Carbohydrate Content and Root Growth in Seeds Germinated Under Salt Stress

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Abstract. Sugars and sugar alcohols have well-documented roles in salt tolerance of whole plants and maturing seeds. Less is known, however, about possible effects of these compounds during germination. Seeds from mannitol-accumulating salt-tolerant celery \textit{[Apium graveolens L. var. dulce (P. Mill.) DC]}, non-mannitol-accumulating salt-tolerant cabbage \textit{(Brassica oleracea L. var. capitata L. ‘Golden Acre’)}, and salt-sensitive non-mannitol-accumulating tobacco \textit{(Nicotiana tabacum L.)} and arabidopsis \textit{[Arabidopsis thaliana (L.) Heyn.]} were placed on vertical Phytagel plates containing 0 to 300 mM NaCl. Germination percentage, root elongation, and carbohydrate content of seeds and seedlings were assessed. With the exception of cabbage, there was no positive relationship between ability to germinate in NaCl and the reported species salt tolerance of the mature plant. For instance, while cabbage seeds germinated in 300 mM NaCl, germination of two celery cultivars was inhibited completely by 150 mM NaCl. In contrast, seeds from salt-sensitive tobacco and arabidopsis germinated in 200 mM NaCl. There was also no obvious relationship between the observed salt tolerance and total soluble carbohydrates in either non-imbibed seeds or in seedlings germinated in salt. For example, the most-salt tolerant species in these studies, cabbage, had the third highest seed and seedling carbohydrate concentration, while the next most tolerant, arabidopsis, had the lowest. However, both species contained significant amounts of the osmoprotective oligosaccharides raffinose or stachyose. In addition, although celery seedling mannitol concentration initially increased at low NaCl concentrations (50 mM), germination increased and mannitol concentration decreased at higher NaCl concentrations (100 mM). Finally, the broadest response observed was a large increase in seedling sucrose at the lowest salt concentration that significantly inhibited germination. Although most seeds, with the notable exception of cabbage, did not germinate at 150 mM NaCl, they were still metabolically active because the sucrose content was two to eight times higher than in non-imbibed seeds, suggesting a possible role for sucrose in salt-stressed germinating seeds. These results not only suggest that mechanisms providing salt tolerance in mature plants are different from those in germinating seeds, but also that, even when the same mechanisms are employed, they may be less effective in seeds.

Soil salinization arising from evaporative concentration of salts in irrigated fields and from incursion of seawater into depleted freshwater aquifers is a growing problem. It is estimated that 33% of irrigated land worldwide is affected by salinization, and land lost for cultivation due to salinization continues to exceed land becoming available (Marshchner, 1998). Many important crops perform marginally even in slightly saline soils (Shannon and Grieve, 1999). Plants have diverse cellular mechanisms to protect against specific ion effects and osmotic stresses imposed by saline soils. These mechanisms include increases in proteins involved in water transport (i.e., aquaporins), ion sequestration and secretion (Baiges et al., 2002; Figueras et al., 2004), as well as increases in osmolytes or compatible solutes (Bray et al., 2000).

Osmolytes balance differences in $\Psi_S$ between the cytosol and vacuole and are essential for maintaining turgor at low tissue water potential. In contrast, compatible solutes, so called due to their ability to accumulate to high concentrations without disrupting cellular processes, are thought to maintain a sphere of hydration around proteins or membranes to allow continued function at low $\Psi_S$ (Jennings and Burke, 1990; Stoop et al., 1996). The same compounds maintain a “positive” water potential gradient to ensure uptake in a hypertonic environment. A number of compounds can function as osmolytes or compatible solutes, including simple sugars (such as sucrose, glucose, and fructose), the raffinose family oligosaccharides [RFOs (such as stachyose and raffinose)], and polyols (such as sorbitol, pinitol, and mannitol). For instance, simple sugars not only act as osmolytes but can also stabilize membranes in desiccated resurrection plants (Norwood et al., 2000). In addition to increased production via induction of the sucrose phosphate synthase activity [SPS (Huber and Huber, 1996; Yang et al., 2001)], sucrose also is generated quickly via gluconeogenesis or from catabolism of polymeric carbohydrates in response to salt or osmotic stress (Levitt, 1980). Analogous carbohydrate conversions appear to be a common phenomenon in desiccation-tolerant seeds (Peterbauer and Richter, 2001; Prado et al., 2000). For instance, RFOs accumulate during seed maturation where they are believed to form, with sucrose, a vitrified matrix where they are believed to form, with sucrose, a vitrified matrix (Obendorf, 1997).

The roles of sugar alcohols in salt tolerance also are well documented (Stoop et al., 1996; Williamson et al., 2002). In salt...
or osmotically stressed celery plants, the activation of mannitol synthesis in leaves combined with inactivation of mannitol catabolism in sink tissues results in increased mannitol concentrations throughout the plant. Physiological and transgenic evidence indicates that, unlike sucrose, mannitol functions as an osmoprotectant rather than as a simple osmolyte. Transgenic experiments likewise have demonstrated the role of mannitol in tolerance to reactive oxygen species in plants and cell cultures (Shen et al., 1997).

A variety of carbohydrates clearly play important roles in salt and osmotic stress tolerance in plants. Left unanswered, however, is the question of how carbohydrates in seed (both pre-existing and those accumulating during germination) affect germination and root elongation under salt stress. We assessed germination and carbohydrate status of seeds from two salt-tolerant species, tobacco and arabidopsis, under increasing levels of salt stress.

Materials and Methods

Plant material and germination conditions. Cultivars and seed sources were as follows: salt-sensitive species, arabidopsis ‘Columbia’ and tobacco ‘K326’ (provided by S. Clouse, North Carolina State University) (Tarczynski et al., 1992; Thomas et al., 1995); a non-mannitol synthesizing salt-tolerant plant, ‘Golden Acre’ cabbage (Wyatt-Quarles Seed Co., Garner, NC) (Maas and Grattan, 1999), and two salt-tolerant mannitol-synthesizing cultivars, ‘Florida’ celery (Asgrow Seed Co., Kalamazoo, MI), and ‘Ventura’ celery (Johnny’s Select Seeds, Winslow, ME) (Stoop et al., 1996). Seeds were surface-sterilized in 70% ethanol for 5 min and then rinsed with sterile distilled water. Next, seeds were agitated gently in a 1.85% sodium hypochlorite solution containing 0.05% Tween 20 (Sigma Aldrich, St. Louis) for 10 min and then rinsed five times with sterile distilled water. The sterilization procedure did not adversely affect seed viability as the percentage germination of sterilized and nonsterilized seeds on moist paper towels was similar within a given species.

For salt treatments, sterilized seeds were suspended in sterile water 0.01% agar solution and pipetted onto growth media in 15-cm-diameter polystyrene petri dishes at equidistant locations, 50 seeds per plate. Growth medium contained 9 g L⁻¹ Phytagel (Sigma Aldrich) and 4.4 g L⁻¹ Murashige and Skoog salts (Murashige and Skoog, 1962) plus vitamins (Caisson Laboratories, Rexburg, ID) at pH 5.8 and 0–17.53 mg L⁻¹ added sodium chloride (0–300 mM). Plates containing seeds were incubated at 22 °C under a 12-h photoperiod of 150 μmol·m⁻²·s⁻¹ provided by white fluorescent lights. To allow accurate measurement of root length, plates were incubated vertically to ensure geotropic root growth. Because time to emergence and radical elongation varied between species, seedlings were harvested at the same developmental stage (i.e., when lateral roots began to form in the 0 mM NaCl treatment). For arabidopsis, celery, and tobacco this occurred 11 d after plating (DAP), while for cabbage this occurred at 6 DAP. Thus, cabbage was grown for 6 DAP while all other species were grown for 11 DAP, at which point both seedlings and any seed that had not germinated (i.e., ungerminated seeds) were collected for carbohydrate analysis.

Data collection. Germination was defined as radicle elongation greater than the seed diameter, and no radicle lengths were recorded unless they were greater than this measure. Each plate was photographed daily using a digital camera. A 1-mm grid was superimposed over the image, and root length was recorded for each seedling on each plate.

Extraction of soluble sugars. All tissues from all replicates for a given species and salt combination were pooled, frozen in liquid nitrogen, and pulverized, and total carbohydrates extracted essentially as described by Hubbard et al. (1989). Up to 200 mg of this material was added to 3 mL 80% ethanol and ground in a mortar with a pestle. The solution was transferred to a 15-mL centrifuge tube, and the process was repeated twice, producing a total of 9 mL. The solution was incubated at 80 °C for 1 h and then centrifuged at 750g, for 15 min to pellet debris. The supernatant fluids were evaporated using a rotary evaporator (Evapo-Mix, Buchler Instruments, Fort Lee, NJ). After evaporation, the remaining solute was dried at 60 °C for 12 h in a drying oven and resuspended in 1 mL of deionized distilled water. Before analysis by high-performance liquid chromatography (HPLC), samples were centrifuged at 10,000g in a microcentrifuge for 5 min to remove any remaining insoluble material.

Analysis of soluble sugars. Carbohydrate content of the samples was determined using a CarboPac PA-1 column (Dionex, Sunnyvale, CA), 250 mm long, 4 mm i.d., fitted with a CarboPac PA-1 guard column (Dionex). The column oven was set at 30 °C. The mobile phase used 200 mM NaOH sparged with helium for 2 h. Ten microliters of sample was injected using water as the carrier solvent. Flow was set at 1.0 mL min⁻¹ (isocratic) using a Dionex GS50 gradient pump. The detector was a Dionex pulsed amperometric (PAD) set to 100 nC (nanocoulombs). Samples were filtered through Dionex OnGuard II H columns to remove free amino acid interference. The samples were run with dilutions of standards to construct five-point curves fitted to zero.

Statistical analysis. Experiments were arranged in a randomized complete block design with five replications. The treatment design was a 5 (plant species/cultivars) × 6 (NaCl concentrations) factorial. Significance of germination and root length measurements for each species was tested using a one-way fixed-effects general linear model with partial sum of squares. Root length means include only those seeds that germinated. Significance of differences between species for a specific treatment was established using least-squares means with a combined replication × species error term. Differences between treatments for specific species were tested using regression analysis tested for lack of fit and a replication × treatment error term.

Given the complex nature of the germination process, we extended our analysis beyond simple comparisons of root length and percent germination to describe more fully the observed responses. The Weibull model was shown to be a superior method for describing the dynamic nature of germination under phytotoxic conditions, giving precise estimates of germination speed, shape of the germination response curve, and germination percentage (Dias, 2001):

\[ Y = a \cdot \left(1 - \exp\left\{-\left[\left(X - I\right)/k\right]^v\right\}\right) \]

where \( Y \) is the proportion of germinated seeds on day \( X \), \( a \) is an estimate of overall germination proportion, \( I \) is an estimate of the latest day when germination was 0, and \( k \) (days) is a scale
parameter, with \( l + k \) estimating the time at which proportion of germination is 0.63. The dimensionless parameter \( c \) estimates the symmetry of the germination distribution around the normal curve (\( c > 3.60 = \) negative asymmetry, \( c < 3.26 = \) positive asymmetry, and \( 3.26 \leq c \leq 3.60 = \) symmetrical). In addition, the Weibull function includes measures of all the more common germination indices and cumulative functions. The \( l \) variable describes the onset of germination, \( k \) the slope of the germination profile (\( 1/k \) estimates germination rate) and the shape (\( c \)) of the germination response curve. Using the Weibull values resulting from our data set, we rearranged the equation to calculate the number of days to 50% final germination (\( G_{50} \)).

**Results**

**Effects of salt stress on germination.** Weibull regression analyses show cabbage with the least reduction in final germination due to increasing NaCl concentration (Fig. 1). Cabbage final germination percentage was not significantly reduced until 200 mM NaCl (data not shown). Final germination percentage of the salt-sensitive species arabidopsis and tobacco at 0 mM was 93% and 94%, respectively by day 11. Somewhat unexpectedly, at 50 and 100 mM NaCl, arabidopsis germination only decreased to 89% and 82%, respectively, and tobacco germination only decreased to 94% and 84%, respectively. In contrast, germination of the salt-tolerant species celery was much more sensitive to salt than arabidopsis and tobacco. ‘Ventura’ celery showed a greater reduction in germination response to increasing NaCl concentration (to 40% and 5% at 50 and 100 mM NaCl, respectively) than Florida (to 60% and 25% at 50 and 100 mM NaCl, respectively).

Generally, increasing NaCl concentration delayed and slowed germination. For example, examining the differences in \( G_{50} \) between the 0 and 50 mM treatments, cabbage was least affected, followed by arabidopsis, where \( G_{50} \) was increased by 1.3 d (Table 1). The celery cultivars were increased by 1.6 d, and tobacco, 2.6 d. Although both arabidopsis and tobacco had similar final germination percentages at each NaCl concentration, germination onset in response to salt was more delayed in tobacco (Fig. 1).

**Effects of salt stress on radicle length.** Increasing NaCl concentration strongly decreased root length in all species except cabbage, which maintained root growth in 200 mM NaCl.
Table 2. Root length and regression analysis of 5 species/cultivars in response to increasing sodium chloride concentration (0–300 mM).

| Species and cultivar | Conc. of added NaCl (mM) | Regression analysis | Final root length (mm) | $F$ value $z$ |
|----------------------|--------------------------|---------------------|------------------------|-------------|
|                      | 0 | 50 | 100 | 150 | 200 | 300 | Linear | Linear slope | Quadratic | Higher-order, linear slope |
| Arabidopsis $y$     | 16.3 $a$ | 10.5 | 5.9 | 1.3 | 1.2 | 0 | 207.50 $***$ | $-0.0775$ | 13.06 $a$ | 1.02 $a$ |
| Cabbage              | 29.8 | 25.7 | 28.1 | 19.8 | 12.9 | 2.2 | 174.02 $***$ | $-0.0599$ | 1.13 $a$ | 1.20 $a$ |
| 'Florida' Celery     | 13.7 | 9.6 | 6.1 | 1.0 | 0 | 0 | 220.71 $***$ | $-0.0560$ | 21.21 $**$ | 0.55 $a$ |
| 'Ventura' Celery     | 14.1 | 9.5 | 8.3 | 3.8 | 0 | 0 | 357.95 $***$ | $-0.0525$ | 95.50 $***$ | 1.28 $a$ |
| Tobacco $y$          | 7.1 | 6.3 | 2.8 | 1.1 | 1.0 | 0 | 266.15 $***$ | $-0.0368$ | 3.70 $a$ | 15.61 $**$ |

Data are means of five independent repetitions.
$y$Denotes salt-sensitive species.
$z$Length data were analyzed by linear, quadratic, and higher-order regression models.
NS,*,**,*** $F$ values reported are nonsignificant, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

Table 3. Carbohydrate concentration in seedlings germinated under increasing NaCl (0–300 mM).

| Species and carbohydrate | NaCl (mM) | Carbohydrate concn. (µg g$^{-1}$ FW) | Regression analysis |
|--------------------------|-----------|-------------------------------------|---------------------|
|                          | 0 | 50 | 100 | 150 | 200 | 300 | Linear | Quadratic | Higher-order |
| Arabidopsis $y$          | Fructose | 6 $a$ | 23 | 0 | 0 | 0 | 0 | 2,944 $***$ | 257 $***$ | 0 $a$ |
| Glucose                 | 91 | 84 | 35 | 26 | 22 | 29 | 2,919 $***$ | 1,125 $***$ | 42 $a$ |
| Sucrose                 | 161 | 247 | 169 | 498 | 613 | 512 | 3,760 $***$ | 329 $***$ | 0 $a$ |
| Myo-inositol            | 34 | 77 | 30 | 98 | 109 | 69 | 3,643 $***$ | 3,129 $***$ | 1,831 $ ***$ |
| Raffinose               | 0 | 0 | 0 | 0 | 0 | 31 | $-a$ | $-a$ | $-a$ |
| Total                   | 292 | 431 | 234 | 856 | 744 | 641 | 45,335 $***$ | 2,611 $***$ | 12,465 $ ***$ |
| Cabbage                 | Fructose | 351 | 334 | 187 | 399 | 510 | 1,011 | 385 $***$ | 159 $***$ | 1 $a$ |
| Glucose                 | 566 | 566 | 298 | 646 | 785 | 1,230 | 4,277 $***$ | 1,596 $***$ | 131 $***$ |
| Sucrose                 | 249 | 315 | 297 | 920 | 1,318 | 3,793 | 141,835 $***$ | 30,306 $***$ | 519 $***$ |
| Myo-inositol            | 130 | 166 | 131 | 305 | 392 | 458 | 9,380 $***$ | 29 $a$ | 706 $***$ |
| Raffinose               | 0 | 0 | 0 | 0 | 0 | 455 | $-a$ | $-a$ | $-a$ |
| Stachyose               | 0 | 0 | 0 | 0 | 0 | 455 | $-a$ | $-a$ | $-a$ |
| Total                   | 1,296 | 1,381 | 913 | 2,270 | 3,029 | 7,129 | 149,201 $***$ | 37,883 $***$ | 67 $a$ |
| 'Florida' Celery        | Fructose | 581 | 1,376 | 1,075 | 799 | 911 | 1,222 | 120 $***$ | 1 $a$ | 728 $a$ |
| Glucose                 | 357 | 961 | 748 | 487 | 538 | 667 | 2 $a$ | 90 $a$ | 2,549 $a$ |
| Sucrose                 | 249 | 315 | 297 | 920 | 1,318 | 3,793 | 141,835 $***$ | 30,306 $***$ | 519 $***$ |
| Mannitol                | 327 | 492 | 395 | 98 | 211 | 151 | 3,618 $***$ | 131 $***$ | 1,440 $ ***$ |
| Myo-inositol            | 16 | 22 | 43 | 70 | 69 | 37 | 788 $***$ | 1,568 $***$ | 345 $ ***$ |
| Raffinose               | 0 | 0 | 0 | 0 | 0 | 455 | $-a$ | $-a$ | $-a$ |
| Stachyose               | 0 | 0 | 0 | 0 | 0 | 455 | $-a$ | $-a$ | $-a$ |
| Total                   | 1,597 | 5,078 | 6,635 | 9,983 | 10,180 | 8,394 | 55,852 $***$ | 31,838 $***$ | 1,567 $***$ |
| 'Ventura' Celery        | Fructose | 350 | 1,167 | 1,049 | 1,189 | 1,919 | 1,412 | 3,979 $***$ | 1,556 $***$ | 64 $a$ |
| Glucose                 | 249 | 766 | 619 | 649 | 1,096 | 728 | 3,204 $***$ | 2,376 $***$ | 43 $a$ |
| Sucrose                 | 641 | 1,917 | 6,178 | 9,589 | 6,770 | 4,915 | 6,131 $***$ | 10,847 $***$ | 150 $ ***$ |
| Mannitol                | 146 | 896 | 112 | 84 | 86 | 62 | 17,522 $***$ | 22 $a$ | 20,285 $a$ |
| Myo-inositol            | 211 | 444 | 221 | 217 | 188 | 112 | 6,080 $***$ | 694 $***$ | 2,654 $ ***$ |
| Raffinose               | 16 | 22 | 43 | 70 | 69 | 37 | 788 $***$ | 1,568 $***$ | 345 $ ***$ |
| Total                   | 1,597 | 5,190 | 8,179 | 11,728 | 10,059 | 7,229 | 65,878 $***$ | 113,826 $***$ | 302 $ ***$ |
| Tobacco $y$             | Fructose | 60 | 22 | 388 | 467 | 102 | 23 | 1 $a$ | 228 $***$ | 5 $a$ |
| Glucose                 | 99 | 90 | 334 | 450 | 117 | 48 | 15 $a$ | 850 $a$ | 18 $a$ |
| Sucrose                 | 284 | 487 | 1,270 | 4,669 | 2,663 | 3,492 | 2,834 $***$ | 515 $***$ | 113 $ ***$ |
| Myo-inositol            | 84 | 180 | 307 | 405 | 195 | 83 | 2 $a$ | 1,200 $***$ | 32 $a$ |
| Total                   | 527 | 779 | 2,299 | 5,991 | 3,077 | 3,646 | 21,070 $***$ | 11,002 $***$ | 544 $ ***$ |

Data were analyzed by linear, quadratic, and higher-order regression models.
$y$Denotes salt-sensitive species.
$z$Hyphens indicate a lack of data for analysis.
$x$Data presented are means of duplicate determinations of a pooled sample from five replications.
NS,$*,**,*** $F$ values reported are nonsignificant, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.
(Table 2). In tobacco and arabidopsis, both selected for their reported salt sensitivity, roots continued to grow at a low level in 200 mM NaCl (mean lengths of 1.2 and 1.0 mm, respectively). In contrast, the two cultivars of celery, selected for their reported salt tolerance, had no root growth at 200 mM NaCl. Root length response to NaCl concentration was predominantly linear in all species. Tobacco radicle length was least affected (0.04 mm less radicle growth per 1 mM NaCl increase), while arabidopsis radicle length was most affected (0.08 mm less radicle growth per 1 mM NaCl increase). Cabbage and the two celery cultivars had similar radicle length responses to increasing salt concentrations (0.05–0.06 mm less radicle growth per 1 mM NaCl increase).

**Assessment of carbohydrate content.** There appeared to be no consistent relationship between the capacity to germinate in elevated salt concentrations and total carbohydrate concentration (Table 3) or content (Fig. 2). For instance, although the most NaCl-tolerant species, cabbage, had high carbohydrate concentrations, the next most tolerant, arabidopsis, had the lowest concentrations. However, a relationship may exist between carbohydrate type and germination salt tolerance, because cabbage and arabidopsis were the only species that contained raffinose or stachyose. Although the two mannitol-containing celery cultivars were among the most salt-sensitive, seedling mannitol concentration increased at 50 mM added NaCl but decreased at higher NaCl concentrations. With the possible exception of cabbage, as germination declined at higher salt concentrations (>100 mM) (Fig. 1), sucrose became the most abundant soluble carbohydrate in all species (Fig. 2). The increase in sucrose amount and concentration was most dramatic in seedlings at the lowest NaCl concentration that completely inhibited root elongation. Although seeds did not germinate at this NaCl concentration, the amount of sucrose in these nongerminated seeds was two to eight times higher than amounts of sucrose in the non-imbibed seeds. Although cabbage still germinated and roots elongated at 300 mM NaCl, cabbage seedling sucrose concentration also began to increase greatly at >150 mM NaCl.

**Discussion**

Mannitol-synthesizing species such as celery have been shown to grow well under high levels of salt stress (Everard et al., 1994; Pharr et al., 1995), as do raffinose oligosaccharide-accumulating plants such as cabbage (Maas and Grattan, 1999; Minorsky, 2003). Conversely, the non-mannitol-synthesizing species arabidopsis and tobacco are reported to be salt-sensitive (Gao et al., 2003; Karakas et al., 1997; Shi et al., 2002, 2003; Tarczynski et al., 1992; Thomas et al., 1995; Xiong and Zhua, 2002). However, our results suggest that the ability of seeds to germinate in the presence of NaCl did not necessarily mirror the salt tolerance of mature plants (Fig. 1). For instance, although salt-tolerant cabbage germinated and seedling roots elongated relatively well under salt stress, the reportedly salt-sensitive species arabidopsis and tobacco germinated and had greater relative root elongation than did salt-tolerant cultivars of celery.

Further, there seemed to be no general relationship between capacity to germinate in high salt and initial non-imbibed seed carbohydrate concentration (Table 3) or content (Fig. 2). For instance, the most tolerant species, cabbage, had the third highest seed and seedling carbohydrate concentration, but the next most tolerant, arabidopsis, had the lowest (Table 3). Both cabbage and arabidopsis seeds did, however, contain significant amounts of raffinose or stachyose. Although there is no evidence for their efficacy in these studies, both of these
Oligosaccharides have been implicated in salt or osmotic stress tolerance (Gupta et al., 1993; Liu et al., 1998; Minorsky, 2003; Obendorf, 1997). In addition, a specific mechanism that is highly effective in the mature plant may be much less effective during germination or may be supplanted by a different response altogether. For instance, accumulation of mannitol is a potent protective mechanism against salt stress in vegetatively growing celery (Stoop et al., 1996): however, celery cultivars were among the most salt-sensitive during germination. Although celery mannitol concentration initially increased in response to low NaCl, at higher NaCl concentrations, mannitol concentration decreased, and sucrose, not mannitol, was the most abundant compatible solute. These results are consistent with work such as that of Dumbroff and Cooper (1974) who showed that tomato (Solanum lycopersicum L.) was far less salt-tolerant at earlier stages of development than at the flowering or fruiting stage.

The broadest response observed in all species was a large increase in seedling sucrose concentration and content at NaCl concentrations that completely inhibited root elongation (Fig. 2). Although seeds failed to germinate at this NaCl concentration, they were still metabolically active because seedling sucrose content was two to eight times higher than in non-imbibed seeds. Further, the inference that the observed increase in sucrose was a specific response to salt, and not simply a result of decreased sucrose utilization, is supported by the observation that the activity of the sucrose biosynthetic enzyme sucrose phosphate synthase is induced by salt stress (Huber and Huber, 1996; Yang et al., 2001).

Our observed salt sensitivity in reportedly salt-tolerant species was unexpected. It seems intuitive that species such as celery that evolved in saline environments (Clapham et al., 1987; Megaloudi, 2005) should also be able to germinate in the presence of salt. However, suspension of germination of salt-adapted species in high salt might represent an alternate strategy, a method of escape or avoidance that facilitates successful completion of the life cycle in a high-stress environment. In fact, seeds of many species can complete the early requisite metabolic events of pregermination in a highly saline or low ψs environment without germinating and then redesiccate without damage. This is the basis of osmotic priming, a technique used widely to increase germination rate by first imbibing seeds in a low-ψs solution (Parera and Cantliffe, 1994). During osmotic priming, seeds complete the early metabolic events necessary for germination, such as membrane repair, enzyme synthesis, and sucrose accumulation (Hong et al., 1997; Singh and Kumar, 1992; Thind, 1991), but germination does not occur. Seeds so treated can be dehydrated without damage and, when rehydrated, germinate more rapidly than nonprimed seeds. These results suggest that an analogous process might be a normal mode of germination for plants such as celery that are adapted to growth in saline environments.

In summary, the results presented here suggest that protective mechanisms against salinity vary not only among species but may also vary ontogenetically. A clearer picture of the salt-tolerance mechanisms employed throughout the entire life cycle of a plant will allow breeders to identify additional sources of germplasm for use in breeding new cultivars. Because development of salt-tolerant cultivars of important crop species will be necessary to maintain productivity on saline soils, continued research on salt and osmotic tolerance mechanisms is critical.

**Literature Cited**

Baiges, I., A.R. Schaffner, M.J. Affenzeller, and A. Mas. 2002. Plant aquaporins. Physiol. Plant. 115:175–182.

Bray, E.A., J. Bailey-Serres, and E. Weretilnyk. 2000. Responses to abiotic stresses, p. 1158–1203. In: B.B. Buchanan, W. Grusscem, and R.L. Jones (eds.). Biochemistry and molecular biology of plants. Amer. Soc. Plant Physiol., Rockville, MD.

Clapham, A., T. Tutin, and E. Warburg. 1987. Flora of the British Isles. Cambridge University Press, Cambridge, England.

Dias, L.S. 2004. Describing phytotoxic effects on cumulative germination. J. Chem. Ecol. 27:411–418.

Dumbroff, E.B. and A.W. Cooper. 1974. Effects of salt stress applied as that of Dumbroff and Cooper (1974) who showed that tomato (Solanum lycopersicum L.) was far less salt-tolerant at earlier stages of development than at the flowering or fruiting stage. In summary, the results presented here suggest that pro-

Evander, J.D., R. Gucci, S.C. Kann, J.A. Flore, and W.H. Loescher. 1994. Gas exchange and carbon partitioning in the leaves of celery (Apium graveolens L.) at various levels of root zone salinity. Plant Physiol. 106:281–292.

Figueiras, M., J. Pujal, A. Saleh, R. Save, M. Pages, and A. Goday. 2004. Maize Rab17 overexpression in Arabidopsis plants promotes osmotic stress tolerance. Ann. Appl. Biol. 144:251–257.

Gao, X., Z. Ren, Y. Zhao, and H. Zhang. 2003. Overexpression of SOD2 increases salt tolerance of Arabidopsis. Plant Physiol. 133:1873–1881.

Gupta, A.K., S. Jagdeep, K. Narinder, and S. Rangil. 1993. Effect of polyethylene glycol-induced water stress on germination and reserve carbohydrate metabolism in chickpea cultivars differing in tolerance to water deficit. Plant Physiol. Biochem. 31:369–378.

Hong, F., C. Ma, X. Wang, and G. Ji. 1997. Effect of polyvinyl alcohol pretreatment on seed germination, metabolism and growth of wheat. Acta Agron. Sinica 23:247–251.

Hubbard, N.L., S.C. Huber, and D.M. Pharr. 1989. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (Cucumis melo L.) fruits. Plant Physiol. 91:1527–1534.

Huber, S.C. and J.L. Huber. 1996. Role and regulation of sucrose phosphate synthase in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:431–444.

Jennings, D.H. and R.M. Burke. 1990. Compatible solutes—the mycological dimension and their role as physiological buffering agents. New Phytol. 116:277–283.

Karakaš, B., P. Ůzias Akins, C. Stushnoff, M. Suerfelder, and M. Rieger. 1997. Salinity and drought tolerance of mannitol-accumulating transgenic tobacco. Plant Cell Environ. 20:609–616.

Levitt, J. 1980. Responses of plants to environmental stresses. 2d ed. Academic Press, New York.

Liu, J.J.J., D.C. Krenz, A.F. Galvez, and B.O. de Lumen. 1998. Galactinol synthase (GS): increased enzyme activity and levels of mRNA due to cold and desiccation. Plant Sci. 134:11–20.

Maas, E.V. and S.R. Grattan. 1999. Crop yields as affected by salinity, p. 55–108. In: R.W. Skaggs and J. van Schilfgaarde (eds.). Agricultural drainage. Agron. Monogr. 38. ASA, CSSA, SSSA, Madison, W1.

Marschner, H. 1998. Mineral nutrition of higher plants. 2nd ed. Academic Press, London.

Megaloudi, F. 2005. Wild and cultivated vegetables, herbs and spices in Greek antiquity. Environ. Archaeol. 10:73–82.

Minorsky, P.V. 2003. Raffinose oligosaccharides. Plant Physiol. 131:1159–1160.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Norwood, M., M.R. Truesdale, A. Richter, and P. Scott. 2000. Photosynthetic carbohydrate metabolism in the resurrection plant, Craterostigma plantagineum. J. Expt. Bot. 51:159–165.

Obendorf, R.L. 1997. Oligosaccharides and galactosyl cyclitols in seed desiccation tolerance. Seed Sci. Res. 7:63–74.

Parera, C.A. and D.J. Cantliffe. 1994. Presowing seed priming. Hort. Rev. (Amer. Soc. Hort. Sci.) 16:109–141.

J. Amer. Soc. Hort. Sci. 132(6):876–882. 2007.
Peterbauer, T. and A. Richter. 2001. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Sci. Res. 11:185–197.

Pharr, D.M., J.M.H. Stoop, M.E. Studer-Feusi, J.D. Williamson, M.O. Massel, and M.A. Conkling. 1995. Mannitol catabolism in plant sink tissues, p. 180–194. In: M.A. Madora and W.J. Lewis (eds.). Carbon partitioning and source-sink interactions in plants. Current Topics in Plant Physiology, Vol. 13. Amer. Soc. Plant Physiol., Rockville, MD.

Prado, F.E., C. Boero, M. Gallardo, and J.A. Gonzalez. 2000. Effect of NaCl on germination, growth, and soluble sugar content in Chenopodium quinoa Willd. seeds. Bot. Bul. Acad. Sinica (Taipei) 41:27–34.

Shannon, M.C. and C.M. Grieve. 1999. Tolerance of vegetable crops to salinity. Scientia Hort. 78:5–38.

Shen, B., R.G. Jensen, and H.J. Bohnert. 1997. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Plant Physiol. 113:1177–1183.

Shi, H., L. Xiong, B. Stevenson, T. Lu, and J.-K. Zhu. 2002. The arabidopsis salt overly sensitive 4 mutants uncover a critical role for vitamin B6 in plant salt tolerance. Plant Cell 14:575–588.

Shi, H., B. Lee, S.-J. Wu, and J.-K. Zhu. 2003. Overexpression of a plasma membrane Na+/H+ antiporter gene improves salt tolerance in Arabidopsis thaliana. Nat. Biotechnol. 21:81–85.

Singh, R.A. and P. Kumar. 1992. Effect of water stress and hardening on metabolic changes in chick pea (Cicer arietinum L.). Indian J. Plant Physiol. 35:252–257.

Stoop, J.M.H., J.D. Williamson, and D.M. Pharr. 1996. Mannitol metabolism in plants: a method for coping with stress. Trends Plant Sci. 1:139–144.

Thind, S.K. 1991. Effects of a long chain aliphatic alcohol mixture on growth and solute accumulation in water stressed wheat seedlings under laboratory conditions. Plant Growth Regulat. 10:223–234.

Tarczynski, M.C., R.G. Jensen, and H.J. Bohnert. 1992. Expression of a bacterial mtlD gene in transgenic tobacco leads to the production and the accumulation of the sugar alcohol, mannitol. Proc. Natl. Acad. Sci. USA 89:2600–2604.

Thomas, J.C., M. Sepahi, B. Arendall, and H.J. Bohnert. 1995. Enhancement of seed germination in high salinity by engineering mannitol expression in Arabidopsis thaliana. Plant Cell Environ. 18:801–806.

Williamson, J.D., D.B. Jennings, W.-W. Guo, D.M. Pharr, and M. Ehrenshaft. 2002. Sugar alcohols, salt stress, and fungal resistance: Polysols-multifunctional plant protection? J. Amer. Soc. Hort. Sci. 127:467–473.

Xiong, L. and J.-K. Zhu. 2002. Salt tolerance, p. 1–22. In: C.R. Somerville and E.M. Meyerowitz (eds.). The arabidopsis book. Amer. Soc. Plant Biologists, Rockville, MD.

Yang, J., J. Zhang, Z. Wang, and Q. Zhu. 2001. Activities of starch hydrolytic enzymes and sucrose-phosphate synthase in the stems of rice subjected to water stress during grain filling. J. Expt. Bot. 52:2169–2179.