

Communication

The Effect of Sodium Chloride on Solute Potential and Proline Accumulation in Soybean Leaves¹

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ABSTRACT

Two cultivars of soybean (*Glycine max* [L.] Merr.) were grown in solution with up to 100 millimolar NaCl. Leaf solute potential was -1.1 to -1.2 megapascals in both cultivars without NaCl. At 100 millimolar NaCl leaf solute potential was -3.1 to -3.5 megapascals in Bragg and -1.7 megapascals in Ransom. The decrease in solute potential was essentially proportional to the concentration of NaCl. In both salt susceptible Bragg and salt semitolerant Ransom, leaf proline was no more than 0.4 micromole per gram fresh weight at or below 20 millimolar NaCl. At 40 and 60 millimolar NaCl, Bragg leaf proline levels were near 1.2 and 1.9 micromoles per gram fresh weight, respectively. Proline did not exceed 0.5 micromole per gram fresh weight in Ransom even at 100 millimolar NaCl. Proline accumulated in Bragg only after stress was severe enough to induce injury; therefore proline accumulation is not a sensitive indicator of salt stress in soybean plants.

With the solute potential of a nutrient solution (about -0.07 MPa) being decreased nearly 0.05 MPa for each 10 mM increase in NaCl, leaves of plants growing in solutions with added NaCl would be expected to respond with lower solute potentials (6, 10). Various solutes could be involved, including ions of the salt. Although leaf solute potentials need not be closely related to growth, if leaf solute potentials did not keep pace with solution solute potentials, growth could be reduced. On the other hand, low leaf solute potentials might be involved directly or indirectly in lowering growth or causing other symptoms of injury.

The accumulation of proline in a wide variety of both halophytes and nonhalophytes from exposure to various stresses and possible ways proline might be involved in adaptive responses have been reviewed (1). Drought-stressed soybean leaves did not accumulate proline without prolonged wilting either in a chamber (11) or the field (12). Bhaskaran *et al.* (3) found no correlation between proline level and stress tolerance in cultured sorghum cells exposed to low water potentials from PEG and concluded proline increase in their system was an incidental consequence of rather than an adaptive response to stress. Hanson and Nelsen (7) suggested that proline accumulation is merely a symptom of injury. Greenway and Munns (6), although not accepting the latter view because of the way in which the stress was imposed, presented evidence that the role of proline is related to survival rather than to growth maintenance.

Roeb *et al.* (9) followed the proline content of various parts of salt sensitive Jackson and salt semitolerant Lee cultivars of

soybean for up to 7 d exposure to one concentration (75 mM) of NaCl in solution culture. They also measured plant transpiration and the Cl^- , Na^+ , ABA, and cytokinin contents of plant parts.

The present study was undertaken to clarify the relationships among salt tolerance, leaf solute potential, and the capacity to accumulate proline in two cultivars of soybean, salt sensitive Bragg and salt semitolerant Ransom. Our results will be compared with those of Roeb *et al.* (9).

MATERIALS AND METHODS

Growth Conditions. Seeds of soybean (*Glycine max* [L.] Merr. cvs Bragg and Ransom) were germinated in sand. Hoagland No. 1 solution (8) was applied initially, with demineralized water used later as necessary. Seven d after sowing uniform plants were transferred to Mason jars. Each jar was wrapped with aluminum foil and contained 850 ml of continuously aerated Hoagland solution, which was renewed every 2nd d. The plants were grown in a chamber. The photoperiod was 16 h of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (400–700 nm) photon flux density from a mixture of fluorescent and incandescent lights, with $27 \pm 1^\circ\text{C}$ air temperature and 70% RH. The dark period had $20 \pm 1^\circ\text{C}$ air temperature and 70% RH.

Treatments. After 7 d in jars, salinity treatments were begun by adding NaCl to the basal nutrient solution. Three levels, 0, 10, and 100 mM constituted a first experiment. Stepwise additions were used to reach 100 mM with reduced osmotic shock. The first two steps were 10 mM d^{-1} ; others were 20 mM d^{-1} . Each treatment was replicated six times. Plants were randomly distributed, with positions changed daily. The third (mature) and sixth (young) leaves, counting from the first trifoliate leaf, were harvested 14 d after beginning treatment. Because of results obtained, 0 and intermediate levels of 20, 40, and 60 mM NaCl were used in a second experiment. The only other difference was that all steps were 20 mM d^{-1} . Plants were exposed to final levels of 10, 20, 40, 60, or 100 mM NaCl for 14, 14, 13, 12, and 9 d, respectively.

Solute Potential Measurement. Two sets of three 6 mm diameter discs, one from each leaflet, were punched from each designated leaf. Sets were wrapped with aluminum foil, sealed in polyethylene tape, and frozen. Using Wescor C-52 thermocouple hygrometers calibrated with NaCl solutions and an HR-33 microvoltmeter in the dew point mode, a mean of three successive measurements was obtained for each thawed disc. These were used to obtain a mean for each set.

Proline Measurement. Immediately after removing discs for solute potential measurements, duplicate 500 mg portions of leaf were homogenized, each in 10 ml of 3% sulfosalicylic acid, and filtered through Whatman No. 2 filter paper. Proline was estimated spectrophotometrically using the ninhydrin method (2). Purified proline was used for standardization.

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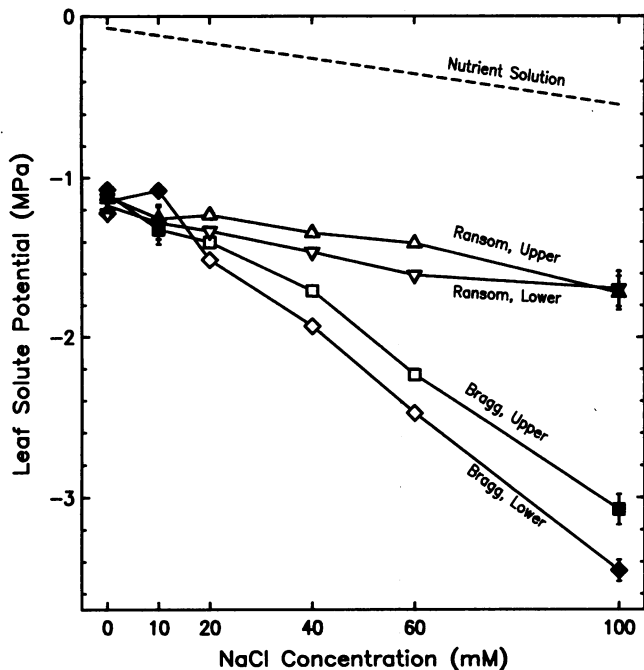


FIG. 1. Solute potentials of lower (third trifoliolate) and upper (sixth trifoliolate) leaves from plants of Bragg and Ransom soybeans grown in nutrient solution supplemented with various levels of NaCl. Plants were exposed to final levels of 10, 20, 40, 60, or 100 mM NaCl for 14, 14, 13, 12, and 9 d, respectively. Solid symbols: experiment 1; open symbols: experiment 2. Error bars show SE. Where unseen, error bars are smaller than symbols.

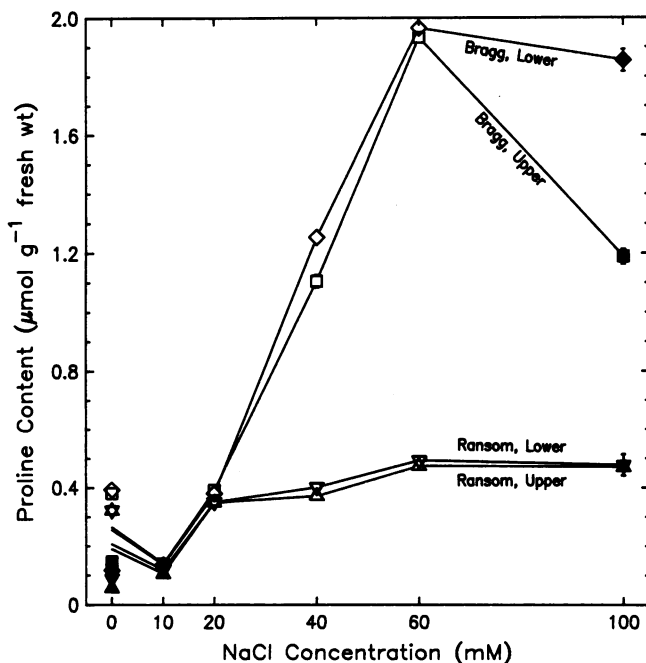


FIG. 2. Proline contents of lower (third trifoliolate) and upper (sixth trifoliolate) leaves from plants of Bragg and Ransom soybeans grown in nutrient solution supplemented with various levels of NaCl. Plants were exposed to final levels of 10, 20, 40, 60, or 100 mM NaCl for 14, 14, 13, 12, and 9 d, respectively. Solid symbols: experiment 1; open symbols: experiment 2. Lines begin at means of means for both experiments. Error bars as for Figure 1.

RESULTS

Solute potentials of leaves from plants grown without NaCl were approximately the same for both cultivars (Fig. 1). With increasing salinity, solute potentials decreased. In Ransom, the decreases nearly paralleled the drop in solution potentials; however, in Bragg, the decreases were much greater.

Without NaCl, free proline appeared to be slightly less in Ransom than in Bragg (Fig. 2). Salinity yielded relatively small increases in proline in Ransom and, at 10 to 20 mM, in Bragg; but, at 40 mM NaCl and beyond, much higher levels were found in Bragg.

The proline content of Ransom increased but slightly with added NaCl (Fig. 2). We cannot explain the consistent differences in proline content of controls for the two experiments; however the levels were low and of little consequence. The important result is the large jump in proline content between 20 and 40 mM NaCl that occurred in Bragg (Fig. 2). The highest proline content found would have contributed no more than 0.005 MPa toward lowering the solute potential.

DISCUSSION

Roeb *et al.* (9) and we found comparable concentrations of proline in the lower trifoliolate leaves of unstressed soybean plants; and, in plants stressed with 75 mM NaCl, their semitolerant cultivar, Lee, had only a little more proline than Ransom at 60 or 100 mM NaCl; but their sensitive cultivar, Jackson, had approximately twice as much proline as Bragg. Moreover, their upper trifoliolate leaves showed hardly any elevation in proline level, while we found little difference between lower and upper leaves (Fig. 2). Besides using different cultivars, their maximum time of exposure was 7 rather than 14 d, their day and night temperatures were 4°C lower, their days were 4 h shorter, and their photon flux density was less. Their plants grew much more slowly as 22 d had elapsed before the second trifoliolate leaves were expanded.

The drop in the solute potential of the leaves of NaCl-treated plants may result from water loss or an increase in dissolved solutes or a combination of both. Much of any increase in dissolved solutes may be from uptake of NaCl (10); but, in those salt tolerant plants that efficiently exclude NaCl from stems and leaves, such as Ransom soybean, other ions or compounds would also have to be involved. Proline might play such a role; however the highest concentration of proline, in Bragg, would have reduced leaf solute potential less than 0.005 MPa. Although proline accumulation was correlated with the solute potential of leaves from salt stressed barley plants, the contribution of proline to leaf solute potential was insignificant (4). Mineral analyses of leaves from plants exposed to 100 mM NaCl, to be presented in detail elsewhere, showed high levels of Na⁺ and Cl⁻ (15–20 mg Na, 45–80 mg Cl g⁻¹ dry weight) in Bragg and much lower levels (5–10 mg Na, 17–25 mg Cl g⁻¹ dry weight) in Ransom. Considered with the percentages of dry weight and without attempting to partition among cell wall, cytoplasmic, and vacuolar regions, the Na⁺ and Cl⁻ might have lowered solute potentials by about 1.0 to 1.6 MPa in Bragg but only 0.4 to 0.6 MPa in Ransom. Thus, these ions could account for most of the solute potential drop in Bragg and all, in Ransom.

Chu *et al.* (5) reported much less proline accumulation in leaves of barley plants with roots in solutions whose solute potentials were reduced by Na₂SO₄ and NaCl than by PEG or MgCl₂. Increased Na⁺ may account for the lower concentrations of proline found in Bragg at 100 as compared with 60 mM NaCl (Fig. 2).

The accumulation of proline in plants exposed to identical salt stresses appears to be cultivar specific in soybean. Proline levels in stressed plants were inversely correlated with capacity to

withstand salinity stress.

Chlorosis and injury were apparent in Bragg plants exposed to 40 mM or more of NaCl. Proline accumulation was associated with this severe damage and did not occur otherwise. Therefore, we may conclude that proline is neither a sensitive indicator of salinity stress nor of protective value. Any benefit toward survival is not obvious. This disagrees with the suggestion (6) that proline may have an adaptive role related to survival and not be just a symptom of injury.

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