

# Growth, Water Relations, and Accumulation of Organic and Inorganic Solutes in Roots of Maize Seedlings during Salt Stress<sup>1</sup>

Humberto González Rodríguez<sup>2</sup>, Justin K. M. Roberts, Wayne R. Jordan, and Malcolm C. Drew\*

Department of Soil and Crop Sciences (H.G.R., W.R.J.), and Department of Horticultural Sciences (M.C.D.), Texas A&M University, College Station, Texas 77843; and Department of Biochemistry, University of California, Riverside, California 92521 (J.K.M.R.)

Seedlings of maize (*Zea mays* L. cv Pioneer 3906), hydroponically grown in the dark, were exposed to NaCl either gradually (salt acclimation) or in one step (salt shock). In the salt-acclimation treatment, root extension was indistinguishable from that of un-salinized controls for at least 6 d at concentrations up to 100 mM NaCl. By contrast, salt shock rapidly inhibited extension, followed by a gradual recovery, so that by 24 h extension rates were the same as for controls, even at 150 mM NaCl. Salt shock caused a rapid decrease in root water and solute potentials for the apical zones, and the estimated turgor potential showed only a small decline; similar but more gradual changes occurred with salt acclimation. The 5-bar decrease in root solute potential with salt shock (150 mM NaCl) during the initial 10 min of exposure could not be accounted for by dehydration, indicating that substantial osmotic adjustment occurred rapidly. Changes in concentration of inorganic solutes (Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>) and organic solutes (proline, sucrose, fructose, and glucose) were measured during salt shock. The contribution of these solutes to changes in root solute potential with salinization was estimated.

Salinity, whether natural or induced by agriculture, is a widespread environmental stress that can limit growth and development of salt-sensitive plants (Greenway and Munns, 1980; McWilliam, 1986). Studies of responses of vegetative plants to salt stress have focused mainly on leaf tissue (Neumann et al., 1988; Thiel et al., 1988; Myers et al., 1990; Cramer and Bowman, 1991), so there is less information pertinent to growing roots. However, the root is the first organ of the plant to become exposed to salinity, and in some instances it plays a role in exclusion of salt from the leaves (Yeo et al., 1977; Läuchli, 1984). Additionally, the response of the root apical zone to salt stress is critical to further growth and development of the root system.

Root responses to salt stress have been less well characterized than responses to relatively impermeant osmotica or to water-deficient media. All three stresses involve some degree of dehydration and therefore the likely loss of  $\Psi_p$ ,

but salinity also exposes roots to high concentrations of ions that are readily transported into cells. Can lowering the  $\Psi_p$  account for inhibition of root extension with all three stresses? With osmotic stress (mannitol), a drop in  $\Psi_p$  appeared to be an important determinant of extension rate in wheat roots (Pritchard et al., 1990), although cell walls in the proximal region of the growing zone became harder, i.e.  $\Psi$  had increased and/or wall extensibility had decreased (Pritchard et al., 1991). With maize (*Zea mays* L.) roots, sudden exposure to mannitol or to KCl (Frensch and Hsiao, 1994) caused an immediate decrease in  $\Psi_p$  and extension rate, followed by their parallel recovery within the next 30 min. However,  $\Psi$  had decreased during that time, so that extension could resume as soon as  $\Psi_p$  exceeded the new (lower) value of  $\Psi$ .

With salinity, slower root extension in maize seedlings was not attributed to changes in  $\Psi_p$ , which remained at control (un-salinized) levels, but to a greater hardening of cell walls (Neumann et al., 1994). However, seedlings were exposed to salinity (100 mM NaCl) for 24 h before observations began, so that early responses to salt shock were not recorded. The concentration of Ca<sup>2+</sup> in the root environment is well recognized as critical to the response to NaCl (Cramer et al., 1988; Eвлagon et al., 1992; Zhong and Lauchli, 1993, 1994); extension rates in salt-shocked maize roots (Cramer et al., 1988), monitored continuously over 6 h, remained at control levels provided that 10 mM Ca<sup>2+</sup> was in the external solution. At low Ca<sup>2+</sup>, inhibition of extension by 75 mM NaCl was almost immediate.

Osmotic adjustment helps cells of higher plants to withstand salt stress and water deficit by maintaining sufficient turgor for growth to proceed (Zimmermann, 1978), and involves transport, accumulation, and compartmentation of inorganic ions and organic solutes (Wyn Jones, 1981; Weimberg et al., 1984; McNulty, 1985; Hajibagheri et al., 1987; Binzel et al., 1988; Gibbs et al., 1988; Premachandra et al., 1989; Voetberg and Sharp, 1991; Spickett et al., 1992). Under saline conditions, osmotic withdrawal of water from enlarging cells can cause their  $\Psi_p$  to drop below  $\Psi$ . Cells must then develop a sufficiently low  $\Psi_s$  to reverse the flow

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<sup>2</sup> Permanent address: Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México.

\* Corresponding author; e-mail mcdrew@tamu.edu; fax 1-409-845-0627.

Abbreviations:  $\Psi_p$ , pressure potential or turgor;  $\Psi_s$ , solute or osmotic potential;  $\Psi_w$ , water potential; RWC, relative water content;  $\Psi$ , yield stress threshold.

of water, either by uptake of ions from the medium or by synthesis and transport of organic osmotica; otherwise, cell enlargement will stop (Kurth et al., 1986). Osmotic adjustment requires the regulation of the intracellular levels of several carbon compounds (carbohydrates, sugar alcohols, and organic acids) and nitrogenous compounds (amino acids, Glycine betaine, choline, and polyamines), many of which are assumed to be compartmented mainly in the cytoplasm, whereas inorganic ions (principally  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ ) are sequestered in the vacuoles or distributed between the vacuole and cytoplasm (Jeschke et al., 1986; Hajibagheri et al., 1987; Voetberg and Sharp, 1991). Organic solutes in the cytosol contribute to intracellular osmotic balance when inorganic ion concentrations are high in the vacuole, and they may also protect cytosolic enzymes when ion concentrations increase (Greenway and Munns, 1980). Osmotic adjustment in the root apical zone in maize seedlings exposed to drying conditions is accompanied by a decline in the rate of tissue expansion and an increase in the rate of Pro deposition, i.e. there is a net addition of Pro to the osmotic pool (Voetberg and Sharp, 1991). The increase in concentration of hexose sugars is without increased deposition and results from the slowing of growth. With salinity, it is evident that maize roots are able to lower the  $\Psi_s$  of the cell sap (Evlagon et al., 1992; Neumann et al., 1994), and in root tips of cotton, osmotic adjustment involved an increased deposition rate of total osmotica and  $\text{Na}^+$  (Zhong and Lauchli, 1994). However, the time course of osmotic changes and the identity of the principal inorganic and organic solutes do not appear to have been examined in the same experimental system in previous reports.

The present research was undertaken to improve understanding of the response of maize primary roots to either a sudden imposition of salt stress (salt shock) or to a more gradual increase in salinity (salt acclimation) that might allow roots to adjust progressively. Specifically, our objectives were to: (a) determine whether salt shock and salt acclimation at the same final concentration of NaCl had equally damaging effects on root extension; (b) quantify changes in osmotic relations of root tips during salt stresses to determine whether there were marked losses in  $\Psi_p$  that alone might account for inhibition of extension; and (c) estimate the contributions of inorganic solutes, including  $\text{Na}^+$  and  $\text{Cl}^-$ , and organic solutes to changes in root  $\Psi_s$ . These were examined specifically in relation to salt shock because of the rapid changes in root extension and  $\Psi_s$  that were found. We focused attention on the apical 10-mm zone, which comprises the zones of cell division and cell elongation, and where cells show a transition from being highly cytoplasmic with small vacuoles close to the root tip to being almost fully vacuolate when cell expansion has ceased.

## MATERIALS AND METHODS

### Plant Material, Germination, and Growth Conditions

Caryopses of hybrid maize (*Zea mays* L. cv Pioneer 3906), generously supplied by Pioneer Hi-Bred International

(Johnston, IA), were treated with Captan fungicide (Gustafson Inc., Plano, TX), surface-sterilized with 1% (v/v) fresh NaOCl solution for 5 min, and rinsed thoroughly with deionized water. Caryopses were allowed to imbibe for about 15 h in aerated, 1.0 mM  $\text{CaSO}_4$ , and then germinated in the dark at 27°C between sheets of absorbent germination paper rolled into cylinders and placed vertically in 3-L plastic containers containing about 150 mL of 1 mM  $\text{CaSO}_4$  for continuous wetting. The tops of the containers were covered with plastic wrap to minimize evaporation from the germination paper. At the end of this period, the primary seminal root of typical seedlings was about 15 cm long.

Eighty-four hours from the start of imbibition, groups of eight seedlings of uniform root length were placed in fresh, full-strength nutrient solution with or without the addition of NaCl in 3-L plastic containers fitted with lids. Circular holes were cut in the lids so that each seedling could be supported around the shoot base with foam rubber. Plastic tubes were passed through the lid to provide aeration with a volume flow of 0.3 L  $\text{min}^{-1}$ . The standard nutrient solution was composed of (in mM): 1.0  $\text{KNO}_3$ , 4.0  $\text{Ca}(\text{NO}_3)_2$ , 1.0  $\text{NH}_4\text{H}_2\text{PO}_4$ / $(\text{NH}_4)_2\text{HPO}_4$  (pH 5.0), 0.5  $\text{MgSO}_4$ , and 0.05 Fe(III)-EDTA (monosodium salt), with the micronutrients (in  $\mu\text{M}$ ) 9.22  $\text{H}_3\text{BO}_3$ , 0.16  $\text{CuSO}_4$ , 14.1 KCl, 3.6  $\text{MnSO}_4$ , 0.016  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ , and 0.77  $\text{ZnSO}_4$ . Seedlings were grown at  $25 \pm 2^\circ\text{C}$  in the dark to minimize transpiration and the transfer of salts to the leaves; this was to reduce the possibility of an indirect effect of salinity on root growth because of damage to shoot metabolism. All experiments described below were repeated at least once.

### Salinization Treatments

NaCl was added to the standard nutrient solution to make concentrations of 50, 100, and 150 mM. The controls received no NaCl additions. Salinization treatments were imposed using two different protocols. In salt acclimation, the NaCl concentration was raised by 25 mM once every 24 h until the desired concentration was attained. In salt shock, NaCl was raised to the final concentration in one step. Values of  $\Psi_w$  ( $=\Psi_s$ ) for the nutrient solutions, determined by thermocouple psychrometry, in order of increasing salt concentration, were  $-0.6$ ,  $-3.0$ ,  $-5.5$ , and  $-8.0$  bar.

### Measurement of Growth

Extension rates of primary seminal roots of intact seedlings were measured by marking the root 10 mm from the tip with charcoal powder using a fine brush and measuring the increase in length from this reference mark. No elongation occurred at distances greater than 10 mm from the tip.

### Measurement of Root $\Psi_w$ and $\Psi_s$

$\Psi_w$  and  $\Psi_s$  of root tips were measured by thermocouple psychrometry using sample chambers (model C-52, Wescor, Logan, UT) and a microvoltmeter (HR-33T, Wescor) attached to a chart recorder to read microvolt output.

Calibration was at 25°C with 50- $\mu$ L volumes of NaCl solutions of known molality. For  $\Psi_w$  determinations, groups of eight root sections were excised with a razor blade on filter paper moistened with the medium in which they had been growing, quickly blotted with dry filter paper, and immediately placed in the sample chamber to equilibrate. Because the root cells may have expanded after excision, values of  $\Psi_w$  could have been affected by cell wall relaxation (Neumann et al., 1994). Steady values were obtained by 75 to 90 min of equilibration, and the 90-min period was used for all reported results. Preliminary trials showed that with fewer than eight root tips per chamber, equilibration was not complete in 90 to 120 min and estimates of  $\Psi_w$  and  $\Psi_s$  were erroneously lower. After readings, the tissue was removed from the chamber, sealed in a vial, and plunged into liquid N<sub>2</sub>. The frozen tissue was allowed to thaw and then quickly returned to the chamber for  $\Psi_s$  determination. Readings were taken after >30 min of equilibration.  $\Psi_p$  was calculated as the difference between  $\Psi_w$  and  $\Psi_s$ . No correction of root  $\Psi_s$  was made for apoplastic water or solutes.

In an initial series of experiments to examine water and osmotic relations over a wide range of NaCl concentrations, root segments were excised at 0 to 5 and 5 to 10 mm from the tip. In subsequent work to examine the accumulation of solutes in the apical zone, we worked with segments excised at 0 to 3 and 3 to 10 mm from the tip. These zones were chosen because they comprise, respectively, a population of cells that are more densely cytoplasmic, with many microvacuoles, and a population of vacuolating to fully vacuolate cells. This distinction is a first approximation because cell structure in the root apical zone is not uniform, either radially or axially.

#### RWC

To determine RWC, 84-h-old seedlings were transferred to fresh standard nutrient solution for about 12 h to ensure full turgidity and uniform root growth rate. Root sections then were excised at 0 to 5 and 5 to 10 mm from the apex and transferred in groups of 20 to fresh, aerated standard nutrient solution. After about 3 h at room temperature under dim light, the turgid weight was determined and root segments were subjected to 100 and 150 mM NaCl salt shock for 5 h. During that time, root fresh weight was monitored by blotting segments and reweighing at intervals of 30 min. At the conclusion of the experiment, root segments were frozen in liquid N<sub>2</sub>, lyophilized at -70°C for at least 36 h, and dry-weighed. RWC was calculated from:

$$\text{RWC (\%)} = [(FW - DW) / (TW - DW)]100,$$

where *FW* is fresh weight, *DW* is dry weight, and *TW* is turgid weight. This protocol can lead to estimates of RWC greater than 100% if cells continue to expand and absorb water during the 5-h period.

#### Root Sampling and Elemental Analyses

Seminal roots were excised from the caryopses and rinsed in deionized water (two rinses each of 1 min), and

each was cut into segments 0 to 3 mm and 3 to 10 mm from the root tip and the remainder of the root axis (>10 mm). Fresh weights were recorded immediately and segments were frozen in liquid N<sub>2</sub>, lyophilized for at least 36 h, and ground to a fine powder. Each sample comprised 24 segments for the 0- to 3-mm and the 3- to 10-mm zones and four segments for the >10-mm zone. To analyze Cl<sup>-</sup>, Na<sup>+</sup>, and K<sup>+</sup>, ground, freeze-dried root segments were placed in a glass vial with 0.4 N HNO<sub>3</sub>, allowed to stand for about 30 min, suspended in an ultrasonic bath for 3 min, and then placed in a water bath at 80°C for 15 min. The suspension was filtered or centrifuged and the supernatant was stored frozen until assayed. Determination of Cl<sup>-</sup> was by potentiometry using a digital chloridometer (model 4425000, Haake Buchler Instruments, Saddle Brook, NJ). Assays of Na<sup>+</sup> and K<sup>+</sup> were done on the same tissue extract as well as on reagent blanks by atomic absorption spectrophotometry.

#### Analyses of Organic Osmolytes

Natural abundance <sup>13</sup>C-NMR was used to identify carbon compounds that accumulated in response to salinization. Analyses were made of root segments excised from plants that had been exposed to 100 mM NaCl shock for 48 h. The two root zones were analyzed to identify osmolytes that might be associated predominantly with the more densely cytoplasmic cells close to the root tip (0-3 mm) or the more vacuolated ones (3-10-mm zone). The fresh weight of the root segment samples was about 3.0 g each. Details of the perchloric acid extraction and NMR methods are given elsewhere (Chang and Roberts, 1989; Roberts et al., 1992).

GC was used to quantify carbohydrates identified by <sup>13</sup>C-NMR. The GC method is more sensitive, allowing smaller samples to be used for quantitative analysis, and it is better suited to analysis of multiple samples. For GC analysis root segments in groups of about 48 per sample were excised from the seminal roots, blotted, fresh-weighed, frozen in liquid N<sub>2</sub>, and stored at -80°C. To prepare ethanol extracts, weighed, lyophilized segments were ground to a powder using a mortar and pestle at room temperature, and then reground in 500  $\mu$ L of 80% (v/v) ethanol. The suspension was quantitatively transferred to an Eppendorf tube by rinsing with small volumes of 80% ethanol, and microfuged for 15 min. The supernatant was transferred to a test tube and the pellets were extracted two more times (1 mL each) with 80% ethanol. The combined supernatant volume was about 3.5 mL, to which was added 3.5 mL each of water and chloroform for lipid/sugar partitioning. The samples were mixed after adding each component and allowed to stand at room temperature for about 1 h to allow phase separation. The aqueous upper phase containing the sugars was removed, transferred to a test tube, frozen in liquid N<sub>2</sub>, and lyophilized for at least 24 h. The sugars were derivatized as described by Ferguson et al. (1979). Separation of silylated sugars by GC was essentially as described by Zimmerman and Cobb (1989).

### Calculated Contribution to $\Psi_s$

$\Psi_s$  for each measured concentration of solute was calculated according to the van't Hoff equation (Nobel, 1991), which applies strictly to ideal dilute solutions:

$$\Psi_s = -nRT.$$

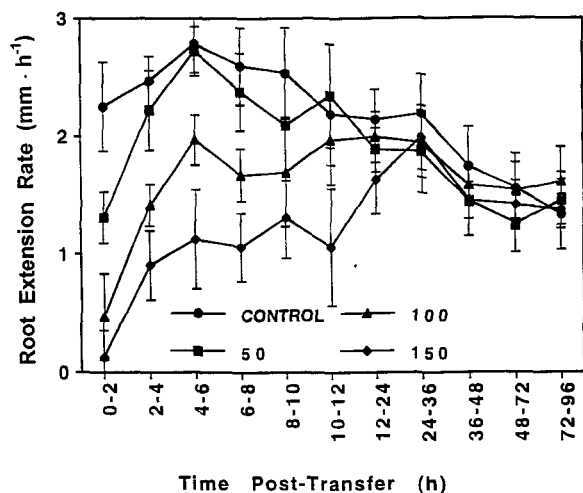
Where  $n = \mu\text{osmol} (\text{g H}_2\text{O})^{-1}$ ,  $R = 8.314 \times 10^{-5} \text{ m}^3 \text{ bar mol}^{-1} \text{ K}^{-1}$ , and  $T = 298.2 \text{ K}$ . For  $n$ , we based values on measurements of  $\mu\text{mol mL}^{-1}$  solution.

## RESULTS

### Effect of NaCl on Root Extension and Root Water Relations

When intact seedlings were salt-shocked by exposure to 50, 100, or 150 mM NaCl, root extension was initially slowed, with greater inhibition at higher NaCl concentrations (Fig. 1). This was followed by recovery of root extension rates, so that by 24 h the roots of NaCl-treated seedlings and controls had reached similar values. The declining extension rate of controls was the consequence of growth in the dark and depletion of seed reserves; light-grown seedlings showed no comparable decline in root extension (data not presented). Salt shock caused an appreciable decrease in the diameter of the new root tissue produced after the start of the treatments. At 96 h the diameters ( $\text{mm} \pm \text{SD}$ ,  $n = 8$ ) measured 10 mm behind the tip for the controls and for the three increasing concentrations of NaCl were  $0.96 \pm 0.16$ ,  $0.87 \pm 0.12$ ,  $0.74 \pm 0.10$ , and  $0.69 \pm 0.06$ , respectively.

In the salt-acclimation experiments, no significant differences in extension rate were observed between unsalinized controls and plants exposed to 50 or 100 mM NaCl for 144 h (Table I); in all three treatments, extension was similar to that of the controls in Figure 1. Because of the time taken to reach the final NaCl concentration and the declining exten-



**Figure 1.** Effect of salt shock on the extension rate of the primary seminal root of maize seedlings during the stated time intervals. NaCl concentrations range from 0 (control) to 150 mM. Values are means  $\pm$  SD ( $n = 16$ ).

**Table I.** Extension rates of primary seminal roots during exposure to gradual increases in NaCl concentration (salt acclimation)

NaCl concentrations were increased by 25 mM once per day until the required final concentration was reached. Values are means and (SD),  $n = 10$ .

Time Interval	Final NaCl Concentration		
	0 mM	50 mM	100 mM
<i>h</i>		<i>mm h<sup>-1</sup></i>	
0-48	2.62 (0.23)	2.29 (0.29)	2.49 (0.16)
48-96	1.69 (0.09)	1.62 (0.21)	1.94 (0.20)
96-144	1.33 (0.09)	1.26 (0.09)	1.37 (0.08)

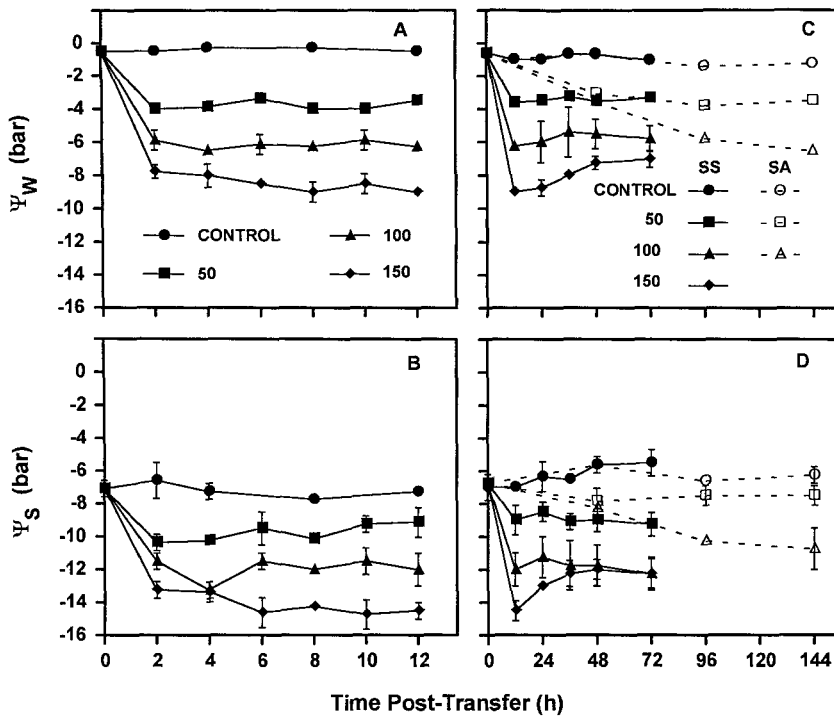
sion rates with time, we did not extend the study to 150 mM NaCl.

Salt shock caused a fall in root tip (0-5-mm zone)  $\Psi_w$  during the first 2 h, reaching values of -4.0, -5.8, and -7.9 bar for the 50, 100, and 150 mM NaCl treatments, respectively, whereas controls averaged  $0.63 \pm 0.18$  bar (Fig. 2, A and C). In the salt-acclimation treatments, the decline in root tip  $\Psi_w$  was more gradual (Fig. 2C), reaching values at 144 h that were similar to those observed at 72 h with salt shock. Thus, root tip  $\Psi_w$  was very close to that of the external solution, indicating that relaxation during the equilibration period was minimal (cf. Neumann et al., 1994).

Salt shock caused a sharp decline in root tip  $\Psi_s$  during the first 2 h (Fig. 2B), to -10.3, -11.5, and -13 bar for the 50, 100, and 150 mM NaCl treatments, respectively. For 150 mM NaCl, the initial decline in root tip  $\Psi_s$  was followed by an increase to reach a steady value from 48 to 72 h. In the salt-acclimation experiments, there was a gradual decrease in root tip  $\Psi_s$  as the concentration of NaCl increased, and the final  $\Psi_s$  values were about 1 bar higher than with salt shock. In a separate experiment, measurements were made of root tip  $\Psi_s$  only, so that the 90-min period usually used for equilibration of tissue  $\Psi_w$  was omitted. Salt shock resulted in a substantial decrease in root  $\Psi_s$  within the first 10 min (Fig. 3), reaching -10 and -11.8 bar for the 100 and 150 mM NaCl treatments, respectively, followed by a slow decline until the end of the experiment. Values for  $\Psi_s$  at 120 min were similar to those determined after prior  $\Psi_w$  estimates (Fig. 2), indicating that the  $\Psi_s$  values in Figure 2 were not appreciably altered during the equilibration period.

Although root tip  $\Psi_w$  and  $\Psi_s$  decreased greatly during salt stress,  $\Psi_p$  during salt shock or salt acclimation appeared in general to be maintained, but at a level a little below that of controls (Table II). With 150 mM salt shock, values were distinctly lower at 24 and 36 h, but by that time extension had increased to the control rate. Similarly, values of  $\Psi_p$  were a little lower for salt-acclimated roots at 100 mM NaCl, although extension was not affected.

To determine whether dehydration could account for the rapid initial lowering of root  $\Psi_s$  during salt shock, the RWC was determined for the apical zones (Fig. 4). RWC of controls in the 0- to 5-mm zone increased steadily from 100% to about 105% during the 5-h experimental period, presumably because of continued cell expansion and water



**Figure 2.** Effect of NaCl on  $\Psi_w$  (A and C) and  $\Psi_s$  (B and D) in the apical 5-mm zone of primary seminal roots during salt shock (SS) and salt acclimation (SA). A and B show the initial 12-h response to salt shock only. Values are means  $\pm$  SD ( $n = 2$ ).

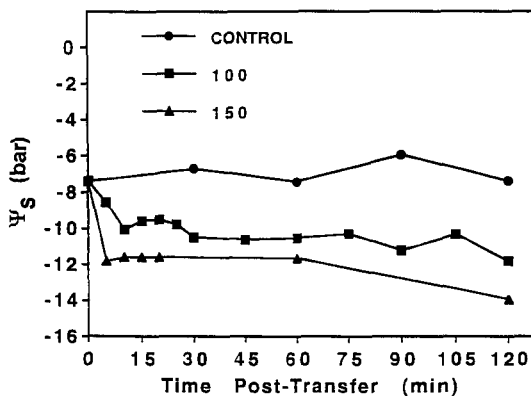
uptake. Imposition of 100 or 150 mM NaCl salt shock resulted in a decrease to 98 and 88%, respectively, within the first 30 min. Thereafter, RWC increased gradually for the 150 mM shock treatment, reaching 94% at the conclusion of the experiment. Similar changes in RWC were found for the 5- to 10-mm zone, except that dehydration was somewhat greater at 150 mM NaCl (Fig. 4B), lowering the RWC to 83%.

To compare the salt-shock response in the highly cytoplasmic cells near the root tip with that of more vacuolated root tissue, and to evaluate the contribution of different osmolytes in these zones, additional water-relation measurements were made for the apical 0- to 3-mm and 3- to 10-mm zones. Cell expansion is complete within the apical 10 mm of maize seminal roots, and even if salt shock

causes cell expansion to become more localized toward the tip, as happens with water deficit (Sharp et al., 1988) or with salt-stressed cotton roots (Zhong and Lauchli, 1993), the two zones would still effectively separate contrasting populations of cells. We chose to work with a single NaCl concentration (100 mM) and to focus on the salt-shock response because of the rapid changes in root  $\Psi_s$  it provoked. With salinization (Fig. 5), a rapid decrease in root  $\Psi_w$  was observed for both zones, with the 0- to 3-mm zone showing transiently a greater decrease, and  $\Psi_s$  was lowered approximately in parallel to  $\Psi_w$ .  $\Psi_p$  remained between 5 and 7 bar in both controls and salinized roots (Fig. 5C), even within the first 2 h, when  $\Psi_s$  of salt-shocked seedlings dropped rapidly.  $\Psi_p$  for the 3- to 10-mm zone was lower at 24 to 72 h, but extension had resumed by that time. However, analysis of variance showed that there was a significant ( $P < 0.01$ ) lowering of  $\Psi_p$  in salt-shocked root tips (5.7 bar) compared with controls (6.5 bar) at 2 h, and throughout the initial 6 h of salinization  $\Psi_p$  averaged 0.77 bar less than in controls ( $P < 0.01$ ). The 3- to 10-mm zone failed to show a significant effect of salt shock on  $\Psi_p$  at any time ( $P = 0.58$ ).

#### Effect of NaCl on Inorganic Solute Accumulation

Concentrations of  $\text{Cl}^-$ , expressed on a tissue water basis, increased rapidly in response to salt shock (100 mM NaCl), with greater accumulation in the 0- to 3-mm and 3- to 10-mm root zones than in the remainder of the root (Fig. 6A). Compared with unsalinized seedlings, the net increases in  $\text{Cl}^-$  content at 12 h were 63, 77, and 43  $\mu\text{mol mL}^{-1}$  for the 0- to 3-, 3- to 10-, and >10-mm zones, respectively. Measurements were made for the >10-mm zone because most literature on ion fluxes in roots is with whole



**Figure 3.** Initial changes in  $\Psi_s$  in the apical 5-mm zone of primary seminal roots of seedlings exposed to salt shock. NaCl concentrations range from 0 (control) to 150 mM. Each value represents a single determination.

**Table II.** Root  $\Psi_p$  with time for the apical 5-mm zone of primary seminal roots of maize seedlings exposed to salt shock or salt acclimationValues are means and (SD),  $n = 2$ .

Treatment	Time h	$\Psi_p$ bar			
		0 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl
Salt shock					
	0	6.3 (0.9)	5.8 (0.7)	5.8 (0.3)	5.8 (0.3)
	2	6.1 (1.1)	6.3 (0.7)	5.8 (0.1)	5.4 (0.1)
	4	6.9 (0.4)	6.3 (0.0)	6.7 (0.9)	5.4 (0.1)
	6	nd <sup>a</sup>	6.2 (0.8)	5.3 (0.1)	6.1 (1.2)
	8	6.6 (0.0)	6.0 (1.0)	5.6 (0.3)	5.3 (0.6)
	10	nd	5.2 (0.5)	5.5 (0.1)	6.2 (0.4)
	12	6.3 (0.3)	5.3 (1.0)	5.8 (0.5)	5.6 (0.5)
	24	5.6 (1.1)	5.0 (0.4)	5.3 (0.0)	4.4 (0.2)
	36	6.0 (0.0)	5.8 (0.3)	6.3 (0.0)	4.4 (0.3)
	48	5.1 (0.7)	5.6 (0.6)	6.4 (0.3)	5.2 (0.3)
	72	4.9 (0.7)	5.9 (0.4)	6.4 (0.0)	5.3 (0.3)
Salt acclimation					
	0	5.7 (0.4)	5.7 (0.4)	5.7 (0.4)	nd
	48	5.4 (0.4)	5.1 (0.3)	4.7 (1.0)	nd
	96	5.4 (0.1)	4.4 (0.9)	4.6 (0.1)	nd
	144	5.1 (0.1)	4.7 (0.3)	4.4 (1.1)	nd

<sup>a</sup> nd, Not determined.

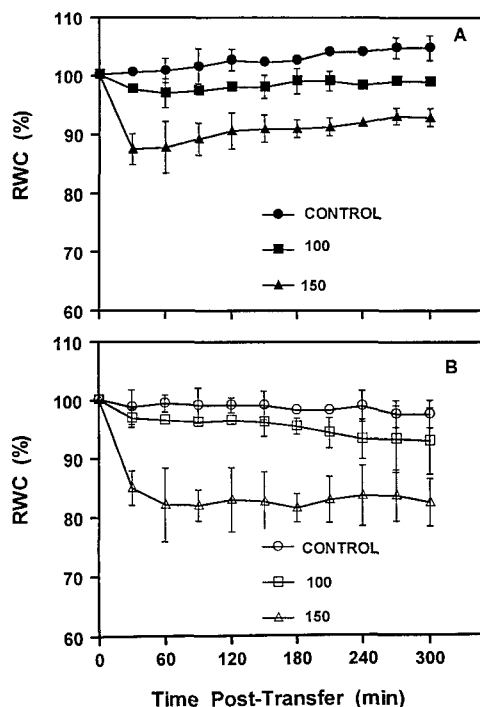
root systems. In the apical 0- to 3-mm zone, the  $\text{Na}^+$  concentration was 160 to 180  $\mu\text{mol mL}^{-1}$ , regardless of treatment, and it remained essentially constant (Fig. 6B). In the 3- to 10-mm zone, the  $\text{Na}^+$  concentration increased over that in controls by about 26  $\mu\text{mol mL}^{-1}$  at 12 h, whereas in the >10-mm zone, the  $\text{Na}^+$  concentration increased by 70

$\mu\text{mol mL}^{-1}$ , a 4.5-fold increase. The high concentration of  $\text{Na}^+$  in the 0- to 3-mm zone and its failure to increase with exposure to NaCl was unexpected, but the same result was obtained in three independent experiments. Analytical artifacts seem very unlikely because the extracts analyzed for  $\text{Na}^+$  were also analyzed for  $\text{K}^+$  and  $\text{Cl}^-$  and showed no unusual features for those ions; furthermore, the effect was not observed in the older root zones, in which, as expected,  $\text{Na}^+$  uptake clearly occurred with time.

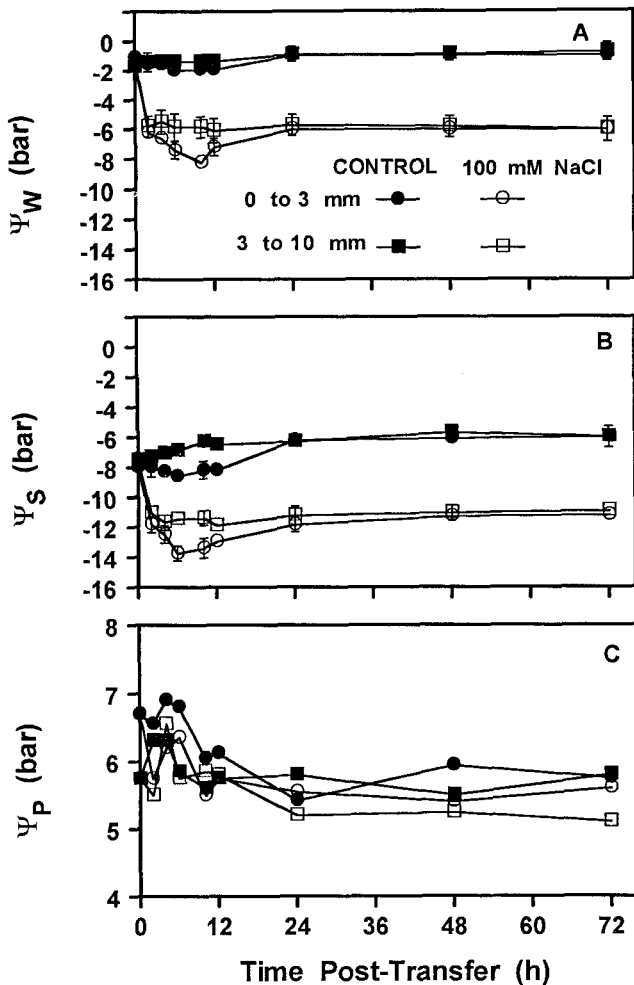
The  $\text{K}^+$  concentration in the 0- to 3-mm zone of both control and salinized roots was about 125  $\mu\text{mol mL}^{-1}$  at 24 h (Fig. 6C). A transient increase in  $\text{K}^+$  concentration occurred during the first 4 to 6 h with salt shock, but thereafter tissue concentrations were similar with time for both treatments. For the 3- to 10-mm zone, the  $\text{K}^+$  concentration also tended to increase during the first 10 h with salt shock. For the >10-mm zone,  $\text{K}^+$  concentration in both treatments (about 32  $\mu\text{mol mL}^{-1}$ ) was lower than for the other zones, and in the salinized roots it did not increase with time as it did in controls.

#### Effect of NaCl on Organic Solute Accumulation

$^{13}\text{C}$ -NMR measurements identified the main organic solutes that accumulated in response to salt shock with 100 mM NaCl. All  $^{13}\text{C}$ -NMR peaks that increased during salinization were assigned. At 48 h, only Fru, Glc, Suc, and Pro showed detectable changes (Fig. 7). Regardless of the treatment, Suc and Pro concentrations were higher in the 0- to 3-mm zone, whereas Fru and Glc accumulated to higher concentrations in the 3- to 10-mm zone. Further experiments measured changes in concentrations of the three sugars over shorter times using GC (Fig. 8). Values obtained by GC were within the range found by NMR. In both treatments, concentrations of Fru and Glc in the 0- to



**Figure 4.** Effect of salt shock on RWC in the apical 0- to 5-mm (A) and 5- to 10-mm (B) zones of primary seminal roots. NaCl concentrations range from 0 (control) to 150 mM. Values are means  $\pm$  SD ( $n = 2$ ).



**Figure 5.** Effect of salt shock with 100 mM NaCl on  $\Psi_w$  (A),  $\Psi_s$  (B), and  $\Psi_p$  (C) in the apical 0- to 3-mm and 3- to 10-mm zones of primary seminal roots. Values are means  $\pm$  SD ( $n = 4$ ). For clarity, error bars are omitted from C.

3-mm zone were relatively low, 2 to 10  $\mu\text{mol mL}^{-1}$ . In the 3- to 10-mm zone, the concentrations of Fru and Glc were always greater with salt shock, but both decreased during the first 12 h and then gradually increased.

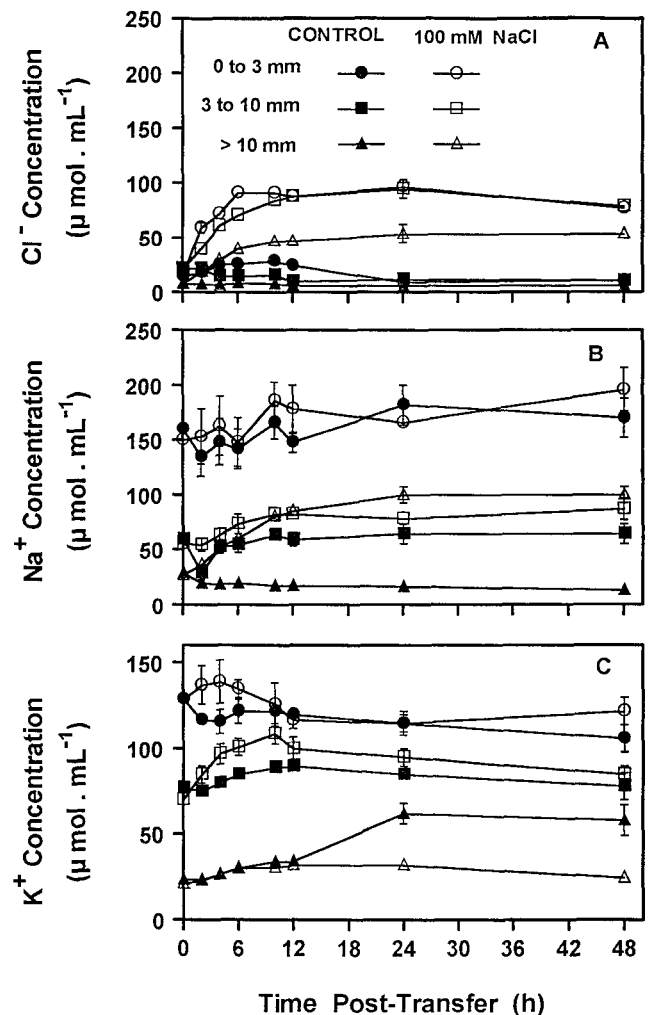
The concentration of Suc in the apical 0- to 3-mm zone of NaCl-treated roots was relatively stable at 25 to 35  $\mu\text{mol mL}^{-1}$ , whereas in controls it decreased during the initial 6 h before increasing again. Suc concentration in the 3- to 10-mm zone was low in controls (near 1.0  $\mu\text{mol mL}^{-1}$ ), but in salinized seedlings it was consistently higher, about 8  $\mu\text{mol mL}^{-1}$ .

#### Solute Contribution to Root $\Psi_s$

To allow comparison with published data, the  $\Psi_s$  arising from the measured concentrations of  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , Fru, Glc, and Suc were calculated initially on the assumption that all were osmotically active and distributed uniformly throughout all cellular compartments (Fig. 9). The second assumption is unlikely to be correct for at least some solutes (see "Discussion"). In the 0- to 3-mm root zone, the

major contributors to  $\Psi_s$  in controls (Fig. 9A) were the inorganic ions, and these, together with the organic solutes, were in excess of those needed to account for the measured  $\Psi_s$ . For the salt-shocked 0- to 3-mm zone,  $\Psi_s$  for inorganic and organic solutes totaled  $-10.0$  bar at 12 h, compared with the psychrometrically measured  $\Psi_s$  of  $-13$  bar, i.e. 77% was accounted for. Numerous organic and inorganic solutes that were not assayed must have been present at relatively low concentrations; however, it was not the aim of this investigation to account for all of them or their contribution to  $\Psi_s$ .

In the 3- to 10-mm zone in controls (Fig. 9B), organic solutes made a slightly larger contribution to  $\Psi_s$ ; the sum of inorganic and organic solutes was calculated to reach  $-5$  bar at 12 h, compared with the psychrometrically determined  $\Psi_s$  value of  $-6.5$  bar. In NaCl-treated seedlings (Fig. 9D), organic solutes made an appreciable contribution, with Fru and Glc together contributing about  $-2.0$  bar. The total contribution of measured solutes to the



**Figure 6.** Accumulation of  $\text{Cl}^-$  (A),  $\text{Na}^+$  (B), and  $\text{K}^+$  (C) in the 0- to 3-mm, 3- to 10-mm, and >10-mm zones of primary seminal roots of seedlings exposed to 100 mM NaCl shock. Values are means  $\pm$  SD ( $n = 3$ ).

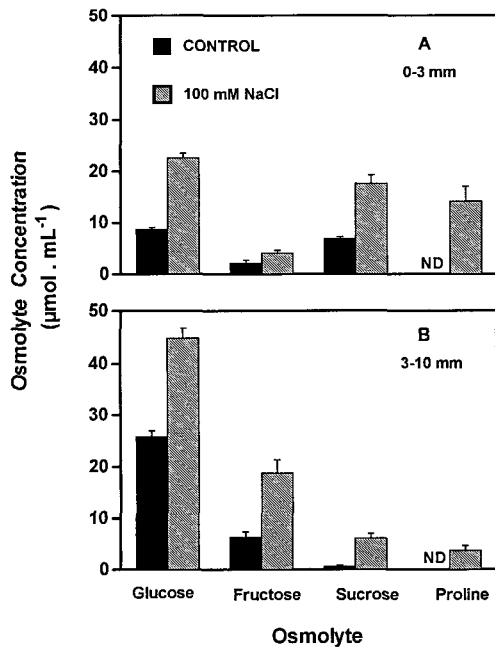
calculated  $\Psi_s$  was  $-9.0$  bar, compared with the measured root  $\Psi_s$  of  $-12$  bar.

## DISCUSSION

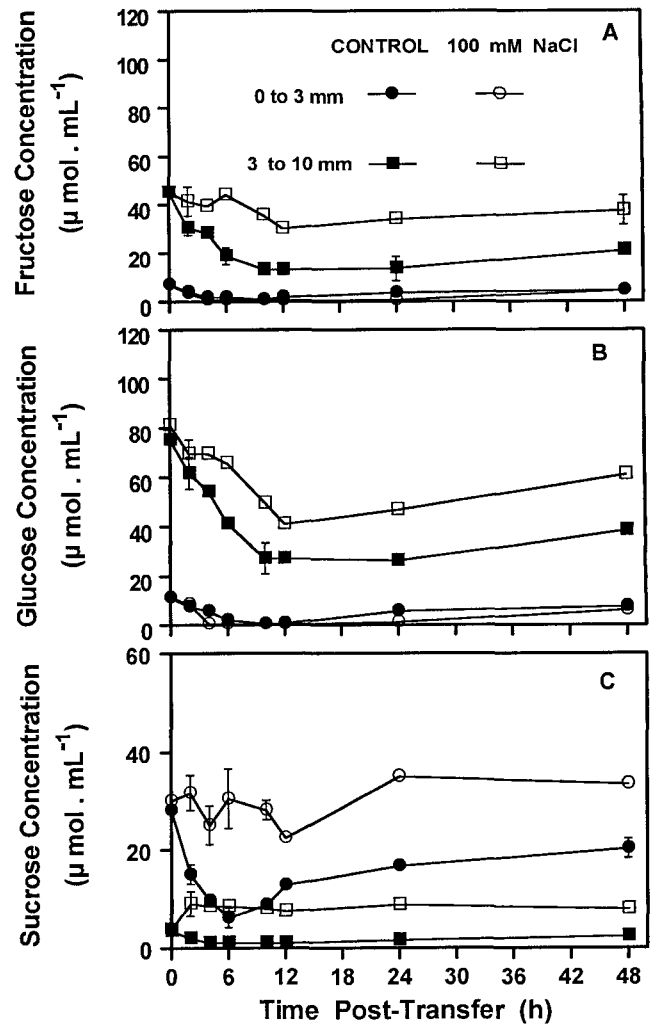
### Root Elongation and Water Relations

The present work reveals the remarkable ability of maize roots to continue extension when gradually exposed to increasing concentrations of NaCl, and to resume extension during salt shock, even at concentrations (100 mM and greater) that eventually prove very injurious to leaves of intact, transpiring plants as salts accumulate in them (Drew et al., 1988). Roots of dark-grown cotton seedlings that had acclimated to 150 mM NaCl were also highly tolerant, extending at 60 to 80% of the rate of unsalinized controls (Zhong and Lauchli, 1994).

The degree and duration of the reduced root growth rate during salt shock, which depended on the concentration of NaCl in the nutrient solution (Fig. 1), are likely to be related to the properties of elongating cells. In the leaves of maize plants with the roots exposed to long-term salinization, inhibition of extension was attributed to increases in  $Y$ , and not to cell wall extensibility, hydraulic conductance, or  $\Psi_p$  (Cramer and Bowman, 1991; Neumann, 1993). However, in bean leaves salinity inhibited leaf expansion, mainly by lowering turgor (Neumann et al., 1988). In our experiments with roots, we could not detect any major losses in turgor, but our technique was inadequate to resolve short-term changes. Using a pressure probe, Frensch and Hsiao (1994) recorded transient changes in  $\Psi_p$  when maize roots were suddenly exposed to KCl or mannitol



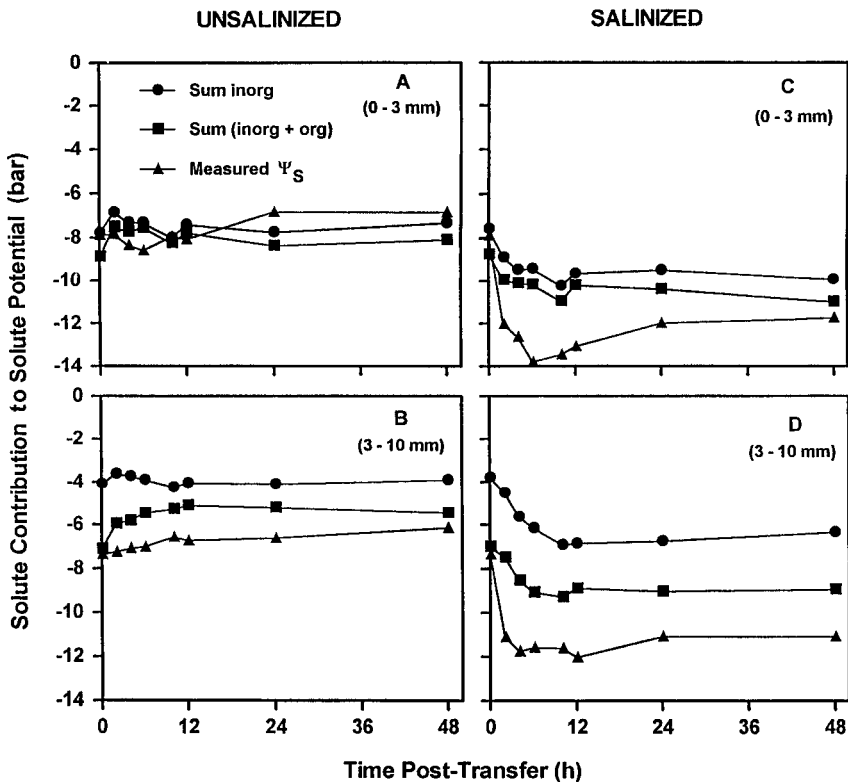
**Figure 7.** Concentrations of soluble sugars and Pro, measured by  $^{13}\text{C}$ -NMR, in the apical 0- to 3-mm (A) and 3- to 10-mm (B) zones of primary seminal roots of seedlings exposed to 100 mM NaCl shock for 48 h. Each value indicates the analysis of extracts from root segments totaling about 3.0 g fresh weight. ND, Not detectable.



**Figure 8.** Fru (A), Glc (B), and Suc (C) concentrations in the apical 0- to 3-mm and 3- to 10-mm zones of primary seminal roots of seedlings with time of exposure to 100 mM NaCl shock. Values are means  $\pm$  SD ( $n = 2$ ). Concentrations are expressed per milliliter of tissue water.

solutions ( $\Psi_s = -3$  bar).  $\Psi_p$  decreased almost immediately upon addition of the osmoticum, but recovered within the next 30 min to a new steady value that was only a little below the original one. During this time,  $Y$  quickly changed from an initial value of 6 to 4.5 bar. Root elongation was inhibited only during the transient loss of  $\Psi_p$ , and recovered as soon as  $\Psi_p$  exceeded the new (lower) value of  $Y$ . Similar changes were observed by Pritchard et al. (1991) in wheat roots exposed to mannitol ( $\Psi_s = -4.8$  bar), in which an initial drop in  $\Psi_p$  in the 2-mm apical zone and inhibition of extension were followed by a gradual recovery in about 3 h. However, at high concentrations of mannitol ( $\Psi_s = -9.6$  bar), extension was strongly inhibited, and at 24 h  $\Psi_p$  was much lower in the apical zone, with signs of wall hardening, i.e. decreased extensibility. The present results are compatible with these earlier studies: with 100 mM NaCl shock, the 0- to 3-mm zone showed a small but distinct decrease in  $\Psi_p$ . It is possible that the driving force for cell expansion ( $\Psi_p - Y$ ) was appreciably lowered. In





**Figure 9.** Contributions of inorganic and organic solutes to  $\Psi_s$  in the apical 0- to 3-mm and 3- to 10-mm zones of control (A and B) and 100 mM NaCl-treated (C and D) seedlings. Values are for the sum of the inorganic solutes and the sum of inorganic and organic solutes together. Measured  $\Psi_s$  values are reproduced from Figure 5.

contrast to our results, Neumann et al. (1994), working with light-grown maize seedlings, found that root extension rates did not recover during exposure to 100 mM NaCl for 6 d. They attributed the long-term inhibition to an increase in  $Y$ .

Reduction in root extension rates might also come from the marked lowering of root radial hydraulic conductivity that occurs with salinization (Joly, 1989; Azaizeh and Steudle, 1991; Azaizeh et al., 1992; Evlagon et al., 1992), but Neumann et al. (1994) concluded that despite the drastic decline in conductivity, water movement into cells was still sufficiently rapid so as not to restrict their rate of expansion.

Salt shock resulted in a rapid reduction in  $\Psi_w$  and  $\Psi_s$  in both "cytoplasmic" and "vacuolated" root zones (Fig. 5), so that there were only small losses of  $\Psi_p$ . The 5% reduction in root RWC due to the 100 mM salt-shock treatment (Fig. 4) would not be sufficient to account for more than a small fraction of the 5-bar decrease in root  $\Psi_s$  during the first 2 h of treatment (Fig. 3). At least a 50% reduction in RWC would have had to occur to double the solute concentration, and thus  $\Psi_s$ , due to root dehydration. This decrease in  $\Psi_s$ , which occurred within 5 to 10 min of the start of salt shock, would help to maintain  $\Psi_p$  above the yield threshold and thus maintain cell expansion.

#### Osmotic Contribution from Inorganic Solutes

The general features of solute accumulation in the present study clearly demonstrate that maize root tip cells are able to adjust osmotically to lower the root  $\Psi_s$  in response to NaCl treatment, accomplished by the accumu-

lation of both inorganic and organic solutes. However, the pattern of changes in concentration of these solutes in response to salt shock is strikingly different between the apical and subapical root zones. There was a marked increase in  $\text{Cl}^-$  concentration in all root zones in response to NaCl, presumably as a result of direct uptake from the external solution. In contrast,  $\text{Na}^+$  in the 0- to 3-mm zone of the same roots failed to increase at all during exposure to NaCl (Fig. 6). One explanation is that  $\text{Na}^+$  was translocated to the apical zone from the caryopsis. Root apical zones typically accumulate high concentrations of cations via the phloem (Jeschke and Wolf, 1988), and it was noticeable that  $\text{K}^+$  and  $\text{Na}^+$  strongly accumulated in the same zone (Fig. 6C) so that the  $\text{Na}^+/\text{K}^+$  ratio was about 1.3 for both the control and salt-shock treatments. An additional possibility is that  $\text{Na}^+$  was strongly excluded from cells in the 0- to 3-mm zone during exposure to NaCl (Schubert and Lauchli, 1988). With roots of cotton (Zhong and Lauchli, 1994),  $\text{Na}^+$  concentrations in the equivalent apical zone were much lower than in maize, reaching only 25 and 50  $\mu\text{mol g}^{-1}$  fresh weight, respectively, in controls (1.0 mM NaCl) and in roots exposed to salt (150 mM NaCl). As expected, the more mature zones in maize roots (>10 mm) accumulated  $\text{Na}^+$  and  $\text{Cl}^-$  when exposed to NaCl, and to higher concentrations than for  $\text{K}^+$ . Thus, the  $\text{Na}^+/\text{K}^+$  ratio changed from 0.88 to 2.8 with salinization (from Fig. 6). Concentrations of  $\text{K}^+$  in cotton root tips (Zhong and Lauchli, 1994) were very similar to those reported here for maize, in the range of 100 to 150 mM for all zones of controls and in the apical 0 to 3 mm of salinized roots. However, in cotton, salinity caused a marked decline in  $\text{K}^+$

concentration at 10 mm from the apex. Differences in inorganic ion accumulation between the different root zones in these species presumably reflect differences in selectivity at the plasma membrane during ion uptake, in phloem transport of ions to the apex, and in ion compartmentation (Yeo et al., 1977; Hajibagheri et al., 1987).

To what extent does the change in concentration of inorganic solutes account for osmotic adjustment? For the 0- to 3-mm zone at 12 h of salt shock, inorganic ions contributed  $-10.0$  bar to  $\Psi_s$ , compared with  $-7.5$  bar at the start of salinization (Fig. 9C). Higher concentrations of inorganic solutes thus accounted for a  $\Psi_s$  change of 2.5 bar, compared with the psychrometrically measured change of about 5 bar. However, assuming that the volume occupied by vacuoles in the 0- to 3-mm zone is 50% (Lee et al., 1990), that  $K^+$  is equally distributed between the cytoplasm and vacuole (Flowers and Läuchli, 1983; Drew et al., 1990), and that  $Na^+$  and  $Cl^-$  are also equally distributed, having not yet been sequestered in the developing vacuoles of cells newly exposed to NaCl in the meristematic zone, the  $\Psi_s$  contributions for the vacuole become (bar):  $Cl^-$  ( $-2.3$ ),  $Na^+$  ( $-4.8$ ), and  $K^+$  ( $-3.4$ ), totaling  $-10.5$  (Table III). Based on the foregoing assumptions, the additional concentrations of inorganic ions could barely account for osmotic adjustment in the vacuolar compartment, but the excess of cations over anions in the vacuole ( $-5.9$  bar) is probably balanced by the dicarboxylic malic acid. Thus, a further  $-2.95$  bar should be included, making a revised calculated  $\Psi_s$  of  $-13.45$  bar. Similarly, for the cytoplasm, the total calculated  $\Psi_s$  for the measured solutes is  $-12.4$  bar. It is difficult to make an allowance for the excess of cations over anions ( $-5.9$  bar) in the cytoplasm because some of the cations will be associated with proteins and organic acids, which would have a smaller osmotic impact than  $-5.9$  bar. The question of concentrations of solutes in the cytoplasm will be discussed further below.

For the 3- to 10-mm zone, the  $\Psi_s$  contribution of the inorganic ions was  $-6.8$  bar at 12 h of salinization compared with  $-4.0$  bar in the controls, a change of 2.8 bar. The

measured change in tissue  $\Psi_s$  was 5 bar (Fig. 9). Because most cells would be fully vacuolated in this root zone, any refinements based on vacuolar compartmentation (about 95% of the volume) would have little effect on calculated  $\Psi_s$  values. Thus, for the more mature root zone, inorganic ion accumulation accounted for the major part of osmotic adjustment of the vacuole (Table III), but a contribution from other solutes, presumably inorganic and organic anions, is implicated because the total calculated  $\Psi_s$  ( $-9.3$  bar) does not fully account for the measured  $\Psi_s$  of  $-12.0$  bar. The conclusion that  $Cl^-$ ,  $Na^+$ , and  $K^+$  accounted for a considerable proportion of the measured decrease in root  $\Psi_s$  is in agreement with the general view that in nonhalophytes, inorganic ions provide the major contribution to cell sap  $\Psi_s$  at high salinity (Greenway and Munns, 1980; Flowers and Läuchli, 1983).

### Osmotic Contribution from Organic Solutes

The analyses of soluble sugars (Fru, Glc, and Suc) and Pro suggested that they play a relatively minor role during osmotic adjustment, based on the initial assumption that solutes were at the same concentration throughout the cell. For instance, in the 0- to 3-mm zone, Suc contributed at the most  $-1$  bar to root  $\Psi_s$ , whereas the contributions of Fru and Glc to osmotic adjustment were negligible. In contrast, in the 3- to 10-mm zone Fru and Glc provided about  $-1$  bar each to root  $\Psi_s$ , with a negligible contribution from Suc. These results are consistent with previous observations (Weimberg et al., 1984; Sharp et al., 1990) that sugars, particularly Suc, do not contribute materially to root osmotic adjustment in the apical zone. However, much higher contents of reducing sugars in the 3- to 10-mm root zone at low vermiculite  $\Psi_w$  ( $-16$  bar) were reported by Sharp et al. (1990), in which concentrations, expressed on a tissue water basis, reached up to 400 mM. In the cell sap of leaves of *Z. mays* plants exposed to water deficits, soluble sugars and  $K^+$  were the major osmotic contributors, totaling about  $-5.4$  bar (Premachandra et al., 1989), although

**Table III.** Calculated solute contributions to  $\Psi_s$  of cytoplasmic and vacuolar compartments of maize roots exposed to 100 mM NaCl (salt shock) for 12 h

Calculations are based on data shown in Figures 7 and 8. Assumptions are listed below.

Solute	Apical Zone (0–3 mm) $\Psi_s$		Subapical Zone (3–10 mm) $\Psi_s$	
	Cytoplasm <sup>a</sup>	Vacuole <sup>b</sup>	Cytoplasm <sup>c</sup>	Vacuole <sup>d</sup>
	bar			
$Cl^-$	-2.3	-2.3	0	-2.4
$Na^+$	-4.8	-4.8	0	-2.2
$K^+$	-3.4	-3.4	-2.8	-2.8
Fru	0	0	0	-0.8
Glc	0	0	0	-1.1
Suc	-1.2	0	-4.4	0
Pro <sup>e</sup>	-0.7	0	-2.0	0
Total calculated $\Psi_s$	-12.4	-10.5	-9.2	-9.3
Measured $\Psi_s$	-13.6		-12.0	

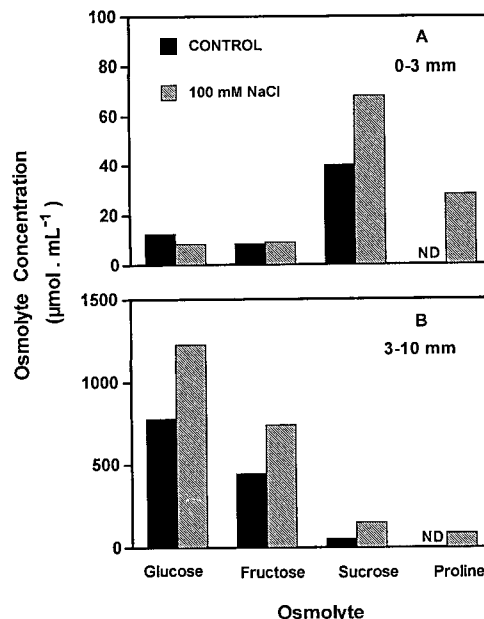
<sup>a</sup> Cytoplasm constitutes 50% of cell volume, Pro and Suc are compartmented in the cytoplasm, and  $Na^+$ ,  $Cl^-$ , and  $K^+$  are distributed at equal concentrations between cytoplasm and vacuole. <sup>b</sup> Vacuole constitutes 50% of cell volume. <sup>c</sup> Cytoplasm constitutes 5% of cell volume. <sup>d</sup> Vacuole constitutes 95% of cell volume and retains Fru and Glc. <sup>e</sup> Pro values are from Figure 7.

the individual sugars were not analyzed. Sugars were also identified as contributing appreciably to  $\Psi_s$  in the leaves of cereal species during water deficit (Jones et al., 1980; Munns and Weir, 1981).

Concentrations of Pro rose markedly to  $>10$  mM in response to salt stress (Fig. 7), but were always much lower than the concentration found in maize root tips by Voetberg and Sharp (1991), in which values averaged about 100 mM for the apical 0- to 3-mm zone in vermiculite at  $\Psi_w$  -16 bar. Pro concentrations decreased strongly at higher  $\Psi_w$  (Voetberg and Sharp, 1991), but even at  $\Psi_w$  of -5.5 bar (the  $\Psi_w$  for the nutrient solution containing 100 mM NaCl), Pro concentrations between 30 and 60 mM could be expected from interpolation of their data. This might suggest that Pro has a more important role in osmotic adjustment in response to water deficit than with salt stress, but a more likely explanation is that Voetberg and Sharp (1991) used much younger maize seedlings, in which roots had reached a length of only 5 cm and seed reserves to provide substrates for growth were more plentiful.

What would be the contribution to osmotic adjustment of the organic solutes if they were compartmented only in the cytoplasm? Without detailed information on cell structure along control and salinized roots (cf. Huang and van Steveninck, 1990; Sánchez-Aguayo and González-Utor, 1992), such a calculation is an approximation at best, but it is essential in forming an opinion regarding the significance of the organic solutes. If it is again assumed that the volume of cytoplasm is 50 and 5% for the 0- to 3-mm and 3- to 10-mm zones, respectively (see Lee et al., 1990), and using data shown in Figures 7 and 8, the calculated cytoplasmic concentrations of Glc, Fru, Suc, and Pro are as shown in Figure 10. In the 0- to 3-mm zone, Suc and Pro accumulated to higher concentrations in response to salt shock and together made an appreciable contribution to osmotic adjustment. By contrast, in the 3- to 10-mm zone, Fru and Glc reached much higher calculated concentrations, equivalent to  $\Psi_s$  values of -29 to -50 bar. Such values are clearly unrealistic in view of the actual root  $\Psi_s$  measurements (Fig. 9) of -7 bar (controls) to -12 bar (salinized). Thus, it seems reasonable to conclude that Suc and Pro could be compartmented within the cytoplasm, whereas Fru and Glc in the vacuolated cells must be primarily in the vacuole or distributed between vacuole and cytoplasm.

Our measurements of the principal organic solutes in immature and mature cells in the apical zone of maize roots agree with those of Sharp and co-workers, who found that at low vermiculite  $\Psi_w$  the concentration of reducing sugars increased greatly in more basal (vacuolated) cells and were the largest component of  $\Psi_s$  (Sharp et al., 1990; Voetberg and Sharp, 1991). However, in their experiments the increase in concentration of reducing sugars (presumably Fru and Glc) derived from a slower volumetric growth rate (i.e. roots had a smaller diameter), resulting in less dilution of solutes by expanding cells. Similar conclusions apply to the salinized roots we studied. Although root extension recovered to control levels by 10 to 12 h of salt shock, the diameter was smaller along



**Figure 10.** Estimated concentrations of soluble sugars and Pro assumed to be compartmented in the cytoplasm in the apical 0- to 3-mm (A) and 3- to 10-mm (B) zones of primary seminal roots of seedlings exposed to 100 mM NaCl shock for 48 h. Values are based on those given in Figure 7 for Pro and Figure 8 for the other organic solutes. ND, Not detectable by  $^{13}\text{C}$ -NMR analysis.

the entire length of the new growth. The ratio of the new root tissue volumes is equal to the ratio of the squares of the radii. Based on the measured root diameters at different salt levels (see "Results"), the ratio of the square of the radius for control/salt-shocked (100 mM NaCl) roots was 1.68. Thus, concentrations of organic solutes must increase by more than 1.68-fold to provide evidence of an increase in deposition. Figure 8 shows that the deposition rates for Fru and Glc did not increase, whereas there was evidence of increased deposition for Suc in the 3- to 10-mm zone and for Pro (Fig. 7) in both the 0- to 3-mm and 3- to 10-mm zones. Increased deposition rates of total osmotica and of  $\text{Na}^+$  and lower deposition rates of  $\text{K}^+$  were found in root tips of cotton exposed to 150 mM NaCl (Zhong and Lauchli, 1994), but the contribution to  $\Psi_s$  by individual solutes was not determined.

## CONCLUSIONS

Inorganic ions contributed relatively more to  $\Psi_s$  than did organic solutes. Rapid uptake of  $\text{Cl}^-$  on exposure to NaCl assisted in adjustment of  $\Psi_s$  during the initial hours of salt shock, a contribution to  $\Psi_s$  that would not normally occur during water deficit alone. Our results differ from those found when roots received an osmotic stress by exposure to mannitol, where it appears that organic solutes alone are involved in osmotic adjustment (Pritchard and Tomos, 1993). Seedling roots of maize responded to NaCl shock by maintaining higher concentrations of Suc and Pro in the 0- to 3-mm apical zone and of Fru and Glc in the subapical 3- to 10-mm zone. Based on the concen-

trations of Pro and Suc in the more cytoplasmic cells of the 0- to 3-mm zone and the higher concentrations of Fru and Glc in the vacuolated cells of the 3- to 10-mm zone, it seems reasonable to conclude that Pro and Suc are compartmented in the cytoplasm and Fru and Glc are compartmented in the vacuole (or distributed throughout the cell), which agrees with the measurements of solute concentration in maize roots at low  $\Psi_w$  (Voetberg and Sharp, 1991). Additionally, making assumptions about vacuolar size (Table III), Pro and Suc would have made a substantial contribution to cytoplasmic  $\Psi_s$  in the apical and subapical zone, whereas Fru and Glc could not possibly have been compartmented in the cytoplasm in the subapical zone. The inability to account for all of the root  $\Psi_s$  can be attributed to the presence of other organic solutes and inorganic ions that were not quantitated.

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