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Effects of Sodium Chloride Treatments on Growth and Ion Accumulation of the Halophyte *Haloxylon recurvum*

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ABSTRACT

Effects of increasing salt concentrations 0, 180, 360 mol m^{-3} sodium chloride (NaCl), on growth, succulence, mineral composition, and glycinebetaine content in *Haloxylon recurvum* was investigated. Fresh and dry weight of plants increased with an increase in salinity. Succulence of shoots increased at low salinity and decreased at high salinity. Root and shoot Ca^{+}, Mg^{+}, and K^{+} content decreased with increasing salinity while both Na^{+} and Cl^{-} content increased, reaching 4,900 and 5,300 mmol kg^{-1} dry weight, respectively. Glycinebetaine (mol m^{-3} tissue water) significantly increased in shoots at 360 mol m^{-3} NaCl, but did not differ significantly in roots treated with from 0 to 360 mol m^{-3} NaCl. *Haloxylon recurvum* is a highly salt tolerant stem succulent plant which accumulate a high quantity of salt, which makes it a good candidate to use for phytoremediation in highly saline areas of the sub-tropics.

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INTRODUCTION

Haloxylon recurvum Bunge ex. Boiss. (Chenopodiaceae) is a stem succulent perennial shrub commonly found in highly saline patches in association with *Suaeda fruticosa* (L.) Forssk., *Salsola baryosma* (R. & S.) Dandy, and *Sporobolus arabicus* (Boiss.). *Haloxylon recurvum* is commonly found on inland salt flats and its seeds maintain a transient seed bank (Khan, 1991). Germination occurs after monsoon rains under field conditions, when it was cooler and soil salinity is reduced (Khan and Ungar, 1996). Recruitment by seeds is rare, unless rainfall during the monsoon season is much higher than normal and persists for a long period of time (Khan, 1990). Seeds require light to germinate and are salt tolerant, and those seeds that remain ungerminated after exposure to high salinities were able to germinate rapidly when returned to distilled water (Khan and Ungar, 1996, 1997a, 1997b).

Salinity may cause a decrease in biomass production because increased soil salinity produces a lowering of plant water potentials, specific ion toxicities, or ionic imbalances (Neumann, 1997). Plants protect themselves from NaCl toxicity by minimizing Na^+ uptake and transport to the shoots (Cramer et al., 1989). Protection from osmotic stress injury is accomplished by the accumulation of organic osmolytes in order to create an osmotic balance in the cytoplasm and they also act as an osmoprotectant for cell proteins (Popp, 1995; Bohnert et al., 1995). Increased production and accumulation of organic compounds in plant tissues and mechanisms to reduce the uptake and the transport of ions in plants carry a considerable energetic cost that results in reduced biomass production, but these mechanisms of protection from salinity stress results in the successful adaptation of halophytes to saline conditions (Greenway and Munns, 1980; Ungar, 1991; Bohnert at al., 1995).

Dry mass of most halophytes usually decreases with an increase in salinity and a high concentration of NaCl is probably not essential for the optimal growth of most halophytes (Ungar, 1991). There are a number of reports that indicate that plant growth is significantly promoted at lower salinities in some halophytes, but growth of these species is usually inhibited at higher salinities (Yeo and Flowers, 1980; Baumeister and Schmidt, 1962; Hekmat-Shoar, 1978; Khan and Aziz, 1998).

Haloxylon recurvum occurs in saline habitats and could be used to reclaim salinized agricultural land if it is capable of accumulating inorganic ions. Specific conductance of soils in this *H. recurvum* habitat range from 19 to 34 dS m^{-1}, indicating a total soil solution salt content in the range of 190 to 340 meq L^{-1} (Rajpurohit and Sen, 1977; Khan, 1990). Little information is available concerning the salt tolerance and biomass production of this species under a range of saline conditions. This study was conducted to determine the effect of salinity on growth, ion accumulation and glycinebetaine content in the halophyte *H. recurvum*. Since *H. recurvum* grows in highly saline habitats, it is hypothesized that dry mass production will be salt stimulated and that Na, Cl, and glycinebetaine will be accumulated so that plants can adjust osmotically to soil salinity levels.

MATERIALS AND METHODS

Plant Material

Haloxylon recurvum Bunge ex. Boiss. seeds were collected during the fall of 1994 from salt flats situated on the Karachi University Campus, Pakistan. These seeds were brought to Ohio University, Athens, OH, and growth studies were started in January 1996. Seeds were surface sterilized using the fungicide phygon active ingredient dichlone (2,3 dichloro-1,4-naphthoquinone).

Growth Conditions

Plants were grown in a growth chamber at a thermoperiod of 25°C:35°C (night:day), and a 12-h photoperiod (300 μmol photons m^{-2} s^{-1}, 400-700 nm). Ten pots in each treatment were grown at 0, 180, and 360 mol m^{-3} NaCl in sand culture. Sand culture was used to determine the direct effects of salinity without the influence of loss of aggregation and correction for cation exchange that would occur in clay substrates. The salinity levels used are in the range found under field conditions at the Karachi location (Khan, 1990). A half-strength Hoagland and Arnon no. 2 nutrient solution was used to supply the macronutrients and micronutrients. Pots were sub-irrigated, and the water level was adjusted daily to correct for evaporation. Salt solutions were completely replaced once a week to avoid build-up of salinity in pots. At initiation of experiment, salinity concentrations were gradually increased by 90 mol m^{-3} at 2-d intervals to reach maximum salinity levels of 360 mol m^{-3} NaCl after 8 days.

Fresh and dry weight of plant shoots and roots were measured 90-d after the highest salt concentration was reached. Dry mass was determined after drying for 48 h in a forced-draft oven at 60°C.

For glycinebetaine and ion measurements 0.5 g of plant material was boiled in 10 mL of water for two hours at 100°C using a dry heat bath. This hot water extract was cooled and filtered using Whatman no. 42 filter paper, and then used directly to measure glycinebetaine using a Hewlett Packard HPLC model HP 1050 modular 3D LC system with diode array detector (Khan et al., 1998). One mL of hot water extract was diluted with distilled water for ion analysis. Chloride ion content was measured with a Beckman specific ion electrode. Cation content of plant organs was analyzed using a Perkin Elmer model 360 atomic absorption spectrophotometer. The Na^+ and K^+ content of plant tissue were assayed by flame emission and Ca^{2+} and Mg^{2+} concentrations by atomic absorption spectrophotometery.

Statistical Analysis

The data for fresh and dry mass, ionic content, and glycinebetaine were analyzed using one-way ANOVA to determine if significant differences were present among

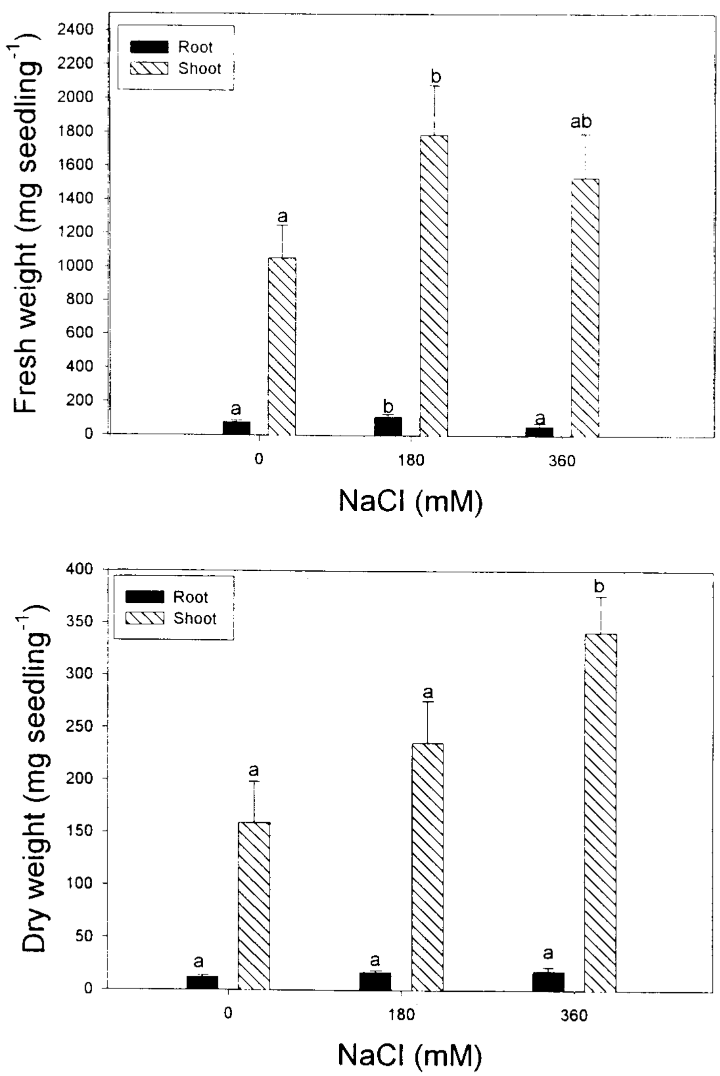

FIGURE 1. Effect of NaCl (0, 180, and 360 mol m^{-3}) on the fresh and dry weight in *Haloxylon recurvum* plants. Bars represent mean ± standard error. Different letters above bars represent a significant difference (P<0.05) between treatments.

means. A post-hoc Bonferroni test was carried out to determine if significant ($P<0.05$) differences occurred between individual treatments (SPSS, 1996).

RESULTS AND DISCUSSION

Growth

A one-way ANOVA on the biomass production of *H. recurvum* indicated that salinity increased root fresh weight ($F=7.23$, $P<0.009$) while root dry weight was not significantly different ($F=1.16$, $P>0.35$). Shoot dry weight ($F=9.92$, $P<0.003$) and fresh weight ($F=0.034$, $P<0.034$) were significantly affected by salinity. Root fresh weight increased significantly at low salinity (Figure 1). Shoot fresh weight increased at low salinity, but there was not a significant difference in the fresh weight between the control and 360 mol m^{-3} NaCl (Figure 1). Root dry weight decreased significantly with increased salinity (Figure 1). Shoot dry weight was significantly higher than the control and low salinity treatment at the highest salinity.

Haloxylon recurvum is one of the most abundant halophytes found in the inland saline flats around Karachi, Pakistan. The inland salt flats with a high water table and high salinity is often dominated by *H. recurvum* in association with *Suaeda fruticosa* and *Sporobolus arabicus*. Field investigations indicate that *H. recurvum* is a salt tolerant species (Khan, 1990), and its growth was salt-stimulated at 360 mM NaCl. Growth of most halophytes is substantially inhibited at this salinity concentration (Ungar, 1991). Most of the halophytic grasses do not survive in more than 300 mM NaCl (Pearen et al., 1997). Some *Atriplex* species are reported to grow at higher salinities, ranging from 600 to 750 mM (Ashby and Beadle, 1957; Priebe and Jäger, 1978). Other dicotyledenous halophytes are reported to have optimal growth in the presence of 100 to 200 mM NaCl (Naidoo and Rughunan, 1990; Rozema, 1991; Ayala and O'Leary, 1995; Khan et al., 1999). Growth studies with co-occurring species such as *Cressa cretica* L. and *Suaeda fruticosa* also indicated that these species had a high degree of salinity tolerance (Khan and Aziz, 1998; Khan et al., unpublished data).

Tissue Water

A one-way ANOVA indicated that salinity significantly affected tissue water content of *H. recurvum* on a unit dry weight basis ($F=10.16$, $P<0.003$), and water per plant basis ($F=4.66$, $P<0.035$). Succulence, when expressed as g tissue water g^{-1} dry weight increased slightly at low salinity but significantly decreased at 360 mol m^{-3} NaCl (Figure 2).

Tissue water when expressed on a per plant basis significantly increased at low salinity and decreased to control levels at higher salinity (Figure 2). Succulence is thought to contribute to salt regulation by increasing the vacuolar volume available for ion accumulation (Greenway and Munns, 1980; Albert, 1982; Ungar, 1991). Salinity is correlated with an increase in water content of *Salsola kali* (Reimann

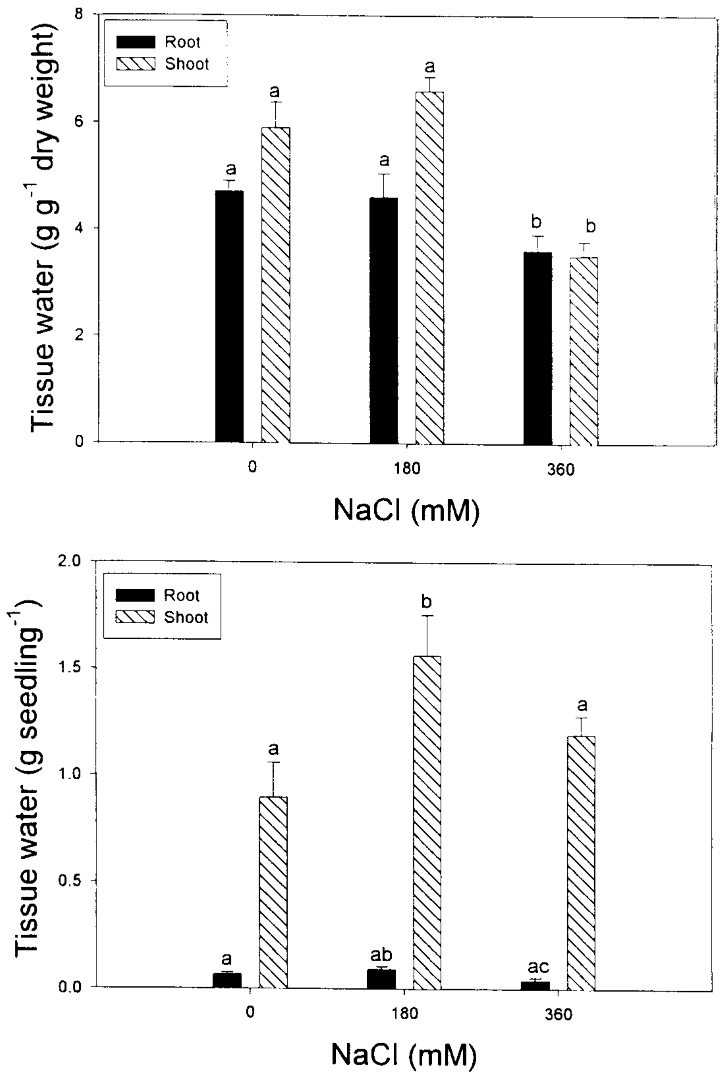

FIGURE 2. Effect of NaCl (0, 180, and 360 mol m^{-3}) on tissue water in *Haloxylon recurvum* plants. Bars represent mean ± standard error. Different letters above bars represent a significant difference ($P<0.05$) between treatments.

TABLE 1. Ionic content (mmol kg^{-1} dry weight) of *Haloxylon recurvum* grown in different NaCl concentrations.*

Salinity	Calcium		Chloride		Magnesium		Potassium		Sodium	
mol m^{-3}	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	321^a	318^a	234^a	215^a	154^a	114^a	169^a	265^a	220^a	278^a
	±34	±23	±44	±18	±5	±12	±41	±56	±68	±15
180	226^b	96^b	526^b	3410^b	61^b	80^b	43^b	64^b	861^b	4520^b
	±12	±12	±34	±311	±4	±9	±22	±5	±54	±171
360	28^c	60^c	866^c	4880^c	13^c	60^b	23^b	27^c	1362^c	5285^c
	±7	±7.5	±73	±300	±19	±18	±38	±5	±106	±128

*Different letters per element in a column indicate a significant difference at $P<0.05$ (Bonferroni test).

and Breckle, 1995) and *Arthrocnemum fruticosum* (Eddin and Doddema, 1986), and this increase in succulence is proposed to be caused by an accumulation of ions. However, our results showed that *H. recurvum* had a significant increase in salt accumulation even though the water content of tissues was significantly reduced in roots and shoots at 360 mM NaCl. It has been suggested that the salt stimulated dry mass production in some halophytes acts as a dilution factor mechanism in plants even though the level of succulence decreases (Ungar, 1991).

Ionic Content

A one-way ANOVA indicated that salinity caused a significant reduction in Ca^{2+} ($F=4.98, P<0.005$), Mg^{2+} ($F=11.46, P<0.0001$), K^+ ($F=14.83, P<0.0001$), and an increase in Na^+ ($F=21.25, P<0.0001$) and Cl^- ($F=43.66, P<0.0001$) of both roots and shoots (Table 1). Both Cl^- and Na^+ content of root and shoots progressively increased with an increase in salinity (Table 1).

Increasing salinity significantly reduced the K^+ content in shoots of *H. recurvum*, whereas Na^+ content increased with an increasing substrate salinity. This pattern of K:Na relations is reported for other relatively salt tolerant species such as *Suaeda maritima*, *Atriplex hortensis*, and *Atriplex prostrata* (Flowers, 1975; Jeshke and Skelter, 1983; Karimi and Ungar, 1984). Jennings (1976) and Flowers and Lauchli (1983) reported that Na^+ can substitute for K^+ in some cellular activities such as in osmotic adjustment, stomatal regulation, and enzyme activation, which may in part explain why growth may be stimulated when K^+ concentrations in plants are reduced while Na^+ concentrations increase at higher salinity. Chloride content also increased with increases in salinity, and this pattern is consistent with other subtropical perennial halophytes like C. *cretica*, *S. fruticosa*, *Atriplex griffithii*, and *Halopyrum mucronatum* (Khan and Aziz, 1998; Khan, unpublished data).

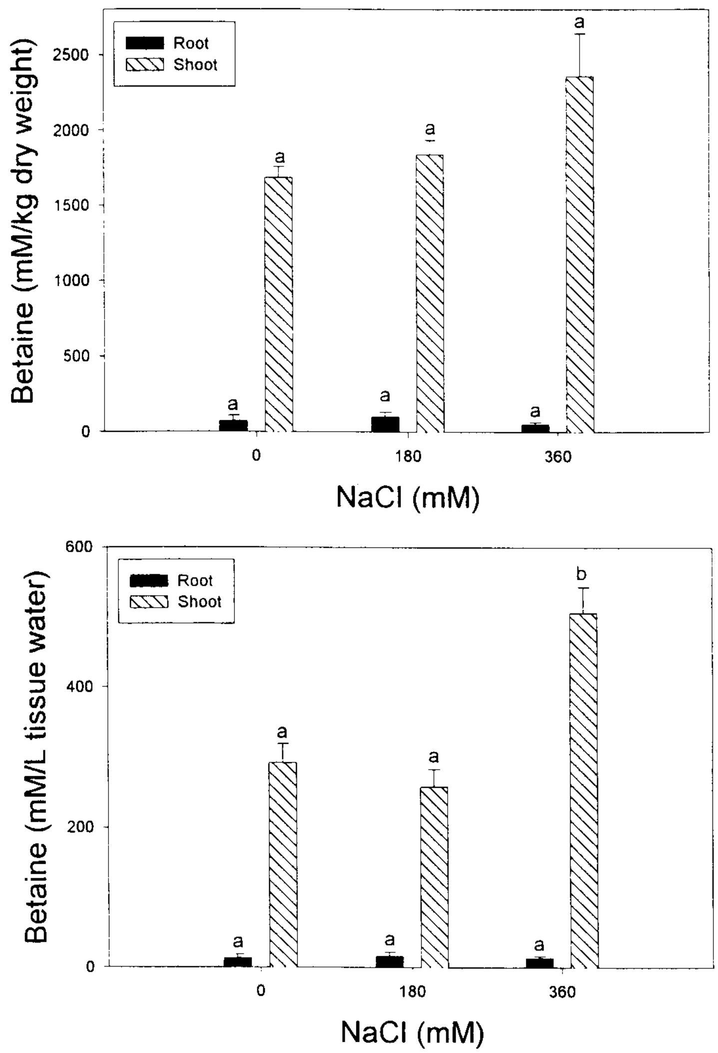

FIGURE 3. Effect of NaCl (0, 180, and 360 mol m^{-3}) on glycinebetaine content of *Haloxylon recurvum* plants. Bars represents mean ± standard error. Different letters above bars represent a significant difference ($P<0.05$) between treatments.

Glycinebetaine

A one-way ANOVA of the glycinebetaine content of *H. recurvum* revealed that salinity significantly affected the concentration of glycinebetaine (mol m^{-3} tissue water) ($F=11.23$, $P<0.009$), but there was not a significant effect on a dry weight basis ($F=3.9$, $P<0.081$) (Figure 3).

Glycinebetaine is considered to be a compatible osmoticum in halophytes (Wyn Jones, 1984; Gorham, 1995). There is evidence that glycinebetaine and other osmoprotectants are located primarily in the cytoplasm (Gorham and Wyn Jones, 1983). The possible contribution of glycinebetaine to cytoplasmic osmotic adjustment assumes that these compounds are located primarily in the cytoplasm. If this assumption is correct, the concentration of glycinebetaine in *H. recurvum* tissues would be sufficient to act in osmotic adjustment of the cytoplasm at both intermediate and high salinities.

CONCLUSIONS

Haloxylon recurvum should be included as one of the highly salt tolerant subtropical desert perennial halophytes. Growth is stimulated in up to 360 mol m^{-3} NaCl and because of this it should be considered an obligate halophyte. It is also an ion accumulator and may be a good candidate for use in phytoremediation of saline soils in the arid saline regions of the world.

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