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Leaf photosynthesis of the mangrove *Avicennia germinans* as affected by NaCl

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Abstract

In leaves of the mangrove species *Avicennia germinans* (L.) L. grown in salinities from 0 to 40 ‰, fluorescence, gas exchange, and $\delta^{13}C$ analyses were done. Predawn values of F_v/F_m were about 0.75 in all the treatments suggesting that leaves did not suffer chronic photoinhibition. Conversely, midday F_v/F_m values decreased to about 0.55-0.60 which indicated strong down-regulation of photosynthesis in all treatments. Maximum photosynthetic rate (P_{max}) was 14.58 ± 0.22 μmol m^{-2} s^{-1} at 0 ‰; it decreased by 21 and 37 % in plants at salinities of 10 and 40 ‰, respectively. Stomatal conductance (g_s) was profoundly responsive in comparison to P_{max} which resulted in a high water use efficiency. This was further confirmed by $\delta^{13}C$ values, which increased with salinity. From day 3, after salt was removed from the soil solution, P_{max} and g_s increased up to 13 and 30 %, respectively. However, the values were still considerably lower than those measured in plants grown without salt addition.

Additional key words: $\delta^{13}C$; carbon discrimination; down-regulation; fluorescence; intercellular CO_2 concentration; photoinhibition; salinity; stomatal conductance; succulence; water use efficiency.

Introduction

Salt tolerance of *Avicennia* species and other halophytes seems to be in concordance with the avoidance of excessive NaCl concentration from compartments of active metabolism (Greenway and Munns 1980, Ball 1996). Indeed, salt entering the leaf is excreted by salt glands (Scholander *et al.* 1962, Waisel *et al.* 1986, Ball 1988), accumulated inside the vacuole (Popp *et al.* 1984), sequestered in hypodermal cells (Tomlinson 1986), and possibly retranslocated through the phloem (Clough *et al.* 1982). Additionally, regulation of water loss could considerably influence the salt

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amount reaching the leaf under high salinity (Ball and Farquhar 1984). Nevertheless, carbon gain would be the ultimate determinant of plant growing capacity and would limit their salinity tolerance.

In natural conditions, *Avicennia germinans* (L.) L. tolerates a range of salinity, from almost freshwater to up three times seawater (Mallery and Teas 1984, Tomlinson 1986). However, its photosynthesis is lowered when salt concentration in the soil increases (Smith *et al.* 1989, Azócar *et al.* 1992, Sobrado 1999). *A. germinans* responses in the field are nevertheless modulated by environmental constraints changing in space and time (Sobrado 1999). Therefore, this study was undertaken to assess the photosynthetic responses of *A. germinans* to different NaCl concentrations under controlled conditions.

Materials and methods

Plants: Seedlings of *A. germinans* (L.) L. were planted in pots with sand and nutrient solution in a glasshouse under natural sunlight and photoperiod (12 h). Temperature ranged from 25 to 35 °C during the day and 15 to 20 °C at night. Pots were placed on trays 7 cm high in order to maintain a constant supply without being waterlogged. NaCl was dissolved in 50 % Hoagland solution. Twelve plants per treatment were grown at salinities of 0, 10, 20, 30, and 40 ‰ for 4 months. After all measurements for each salinity were completed, plants were removed from salinity by washing the sand repeatedly, and afterwards each pot was watered with a 100 % Hoagland solution. Leaves for measurements were young, fully expanded, and selected from the upper part of the plant canopy where irradiance was maximal. Five replicate plants per treatment were used for fluorescence and all the destructive measurements. Gas exchange measurements were taken in 15-20 leaves from the 12 replicate plants per treatment.

Leaf characteristics: Leaf areas were determined immediately after collection. In ground and homogenised oven-dried (80 °C) leaves, nitrogen content was determined by micro-Kjeldahl method. Chlorophyll (Chl) ($a+b$) was extracted with 80 % acetone and spectroscopic determinations using the equations given by Lichtenthaler and Wellburn (1983) were performed. For light microscopy, leaf sections following ethanol dehydration were embedded in *Araldite*, cut to 1 μm using an ultramicrotome, and stained with toluidine blue.

Chl *a* fluorescence was measured with a portable Chlorophyll Fluorescence System model *CF 1000* (*Morgan Scientific*, USA). Measurements were done in the same leaves at predawn and at midday. The parameters obtained were initial fluorescence (F_0), variable fluorescence (F_v), and variable to maximum fluorescence (F_v/F_m) ratio. Standard conditions for measurements were 30 min dark adaptation and 3 min irradiance of 1000 μmol m^{-2} s^{-1}.

Gas exchange was measured on 15 to 20 leaves per salinity treatment using a portable gas analyser model *LCA-2* (*Analytical Development Co.*, UK). Plants were measured in pots where they were grown, and also for 10 successive days following salt

removal. This permitted to detect the degree of recovery in gas exchange. During the measurements the irradiance was greater than 1900 μmol m^{-2} s^{-1}, leaf temperature about 30 °C, relative humidity 85 %, and ambient CO_2 concentration 350 μmol mol^{-1}. The parameters determined were P_{max}, g_s, and intercellular CO_2 concentration (C_i) following Caemmerer and Farquhar (1981).

Carbon isotope analyses ($\delta^{13}C$) were performed in leaf samples taken from each plant. The analyses were done in the Stable Isotope Research Facility for Ecological Research of the University of Utah (Salt Lake City, USA) by using an isotope ratio mass spectrometer (model *Dèlta S, Finnigan MAT*, San Jose, CA, USA). For detailed description of the procedures see Ehleringer *et al.* (1992). Trends of $\delta^{13}C$ were used to estimate long-term water use efficiency (WUE).

Statistics: Parameters obtained in the experiments were compared by using a one-way Anova; afterwards, the least significant differences (LSD) at $p<0.05$ were determined.

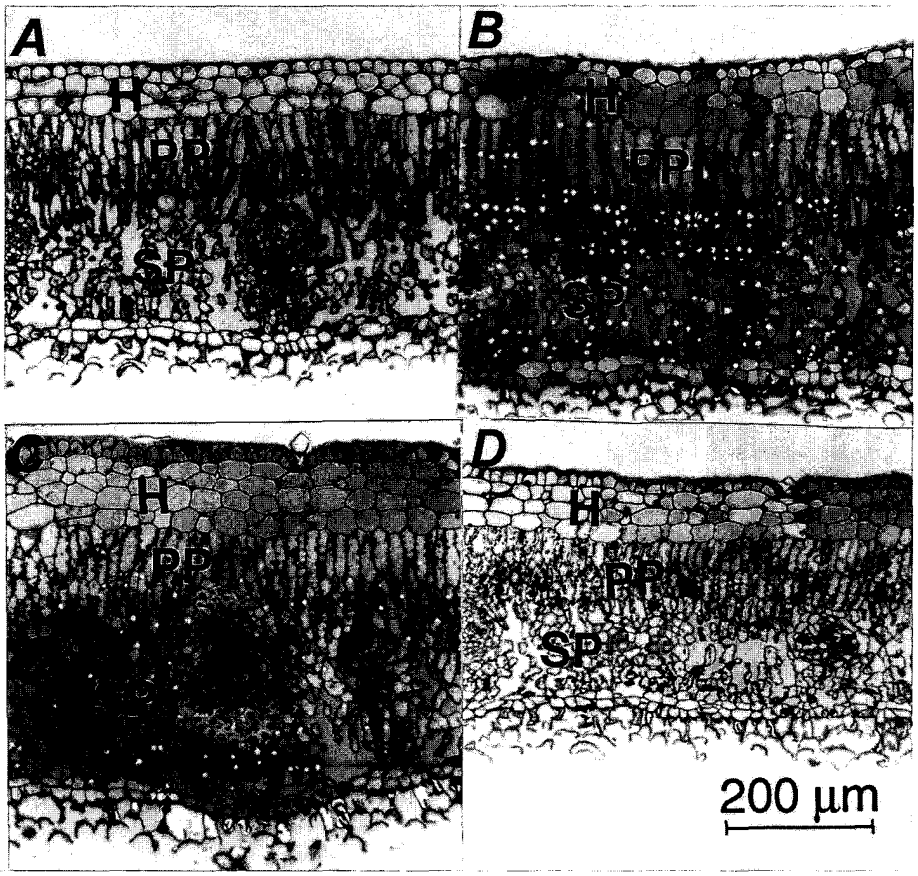

Fig. 1. Typical leaf cross sections from plants of *A. germinans* grown at salinities of 0 (*A*), 10 (*B*), 30 (*C*), and 40 ‰ (*D*). H, hypodermis; PP, palisade parenchyma; SP, spongy parenchyma. Bar indicates the scale for all treatments.

Results and discussion

Leaf characteristics: Leaf cross sections observed under light microscope showed that 10 to 30 ‰ salinity induced increased succulence in comparison to plants grown at 0 ‰ (Fig. 1). Leaf water contents [g m^{-2}] under salinity were: 274 ± 7 (10 ‰), 306 ± 2 (20 ‰), and 303 ± 4 (30 ‰); in control plants 232 ± 6 (0 ‰). Changes included considerable enlargement of the hypodermis (cell size and layer number) and thicker palisade parenchyma. Increasing succulence is an additional salt regulation mode inside leaf tissue, achieved by salt sequestration into hypodermal tissue (Werner and Stelzer 1990). In contrast, at 40 ‰ salinity the leaves contained 255 ± 3 g(water) m^{-2} and more tightly packed cells (Fig. 1*D*). Severe osmotic stress may limit cell enlargement and division, required to maintain such storage tissue. *A. germinans* growing under extreme environments in the field has a less succulent leaf tissue (Smith *et al.* 1989, Suárez *et al.* 1998). A gradual but significant decrease in leaf area/dry mass ratio as well as increase in nitrogen content was observed with increasing salinity (Fig. 2).

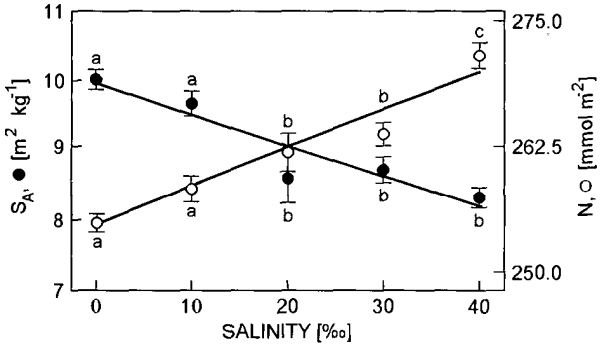

Fig. 2. Leaf area to dry mass ratios (S_A, ●) and nitrogen concentration (N, ○) as a function of salinity in leaves of *A. germinans*. Values are means ± SE. Different letters on top of symbols indicate statistically different means at $p<0.05$.

Chl (*a*+*b*) contant [μmol m^{-2}] increased slightly with salinity from 708 ± 16 (0 ‰) to 740 ± 13 (40 ‰). However, differences among treatments were not statistically significant. The Chl (*a*+*b*) and N concentrations were higher than those found in the field where nutritional status is low (Sobrado 1999). The Chl (*a*+*b*) to N ratios were about 2.75 mol m^{-2}, and those of Chl *a*/*b* were about 3.12. In this experiment, leaves from control plants grown without NaCl additions did not show any symptoms of necrosis, chlorosis, or leaf tissue damage indicating leaf dysfunction. This is contrary to results for other mangrove species that show anomalous leaf development or poor growth in fresh water (Downton 1982, Ball and Pidsley 1995).

Fluorescence of Chl *a*: Variable fluorescence (F_v) decreased from predawn to midday in all treatments (Fig. 3*A*). However, initial fluorescence (F_0) remained about the same with treatment and time of the day. In consequence, F_v/F_m ratios measured

at predawn were about 0.75, which is normal for a leaf without symptoms of chronic photoinhibition. Conversely, midday F_v/F_m decreased to 0.55-0.60, which suggested strong down-regulation of photosynthesis in all treatments. Midday down-regulation may be related to the xanthophyll cycle through de-epoxidation of violaxanthin to zeaxanthin (Demmig-Adams and Adams 1992). This mechanism is photoprotective and allows excess energy to be dissipated harmlessly in the pigment bed. At high irradiance and salinity, conversion of violaxanthin to zeaxanthin has been observed in other mangrove species (Lovelock and Clough 1992, Sobrado and Ball 1999). The fluorescence parameters did not show different response for control and salt-treated plants. This is in accordance with studies in several species (Larcher *et al.* 1990, Brugnoli and Björkman 1992, Sobrado 1999, Sobrado and Ball 1999), where no enhancement of down-regulation with salinity has been observed.

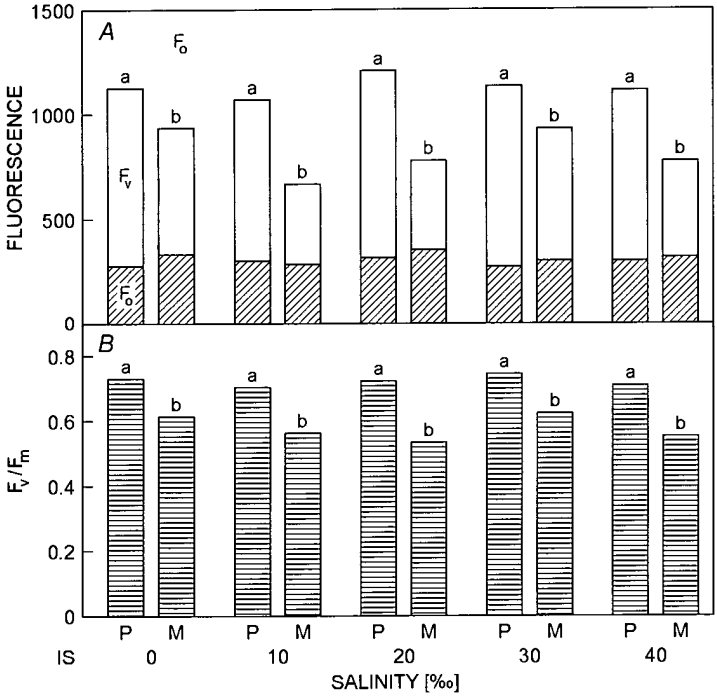

Fig. 3. Fluorescence of chlorophyll *a* parameters as a function of salinity measured in *Avicennia germinans*, at predawn (P) and again at midday (M). The parameters were: (*A*) initial (F_0) and variable (F_v) fluorescence, and (*B*) variable to maximum fluorescence ratio (F_v/F_m). Values are means. Different letters on top of each column indicate statistically different means at $p<0.05$.

Gas exchange characteristics and carbon isotopic composition: Maximum photosynthetic rates (P_{max}) were strongly reduced by salinity, starting from 14.58 ± 0.22 μmol m^{-2} s^{-1} at 0 ‰. Thus, 21 and 37 % reductions were observed at 10 and 40 ‰, respectively, in comparison to control plants (Fig. 4*A*). Therefore, per unit of salinity increase, the P_{max} decrease between 0 (control) to 10 ‰ was the largest, and further

salinity increase (>10 ‰) led to more gradual decline in P_{max}. This decrease was not related to decline in pigment and/or nitrogen composition (Fig. 2), nor to differential down-regulation (Fig. 3). In a number of species, P_{max} is adversely affected by salinity. This is partially due to low diffusion caused by stomatal closure and subsequent reduction in C_i, and also due to non-stomatal limitation of photosynthesis (Ball and Farquhar 1984, Seemann and Critchley 1985, Seemann and Sharkey 1986, Brugnoli and Björkman 1992). Here, the relative reduction in g_s exceeded that of P_{max} (Fig. 4*A,B*). The extent to which stomatal closure affects photosynthetic capacity is indicated by the magnitude of reduction of C_i (Fig. 4*C*) and concomitant increase in the P_{max}/g_s ratio (Fig. 5*A*). Thus, photosynthetic WUE was improved by salinity that was further confirmed by using carbon isotope analysis ($\delta^{13}C$; Fig. 5*B*). The leaf $\delta^{13}C$ increased with salinity in sigmoidal fashion from *ca.* –28.3 ‰ in control plants to –26.8 ‰ at salinity of 40 ‰. Lowered leaf $\delta^{13}C$ is consistent with higher WUE in many plant species (Farquhar *et al.* 1982, 1989). Thus, the tendency of $\delta^{13}C$ was

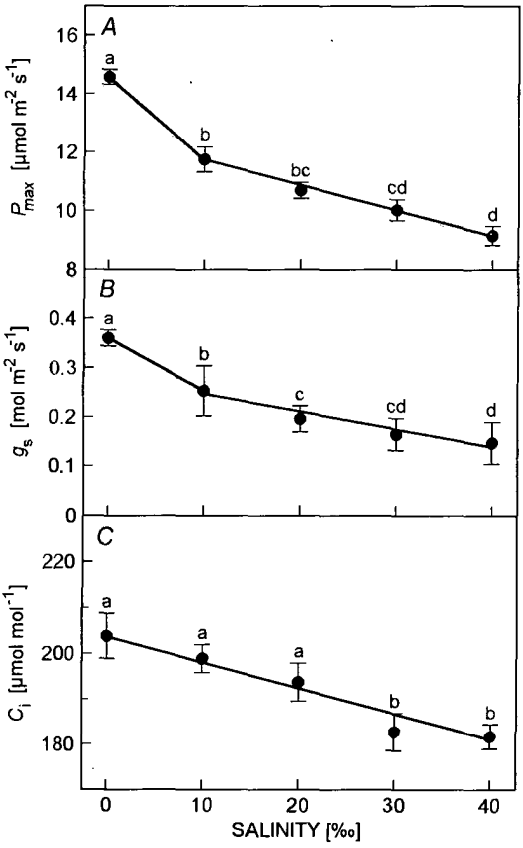

Fig. 4. Gas exchange parameters measured at midday in leaves of *A. germinans* grown in a salinity gradient. The parameters were: (*A*) maximum CO_2 assimilation (P_{max}), (*B*) stomatal conductance (g_s), and (*C*) internal CO_2 concentration (C_i). Values are means ± SE. Different letters on top of symbols indicate statistically different means at $p<0.05$.

consistent with the increased WUE estimated from gas exchange (P_{max}/g_s). In mangrove species, water loss is minimised in relation to carbon gain with increasing salinity (Ball and Farquhar 1984, Ball *et al.* 1988, Clough and Sim 1989, Lin and Sternberg 1992, Ball and Sobrado 1999).

Despite the fact that salt-induced reduction of P_{max} and g_s has been previously reported in a number of species, the extent to which stomatal and non-stomatal factors reduce P_{max} is not clear. I tried to assess the diffusive limitations on the photosynthetic function of *A. germinans* by following the gas exchange parameter for 10 successive days after salt was removed. Values were compared with those measured prior to removal of the salt (Fig. 3). From day 3 up to day 10, in plants grown at 10 ‰, P_{max} and g_s increase was not statistically significant in comparison to those prior salinity relief. In contrast, in plants grown at higher salinities, g_s increased significantly by 30 % and P_{max} from 10 to 13 % upon salt removal. Diffusive limitations due to stomatal closure seemed to be eliminated in all treatments, as indicated by similar C_i (211 ± 13) in all treatments. The better recovery of g_s in comparison to P_{max} was not surprising given the high WUE observed before salt was eliminated from the soil (Fig. 5). For all treatments, P_{max} after salt relief was still much lower than in control plants. Once salt is accumulated inside leaves, it is not thoroughly mobilized out of the tissues after salinity is eliminated (Suárez and Sobrado, unpublished). Thus, remaining inhibition of photosynthesis upon salinity relief would be related to non-diffusive factors.

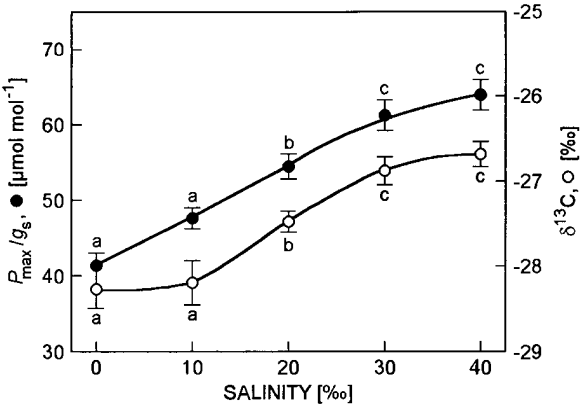

Fig. 5. Ratio of maximum CO_2 assimilation to stomatal conductance (P_{max}/g_s, ●) and carbon isotope composition ($\delta^{13}C$, ○), measured in leaves of *A. germinans* grown in a salinity gradient. Values are means ± SE. Different letters on top of symbols indicate statistically different means at $p<0.05$.

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