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GROWTH AND PHYSIOLOGY OF SALICORNIA BIGELOVII TORR. AT SUBOPTIMAL SALINITY

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Salicornia bigelovii Torr. plants were grown in 5, 200, or 600 mol m^{-3} NaCl, representing suboptimal, optimal, and supraoptimal salinities, respectively. Shoot fresh and dry mass of plants grown at optimal salinity were more than 2× higher than those grown at the other two salinities. In spite of the comparable growth reductions at sub- and supraoptimal salinities, the physiological responses, and presumably the causes of the growth reductions, were not the same at those two salinities. Water and osmotic potentials of the shoots decreased significantly with increasing salinity, but turgor potentials did not differ significantly among treatments. Differences in photosynthetic rates were not consistent with the differences in growth. Rates were significantly higher in plants grown at 5 mol m^{-3} NaCl when expressed relative to photosynthetic area, but when photosynthesis was expressed relative to the amount of chlorophyll, no significant differences were found among salinities. Stomatal conductance decreased with increasing salinity, resulting in a significantly higher transpiration rate at the lowest salinity than at the other two levels. Dark respiration was not significantly affected by salinity. Sodium concentration in shoots and roots increased with salinity. Potassium, calcium, and magnesium were highly concentrated in shoots and roots of plants grown at 5 mol m^{-3} NaCl. Excessive NaCl, however, induced calcium and magnesium deficiencies in plants grown at supraoptimal salinity.

Introduction

Some of the more extreme halophytes, often called euhalophytes, are capable of growth rates and total biomass productivities at salinity concentrations exceeding that of seawater (O'Leary et al. 1985; Glenn et al. 1991) that equal those of crop plants and other glycophytes grown with fresh water. Euhalophytes and glycophytes are similar in that they both exhibit growth reduction with increasing salinity, but this response differs in two important ways. First, glycophytes in general have reduced growth with all salinities beyond the range of zero to about 50 mol m^{-3}, but euhalophytes typically do not exhibit reduced growth until the salinity exceeds the range of about 100–200 mol m^{-3} (Flowers et al. 1986). Second, in those glycophytes that do not exhibit reduced growth until the salinity exceeds ca. 50 mol m^{-3}, the response curve is flat between that level and zero, but in euhalophytes, growth decreases considerably as salinity decreases from the 100–200 mol m^{-3} range to zero (Greenway and Munns 1980; Munns et al. 1983). That is, those plants have adapted so well to functioning at high salinities that the optimal salinity for maximum growth is in the range of 100–200 mol m^{-3}, and growth is significantly reduced as salinity increases or decreases beyond that range. A substantial amount of research has addressed the response of both glycophytes and halophytes to supraoptimal salinity, but comparatively little attention has been devoted to the question of why those plants that are highly adapted to salinity have lost the ability to grow as well at salinities lower than 100–200 mol m^{-3}. Answers to that question would contribute to increasing our understanding of how plants have adapted to highly saline environments and would also provide helpful insight into determining which physiological processes should be targeted in attempts to improve genetically the ability of crop plants to tolerate higher salinities.

Salicornia bigelovii Torr. is a succulent, C_3 annual species that occurs in coastal estuaries and is, arguably, the most salt tolerant vascular plant. It has been reported to have maximum growth at about 170–200 mol m^{-3} NaCl (Webb 1966; Weeks 1986). Based on those reports and preliminary studies of our own, we identified 5, 200, and 600 mol m^{-3} NaCl as representative of suboptimal, optimal, and supraoptimal ranges of salinity, respectively. These concentrations gave equivalent reductions in growth at both sub- and supraoptimal salinities and allowed us to compare and contrast the responses to both sub- and supraoptimal salinities. We found that, in spite of the similar growth reductions under those salinities, the physiological responses under those salinities, and presumably the causes of the growth reductions, were very different.

Material and methods

Plants and experimental conditions

Salicornia bigelovii seeds were collected in the autumns of 1991 and 1992 from plants growing in Estero Morúa, a coastal estuary located 7.5 km east of Puerto Peñasco, Sonora, Mexico (31°17′N, 113°24′W). A series of greenhouse experiments was carried out from May 1992 to July 1993. Night temperatures ranged from 15° to 26°C and day temperatures ranged from 26° to 32°C. Photosynthetically active radiation measured at noon ranged from 450 to 1,350 μmol m^{-2} s^{-1}, and

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relative humidity varied from 25% to 50% during the day and from 70% to 85% at night. To prevent plants from flowering, photoperiod was extended to 24 h using cool white fluorescent lamps at night for two experiments conducted from January to May 1993. Seeds were sown in a mixture of organic soil and sand in plastic flats without drainage. After 48–55 d, seedlings that were about 2 cm tall were transplanted to 1-L containers, where they were grown in aerated nutrient solution (composition in mol m^{-3}: 3 $Ca(NO_3)_2$, 2 KNO_3, 2 KH_2PO_4, 2 $MgSO_4 \cdot 7H_2O$; in mmol m^{-3}: 7.6 $MnCl_2$, 40 H_3BO_3, 0.3 $CuCl_2$, 1.3 $ZnSO_4$, 3 MoO_3; iron was supplied as the EDTA complex at 4.2 mg Fe L^{-1}) with 5, 200, or 600 mol m^{-3} NaCl (analytical reagent). NaCl was added at 50 mol m^{-3} per day until final concentration of 200 or 600 mol m^{-3} was reached. The water used to prepare the nutrient solutions contained (in mol m^{-3}) 1.1 Na^+, 0.04 K^+, 0.8 Ca^{2+}, and 0.08 Mg^{2+}. Solutions were replaced every 5–7 d to avoid nutrient deficiencies and salt dilution or accumulation. Solute potentials were verified by psychrometry immediately after solutions were made and before replacing solutions. Solute potential changed during that time from −0.13 to −0.24 MPa for the suboptimal salinity, from −1.1 to −2.1 MPa for optimal salinity, and from −2.7 to −3.4 MPa for supraoptimal salinity. The pH measured immediately after the solutions were made was 6.8, and just before solutions were replaced, pH was 6.42 ± 0.04, 7.18 ± 0.07, and 6.92 ± 0.02 for suboptimal, optimal, and supraoptimal salinities, respectively. A completely randomized design was used for each experiment with three salinity treatments.

Growth measurements

Fresh mass (FM) and dry mass (DM) of shoots and DM of roots were measured at 5–7-d intervals after the highest salt concentration was reached. Dry mass was determined after oven drying at 65°C to constant mass. Mineral content (ash) of shoots and roots was determined by heating at 600°C for 2 h. To determine relative growth rates (RGR), 15 plants were harvested immediately prior to adding the salt treatments. Thereafter, successive harvests were taken on days 24, 32, and 40, with five plants per treatment harvested. Dry mass at each harvest was used for calculation using the formula RGR = (ln $mass_2$ − ln $mass_1$)/time. Analysis of RGR was done using the method of Venus and Causton (1979). Number of nodes, height, and stem diameter at the fourth internode from the shoot apex were determined at weekly intervals.

Plant water relations

Predawn water and osmotic potentials were measured psychrometrically on plants 42 d after the highest salinity was reached using a Wescor HR-33T microvoltmeter. Samples were taken by excising 3–4 mm sections from the second internode from the shoot apex and placed in thermocouple psychrometer chambers (Model 75-2VC, J. R. D. Merrill Specialty Equipment Co., Logan, Utah). Sealing of the cut ends of the segments with petroleum jelly to prevent erroneous readings from leakage of saline solution was compared with unsealed segments in preliminary trials. However, the cut ends dried so rapidly that there were no differences in readings between the two, therefore, the cut ends were not covered in subsequent measurements. Water potentials were determined after 6 h equilibration at 25°C. After measurements were taken, samples were frozen in liquid N_2. They were then thawed and osmotic potentials measured after 1 h equilibration at 25°C. Turgor potentials were estimated as the difference between water potential and osmotic potential. Five plants per treatment were sampled.

Gas exchange measurements

Instantaneous measurements of net CO_2 assimilation rate (A), dark respiration (R), transpiration (E), stomatal conductance (g), and intercellular CO_2 concentration (C_i) were made on intact shoots using an LCA-3 ADC portable infrared open gas-exchange system (Analytical Development Company, Hoddesdon, England). A cuvette designed to accommodate a portion of the *Salicornia* shoot was constructed from 3-mm thick polycarbonate plastic and had a volume of 343 cm^3. Testing of the cuvette was performed following guidelines described by Parkinson and Day (1990). "Leaf" area was calculated as half of the total area of the cylindrical branch. Measurements were made by enclosing part of the plant in the cuvette allowing approximately 5 min for adjustment of the chamber, equilibration, and recording the data. Dark respiration was estimated by darkening the cuvette for approximately 5 min after recording the photosynthesis data. Gas exchange measurements were made on several different days from 21 to 61 d after the highest salinity was reached. Measurements were performed on plants at three times during the day: morning, from 08:30 to 11:30, midday, from 12:00 to 13:30, and afternoon, from 15:00 to 17:00. Differences among salinities were similar at all three times of the day, so only morning data are presented. Four to six plants per treatment were sampled at every measurement. These experiments were replicated four times.

Stable isotope analysis

At 46 d after the highest salinity was reached, shoot samples from eight plants of each treatment were dried at 65°C to constant mass. Approxi-

mately 1 g was ground to enable passage through a 40-mesh screen, and 1–2 mg of each sample were used for analysis. Natural abundance ^{13}C ratios were measured at the Duke University Phytotron on a SIRA Series II isotope ratio mass spectrometer (VG ISOGAS, Middlewich, United Kingdom), operated in automatic trapping mode after combustion of samples in an elemental analyzer (NA1500, Carlo Erba Instrumentazione, Milan). The reference CO_2, calibrated against the standard Pee Dee belemnite, was obtained from OZTECH (Fremont, Calif.). Internal precision of individual measurements was greater than 0.01‰. Data are expressed as $\delta^{13}C$ values after correction for oxygen isotope contribution.

Chlorophyll and protein determinations

Chlorophyll was extracted with 100% methanol. Chlorophylls *a, b,* and total were calculated using the equations derived from the specific absorption coefficients given by Mackinney (1941): chlorophyll $a = 16.5A_{665} - 8.3A_{650}$, chlorophyll $b = 33.8A_{650} - 12.5A_{665}$, and total chlorophyll = $25.5A_{650} + 4.0A_{665}$. The plant material, left on the filter after chlorophyll was extracted, was recovered and dried. Protein was extracted from the dried material with 0.8 N NaOH. Determination of protein concentration was done using the Pierce BCA Protein Assay (Pierce, Rockford, Ill.).

Determination of cations

Shoot samples, from plants 39 d after highest salinity was reached, were dried and digested with nitric/perchloric acid, and Na^+, K^+, Ca^{2+}, and Mg^{2+} contents were determined by inductively coupled plasma emission spectroscopy. Shoot and root samples from another experiment, with plants exposed to salinity for the same time as before, were dried and digested with nitric acid and Na^+, K^+, Ca^{2+}, and Mg^{2+} determined by atomic absorption spectroscopy (Association of Official Analytical Chemists 1984). Since results from both experiments were similar, combined data are presented.

Data analysis

One- or two-way analyses of variance were done using CoStat (CoHort Software, Berkeley, Calif.) when appropriate for some of the variables studied. Statistical analyses of gas exchange variables were performed with the GLM procedure in SAS (SAS Institute 1988). Throughout, $P \leq .05$ was used to define statistical significance. Whenever significant effects of salinity treatments were found, mean separations were done using Duncan's multiple range test.

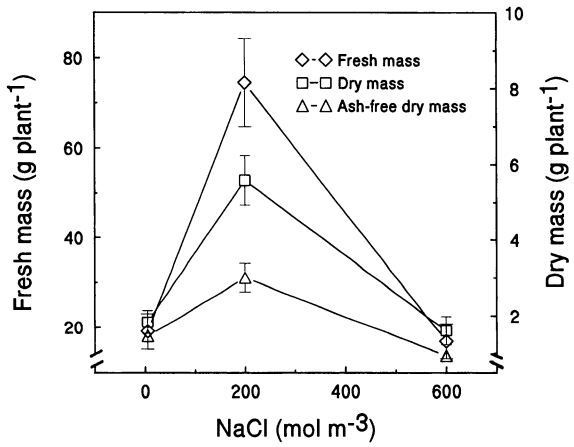

Fig. 1 Effect of added NaCl on shoot fresh, dry, and ash-free dry mass in *Salicornia bigelovii* plants grown for 39 d after the highest salinity was reached. Data points represent means ± standard errors (n = 5–7 plants).

Results

Growth

Growth was reduced significantly by both sub- and supraoptimal salinity, and the response to salinity was the same whether expressed on a fresh, dry, or ash-free dry mass basis (fig. 1). Even though the total plant mass was reduced similarly by both sub- and supraoptimal salinity levels, there were some apparent differences between the responses of shoots and roots. Shoot FM and DM and total DM of plants grown in 200 mol m^{-3} NaCl were significantly higher than plant mass grown in 5 or 600 mol m^{-3} NaCl at every time measured 25 d or more after highest salinity was reached (fig. 2*a, b, d*). Root DM for plants grown at 200 mol m^{-3} NaCl did not differ significantly from plants grown at 5 mol m^{-3} NaCl at 20, 25, or 46 d after highest salinity was reached (fig. 2*c*). In general, roots were less affected by salinity than were shoots, except for plants grown at the highest salinity.

Shoot RGR was calculated at different times during the growing season to determine the time interval during which plants growing in optimal salinity had the greatest mass accumulation. Shoot RGR was highest for plants grown at optimal salinity between 24 and 32 d after the highest salinity was reached. Before and after that time interval, RGRs were similar for all salinities (table 1).

Differences in shoot growth could result from increased production of nodes or increased size (length and width) of internodes as a result of increased cell division and/or cell elongation. To test this, plant size, number of nodes, and stem diameter were measured. Plants were significantly taller when grown at optimal salinity at every time measured except at 32 d after highest

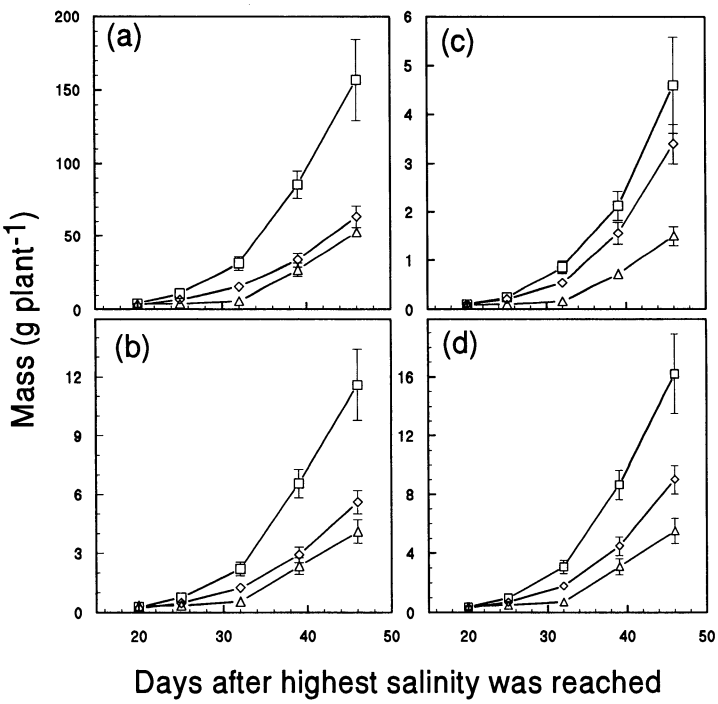

Fig. 2 Effect of added NaCl (◇ = 5, □ = 200, △ = 600 mol m^{-3}) on shoot fresh mass (*a*), shoot dry mass (*b*), root dry mass (*c*), and total dry mass (*d*) in *Salicornia bigelovii* plants at different times during the growing season. Data points represent means ± standard errors combined from 1 to 3 experiments (n = 4 plants at 20 d, n = 9 plants at 25 and 32 d, n = 17 plants at 39 d, and n = 8 plants at 46 d).

salinity was reached (fig. 3*a*). However, the number of nodes per plant was not significantly different in plants grown at sub- and optimal salinities (fig. 3*b*), indicating that the size difference was due to increased length of internodes. Also, the diameters of the internodes were significantly greater in the plants grown at optimal salinity when compared with those grown at suboptimal salinity (data not shown).

Plant water relations

Water relations parameters of the plants were measured to see if they might be related to the differences in growth. Predawn water and osmotic potentials of shoots decreased significantly with increasing salinity. Calculated turgor potentials did not differ significantly among treatments, but the trend was from very low at the suboptimal salinity to higher with each level of increasing salinity (table 2).

Table 1

Effect of added NaCl on relative growth rates (RGR) of Salicornia bigelovii shoots at three time intervals during the growing season: between transplanting (day 0) and 24 d, between 24 and 32 d, and between 32 and 40 d after the highest salinity was reached

NaCl (mol m^{-3})	RGRs (g g^{-1} d^{-1}) for 3 time periods		
	0–24 d	24–32 d	32–40 d
5 ..	.107 ± .007	.103 ± .033	.066 ± .039
200 ..	.120 ± .006	.160 ± .032	.096 ± .032
600 ..	.103 ± .006	.066 ± .031	.124 ± .030

Note. Values represent means ± standard errors (n = 5).

Gas exchange measurements

Measurements were made to see if differences in gas exchange might explain the differences in growth. Stomatal conductance (*g*) was significantly higher at the suboptimal salinity than at the two other salinities, which did not significantly differ from each other (table 3). This resulted in a significantly higher transpiration rate (*E*) and a significantly higher photosynthetic rate per unit of surface area (*A*) at the lowest salinity than at the other two salinities, but the instantaneous water use efficiency (μmol CO_2 fixed per mmol H_2O transpired) was significantly lower at the suboptimal salinity than at the other two levels. The internal CO_2 concentration also was highest at the suboptimal salinity. The $\delta^{13}C$ values for the plants also indicated that the water use efficiency increased with increasing salinity. The values here, which differed significantly between salinity levels, were −29.5‰, −28.6‰, and −27.6‰ for plants grown at 5, 200, and 600 mol m^{-3}, respectively. Dark respiration (*R*) did not differ significantly between salinity levels (table 3).

Table 2

EFFECT OF ADDED NaCl ON WATER AND OSMOTIC POTENTIALS IN SALICORNIA BIGELOVII PLANTS GROWN FOR 42 d AFTER HIGHEST SALINITY WAS REACHED

NaCl (mol m^{-3})	Water potential (MPa)	Osmotic potential (MPa)	Calculated turgor (MPa)
5..	−1.20 ± .09c	−1.29 ± .08c	.09 ± .03
200..	−2.25 ± .10b	−2.42 ± .09b	.17 ± .02
600..	−3.25 ± .17a	−3.48 ± .23a	.23 ± .09

Note. Means in the same column followed by the same letter are not significantly different ($P = .05$) according to Duncan's multiple range test. If there are no letters, there were no significant differences in the ANOVA. Values represent means ± standard errors ($n = 5$ plants).

CHLOROPHYLL, SUCCULENCE, PROTEIN CONTENT, AND PHOTOSYNTHESIS EXPRESSED ON CHLOROPHYLL BASIS

Color differences in shoots were obvious among salinity treatments, indicating differences in chlorophyll content. Direct measurement of chlorophyll content supported that observation. Total chlorophyll content of the shoots was significantly higher at the suboptimal salinity than at the optimal salinity, which was significantly higher than that of plants at the supraoptimal salinity (table 4). The differences in chlorophyll *a* were primarily responsible for these differences.

Since differences in chlorophyll content were found, we repeated the photosynthesis measurements, but this time we also measured chlorophyll content of the same tissue. Again, total chlorophyll content was highest in plants grown at suboptimal salinity. However, the FM per cm^2 of leaf surface (succulence) was lowest for these plants. This resulted in no significant differences in photosynthesis expressed in μmol CO_2 per gram chlorophyll per second (table 5).

No significant differences in protein content were found among salinity levels (data not shown), and expressing the photosynthetic rates on that basis did not provide any new insights. However photosynthesis was expressed, it never was lower in plants growing at suboptimal salinity than in plants growing at optimal salinity.

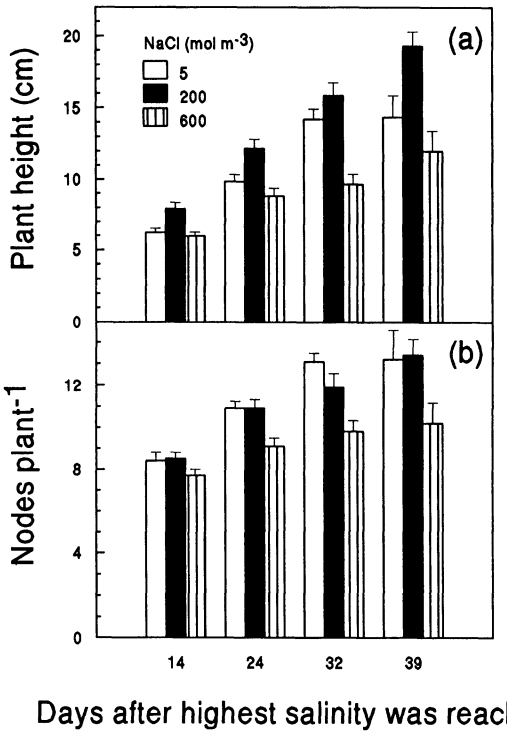

Fig. 3 Effect of added NaCl on plant height (*a*) and nodes per plant (*b*) at different times during the growing season of *Salicornia bigelovii* plants. Bars represent means ± standard errors ($n = 16$ plants at 14 and 24 d, $n = 11$ plants at 32 d, and $n = 6$ plants at 39 d).

CATIONS

Inorganic solutes constitute a major component in tissue osmotic potentials. Total concentration of cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) increased with increases in salinity (table 6). At optimal and supraoptimal NaCl, the increase in total inorganic ions resulted from increased so-

Table 3

EFFECT OF ADDED NaCl ON CONDUCTANCE (g), TRANSPIRATION (*E*), NET PHOTOSYNTHESIS (*A*), DARK RESPIRATION (*R*), INSTANTANEOUS WATER USE EFFICIENCY (WUE) (*E*/*A*), AND INTERNAL CO_2 (C_i)

NaCl (mol m^{-3})	g (mol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	*A* (μmol m^{-2} s^{-1})	*R* (μmol m^{-2} s^{-1})	WUE (μmol CO_2/ mmol H_2O)	C_i (μg cm^{-3})
5..	1.35 ± .22a	10.66 ± .41a	22.14 ± .72a	10.7 ± .9	2.2 ± .1b	256 ± 4a
200..	.57 ± .03b	7.18 ± .27b	17.87 ± .54b	9.5 ± .6	2.6 ± .1a	230 ± 5b
600..	.37 ± .02b	6.33 ± .27b	17.42 ± .58b	10.6 ± .9	2.9 ± .1a	211 ± 4c

Note. Values represent means ± standard errors ($n = 39$ plants for all variables measured except *R* [$n = 10$] for plants grown in 5 or 200 mol m^{-3} NaCl; for 600 mol m^{-3} NaCl plants, $n = 33$ plants for all variables except *R* [$n = 5$]). Means in the same column followed by the same letter are not significantly different ($P = .05$) according to Duncan's multiple range test. If there are no letters, there were no significant differences in the ANOVA.

Table 4

EFFECT OF ADDED NaCl ON CHLOROPHYLL CONTENT IN SALICORNIA BIGELOVII

NaCl (mol m^{-3})	Chlorophyll (mg g^{-1} DM)			
	a	*b*	Total	*a*:*b*
5	42.3 ± 1.3a	21.4 ± 2.3a	63.7 ± 3.4a	2:1
200	34.2 ± 1.3b	20.8 ± 2.4a	55.0 ± 3.5b	1.6:1
600	21.0 ± 1.1c	12.9 ± 1.7b	33.9 ± 2.5c	1.6:1

Note. Values represent means ± standard errors (n = 20–22 plants) from three different experiments. Means in the same column followed by the same letter are not significantly different (P = .05) according to Duncan's multiple range test.

Table 5

EFFECT OF ADDED NaCl ON TOTAL CHLOROPHYLL, SUCCULENCE, AND NET PHOTOSYNTHESIS (*A*) (expressed on a chlorophyll basis) IN SALICORNIA BIGELOVII

NaCl (mol m^{-3})	Total chlorophyll (mg g^{-1} FM)	Succulence (mg FM cm^{-2})	Total chlorophyll (g m^{-2})	*A* (chlorophyll basis) (μmol g^{-1} s^{-1})
5 ..	5.1 ± .4a	225 ± 16b	11.6 ± 1.8	2.2 ± .2
200 ..	4.1 ± .3b	296 ± 14a	11.2 ± 1.2	1.9 ± .2
600 ..	3.5 ± .3b	260 ± 9a	9.1 ± .7	2.2 ± .2

Note. Values represent means ± standard errors (n = 15 plants) from three different experiments. Means in the same column followed by the same letter are not significantly different (P = .05) according to Duncan's multiple range test. If there are no letters, there were no significant differences in the ANOVA.

dium. Calcium and magnesium concentrations were reduced with increase in salinity. These reductions were proportionately larger in shoots than in roots. Potassium concentration in shoots was significantly reduced with salinity. However, potassium in roots was not affected by salinity.

Discussion

Salicornia bigelovii showed optimal growth at 200 mol m^{-3} NaCl. Similar results have also been found by Webb (1966) and Weeks (1986) for this same species. Comparable results have been reported for other *Salicornia* species as well (Halket 1915; Webb 1966; Tiku 1976; Flowers et al. 1977; Abdulrahman and Williams 1981; Pearcy and Ustin 1984). In fact, many dicotyledonous halophytes show optimal growth in the presence of salt (Flowers et al. 1977; Glenn and O'Leary 1984; Naidoo and Rughunanan 1990; Warne et al. 1990; Rozema 1991). Fresh and dry mass of the plants were reduced to a similar extent at sub- and supraoptimal salinities, compared to plants grown at optimal salinity. However, it appears that the cause for the reductions in growth between sub- and supraoptimal salinity plants may not be similar.

Decreased growth at supraoptimal salinity

The deleterious effects of salinity are thought to result from water stress, ion toxicities, ion imbalance, or a combination of these factors. Ion imbalances in plants can, for example, occur when high concentrations of Na^+ in the soil reduce the amounts of available K^+, Mg^{2+}, and Ca^{2+} (Epstein 1972) or when Na^+ displaces membrane-bound Ca^{2+} (Cramer et al. 1985). In addition, Na^+ may have direct toxic effects, as when it interferes with enzyme structure and function. It may also interfere with the function of potassium as a cofactor in various reactions. Many of the deleterious effects of Na^+, however, seem to be related to the structural and functional integrity of membranes (Kurth et al. 1986). As expected, our results indicated that sodium concentration in shoots and roots increased with salinity (table 6). Potassium, calcium, and magnesium concentration of the shoots of plants grown at optimal salinity represent levels adequate for growth (Epstein 1972), but the calcium and magnesium concentrations were extremely low in shoots of plants grown at supraoptimal salinity (table 6), agreeing with results found for other halophytes (Flowers 1972; Glenn and O'Leary 1984; McNulty 1985; Naidoo and Rughunanan 1990). Deficiencies of Ca^{2+} and Mg^{2+} might be involved in the reduced growth of these plants. It is not possible to conclude unequivocally that such is the case, however, without information about the distribution of these ions within the cell. For example, cytoplasmic Ca^{2+} levels normally are maintained very low in most cells (Salisbury and Ross 1992), and if much of the Ca^{2+} in these plants were in the cytosol, then there might be no problem. However, Mg^{2+} reportedly is required in relatively higher concentrations in the cytosol of some halophytes than in glycophytes (Flowers and Dalmond 1992), so there may be a higher probability of a problem with these low tissue concentrations of Mg^{2+}. The plants in this study did exhibit some symptoms at the whole plant level that were consistent with deficiencies. Plants grown at supraoptimal salinity showed malformed and necrotic tips. They also had a light green color, and chlorophylls *a* and *b* were low in these plants (table 4).

Decreased growth at suboptimal salinity

One of the major differences between the plants at sub- and those at supraoptimal salinity was in their photosynthetic responses. In the latter, reduced growth was accompanied by reduced photosynthetic rates, but in the former, similar growth reduction was accompanied by photosynthetic rates that were equal to, or greater than, those of plants growing at optimal salinity. Thus, the re-

Table 6

EFFECTS OF ADDED SALT ON THE CONCENTRATION OF CATIONS IN SHOOTS AND ROOTS OF SALICORNIA BIGELOVII PLANTS GROWN FOR 39 d AFTER HIGHEST SALINITY WAS REACHED

NaCl (mol m^{-3})	Tissue ion concentration (μmol g^{-1} dry mass)							
	Na^+		K^+		Ca^{2+}		Mg^{2+}	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
5	1,905 ± 86c	373 ± 100b	1,052 ± 62a	802 ± 32	482 ± 17a	462 ± 26a	410 ± 15a	151 ± 10a
200	5,727 ± 349b	2,384 ± 182a	390 ± 22b	950 ± 58	62 ± 4b	283 ± 48b	54 ± 2b	81 ± 2b
600	7,177 ± 464a	2,644 ± 50a	213 ± 21c	830 ± 60	32 ± 3c	87 ± 11c	31 ± 3b	52 ± 2c

Note. Values represent means ± standard errors ($n = 13$ for shoots and $n = 6$ for roots). Means in the same column followed by the same letter are not significantly different ($P = .05$) according to Duncan's multiple range test. If there are no letters, there were no significant differences in the ANOVA.

duced growth at suboptimal salinity apparently did not result from insufficient production of photosynthate. This poses an interesting question. If the photosynthetic rates are reflective of total daily photosynthate production, and that production at suboptimal salinity is equal to, or greater than, production at optimal salinity, why is the biomass accumulation only about half as much at suboptimal as it is at optimal salinity? In spite of the higher photosynthetic rate at suboptimal salinity, the water use efficiency was significantly lower than at the other salinities, whether expressed on an instantaneous basis (table 3) or on a long-term average basis using carbon isotope measurements. In C_3 plants, more positive values of $\delta^{13}C$ are correlated with increased water use efficiency (O'Leary 1988), and the values here increased significantly with salinity.

It is clear that halophyte cells must have lower water potentials within than outside the plasmalemma to retain cellular water and that the necessary osmotic adjustment in dicotyledons is largely achieved by Na and Cl ions (Flowers 1985). Our results indicated that water and osmotic potentials of the plants reflected the osmotic potentials of the external solution, especially at optimal and supraoptimal salinities, but turgor potential was not significantly different, even though plants grown at suboptimal salinity had a comparatively low turgor potential (0.09 MPa). However, it would not be appropriate to conclude that this low turgor is the cause of reduced growth. In another succulent halophyte (*Suaeda maritima*), in which turgor was measured directly with a pressure probe, the leaf turgor was found to be higher in the plants growing poorly at suboptimal salinity (Clipson et al. 1985). We agree with the recent conclusion by Munns (1993) that the earlier suggestion (Munns et al. 1983) of low turgor being responsible for reduced growth at suboptimal NaCl probably should be disregarded or, at least, modified.

Shoots of plants grown at suboptimal salinity compensated for the sodium deficiency in the external solution by accumulating K^+, Ca^{2+}, and Mg^{2+} together in an amount equaling that of sodium alone (table 6). Concentrations of these cations were about four times the adequate level for shoots (Epstein 1972). Depending on how those ions were distributed among the cellular compartments, it is possible that toxic effects may have contributed to the reduced growth in those plants.

In addition, plants grown at suboptimal salinity were less succulent and had a significantly smaller stem diameter because of a reduction in cell size. Comparable results were also found by Batalin (cited in Strogonov 1964) and Strogonov (1964) in *Salicornia herbacea* L. (= *Salicornia europaea* L.). Similarly, when *S. maritima* was grown under saline conditions, root diameter, and the cross-sectional area of the cortical cells, became much larger than in nonsalinized plants, but the number of layers of cells in the cortex and total number of cortical cells remained constant (Hajibagheri et al. 1985). Furthermore, Kurth et al. (1986) noted that while Na^+ and Ca^{2+} appeared to have interactive effects on cell shape and the rate of cell production within files of cortical cells, they did not seem to affect the basic organization of the cortex, as indicated by the essentially constant number of layers of cortical cells in cotton (*Gossypium hirsutum* L.) roots.

More than half of the calcium in plants exists in the apoplast (Cleland et al. 1990). Ca^{2+} increases the rigidity of plant cell walls by complexing with wall matrix polysaccharides (Cleland et al. 1990). Protons, and sometimes K^+ and Na^+, have been shown to displace Ca^{2+} from cell walls, thereby increasing wall extensibility (Cleland et al. 1990). One might expect, therefore, increased growth under saline conditions (Kurth et al. 1986), as observed for plants grown at optimal salinity. Cleland et al. (1990), however, concluded that calcium crosslinks were not the major load-bearing bonds in soybean (*Glycine max* L.) hypocotyl cell walls, and protons caused

wall loosening by some mechanism other than displacement of wall calcium. However, exogenous Ca^{2+} inhibited growth by releasing H^+ from the Donnan Free Space (close to cell wall) to the Free Space, raising the pH of the Donnan Free Space and inhibiting wall loosening enzymes with acidic pH optima (Cleland et al. 1990). Sodium might also have an effect on membrane function (Läuchli 1990). At lower Ca^{2+} concentration, mild salt stress (25 mol m^{-3}) depolarized the membrane within 1–2 min, followed by slower recovery. The depolarization was presumably related to Na^+ influx, while recovery may be ascribed to increased H^+ efflux from stimulation of H^+-ATPase activity. As might be expected, the membrane potential was depolarized less by Na^+ at a 10 times greater Ca^{2+} supply.

In summary, our results lead us to conclude that reduced growth at suboptimal salinity apparently is not due to an insufficient supply of photosynthate to support growth nor is it due to less than favorable water relations in the shoots as had been suggested earlier (Munns et al. 1983). Rather, it seems as if the growth differences may be more closely related to differences in ionic relations.

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