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# Rapid Osmotic Adjustment by a Succulent Halophyte to Saline Shock[1]

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## ABSTRACT

**The objective of this research was to measure the short term osmotic adjustment of *Salicornia europaea* L. ssp. *rubra* (A. Nels) Breitung when suddenly exposed to 100 millimolar NaCl. Plants were grown hydroponically, shocked with 100 millimolar NaCl added to the culture solution, and stem tips analyzed for free inorganic ions and small organic molecules at intervals up to 72 hours. In the first 2 hours, the calculated leaf osmoticum showed a net increase of 158.8 millimolar most of which was free $Mg^{2+}$ (+135.3 millimolar). Total sugars increased almost 5-fold by the 6th hour, enough to provide sufficient osmoticum for the cytoplasm if only partially confined there. By 24 hours, all measured osmotica had decreased except $Na^+$, $Mg^{2+}$, $Cl^-$, and proline, with the net increase being 208 millimolar. By 72 hours, there was a net gain of 356 millimolar in osmotica of the stem tips, due to $Na^+$ (+233.3 millimolar), $Cl^-$ (+306.7 millimolar), and a small increase in sugar and proline (+3.5 millimolar), with all other osmotica decreasing in concentration. Compatible osmotica did not change sufficiently to account for osmotic balance between vacuole and cytoplasm; consequently, there must have been a reapportionment of osmotica within the cell in the short time duration of this experiment.**

Most succulent terrestrial halophytes avoid physiological drought by absorbing sufficient ions to maintain a favorable water potential gradient between the soil solution and the plant, *i.e.* these halophytes adjust osmotically by the absorption of ionic solutes (9, 13, 31). Greenway (12) referred to plants with this mode of salt tolerance as salt-accumulating halophytes. Leaf osmotic potentials are commonly −2 to −5 MPa (9), although Kuramato and Best (23) reported $\psi_\pi$ more negative than −5 MPa for *Salicornia europaea* growing in a coastal salt marsh, while Harward and McNulty (19) reported $\psi_\pi$ more negative than −11 MPa for *S. europaea* ssp. *rubra* collected from inland playas, and Hansen and Weber (17) measured $\psi_\pi$ more negative than −14 MPa for *Salicornia pacifica* var *utahensis* collected in the field. Such high concentrations of ions would severely affect metabolism if present in the cytoplasm (8, 9, 14, 31), which has led to the generally accepted concept that the extraneous ions are compartmentalized into the vacuole. At present, evidence for the sequestering of excess ions by the vacuole is circumstantial, but strongly supportive (16, 18, 20, 28, 32). Compartmentation of extraneous ions in the vacuole would necessitate an equal amount of compatible solutes in the cytoplasm to balance the water potential. Small organic molecules that could act alone as osmotica in the cytoplasm without disruption of metabolic reactions have been reported (4, 10, 21, 24, 26, 27). Another possible mechanism for cytoplasmic osmotic adjustment is a general increase in osmotica as suggested by Greenway and Sims (15) or an increase in two or more organic solutes together (10, 24).

The experimental design used by most investigators of salt tolerance mechanisms in terrestrial halophytes has been to grow them in ranges of salinity, followed by analyses of potential osmotica. There is little experimental evidence for the changes that occur in the leaves of halophytes when subject to salinity shock, *i.e.* a sudden increase in exogenous salt concentration (13). The objective of this study was to investigate the short term osmotic adjustment by a succulent, $C_3$, salt-accumulating halophyte when subjected to a saline shock, measuring the degree of osmotic adjustment achieved, and ascertaining which solutes might be responsible.

## MATERIALS AND METHODS

Seeds of *Salicornia europaea* L. ssp. *rubra* (A. Nels.) Breitung were collected locally and germinated in vermiculite wet with Hoagland solution. When about 2 cm high, the seedlings were transferred to large hydroponic tanks containing Hoagland solution plus 10 mM NaCl. The tanks were continuously aerated and the culture solution was changed at 2-week intervals. Sixty d after transplanting, the plants were still vegetative, profusely branched, and about 30 cm tall. At this time (time 0), 4 h after sunrise, 1- to 2-cm samples were taken from the terminal ends of the succulent stems. NaCl was then added to the culture solution to bring it to 100 mM NaCl, and samples were again taken at 2, 4, 6, 8, 12, 16, 20, 24, 48, and 72 h after the addition of salt. Sampling was terminated at 72 h, because previous experiments indicated that 96% of the osmotic adjustment had occurred by this time, complete adjustment required 195 h. The samples were all taken from the terminal ends of the stems to insure similar ages and stages of development of the tissues. They were rapidly rinsed in distilled $H_2O$, damp dried, sealed in bottles, and frozen in liquid $N_2$.

**Extraction and Analysis.** Osmotic potential of the terminal shoots was measured cryoscopically with an osmometer. Pressure extraction of the frozen and thawed shoots did not provide satisfactory reproducible results, so the following procedure was designed and proved to be very reproducible. Five g of frozen shoots were ground with a pestle in a prechilled mortar into a fine powder. The powder was allowed to thaw in a centrifuge tube which was then centrifuged 15 min at 10,000 relative centrifugal force. Two ml of the supernatant was used for the cryoscopic measurement of osmotic potential. The supernatant was also used for the analyses of inorganic ions and organic molecules, diluted as necessary. Sodium and potassium were measured by flame emission spectroscopy, calcium and magnesium by atomic absorption spectroscopy, and chlorides with a chloridometer. Sulfates were measured turbidometrically according to the method of DeJorge *et al.* (5), and phosphates by the

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procedures of Fiske and Subbarow (7). Total carboxylic acids were separated and determined by titration to pH 7.0 with standard KOH (22). Qualitative analysis of the carboxylic acid extract by paper chromatography revealed that malate and soluble oxalate were most abundant; consequently, total organic acids were calculated as dicarboxylates. Total amino acids were separated as described by Jones, Osmond, and Turner (22), evaporated to dryness, dissolved in water, and titrated with standard NaOH. Total free sugar analyses also followed the procedure of Jones, Osmond, and Turner (22). Proline was measured according to the procedure of Bates *et al.* (3), and betaine according to the procedure of Storey and Wyn-Jones (26). All analyses were made in triplicate from separate aliquots of the sample.

## RESULTS AND DISCUSSION

The final results of the analyses are presented in Table I. The culture solution in which the plants were grown contained 10 mM NaCl as they do not grow well at lower NaCl concentrations. Even at that relatively low level of salt, the plants accumulated considerable $Na^+$ and $Cl^-$, characteristic of salt-accumulating halophytes (12, 33). Consequently, these two ions accounted for almost 25% of the total osmotic potential before the 100 mM NaCl treatment was initiated (Figs. 1 and 2). The addition of 100 mM NaCl to the culture solution increased the measured osmotic potential of the solution from 30 to 198 mOsm; thus, the saline shock amounted to 168 mOsm.

Two h after the addition of the NaCl, the measured leaf osmotica showed a net increase of 158.8 mM (25%), and there was less than 1% loss in water content (Table II). Most of the increase was due to the large increase in free $Mg^{2+}$ (+135.3 mM), although all inorganic ions except $PO_4^{3-}$ showed some increase, particularly $Cl^-$ (+28.2 mM) and $SO_4^{2-}$ (18.1 mM) (Figs. 1 and 2). Except for total sugars which doubled in concentration (+13.1 mM), the organic molecules mostly decreased in concentration or showed little change (Fig. 3). Organic acids decreased 18 mM (−18%), amino acids 16.1 mM (−52%), and glycinebetaine 9.1 mM (−40%). The increase in free $Mg^{2+}$ could have occurred at any time in the first 2 h and thus could have been a rapid response to the salinity shock. If most of the $Mg^{2+}$ had been limited to the cytoplasm, it could have provided sufficient osmotic potential to prevent water stress in the cytoplasm. Only 10% of the magnesium in leaf tissue is bound in Chl (25), leaving most of the magnesium as free ions or in a labile, readily dissociable state, which could account for the rapid increase in free $Mg^{2+}$, presumably as a shock response. The concentration of $Mg^{2+}$ in the leaf tissue, in excess of 100 mM, was high when compared to that of glycophytes, which are usually considerably less than 50 mM. However, high concentrations of $Mg^{2+}$ have been reported for a variety of halophytes and often exceed the concentration of $K^+$ (*e.g.* species of Salicornia, Suaeda, Limonium, Armeria) (11, 27). Albert and Popp (2) maintain that concentrations of $Mg^{2+}$ exceeding that of $K^+$ are found only in plants from saline habitats.

There was considerable fluctuation in the concentration of organic molecules in the first 12 h, indicating a broad disruption of metabolic activities (Fig. 3). This was probably a result of the salinity shock treatment rather than a diurnal cycle, as the treatment was not initiated until 4 h after sunrise to separate the effects of the salinity shock from those of the initiation of photosynthesis. The concentration of total sugars showed the greatest change, increasing from 12.9 to 63 mM in 6 h. If only 25% of the increased amount of sugar was limited to the cytoplasm (estimated at 5% of the cell volume [9]), it would be more than enough osmoticum to compensate for the salinity shock. Although sugars are not usually considered to be important osmotica in terrestrial halophytes (10), they may have contrib-

Table I. *Effect of 100 mM Salinity Shock on the Concentrations of Selected Free Ions and Small Organic Molecules in the Shoot Tips of S. europaea*

Results are given in mmols/L of extracted plant sap (mM ± SE, $n = 3$; ND, not determined).

| Time | $Na^+$ | $K^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $Cl^-$ | $SO_4^{2-}$ | $PO_4^{3-}$ | Organic Acids | Amino Acids | Sugars | Glycine-betaine | Proline | Total | Measured $\psi_\pi$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *h* | | | | | | *mM* | | | | | | | | *mOsm* |
| 0 | 104.3 ± 13.2 | 116.9 ± 11.1 | 102.7 ± 4.1 | 23.7 ± 4.3 | 64.8 ± 3.1 | 91.6 ± 7.4 | 35.7 ± 4.3 | 98.0 ± 4.7 | 30.7 ± 3.1 | 12.9 ± 2.3 | 23.4 ± 1.02 | 0.1 ± 0.01 | 704.2 | 744 |
| 2 | 112.3 ± 8.7 | 119.1 ± 4.5 | 238.0 ± 6.4 | 25.5 ± 4.1 | 93.0 ± 2.4 | 109.7 ± 5.2 | 31.0 ± 3.6 | 80.0 ± 7.3 | 14.6 ± 2.4 | 26.0 ± 1.4 | 14.3 ± 1.28 | 0.1 ± 0.02 | 863.0 | 753 |
| 4 | 127.2 ± 7.0 | 125.0 ± 6.2 | 198.8 ± 6.2 | 28.3 ± 6.7 | 117.6 ± 2.3 | 111.3 ± 6.0 | 34.8 ± 2.1 | 79.0 ± 6.2 | 20.0 ± 1.2 | 56.0 ± 3.7 | 9.02 ± 0.97 | 0.2 ± 0.01 | 906.6 | 769 |
| 6 | 133.5 ± 14.3 | 137.0 ± 9.3 | 202.0 ± 5.3 | 22.8 ± 7.2 | 117.2 ± 5.1 | 106.3 ± 4.3 | 35.5 ± 6.7 | 88.0 ± 5.8 | 25.3 ± 5.4 | 63.0 ± 4.2 | 24.5 ± 0.71 | 0.5 ± 0.02 | 955.1 | 773 |
| 8 | 148.3 ± 5.6 | 104.7 ± 3.5 | 169.9 ± 4.8 | 28.7 ± 9.8 | 149.8 ± 4.8 | 90.4 ± 4.9 | 36.5 ± 8.3 | 83.0 ± 6.1 | 13.2 ± 3.8 | 56.0 ± 5.0 | 21.7 ± 0.32 | 0.8 ± 0.03 | 902.6 | 794 |
| 12 | 154.7 ± 18.4 | 104.8 ± 4.0 | 185.0 ± 16.0 | 27.8 ± 5.8 | 152.1 ± 3.9 | 91.7 ± 5.8 | 31.6 ± 3.5 | 76.0 ± 4.3 | 12.2 ± 1.3 | 28.6 ± 3.1 | 19.8 ± 0.68 | 1.0 ± 0.01 | 865.1 | 751 |
| 16 | 175.9 ± 11.2 | 112.2 ± 7.3 | 159.0 ± 11.4 | 22.7 ± 6.7 | 184.4 ± 7.0 | 84.1 ± 4.7 | 28.3 ± 4.8 | 60.0 ± 8.4 | 9.8 ± 2.8 | 15.9 ± 2.8 | ND | ND | 852.3 | 805 |
| 20 | 176.7 ± 6.2 | 100.5 ± 6.8 | 160.0 ± 5.5 | 22.8 ± 8.2 | 194.9 ± 6.8 | 89.1 ± 10.0 | 30.6 ± 7.6 | 73.0 ± 3.2 | 7.5 ± 1.4 | 14.3 ± 1.3 | ND | ND | 869.4 | 810 |
| 24 | 208.2 ± 13.0 | 98.5 ± 3.2 | 159.0 ± 13.0 | 21.1 ± 4.3 | 195.0 ± 4.7 | 77.4 ± 7.6 | 32.1 ± 3.2 | 61.0 ± 8.1 | 25.4 ± 2.5 | 12.9 ± 4.2 | 21.4 ± 0.97 | 1.0 ± 0.03 | 912.4 | 845 |
| 48 | 267.7 ± 24.1 | 92.6 ± 2.0 | 139.0 ± 8.9 | 20.0 ± 3.6 | 295.9 ± 3.6 | 67.6 ± 3.4 | 25.4 ± 2.1 | 46.0 ± 2.1 | 7.3 ± 1.8 | 10.4 ± 2.9 | 23.6 ± 0.84 | 0.2 ± 0.01 | 995.3 | 900 |
| 72 | 337.6 ± 19.3 | 73.8 ± 5.3 | 89.3 ± 7.6 | 12.3 ± 3.4 | 371.5 ± 5.0 | 59.8 ± 4.6 | 20.0 ± 4.0 | 40.0 ± 4.3 | 26.1 ± 3.1 | 16.3 ± 2.3 | 15.7 ± 0.67 | 0.2 ± 0.01 | 1060.2 | 919 |

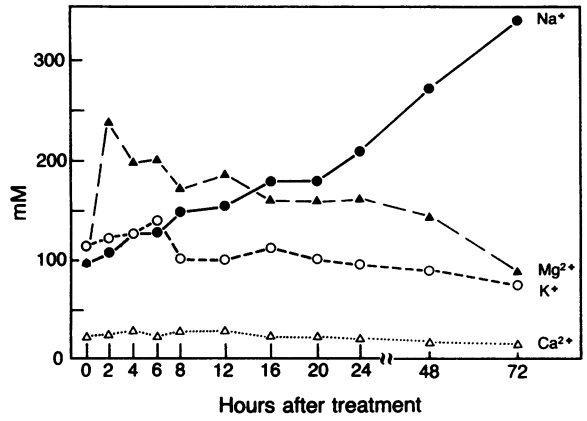


FIG. 1. Concentrations of selected cations in stem tips of *S. europaea* before and for 72 h after adding 100 mM NaCl to the culture solution.

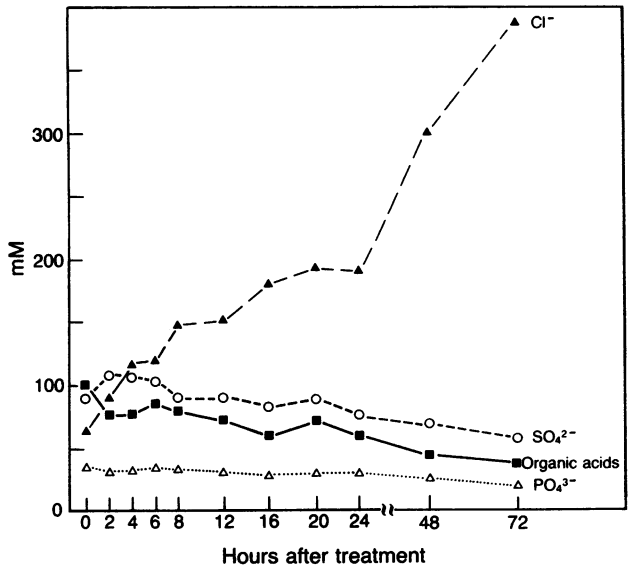


FIG. 2. Concentrations of selected anions in stem tips of *S. europaea* before and for 72 h after adding 100 mM NaCl to the culture solution.

Table II. *Changes in Water and Salt Content Induced by Salinity Shock*

Figures in per cent of wet weight (w/w)

| Sample Hour | Water | Dry Wt | Free Salts (Calculated) | Salts (Per Cent Dry Wt) |
|---|---|---|---|---|
| | | | % | |
| 0 | 86.34 | 13.66 | 2.16 | 15.82 |
| 2 | 85.45 | 14.55 | 2.47 | 16.95 |
| 4 | 84.99 | 15.01 | 2.53 | 16.83 |
| 6 | 85.05 | 14.95 | 2.73 | 18.26 |
| 8 | 85.55 | 14.45 | 2.58 | 17.87 |
| 12 | 85.92 | 14.08 | 2.63 | 18.65 |
| 16 | 86.82 | 13.18 | 2.72 | 20.65 |
| 20 | 86.77 | 13.23 | 2.67 | 20.56 |
| 24 | 87.41 | 12.59 | 2.76 | 21.90 |
| 48 | 88.88 | 11.12 | 2.97 | 26.71 |
| 72 | 90.62 | 10.38 | 3.06 | 30.42 |

uted significantly under the rapid osmotic adjustment necessary under the conditions of this experiment.

By the end of 24 h, all measured osmotica had decreased in concentration except $Na^+$ (+103.9 mM), $Mg^{2+}$ (+56 mM), $Cl^-$ (+130.2 mM), proline (+0.9 mM), and total sugars which returned

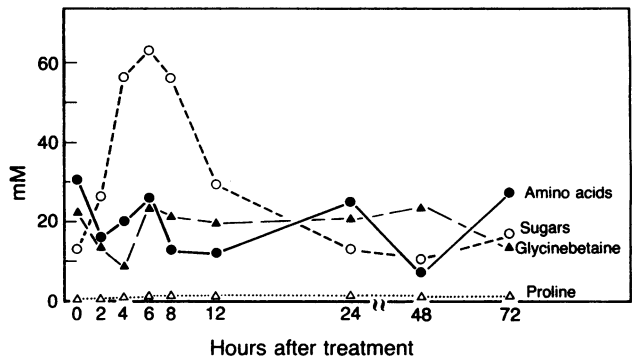


FIG. 3. Concentrations of selected organic molecules in the stem tips of *S. europaea* before and for 72 h after adding 100 mM NaCl to the culture solution.

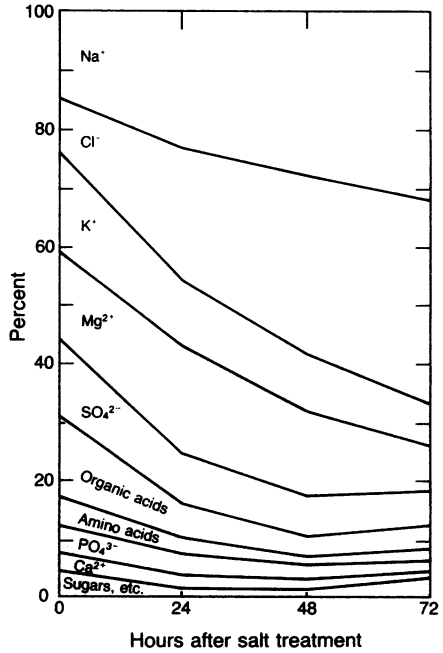


FIG. 4. Changes in the percentage contribution of osmotica to the total calculated osmotic potential at 0, 24, 48, and 72 h.

to their original level (Figs. 1–3). The net total increase was 208 mM, adequate for osmotic balance. The osmotica that were reduced in concentration, decreased from 9% to 38%, and hence this reduction cannot be explained by dilution due to water uptake, which increased only 1% of wet weight in the 24 h (Table II). Between 8 and 24 h, there was a decrease in the total measured osmoticum below the 6-h level (Table I). This was probably due to the diurnal cycle of stomatal closure, and a subsequent increase in water content of the plant (Table II).

Seventy-two h after imposing the salinity shock, $Na^+$ had increased 233.3 mM (+224%) and chloride 306.7 mM (473%), much more than needed to satisfy the 168 mOsm stress (Figs. 1 and 2). Initially, $Na^+ + Cl^-$ accounted for 24% of the calculated osmotic potential and by 72 h these two ions accounted for 67% (Fig. 4). Except for the large increase in $Na^+$ and $Cl^-$ (540 mM) and a small increase in sugar and proline (3.5 mM), all other inorganic ions, organic acids, amino acids, and betaine decreased in concentration (−187.5 mM) (Figs. 1–3), leaving a net total gain of 356 mM (Table I). This supports the generalization that halophytes adjust osmotically by salt accumulation (1, 13). The decrease in some osmotica ranged from 15% to 59% and cannot be entirely accounted for by dilution due to the absorption of water, which increased only 4%, from 86% to 90% of the wet

weight.

Although the osmotic adjustment was almost entirely a function of $Na^+$ and $Cl^-$ absorption, the increase in these ions exceeds the limits of toleration by the cytoplasm (9). Most of the $Na^+$ and $Cl^-$ must have been absorbed into the vacuole as the concentration limits compatible with metabolic activity are probably well below 200 mM (9). As there was no significant increase in any compatible osmotica, the osmotic stabilization of the cytoplasm must have been largely a reapportion of compatible osmotica within the cell, *e.g.* transfer from the vacuole to the cytoplasm. Halophytes are characterized by considerable asymmetry in the distribution of osmotica within their cells (9, 32), particularly between the vacuole and the cytoplasm. The reapportionment of specific ions and molecules between the vacuole and cytoplasm apparently occurs readily in halophytes, accounting in part for their capacity to tolerate large concentrations of extraneous ions within their cells.

These data on extracted leaf sap do not provide information as to the allocation of ions or other osmotica within the cell. None of the potentially compatible osmotica increased sufficiently in concentration to account for adequate osmotic potential of the cytoplasm, even if they were wholly restricted to the cytoplasm. However, if one assumed the cytoplasm to contain a maximum 150 mM each of $Na^+$ and $Cl^-$ (9), 60% of the sugars (30), 20% of the amino acids (29), all of the betaine (16) and proline, and 5% of the organic acids and the other inorganic ions, there would be 345.07 mM osmoticum in the cytoplasm and 717.33 mM in the vacuole. However, crowding the cytoplasmic osmotica into 5% of the cell volume (9) would raise the concentration of osmotica to 759.01 mM, only 6% higher than that of the vacuole, and well within the range of speculative and experimental error.

These results do not explain the mechanism of long term seasonal osmotic adjustment as must occur in the natural habitat of this species. However, they do reveal some of the possibilities by demonstrating the capabilities of a succulent terrestrial halophyte to adjust to saline shock. The rapid increase in free $Mg^{2+}$ was the first response, followed by a 5-fold increase in sugar concentration, either of which could have provided adequate osmoticum to prevent excessive water stress if mostly confined to the cytoplasm. Subsequent adjustment can only be explained by a reapportionment of compatible osmotica within the cells. Such reapportionment would permit the osmotic adjustment in halophytes to occur primarily by the absorption of extraneous ions.

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