

Growth, ion accumulation, and nitrogen fractioning in *Atriplex barclayana* grown at various salinities

AVINOAM NERD AND DOV PASTERNAK

Authors are research agronomist and associate professor, respectively, The Institutes for Applied Research, Ben-Gurion University of the Negev, Beer-Sheva, P.O. Box 1025, Israel.

Abstract

Effects of varying NaCl levels (50–400 mol/m³) on growth, ion accumulation, and nitrogen fractioning in *Atriplex barclayana* were studied in a greenhouse experiment using a water culture method. Relative growth rate of shoots was maintained at a high constant level at NaCl concentrations not exceeding 200 mol/m³, but fell to less than half when salt concentration was increased to 400 mol/m³. Potassium and calcium concentrations in shoots were unaffected by root media salinities up to a concentration of 200 mol/m³ but declined at 400 mol/m³. Sodium and chloride concentrations in shoots demonstrated an increase with rising salinity, particularly when NaCl level was increased from 50 to 100 mol/m³. Total nitrogen concentration in leaves was relatively high (3.51–3.72% of dw) at salinities between 50 to 200 mol/m³ NaCl but decreased significantly at 400 mol/m³ NaCl. Glycinebetaine in leaves rose slightly when culture salinity was raised from 50 to 100 mol/m³ NaCl and then remained constant up to an NaCl level of 400 mol/m³. Our results indicate that *A. barclayana* is a highly salt-tolerant plant with leaves rich in nitrogen, but high salt concentrations in the leaves and stems even at low salinities markedly reduce its potential as a fodder plant.

Key Words: halophyte, water culture, nonsoluble N, soluble N, glycinebetaine

Many arid and semiarid regions in the world contain soils and water sources too saline for most of the common economic crops. The utilization of halophytic plants in pasture and fodder production in saline soils and in fodder production with saline water was proposed by Malcolm (1969), O'Leary (1984, 1985), and Pasternak

et al. (1985). One candidate proposed for this purpose is *Atriplex barclayana*, a small perennial dioecious shrub native to the Baja California seashore (Wiggins 1980). O'Leary examined the productivity of various halophytes irrigated with seawater (4.0% dissolved solids) and reported that *A. barclayana* exhibited high tolerance to repeated cuttings and produced the highest yield, 2,336 g of dry matter/m² per year (O'Leary 1985). Plants of *A. barclayana* grown on seawater with 3.7% dissolved solids demonstrated high ash content, 32% of shoot dry matter (Pasternak et al. 1985). In a feeding trial, both weight gain and energy utilization in sheep fed on seawater-irrigated *A. barclayana* forage proved to be low (Arieli et al. 1989). This performance was attributed to the high ash content and relatively low total digestibility of the forage, and it was suggested that lowering the ash content would elevate the nutritional value of *A. barclayana* fodder. Benjamin et al. (1987) and Arieli et al. (1989) showed that the nitrogen of *A. barclayana* forage is readily available to ruminant livestock fed high-salt diets. However, despite the plant's high crude protein content of up to 20% not all the available N can be utilized. The reason appears to be that a high percent of the total N is in the form of soluble nonprotein N (NPN), which is not necessarily utilized by ruminants as a nitrogen source unless a highly digestible energy material is also present in the rumen during fermentation and digestion (Hassan and Abdel Aziz 1979, Benjamin et al. 1987).

In light of these findings it was decided to study the performance of *A. barclayana* at different salt concentrations in the root medium and to attempt to describe the changes which take place in mineral and nitrogen fractions in the shoots, in order to find ways of improving their nutritional value. A second objective was to follow changes occurring in the betanines, soluble nitrogenous compounds of low molecular weight, at increasing salinity levels. At elevated salinities the content of these compounds increases in

This research was supported by the Dutch Ministry for Development Cooperation. Manuscript accepted 10 July 1991.

shoots of many plant species and can account for over 20% of the total nitrogen content (Storey and Wyn Jones 1977, Storey et al. 1977, Storey and Wyn Jones 1979).

Materials and Methods

Cuttings were prepared from female seedlings of *Atriplex barclayana* grown in our introduction plot in Beer-Sheva. Semihard segments of shoots, 5–8 cm in length, were treated with 0.3% IBA and planted in vermiculite in a misted greenhouse. Temperature fluctuations were between 19 and 32° C. After 2 months well-developed rooted cuttings were transferred to 1 liter pots containing continuously aerated half-strength Hoagland solution (Hoagland and Arnon 1950). The pots were placed in a greenhouse where mean total daily photosynthetically active radiation (PAR) was 32 mol/m² per day and day/night air temperatures and relative humidity averaged 36° C/19° C and 35%/60%, respectively.

After 2 weeks, the salt concentration of the nutrient solution was raised by 50 mol/m³ daily so as to gradually produce treatments of 50, 100, 200, and 400 mol/m³, highest level being reached after 8 days. Constant volume was maintained by adding distilled water daily, and solutions were replaced with fresh ones once a week. The experiment was a completely random design with 12 plants per treatment. First harvest of 6 plants per treatment took place 14 days after the end of salinization. The remaining 6 plants per treatment were harvested 28 days following treatment.

Harvested shoots were separated into stems and leaves and both fresh and dry weights were recorded after drying at 70° C for 72 hours. The relative growth rate (RGR) was estimated as $(\ln w_2 - \ln w_1) / (t_2 - t_1)$, where w_2 and w_1 are the shoot dry weight (dw) at the end of the second and first harvest, respectively, and $t_2 - t_1$ is the time in days. Water content was expressed on a dry weight basis. Pulverized dried tissue was used for ion, N, and glycinebetaine determinations. The powder was digested in 70% HClO₄ and concentrated HNO₃ (1:2 v/v) for Na⁺, K⁺ and Ca⁺⁺ determinations or extracted with 1% H₂SO₄ for Cl⁻. The cations were measured with a Corning-EEL flame photometer and Cl⁻ was measured with a Buchler-Cotlove chloridometer. The micro Kjeldahl method was used to measure total N (crude protein) and nonsoluble N (proteins) after extracting the soluble N compounds with trichloroacetic acid. Soluble N was calculated as the difference between total N and nonsoluble N. Glycinebetaine was determined according to the modified periodide method developed by Grieve and Gratten (1983).

Data were subjected to variance analysis. When significant treatment differences at the 0.05 level of probability were identified, a Duncan-Waller multiple range test was used to separate means (Steel and Torrie 1960).

Since growth and ion accumulation exhibited similar trends in the first and last 2 weeks of the experiment, results are cited only for the second harvest.

Table 1. Influence of NaCl concentrations on shoot relative growth rate (RGR), leaves/stems ratio and water content in *A. barclayana*. The values represent means for 6 plants in each treatment.¹

NaCl (mol/m ³)	Relative Growth Rate		Water content	
	Shoots	Leaves/Stem	Leaves	Stems
	(g/g/day)	(dw/dw)	(g/g dw)	
50	0.073a	1.50c	7.64b	4.68b
100	0.073a	1.94a	9.67a	6.50a
200	0.063b	1.70b	9.45a	6.15a
400	0.029c	1.51c	8.74b	5.05b

¹Values within the same column followed by the same letter were not significantly different by the Duncan-Waller multiple range test ($P > 0.05$).

Results and Discussion

Growth in terms of Relative Growth Rate (RGR) was vigorous at 50, 100, and 200 mol/m³ NaCl but declined markedly at 400 mol/m³, which is equivalent to the salinity of 80‰ seawater (Table 1). These results point to a salt tolerance lower than that of *A. lentiformis* and *A. occidentalis* (Glenn and O'Leary 1984) but similar to that reported for *A. spongiosa* (Storey and Wyn Jones 1979). Plants grown at 100 and 200 mol/m³ (moderate salinity) had the highest leaf to stem ratio and water content (Table 1). The vigorous growth of leaves as compared with stems in these 2 treatments may have been a result of improved shoot water status. Increased water content at moderate salt levels coupled with decreased water content at high levels is widespread among halophytes (Glenn and O'Leary 1984, Storey and Wyn Jones 1979).

Shoot ion concentration (dw basis) was variously affected by salinity (Table 2). In both leaves and stems Na⁺ and Cl⁻ increased significantly as salinity rose to 100 mol/m³ NaCl. While Na⁺ exhibited only slight changes with further increases in salinity, the Cl⁻ concentration showed a very distinct rise when the highest salinity was applied. In contrast, the levels of K⁺ and Ca⁺⁺ dropped significantly in both leaves and stems at the highest salinity. The calculated total ion concentration in the shoots increased significantly from 50 to 100 mol/m³ NaCl, thereafter rising slightly with increasing salinity. This was mainly due to changes in Na⁺ and Cl⁻ levels in shoots. Although Na⁺ and Cl⁻ concentrations obtained at 50 mol/m³ were relatively low, they were higher than levels determined for nonhalophytic agricultural crops such as barley and wheat growing under similar or higher salinities (Greenway and Munns 1980, Termaat and Munns 1986). A tendency to accumulate NaCl has been reported for many other halophytes and is associated with salt tolerance (Greenway and Munns 1980, Storey and Wyn Jones, 1977, Storey and Wyn Jones 1979).

The concentration of nitrogen in the leaves in the various nitrogenous fractions is presented in Table 3 as a percentage of dry weight. Nonsoluble N decreased gradually with rising salinization while soluble N increased up to the moderate salinities, then decreased when 400 mol/m³ was applied. Soluble N amounted to

Table 2. Influence of NaCl concentrations on ion content of leaves and stems of *A. barclayana*. The values represents means for 6 plants in each treatment.¹

NaCl (mol/m ³)	Ion concentration								Total ions in shoot
	Na ⁺		K ⁺		Ca ⁺		Cl ⁻		
	leaves	stems	leaves	stems	leaves	stems	leaves	stems	
	(mol/kg dw)								
50	3.31c	1.85b	0.93a	1.18a	1.20a	1.11a	1.84d	1.44b	6.59
100	5.34b	2.88a	0.91a	1.23a	1.31a	1.14a	2.25c	2.02a	8.93
200	5.71a	2.59a	1.03a	1.20a	1.15a	0.90a	2.63b	2.03a	9.11
400	5.88a	2.43a	0.82b	1.10b	0.65b	0.63b	3.67a	2.24a	9.29

¹Values within the same column followed by the same letter were not significantly different by the Duncan-Waller multiple range test ($P > 0.05$).

Table 3. Influence of NaCl concentrations on non soluble N (protein) and soluble N and glycinebetaine N in leaves of *A. barclayana*. The values represent means for 6 plants in each treatment.¹

NaCl (mol/m ³)	N concentration			Glycinebetaine (% dw)
	Nonsoluble	Soluble	Total	
50	2.43a	1.16b	3.60a	0.49b
100	2.33a	1.39a	3.72a	0.54a
200	2.19b	1.32a	3.51a	0.53a
400	1.92c	1.16b	3.08b	0.54a

¹Values within the same column followed by the same letter were not significantly different by the Duncan-Waller multiple range test ($P>0.05$).

32.2% of total N at the lowest salt treatment and about 37.5% of total N at the other salinities.

The highest total N (nonsoluble and soluble), equivalent to 23.25% crude protein ($\% N \times 6.25$), was measured under 100 mol/m³ NaCl. The lower figure obtained at the highest salinity, 83% of the maximum level, reflects the drop in soluble and nonsoluble N. In general the levels of total N reported are higher than figures demonstrated for pea hay and barley grains (Arieli et al. 1989) but are supported by other data obtained for halophytes (Malcolm 1969, Pasternak 1985).

The glycinebetaine content was 11% at salinity levels of 100, 200, 400 mol/m³ NaCl as compared with 50 mol/m³ NaCl (Table 3). The order of magnitude of the concentrations obtained is the same as reported by Storey et al. 1977 for other *Atriplex* species.

The proportion of glycinebetaine N to total N increased significantly ($P<0.05$) with the increase in salinity (Fig. 1). This result

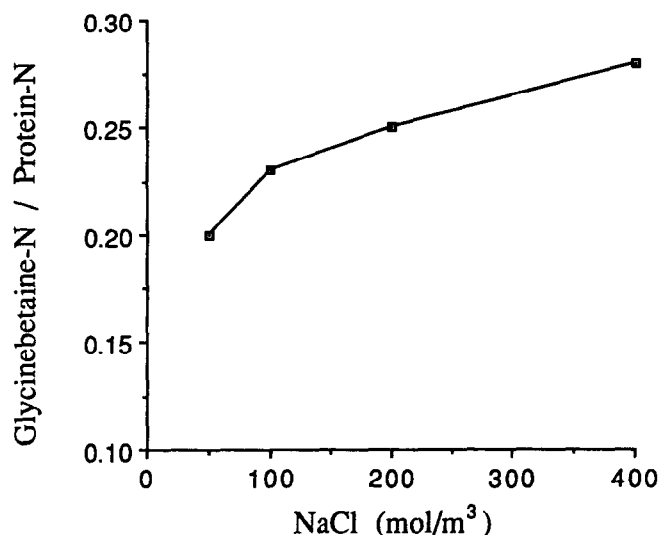


Fig. 1. Glycinebetaine N/total N ratio in leaves of *A. barclayana* grown at various salinities.

may be explained by the stability of the glycinebetaine component under high salinity and the concomitant decline in the other soluble and nonsoluble nitrogen components.

Conclusions

Atriplex barclayana is a highly salt tolerant species capable of growing at salinity approaching that of seawater although at reduced rates. Since the plants accumulate salts at low salinities the feed value of *A. barclayana* irrigated with mildly saline water or grown on mildly saline soil may be expected to be low. The main contribution of *A. barclayana* fodder, particularly if grown at low and moderate salinities, could be its nitrogen content, which is relatively high. Soluble N, which is not efficiently utilized by ruminants, demonstrated an increase at moderate salinities, however, this increase was not correlated with increase in glycinebetaine.

Literature Cited

- Arieli, D., E. Naim, R.W. Benjamin, and D. Pasternak. 1989. The effect of feeding saltbush and sodium chloride on energy metabolism in sheep. *Anim. Prod.* 49:452-457.
- Benjamin, R.W., E. Oren, and E. Katz. 1987. Apparent digestibility of *Atriplex barclayana* and the nitrogen balance of sheep consuming this shrub, p 7-35. *In: Analysis of animal nutrition in shrub-grassland grazing systems with special reference to semiarid Africa*. Prog. Rep. BGUN-ARI-41-87. The Inst. for Appl. Res. Ben-Gurion Univ. of the Negev. Beer-Sheva, Israel.
- Glenn, E.P., and J.W. O'Leary. 1984. Relationship between salt accumulation and water content of dicotyledonous halophytes. *Plant Cell Envir.* 7:253-261.
- Greenway, H., and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* 31:149-190.
- Grieve, C.M., and S.R. Gratten. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and Soil* 70:303-307.
- Hassan, N.I., and H.M. Abdel Aziz. 1979. Effect of Barley supplementation on the nutritive value of saltbush, *Atriplex nummularia*. *World Rev. Animal Prod.* 15:47-55.
- Hoagland, D.R., and D.I. Arnon. 1950. The water culture method for growing plants without soil. *Calif. Agr. Exp. Sta. Circ.* 347:1-32.
- Malcolm, C.V. 1969. Use of halophytes for forage production on saline Westlands. *J. Australian Inst. Agr. Sci.* 35:38-49.
- O'Leary, J.W. 1984. The role of halophytes in irrigated agriculture. p. 285-300. *In: R.C. Staples (ed.) Salinity tolerance in plants: strategies for crop improvement*. John Wiley and Sons, New York, N.Y.
- O'Leary, J.W. 1985. Saltwater crops. *Chemtech* 15:562-566.
- Pasternak, D., A. Danon, and A. Aronson. 1985. Developing the seawater agriculture concept. *Plant and Soil* 89:337-348.
- Steel, R.G.D., and J.H. Torrie. 1960. *Principle and procedure of statistics*. McGraw-Hill, New York, N.Y.
- Storey, R., N. Ahmad, and R.G. Wyn Jones. 1977. Taxonomic and ecological aspects of the distribution of Glycinebetaine and related compounds in plants. *Oecologia* 27:319-332.
- Storey, R., and R.G. Wyn Jones. 1977. Quaternary ammonium compounds in plants in relation to salt resistance. *Phytochemistry* 16:447-453.
- Storey, R., and R.G. Wyn Jones. 1979. Responses of *Atriplex spongiosa* and *Sueda menziesii* salinity. *Plant Physiol.* 63:156-162.
- Termaat, A., and R. Munns. 1986. Use of concentrated macronutrient solution to separate osmotic from NaCl-specific effect on plant growth. *Australian J. Plant Physiol.* 13:529-522.
- Wiggins, I. 1980. *Flora of Baja California*. Stanford Univ. Press, Stanford, Calif.