugars and synthesis of organic ions play important roles in the capacity of *A. germinans* to maintain a positive pressure potential and long-term growth in plants cultivated without added NaCl.

A. germinans was able to adjust its leaf Ψ_s below the Ψ_{sol} (Table 1) through ion uptake. Na^+ and K^+ explained about 78 % of Ψ_s, in all treatments, a value similar to the 75 % contribution of Na^+ to Ψ_s found by Medina and Francisco (1997) in the same species under natural conditions. However, nutrient uptake alone was not enough to explain the reduction in osmotic potential. The accumulation of organic osmolytes can not be ruled out. Osmotic adjustment generates a gradient of water potential between the plant and the substratum, necessary to maintain water uptake and turgor pressure (Clough *et al*. 1982, Downton 1982, Clough 1984, Rada *et al*. 1989, Suárez *et al*. 1998, Suárez and Sobrado 2000). The reduction of Ψ_w by salinity was within the range found for different mangrove species growing under similar salt concentrations in the field (Downton 1982, Clough 1984, Naidoo 1987, Rada *et al.* 1989, Suárez *et al.* 1998, Suárez and Sobrado 2000). At high salinity, small decreases of leaf Ψ_w during the day might result in turgor loss, unless water loss is effectively regulated. In fact, midday turgor loss has been found in mangrove species under natural conditions (Rada *et al.* 1989, Suárez and Sobrado 2000).

In general, as salinity increased, Na^+ concentration remained similar in all tissues, suggesting a lower capacity for ion accumulation in the tissues, or an effective regulation of further Na^+ uptake under high salinity (Fig. 2). At harvest t_2 (Fig. 2*D*) Na^+ concentration in stem tissue increased noticeably, compared with harvest t_1 (Fig. 2*B*). While Na^+ concentration in stem per dry mass unit increased by about 63–138 %, the water content per dry mass decreased by about 32–52 % at harvest t_2 as compared with harvest t_1. Consequently, the lower water content per dry mass was not sufficient to compensate for the increase in ion accumulation, and stem Na^+ concentration increased at harvest t_2. As the stem compartment increases in size, there is a tendency for salt accumulation in this tissue, preventing further transport from the roots to the leaves. Similarly, Joshi *et al*. (1975) reported relatively high salt contents in the stem and bark of some mangrove species. The location of this ion in the stem tissue and its accumulation mechanism deserve further research.

$S_{K,Na}$ tended to increase with salinity. In spite of the reduction in leaf K^+ contents induced by salinity treatments, it remained considerably higher than the K^+ concentration of the nutrient solution (Fig. 2*A*,*C*). In addition, increases in tissue Na^+ content were much smaller than the increases in the nutrient solution, as a result of ion uptake regulation. Similar observations had been reported for other mangrove species (Downton 1982, Clough 1984, Ball *et al*. 1987, Naidoo *et al*. 2002, Paliyavuth *et al*. 2004). The maintenance of a high $S_{K,Na}$ in leaves is a significant factor in salt tolerance, maintaining adequate concentrations of K^+ in chloroplasts, and assuring the photosynthetic requirements of K^+ (Ball and Farquhar 1984, Ball *et al.* 1987, Chow *et al*. 1990).

Changes in leaf water relations and internal ion concentrations associated with salinity may influence photosynthetic response (Ball and Farquhar 1984). Maximum P_N values were not significantly different between 0 and 25 ‰ treatments, but they decreased rapidly at higher

salinities. Pezeshki *et al.* (1990) also found that in *A. germinans* a salinity increase from 0 to 20 ‰ did not cause significant changes in the assimilation rate. The values reported here for maximum P_N, g_s, and C_i are similar to those reported for species of *Avicennia* grown under different salinities (Ball and Farquhar 1984, Naidoo 1987, Ball 1988, Clough and Sim 1989, Sobrado 1999). Thus, in *A. germinans* growth reduction associated with a long-term salinity stress is likely to be a consequence of the combined effects of salt on P_N and the reduction in leaf expansion rates (Ball and Farquhar 1984, Ball 1988, Burchett *et al*. 1989).

The decline in C_i/C_a and the increase in P_N/g_s are both indications of the increase in water use efficiency induced by the salinity treatments (Wong *et al.* 1979, Hall and Schulze 1980, Clough and Sim 1989, Sobrado 1999, Cheeseman and Lovelock 2004). Reductions of photon-saturated P_N in the 40 and 55 ‰ treatments suggest a lower capacity of the biochemical reactions responsible for CO_2 fixation at very high salinity (Fig. 3*A*). However, Φ_c remained relatively constant in all treatments indicating that the photochemical efficiency of photosystem 2 in mangrove leaves is unaffected by high salinity (Björkman *et al*. 1988, Sobrado and Ball 1999). R_D was maximal at 25 ‰ and decreased in control plants and at high salinities (Table 2). Similarly, in *A. marina* R_D decreased in 100 % sea water as compared with plants grown in tap water, while the inverse tendency was observed in *Aegiceras corniculatum* (Burchett *et al*. 1989). The CO_2-compensation concentration (Γ) showed a slight tendency to increase with salinity, suggesting that carbon loss *via* photorespiration is enhanced at high salinity. The Γ values reported here are similar to those reported in other mangroves species (Ball and Farquhar 1984, Naidoo and Willert 1999). Higher photorespiration rates at high salinity may be important in photochemical dissipation of absorbed photon energy in mangrove species (Sobrado and Ball 1999).

In *A. germinans*, the operational C_i (C_{io}) remained near the region of transition between the lower linear and upper plateau portion of the P_N *vs*. C_i curve (Fig. 3*B*), suggesting again that stomatal and biochemical factors co-limited the maximum P_N with salinity (Ball and Farquhar 1984). These results are in agreement with those for other mangroves species (Ball and Farquhar 1984, Naidoo and Willert 1999). We attempted to discriminate diffusive and biochemical effects of salinity on P_N by analyzing the P_N *vs*. C_i curve (Jones 1985). If stomatal conductance had not been affected by salinity (g_s of plants cultivated at 55 and 0 ‰ were identical), the C_i of plants growing at 55 ‰ would have increased from 196 to 283 μmol mol^{-1}, but the photosynthetic rate would have increased only from 6.4 to 9.9 μmol m^{-2} s^{-1}; that is by 35 %. This means that stomatal limitation is relatively smaller than the reduction in intrinsic biochemical capacity caused by salinity.

The decrease in the initial slope of the P_N *vs*. C_i curve with salinity suggests that the Vc_{max} was lower at high salinity (Fig. 3*B*) and was assumed to be a function of both the amount and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), while the reduction of P_N at high C_i was a function of the capacity for regeneration of ribulose-1,5-bisphosphate (RuBP; Caemmerer and Farquhar 1981). The reduction in the initial slope of the P_N *vs*. C_i curve may be a consequence of inactivation in RuBPCO, possibly in response to a decrease of the RuBP regeneration rate as salinity increases and/or in response to stomatal closure associated with development of water stress (Ball and Farquhar 1984, Seemann and Critchley 1985, Cheeseman and Lovelock 2004). Additionally, decrease in the initial slope of the P_N *vs*. C_i curve may also be a consequence of an increased photorespiration (Naidoo and Willert 1999).

Reduced photosynthetic capacity at high salinity may have been a consequence of K^+ deficiency induced by salinity rather than by NaCl toxicity in the leaves (Ball and Farquhar 1984, Ball *et al.* 1987). However, in our study maximum P_N and Vc_{max} values were negatively correlated with Na^+ and not with K^+ content in leaves. Additionally, the deficiency of K^+ in leaves induced the depletion of atrazine-binding sites in PS2 complexes, which in intact leaves would be expressed as a decline in Φ_c. Contrarily, in *A. germinans* Φ_c remained relatively constant with salinity, despite of K^+ concentrations ranging from 115 to 548 mM (Fig. 2*A*,*C*).

Thus, ion accumulation in leaves affects gas exchange properties in *A. germinans* in a direct manner due to toxicity and indirectly by their effect on stomatal closure. The physiological and biochemical origins of the observed reductions in photosynthetic capacity and Vc_{max} require knowledge of the specific localization of ions within the plant cells (Seemann and Critchley 1985). In halophytes, NaCl concentration was relatively low in the cytoplasm and direct inhibition of RuBPCO was not likely (Flowers *et al*. 1977). However, we found that the effect of salinity on P_N was determined over a larger range than that at which the cytoplasm concentration of ions has been measured (10–100 % of seawater). Then, at 40 and 55 ‰ salinities the observed reduction in photosynthetic rate may be a consequence of unsuccessful compartmentation or of other processes associated with source-sink relationships inhibiting some carbon metabolism reactions.

References

Andrews, T.J., Muller, G.J.: Photosynthetic gas exchange of the mangrove, *Rhizophora stylosa* Griff., in its natural environment. – Oecologia **65**: 449-455, 1985.

Ball, M.C.: Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina*. I. Water use in relation to growth, carbon partitioning, and salt balance. – Aust. J. Plant Physiol. **15**: 447-464, 1988.

Ball, M.C., Chow, W.S., Anderson, J.M.: Salinity-induced potassium deficiency causes loss of functional photosystem II in leaves of the grey mangrove, *Avicennia marina*, through depletion of the atrazine-binding polypeptide. – Aust. J. Plant Physiol. **14**: 351-361, 1987.

Ball, M.C., Farquhar, G.D.: Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina*, to long term salinity and humidity conditions. – Plant Physiol. **74**: 1-6, 1984.

Ball, M.C., Passioura, J.B.: Carbon gain in relation to water use: photosynthesis in mangroves. – In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 247-259. Springer-Verlag, Berlin – Heidelberg – New York 1995.

Björkman, O., Demmig, B., Andrews, T.J.: Mangrove photosynthesis: response to high-irradiance stress. – Aust. J. Plant Physiol. **15**: 43-61, 1988.

Burchett, M.D., Clarke, C.J., Field, C.D., Pulkownik, A.: Growth and respiration in two mangrove species at a range of salinities. – Physiol. Plant. **75**: 299-303, 1989.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. – Planta **153**: 376-387, 1981.

Creeseman, M., Lovelock, C.E.: Photosynthetic characteristics of dwarf and fringe *Rhizophora mangle* L. in a Belizean mangrove. – Plant Cell Environ. **27**: 769-780, 2004.

Chow, W.S., Ball, M.C., Anderson, J.M.: Growth and photosynthetic responses of spinach to salinity: implications of K^+ nutrition for salt tolerance. – Aust. J. Plant Physiol. **17**: 563-578, 1990.

Clough, B.F.: Growth and salt balance of the mangroves *Avicennia marina* (Forsk.) Vierh. and *Rhizophora stylosa* Griff. in relation to salinity. – Aust. J. Plant Physiol. **11**: 419-430, 1984.

Clough, B.F., Andrews, T.J., Cowan, I.R.: Physiological processes in mangroves. – In: Clough, B.F. (ed.): Mangrove Ecosystems in Australia: Structure, Function and Management. Pp. 193-210. Australian National University Press, Canberra 1982.

Clough, B.F., Sim, R.G.: Changes in gas exchange characteristics and water use efficiency of mangroves in response to salinity and vapour pressure deficit. – Oecologia **79**: 38-44, 1989.

Downton, W.J.S.: Growth and osmotic relations of the mangrove *Avicennia marina*, as influenced by salinity. – Aust. J. Plant Physiol. **9**: 519-528, 1982.

Flowers, T.J., Troke, P.F., Yeo, A.R.: The mechanism of salt tolerance in halophytes. – Annu. Rev. Plant Physiol. **28**: 89-121, 1977.

Hall, A.E., Schulze, E.-D.: Stomatal response to environment and possible interrelation between stomatal effects on transpiration and CO_2 assimilation. – Plant Cell Environ. **3**: 467-474, 1980.

Jones, H.G.: Partitioning stomatal and non-stomatal limitations to photosynthesis. – Plant Cell Environ. **8**: 95-104, 1985.

Joshi, G.V., Jamale, B.B., Bosale, L.: Ion regulation in mangroves. – In: Walsh, G., Snedaker, S., Teas, H. (ed.): Proceedings of the International Symposium on the Biology and Management of Mangroves. Pp. 8-11. University of Florida, Gainesville 1975.

Medina, E., Francisco, M.: Osmolality and $\delta^{13}C$ of leaf tissues of mangrove species from environments of contrasting rainfall and salinity. – Estuar. coast. Shelf Sci. **45**: 337-344, 1997.

Naidoo, G.: Effects of salinity and nitrogen on growth and water relations in the mangrove, *Avicennia marina* (Forsk.) Vierh. – New Phytol. **107**: 317-325, 1987.

Naidoo, G., Tuffers, A.V., Willert, D.J. von: Changes in gas exchange and chlorophyll fluorescence characteristics of two mangroves and a mangrove associate in response to salinity in the natural environment. – Trees **16**: 140-146, 2002.

Naidoo, G., Willert, D.J. von: Gas exchange characteristics of the tropical mangrove, *Pelliciera rhizophoreae.* – Mangr. Salt Marsh. **3**: 147-153, 1999.

Pezeshki, S.R., DeLaune, R.D., Patrick, W.H., Jr.: Differential response of selected mangroves to soil flooding and salinity: gas exchange and biomass partitioning. – Can. J. Forest Res. **20**: 869-874, 1990.

Pitman, M.G.: Sodium and potassium uptake by seedlings of *Hordeum vulgare*. – Aust. J. biol. Sci **18**: 10-24, 1965.

Rada, F., Goldstein, G., Orozco, A., Montilla, M., Zabala, O., Azocar, A.: Osmotic and turgor relations of three mangrove ecosystem species. – Aust. J. Plant Physiol. **16**: 477-486, 1989.

Paliyavuth, C., Clough, B., Patanaponpaiboon, P.: Salt uptake and shoot water relations in mangroves. – Aquat. Bot. **78**: 349-360, 2004.

Seemann, J.R., Critchley, C.: Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. – Planta **164**: 151-162, 1985.

Sobrado, M.A.: Leaf photosynthesis of the mangrove *Avicennia germinans* as affected by NaCl. – Photosynthetica **36**: 547-555, 1999.

Sobrado, M.A., Ball, M.C.: Light use in relation to carbon gain in the mangrove, *Avicennia marina*, under hypersaline conditions. – Aust. J. Plant Physiol. **26**: 245-251, 1999.

Sokal, R.R., Rohlf, F.J.: Biometry. – W.H. Freeman, San Francisco 1969.

Suárez, N., Medina, E.: Salinity effect on plant growth and leaf demography of the mangrove, *Avicennia germinans* L. – Trees **19**: 728-734, 2005.

Suárez, N., Sobrado, M.A.: Adjustment in leaf water relations of mangrove (*Avicennia germinans*) seedlings grown in a salinity gradient. – Tree Physiol. **20**: 277-282, 2000.

Suárez, N., Sobrado, M.A., Medina, E.: Salinity effects on the leaf water relations components and ion accumulation patterns in *Avicennia germinans* (L.) L. seedlings. – Oecologia **114**: 299-304, 1998.

Werner, A., Stelzer, R.: Physiological responses of the mangrove *Rhizophora mangle* grown in the absence and presence of NaCl. – Plant Cell Environ. **13**: 243-255, 1990.

Wong, S.C., Cowan, I.R., Farquhar, G.D.: Stomatal conductance correlates with photosynthetic capacity. – Nature **282**: 424-426, 1979.