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 [*Apium graveolens* L. var. *dulce* (P. Mill.) DC], non-mannitol-accumulating salt-tolerant cabbage (*Brassica oleracea* L. var. *capitata* L. 'Golden Acre'), and salt-sensitive non-mannitol-accumulating tobacco (*Nicotiana tabacum* L.) and arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] 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 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 (Marschner, 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 ψ_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 ψ_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 environment that stabilizes desiccated seed membranes. Upon germination, RFOs are converted rapidly to monomeric sugars (Obendorf, 1997).

The roles of sugar alcohols in salt tolerance also are well documented (Stoop et al., 1996; Williamson et al., 2002). In salt

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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, celery and cabbage, and two salt-sensitive 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 5 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 replications 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_n 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_n 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):

$$Y = a \cdot (1 - \exp\{-[(X - l)/k]^c\})$$

where Y is the proportion of germinated seeds on day X , a is an estimate of overall germination proportion, l 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 arabisopsis and tobacco at 0 mM was 93% and 94%, respectively by day 11. Somewhat unexpectedly, at 50 and 100 mM NaCl, arabisopsis 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 arabisopsis 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 arabisopsis, 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 arabisopsis and tobacco had similar final germination percentages at each NaCl concentration, germina-

Fig. 1. Seed germination percentages of salt-tolerant species (‘Florida’ celery, ‘Ventura’ celery, and cabbage) and salt-sensitive species (arabisopsis and tobacco) in response to 0–150 mM NaCl. Weibull-generated curves are superimposed on the means of data points from five independent replications. Error bars represent ± 1 SE. NaCl (mM): (■) 0; (□) 50; (●) 100; (○) 150.

Table 1. Time to 50% final germination (G_{50}) under increasing sodium chloride concentrations (0–300 mM) as predicted using Weibull nonlinear regression.

	G_{50} (d)					Regression analysis	
	Concn. of added NaCl (mM)					Linear	Higher order
	0	50	100	150	200	F value ^z	
Arabidopsis ^x	1.9	3.2	4.5	6.7	— ^y	141.99***	2.85 ^{ns}
Cabbage	1.6	1.6	—	—	—	23.17**	6.68 ^{ns}
‘Florida’ Celery	4.0	5.7	7.8	—	—	129.43***	0.31 ^{ns}
‘Ventura’ Celery	4.9	6.4	—	—	—	38.65**	—
Tobacco ^x	2.7	5.3	7.2	10.3	—	240.65***	13.55**

^zData from five independent replications and were analyzed by linear, quadratic, and higher-order regression models.

^yHyphens indicate no germination or integration failure of the Weibull model.

^xDenotes salt-sensitive species.

^{ns}, **, *** F values reported are nonsignificant, $P < 0.001$, and $P < 0.0001$, respectively.

tion 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).

	Concn. of added NaCl (mM)						Regression analysis			
	0	50	100	150	200	300	Linear	Linear slope	Quadratic	Higher-order, linear slope
	Final root length (mm)						<i>F</i> value ^z			
Arabidopsis ^y	16.3 ^x	10.5	5.9	1.3	1.2	0	207.50***	−0.0775	13.06*	1.02 ^{NS}
Cabbage	29.8	25.7	28.1	19.8	12.9	2.2	174.02***	−0.0599	1.13 ^{NS}	1.20 ^{NS}
‘Florida’ Celery	13.7	9.6	6.1	1.0	0	0	220.71***	−0.0560	21.21**	0.55 ^{NS}
‘Ventura’ Celery	14.1	9	8.5	3.8	0	0	357.96***	−0.0525	95.50***	1.28 ^{NS}
Tobacco ^y	7.1	6.3	2.8	1.1	1.0	0	266.15***	−0.0368	3.70 ^{NS}	15.61**

^xData are means of five independent repetitions.^yDenotes salt-sensitive species.^zLength 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)						Regression analysis ^z		
	0	50	100	150	200	300	<i>F</i> value		
	Carbohydrate concn. (μg·g ^{−1} FW)						Linear	Quadratic	Higher-order
Arabidopsis ^y									
Fructose	6 ^w	23	0	0	0	0	2,944***	257***	0 ^{NS}
Glucose	91	84	35	26	22	29	2,919***	1,125***	42**
Sucrose	161	247	169	498	613	512	3,760***	329***	0 ^{NS}
Myo-inositol	34	77	30	98	109	69	3,643***	3,129***	1,831***
Raffinose	0	0	0	0	0	31	— ^x	—	—
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 ^{NS}
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*	706***
Raffinose	0	0	0	0	24	182	16,018***	7,698***	727**
Stachyose	0	0	0	0	0	455	—	—	—
Total	1,296	1,381	913	2,270	3,029	7,129	149,201***	37,883***	67*
‘Florida’ Celery									
Fructose	581	1,376	1,075	799	911	1,222	120***	1 ^{NS}	728***
Glucose	357	961	748	487	538	667	2 ^{NS}	90***	2,549***
Sucrose	681	1,208	3,673	8,217	8,378	6,224	8,604***	3,532***	1,652***
Mannitol	220	1,019	701	168	73	93	8,912***	964***	16,227***
Myo-inositol	327	492	395	242	211	151	3,618***	131***	1,440***
Raffinose	16	22	43	70	69	37	788***	1,568***	345***
Total	2,182	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**
Glucose	249	766	619	649	1,096	728	3,204***	2,376***	43**
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*	20,285***
Myo-inositol	211	444	221	217	188	112	6,080***	694***	2,654***
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 ^{NS}	228***	5 ^{NS}
Glucose	99	90	334	450	117	48	15*	850***	18*
Sucrose	284	487	1,270	4,669	2,663	3,492	2,834***	515***	113***
Myo-inositol	84	180	307	405	195	83	2 ^{NS}	1,200***	32**
Total	527	779	2,299	5,991	3,077	3,646	21,070***	11,002***	544***

^zData were analyzed by linear, quadratic, and higher-order regression models.^yDenotes salt-sensitive species.^wHyphens indicate a lack of data for analysis.^wData 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

Fig. 2. Carbohydrate content (bars) and mean root length (lines) of non-imbibed seeds and seedlings under increasing NaCl stress of salt-tolerant species ['Florida' and 'Ventura' celery (both harvested at 11 d), and cabbage (harvested at 6 d)] and salt-sensitive species [arabidopsis and tobacco (both harvested at 11 d)]. Error bars represent ± 1 SE.

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 ψ_s 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- ψ_s 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 re-imbibed, 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.

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