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BRIEF COMMUNICATION

The effect of different salts of sodium and potassium on the accumulation of glycinebetaine in *Atriplex prostrata*

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Abstract

Atriplex prostrata was grown for one month in nutrient solutions with NaCl, KCl, Na_2SO_4, and K_2SO_4 (at osmotic potentials of 0, -0.75, -1.00, and -1.50 MPa). Plants treated with K_2SO_4 had less glycinebetaine at -1.0 and -1.50 MPa than those treated with Na^+ salts, probably due to the inhibitory effects of K^+ on glycinebetaine accumulation.

Additional key words: *Chenopodiaceae*, compatible osmotica, halophyte, HPLC, salt tolerance.

Some plant species adjust ionically to their surrounding by taking up Na^+ and Cl^- (Ungar 1991). Sodium ions accumulated by *Hordeum vulgare* and *Atriplex hortensis* are transported from the cytoplasm into the vacuole (Flowers 1975, Jeschke and Stelter 1976, Matoh *et al.* 1987, McCue and Hanson 1990). Because Na^+ is transported into the vacuole, the concentration of ions in the vacuole leave the cytoplasm in a hypotonic state, and to adjust to this differential in osmotic potential the cytoplasmic glycinebetaine concentration increases (Wyn Jones *et al.* 1977).

Glycinebetaine is often localized in the cytoplasm of leaf cells in salt tolerant species of the *Chenopodiaceae* (Hall *et al.* 1978, Matoh *et al.* 1987, Rhodes and Hanson 1993). One of the first studies to assess the role of glycinebetaine in halotolerance compared its accumulation in NaCl stressed versus non-stressed plants (Storey and Wyn Jones 1975). The accumulation of glycinebetaine in shoots of several halophyte species increased directly with their ability to tolerate salinity (Storey and Wyn Jones 1975).

Most studies with halophytes have concentrated on the effects of NaCl on plant growth with only a few investigations evaluating the effects of Na_2SO_4 on the growth of halophytes (Warne *et al.* 1989). Determining the effects of salts other than NaCl are important because many different types of salts occur naturally in soils from different regions of North America (Frey 1966), and the predominant forms of salinity in Canadian prairie soils are SO_4^{2-} salts (Curtain *et al.* 1993).

The purpose of this investigation was to determine the effect of different concentrations of NaCl, KCl, Na_2SO_4 and K_2SO_4 on glycinebetaine accumulation in the salt marsh species *Atriplex prostrata*, which has a cosmopolitan distribution in coastal and inland saline habitats of Africa, Asia, Australia, Europe, and North America (Osmond *et al.* 1980, Gleason and Cronquist 1991).

Atriplex prostrata Boucher ex DC (syn: *A. triangularis* Willd.) plants were grown from seeds collected in a salt marsh at Rittman, Ohio (Wayne County) on 17 November 1995. Medium sized (1.5 - 2.0 mm diameter) seeds were germinated in sand in an incubator with a 12-h photoperiod with an irradiance of 20.0 μmol(photons) $m^{-2} s^{-1}$, 400 - 700 nm and a dark/light temperature of 5/25 °C. Plants were acclimated to

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greenhouse conditions for 2 d and placed in individual square plastic pots (9 × 9 cm) in sand and grown for 15 d (9 October to 24 October) under natural light.

Solutions of NaCl, KCl, Na_2SO_4, and K_2SO_4 dissolved in half strength Hoagland and Arnon's No. 2 solution (Moore 1960) in concentrations corresponding to osmotic potentials of 0.00, and -0.75, -1.00, and -1.50 MPa. To prevent wilting, plants were placed in above solutions with successively lower osmotic potentials every 2 d until the final osmotic potential was reached. Plants were allowed to grow for one month after treatment conditions were reached. There were ten replicates of each of the 16 treatments, and these were randomized weekly.

For glycinebetaine measurements, 0.5 g of whole plant dry tissue was boiled in 10 cm^3 of distilled water for 2-h at 100 °C in a dry heat bath. Samples were diluted with a 50 mM potassium dihydrogen phosphate buffer adjusted to pH 4.6. The sample was filtered using a 0.45 μm membrane filter, and then used directly to measure glycinebetaine with a *Hewlett Packard HP 1050* molecular 3D HPLC (Boise, USA). Separations were performed on a 250 × 4 mm stainless steel column packed with 10 μm *Nucleoside 100-10SA* at a flow rate of 1.2 cm^3 min^{-1}.

When significant differences ($P < 0.05$) were found among treatments using analysis of variance (*ANOVA*), a Fisher's LSD post hoc analysis was used to demonstrate whether or not plants treated with the potassium salts accumulated significantly more or less glycinebetaine than those treated with sodium salts.

The ANOVA analysis indicated that there was a significant effect of salt type ($F = 24.35$, $P < 0.001$), concentration ($F = 16.87$, $P < 0.001$), and salt type × concentration interaction ($F = 7.63$, $P < 0.001$) on the glycinebetaine content of *Atriplex prostrata* plants. A significant interaction occurred because lowering of the osmotic potential did not cause a concomitant decrease in betaine for some salt types.

Glycinebetaine concentration of plants in the -0.75 MPa treatments was highest in NaCl and lowest in KCl (Fig. 1). At an osmotic potential of -1.00 MPa, a higher glycinebetaine concentration was found in the NaCl treatment than in the Na_2SO_4 treatment (Fig. 1). Plants grown in K^+ salts were significantly lower in glycinebetaine than Na^+ treated plants at -1.00 MPa, with K_2SO_4 treated plants having the lowest glycinebetaine concentration. A higher glycinebetaine concentration was found in the Na^+ than in the K^+ treated plants at -1.50 MPa. Plants treated with KCl had a glycinebetaine concentration that was lower than in Na^+ treated plants, and the K_2SO_4 treated plants had the lowest glycinebetaine concentration (Fig. 1). Sodium chloride treated plants at -0.75 and -1.00 MPa had higher

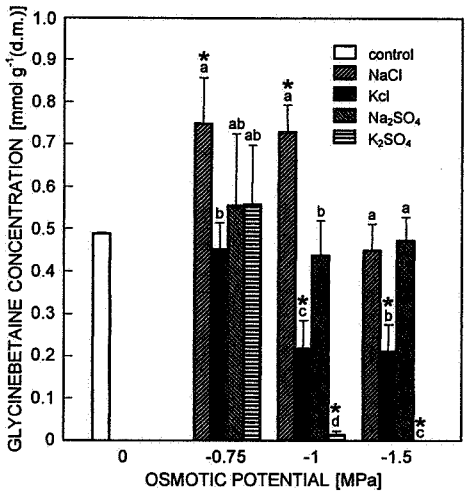

Fig. 1. Glycinebetaine concentration in *A. prostrata* plants treated with NaCl, KCl, Na_2SO_4, and K_2SO_4 at osmotic potentials of 0.00, -0.75, -1.00, and -1.50 MPa for 30 d. Means ± SE, $n = 10$. Different letters denote significant differences ($P < 0.05$) within an osmotic potential, and an asterisk indicates a significant difference from control using the Fisher's LSD post-hoc test.

glycinebetaine concentration than the control (Hoagland's solution only), while plants treated with KCl and K_2SO_4 at -1.00 and -1.50 MPa had a significantly lower glycinebetaine concentration than that of the control (Fig. 1). The inability to adjust to salt stress by accumulating glycinebetaine in the cytoplasm as an osmoticum or osmoprotectant may explain why plants grown in K^+ salts produced less biomass and had lower survival percentages at higher salt concentrations than plants treated with Na^+ salts (Egan and Ungar 1998). It is possible that plants exposed to K^+ salts, in contrast to Na^+ salt treatments, were not able to transport K^+ into the vacuoles, causing a specific ion toxicity in the cytoplasm that inhibited both growth and glycinebetaine production.

From these results, we can conclude that high concentrations of Na^+ salts increased glycinebetaine accumulation in plants, but that K^+ salts were inhibitory to the production of glycinebetaine at all salt concentrations. Glycinebetaine accumulation may play an important role in maintaining osmotic balance or may act as an osmoprotectant in *Atriplex prostrata* plants treated with Na^+ salts.

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