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Myo-Inositol-Dependent Sodium Uptake in Ice Plant[1]

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In salt-stressed ice plants (*Mesembryanthemum crystallinum*), sodium accumulates to high concentrations in vacuoles, and polyols (*myo*-inositol, D-ononitol, and D-pinitol) accumulate in the cytosol. Polyol synthesis is regulated by NaCl and involves induction and repression of gene expression (D.E. Nelson, B. Shen, and H.J. Bohnert [1998] Plant Cell 10: 753–764). In the study reported here we found increased phloem transport of *myo*-inositol and reciprocal increased transport of sodium and inositol to leaves under stress. To determine the relationship between increased translocation and sodium uptake, we analyzed the effects of exogenous application of *myo*-inositol: The NaCl-inducible ice plant *myo*-inositol 1-phosphate synthase is repressed in roots, and sodium uptake from root to shoot increases without stimulating growth. Sodium uptake and transport through the xylem was coupled to a 10-fold increase of *myo*-inositol and ononitol in the xylem. Seedlings of the ice plant are not salt-tolerant, and yet the addition of exogenous *myo*-inositol conferred upon them patterns of gene expression and polyol accumulation observed in mature, salt-tolerant plants. Sodium uptake and transport through the xylem was enhanced in the presence of *myo*-inositol. The results indicate an interdependence of sodium uptake and alterations in the distribution of *myo*-inositol. We hypothesize that *myo*-inositol could serve not only as a substrate for the production of compatible solutes but also as a leaf-to-root signal that promotes sodium uptake.

Current strategies for improving tolerance to sodium stress, whether based on breeding or transformation, rely primarily on the production of low-M_r solutes and on enhancing radical-scavenging enzyme systems (Tarczynski et al., 1993; Kishor et al., 1995; Pilon-Smits et al., 1995; Holmstrøm et al., 1996; Hayashi et al., 1997; Roxas et al., 1997; Shen et al., 1997; Sheveleva et al., 1997). These strategies attempt to alter the cytoplasmic osmotic potential and prevent oxidative damage. However, knowledge of the most important process, how plants deal with the sodium ion itself, is largely physiological and biochemical (Rausch et al., 1996; Serrano, 1996) and almost nonexistent at the gene level. We are still far from having identified the sets of genes that encode the essential elements that accomplish (a) exclusion or export of sodium, (b) sodium uptake into the vascular system and export to the shoot, and (c) transport of sodium from the leaf vasculature to mesophyll cell vacuoles.

In the case of exclusion, a plasma membrane sodium/proton antiporter has yet to be found and a functionally homologous vacuolar Na^+/H^+-antiporter, important for compartmentation, has only been described physiologically (Barkla and Blumwald, 1991; Rausch et al., 1996). Recently, it was demonstrated that a wheat high-affinity potassium transporter, HKT1, may significantly contribute to sodium influx (Rubio et al., 1995; Gassmann et al., 1996; Maathuis et al., 1996). As more information accumulates, views of ion-transporter functions are changing. There are, it seems, few strictly ion-specific transporters, and there seem to be many transport systems with different ion uptake specificities.

We report that a portion of the pathway for sodium transport from root to leaf involves an interaction between *myo*-inositol and sodium, i.e. we suggest that *myo*-inositol acts as a facilitator of sodium uptake and long-distance transport. The results extend our previous studies of the expression of genes for the inositol biosynthetic pathway, *INPS* (*myo*-inositol-1-phosphate synthase, EC 5.5.1.4), *IMP* (*myo*-inositol monophosphatase), and *IMT* (*myo*-inositol *O*-methyltransferase, EC 2.1.1.129; Vernon and Bohnert, 1992; Ishitani et al., 1996; Nelson et al., 1998a). By the induced expression of IMT, increased *myo*-inositol then serves as the substrate for accumulating metabolic end products that facilitate sodium sequestration and protect photosynthesis (Bohnert et al., 1998; Nelson et al., 1998b).

Myo-inositol and its derivatives are typically examined with regard to cell signaling and membrane biogenesis, but they also participate in responses to salinity in animals and plants. In animal cells membrane transport of hexoses, including the action of SMIT, is linked to sodium uptake. The physiological purpose seems to be for *myo*-inositol to act as a compatible solute (Garcia-Perez and Burg, 1991; Kwon et al., 1992; Mallee et al., 1997). In yeast two ITR genes exist. Their encoded proteins are described as proton/*myo*-inositol symporters (Nikawa et al., 1991). Inositol metabolism and long-distance transport in the phloem have been demonstrated in a number of plant species and for a number of biochemical pathways (Kollar and Seemüller, 1990; Bachmann and Keller, 1995; Gillaspy et al., 1996; Hübel and Beck, 1996; Wang and Nobel, 1998). The pool size of phloem-transported inositol synthesized de novo, but not yet part of any of the recycling pathways, is not

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Abbreviations: ITR, *myo*-inositol transporter; SMIT, sodium/*myo*-inositol symporter.

known. Therefore, measurements of *myo*-inositol content in phloem exudates (Kollar and Seemüller, 1990; Nelson et al., 1998a) probably underestimate the flux of *myo*-inositol (either in the free or derivatized form). Reports are missing that describe a relationship between *myo*-inositol transport and the metabolism of phosphoinositides, which could have implications for signaling throughout the plant. In contrast, phosphoinositides are found extracellularly in animal cells (Roberts et al., 1997) where they may increase to levels similar to those observed intracellularly.

A relationship between ion uptake and *myo*-inositol biosynthesis in cell cultures was previously reported (Wood and Braun, 1961, 1965; Braun and Wood, 1962). Although *myo*-inositol is included in most tissue-culture media, the basis for its requirement is unknown; equally unknown is why some cultures and animal cells in culture do not require *myo*-inositol (Duffy and Kane, 1996; Hrib et al., 1997) or produce excess inositol (Biffen and Hanke, 1991). Wood and Braun (1961, 1965) showed facilitated ion uptake and/or utilization dependent on *myo*-inositol availability and suggested changes in inositol-induced ion transport. In spite of their interesting conclusions, the point has not been pursued in subsequent investigations.

We have characterized *myo*-inositol transport in the salt-stressed ice plant (*Mesembryanthemum crystallinum*). The results build on our previous findings (Vernon and Bohnert, 1992; Ishitani et al., 1996; Nelson et al., 1998a). They allow us to suggest a function for *myo*-inositol synthesis and transport in salt-stress tolerance. First, feedback inhibition of salt-inducible INPS by *myo*-inositol is demonstrated; second, stimulation of sodium uptake by *myo*-inositol is shown; and third, *myo*-inositol plays a vital role in the transition of the ice plant from the nontolerant to the salinity-tolerant state. We have taken the observations by Braun and Wood (1962) about the relationship between *myo*-inositol and ion uptake in cells and extended them to the whole plant. The results also point to a mechanism of sodium uptake, which could be an essential part of the successful adaptation of the ice plant to salinity stress.

MATERIALS AND METHODS

Plant Growth Conditions

Ice plant (*Mesembryanthemum crystallinum* L.) seeds were germinated on vermiculite and irrigated with one-half-strength Hoagland solution. Plants were kept in growth chambers (Conviron, Winnipeg, Canada) illuminated with 300 μE derived from fluorescent and incandescent lights. Standard conditions were 12 h of light/12 h of dark at 23°C/17°C without humidity control. For hydroponically grown plants, 2-week-old seedlings were kept in aerated one-half-strength Hoagland solution. For soil-grown plants, seedlings were transplanted to a 1:1:2 mixture of sand:vermiculite:soil. HPLC measurements of one-half-strength Hoagland solutions, which were occasionally conducted, indicated a concentration range for sodium of 0.4 to 0.8 mM, likely from impurities in the reagents used.

Protein Analysis

Protein from leaf and root tissue was extracted directly in Laemmli buffer containing protease inhibitors (1 mM PMSF, 1 μg/mL leupeptin, and 1 μg/mL E-64). Bradford assays were used to determine protein, followed by protein separation on SDS-PAGE (Nelson et al., 1998a). Protein was transferred to nitrocellulose by electroblotting and detected using a 1:5000 dilution of primary antibodies diluted in TBS buffer containing 1% Tween 20 and 1% powdered milk. Goat anti-rabbit antibody conjugated to horseradish peroxidase diluted 1:5000 in the same buffer was used to detect the primary antibodies. Detection of the complex was by chemiluminescence using ECL reagents (Amersham).

Collection of Phloem Exudate, Xylem Sap, and Measurement of Solutes

The collection of phloem exudate was used according to the method of King and Zeevaart (1974). Side shoots bearing two leaf pairs of 4-week-old plants were excised 4 h after the start of the light period. The cut surface was submerged in 2 mL of 10 mM EDTA, pH 8.0, contained in liquid-scintillation vials. After a 2-h incubation period to wash out solutes released directly at the cut surface, the side shoots were placed in fresh medium for an additional 4 h. For xylem sap (extracellular sap) collection, side shoots were placed in a pressure chamber (PMS Instrument Co., Corvallis, OR). The chamber was slowly pressurized with nitrogen. Initial exudate from the cut surface was discarded and exudate for sampling was collected. Methods used for solute analysis have been described (Adams et al., 1992, 1993, 1998).

RESULTS

Correlation of *Myo*-Inositol Synthesis and NaCl Accumulation

The illustration in Figure 1 is based on immunocytology and solute measurements (Nelson et al., 1998a) and relates the data of previous experiments (Ishitani et al., 1996; Nelson et al., 1998a) to the hypothesis that we present here. In the ice plant prior to NaCl treatment, de novo *myo*-inositol synthesis seems to be cell autonomous, i.e. all cells are able to synthesize *myo*-inositol, although INPS amounts vary between cell types. This autonomy is lost in response to salt stress: following NaCl treatment, INPS increases in leaf mesophyll tissue and decreases in all parts of the root. Within the limits of detection by immunoblotting and immunocytology, INPS seems to disappear completely from the roots. Based on NaCl-induced increased translocation of *myo*-inositol and ononitol through the phloem, Figure 1 points to the difference in *myo*-inositol synthesis between the shoot and the root. The induction of INPS in leaf cells is consistent with a role in supplying a substrate for pinitol, which accumulates in the cytosol along with sodium in vacuoles of cells in aerial tissues. The repression of INPS in roots (Nelson et al., 1998a) was unexpected and did not fit

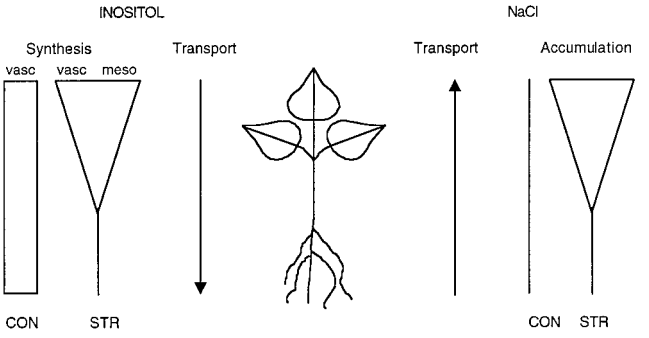

Figure 1. Schematic presentation of *myo*-inositol and NaCl transport relationships. Under stress *myo*-inositol synthesis increases in leaf mesophyll (meso), remains constitutive in the vasculature (vasc), and decreases in the root, increasing translocation to the root. NaCl uptake in the root is matched by transport to the shoot, resulting in accumulation only in the shoot. As NaCl is accumulated in the shoot, provision of *myo*-inositol for pinitol synthesis should only be necessary. The increased flux toward the root suggested a second relationship between *myo*-inositol translocation and sodium uptake. CON, Control plants; STR, salt-stressed plants.

a model in which INPS regulation would solely provide the substrate for methylation reactions, suggesting the possibility of *myo*-inositol acting as a shoot-to-root signal for sodium uptake. A hydroponic system was used in the experiments described here and showed a correlation between the presence of *myo*-inositol in roots and sodium uptake into leaves.

Feedback Inhibition of *Myo*-Inositol Synthesis

In yeast *myo*-inositol synthesis is feedback inhibited by exogenous *myo*-inositol (Culbertson et al., 1976; Ashburner and Lopes, 1995). Since NaCl treatment resulted in the repression of INPS accumulation in roots (Nelson et al., 1998a), we tested the effect of exogenous *myo*-inositol on INPS amounts in seedling roots (Fig. 2). *Myo*-inositol at 5 to 10 mM completely repressed INPS expression (data not shown), confirming that the NaCl-inducible INPS in the ice plant is feedback inhibited by *myo*-inositol.

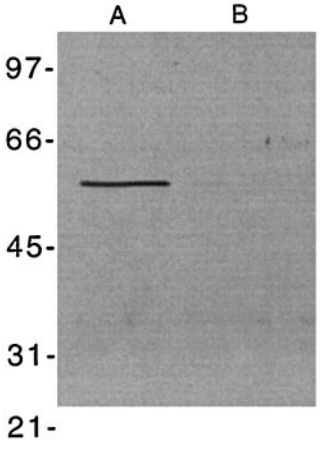

Figure 2. Feedback repression of INPS accumulation. Immunological analysis of INPS in roots of plants irrigated with *myo*-inositol. Lane A, Thirty micrograms of total protein from control roots; lane B, 30 μg of total protein of roots treated with 25 mM *myo*-inositol. The abscissa represents M_r in thousands.

Synergy of NaCl and *Myo*-Inositol Treatment

Because *myo*-inositol translocation to roots increased after salt stress, and since young seedlings have not yet developed the degree of NaCl tolerance exhibited by older plants (Cushman et al., 1990), we tested the effect of exogenous *myo*-inositol application to roots on NaCl accumulation in the leaves. Hydroponically grown seedlings were treated with combinations of NaCl and *myo*-inositol, and leaves were harvested for solute analysis after 3 d. Little sodium was found in the leaves of control plants and in plants treated with 1 to 25 mM *myo*-inositol (Fig. 3A, 10 mM). Treatment of seedlings with 100 mM NaCl alone resulted in an increase in sodium in leaves. Finally, the combination of NaCl and *myo*-inositol resulted in a higher accumulation of sodium than the treatment with only NaCl. None of these treatments altered the amount of leaf potassium in comparison to the controls (Fig. 3B), indicat-

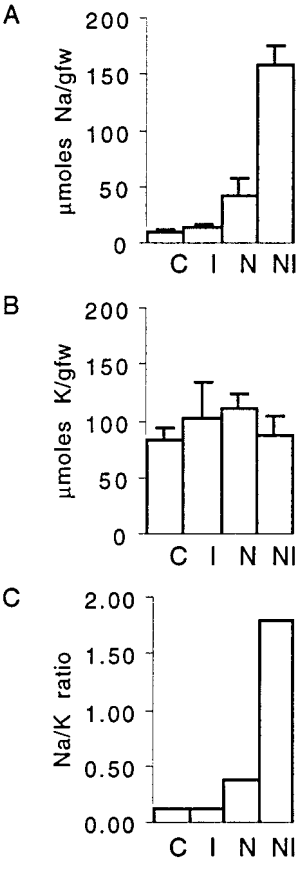

Figure 3. Inositol stimulation of sodium uptake. Analysis of sodium and potassium in leaves of hydroponically grown 2-week-old seedlings treated for 3 d (n = 5 for each treatment). C, Control; I, 10 mM *myo*-inositol; N, 100 mM NaCl; and NI, 10 mM *myo*-inositol plus 100 mM NaCl. A, Sodium accumulation in leaf; B, potassium accumulation in leaf; C, sodium/potassium ratio. gfw, Grams fresh weight. Results are means ± SD.

ing that the effect was specific to sodium. From these data, the ratio of sodium to potassium was calculated (Fig. 3C). Simultaneous treatment with *myo*-inositol and NaCl dramatically increased the ratio to a level similar to that in sodium-treated, mature ice plants (Adams et al., 1992, 1998) and in adapted tobacco cells (Binzel et al., 1988). In the ice plant the ratio varied from 11 to 13 in leaves of treated plants, whereas in control plants the ratio was 0.5. In the adapted tobacco cells the ratio ranged from 0.27 in control cells to 8.2 in cells adapted to 428 mM NaCl. When the data of Binzel et al. (1985) are regressed to 100 mM NaCl, the expected ratio is near 2, similar to the ratio of 1.7 observed in ice plant seedlings treated with 100 mM NaCl and *myo*-inositol. In other unadapted glycophytes such ratios are typically <1 (Garcia et al., 1997). The results show that *myo*-inositol treatment converted intolerant seedlings to a physiological condition indicative of more mature ice plants (Adams et al., 1998).

Titration of *Myo*-Inositol

Next, the concentration of *myo*-inositol necessary to stimulate sodium accumulation was determined. A concentration of 100 μM caused an accumulation of sodium to a significantly higher level than the controls but no different from treatment with NaCl alone (Fig. 4). At 300 μM stimulation was significantly greater than both controls, but the effective concentration for stimulation of sodium uptake is likely lower because other synthetic pathways undoubtedly consume some of the exogenously applied *myo*-inositol.

Myo-Inositol Does Not Stimulate Growth

Cell expansion could have diluted the effect of salt uptake, and an inositol-dependent stimulation of growth might lead to an increased accumulation of sodium. To determine whether *myo*-inositol stimulated seedling growth, plants were treated with 10 mM *myo*-inositol. The fresh weights of the roots and leaves were taken at the beginning of the experiment. After 3 d control and *myo*-inositol-treated plants were compared. During the 3-d period the fresh weight of control and stressed plants doubled (data not shown), indicating that *myo*-inositol did not stimulate sodium accumulation indirectly through a stimulation of growth. In older plants, such as those used for obtaining phloem and xylem contents (Nelson et al., 1998a; Table I), salinity stress accelerates growth for several weeks (Adams et al., 1998) and no significant reduction in xylem sap recovery was observed, whereas xylem xylem is reduced when the plants switch from C_3 to CAM.

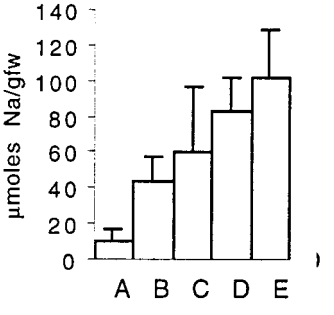

Figure 4. Titration of inositol concentration in its effect on sodium uptake. Analysis of sodium in leaves of hydroponically grown 2-week-old seedlings treated for 3 d (n = 5 for each treatment). Treatments consisted of the following: A, Control; B, 100 mM NaCl; C, 100 mM NaCl plus 0.1 mM *myo*-inositol; D, 100 mM NaCl plus 0.3 mM *myo*-inositol; E, 100 mM NaCl plus 1.0 mM *myo*-inositol. gfw, Grams fresh weight. Results are means ± SD.

Stimulation of IMT Accumulation by *Myo*-Inositol

It had been shown that ice plant seedlings are not salt tolerant and do not express the *Imt1* gene or accumulate IMT protein following NaCl treatment as older plants do (Cushman et al., 1990; Vernon and Bohnert, 1992; Ishitani et al., 1996). Since *myo*-inositol stimulated sodium uptake (Fig. 3), the effect of *myo*-inositol on IMT accumulation was tested. In fact, the addition of sodium and *myo*-inositol together caused the accumulation of IMT in leaves (Fig. 5A) and roots (Fig. 5B), converting the seedlings to a state that is characteristic of older plants. The addition of either NaCl or *myo*-inositol alone did not stimulate IMT, indicating a synergistic interaction between the compounds.

Myo-Inositol Stimulates the NaCl Induction of Polyol Accumulation

To confirm the results of the protein analysis, the accumulation of leaf polyols was measured following treatment with two concentrations of NaCl and three concentrations of *myo*-inositol. In the absence of stress, the seedlings did not contain more total polyols until at least 10 mM *myo*-inositol was added (Fig. 6). In the absence of *myo*-inositol, there was no increase in polyol accumulation even under stress. Treatment with as low as 1 mM *myo*-inositol in the presence of 150 mM NaCl increased the amount of total polyols significantly and caused the seedlings to respond like mature plants.

Maintenance of the Sodium-to-Polyol Ratio

Following NaCl treatment, ice plants establish a similar ratio of sodium to pinitol throughout their life cycle (Ad-

Table I. *Solute concentrations in leaf extracellular (xylem) sap of ice plant side shoots*

The side shoots, which develop only in mature ice plants (Adams et al., 1998), were from plants 8 weeks of age. A single experiment is shown; repeat experiments (eight times with side shoots from different plants in separate stress experiments) demonstrated the same relative increases for *myo*-inositol/ononitol and similar increases in sodium. The plants were stressed by the addition of NaCl (100 mM) for 24 h.

Solute	Control	NaCl Stress
	mM	
Sodium	0.30	56.22
Myo-Inositol	0.04	0.44
D-Ononitol	0.11	1.33
Suc	0.0	0.0

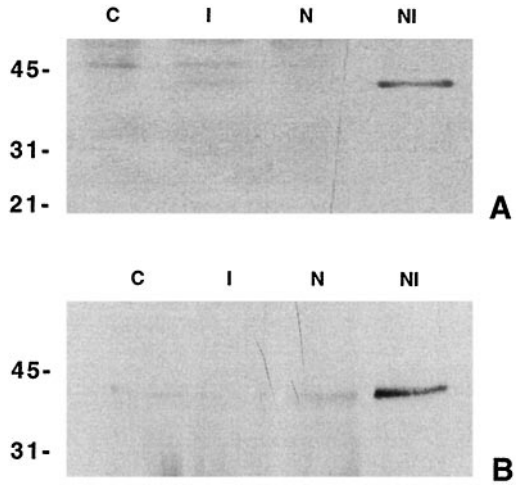

Figure 5. Stimulation by *myo*-inositol of NaCl-induced IMT accumulation. A, Immunological analysis of IMT in leaves of hydroponically grown 2-week-old seedlings treated for 3 d (n = 5 for each treatment). C, Control; I, 10 mM *myo*-inositol; N, 100 mM NaCl; NI, 10 mM *myo*-inositol plus 100 mM NaCl. B, Immunological analysis of IMT in roots of hydroponically grown 2-week-old seedlings treated for 3 d (n = 5 for each treatment). Treatments were as in A. The abscissa represents M_r in thousands.

ams et al., 1998) with values of approximately 100 in juvenile leaves (5 d), 76 in adult leaves (35 d), and 83 in adult leaves (61 d). The ratio of sodium to total polyols (*myo*-inositol plus ononitol plus pinitol) was determined for leaves of hydroponically grown 2-week-old seedlings treated for 3 d (n = 5 for each treatment) in the presence or absence of 10 mM *myo*-inositol, 100 mM NaCl, and 10 mM *myo*-inositol plus 100 mM NaCl. Control plants had a ratio of approximately 30, which became highly skewed following treatment by either *myo*-inositol (7) or NaCl (120), and both compounds together returned the ratio to near the control value (37). Furthermore, the absolute amounts of NaCl and polyols increased equally, approximately 10-fold, from the control to the double-treated plants.

Extracellular Location of *Myo*-Inositol and Ononitol

Sodium seems to enter the plant initially through the root apoplastic space. In maize, for example, sodium is detected in the xylem prior to its appearance in vacuoles of cortical cells (Frensch et al., 1992) and does so by passive movement. Similarly, apoplastic movement of sodium has also been observed in rice, but wheat seems to utilize another mechanism recognizable by a different potassium: sodium discrimination (Garcia et al., 1997). In the ice plant, *myo*-inositol is translocated to the root and this facilitates sodium uptake. *Myo*-inositol may play an indirect role in sodium uptake by exerting its effect from the cytoplasm. Alternatively, if it were to be found in the extracellular space, it may play a direct role. To test whether *myo*-inositol can be found extracellularly, side shoots were used to analyze xylem contents (Nelson et al., 1998a). Side shoots were pressurized in a pressure chamber and sap was collected. These samples may not contain xylem sap exclusively, but, based on the absence of Suc, the samples were not significantly contaminated by either phloem or cytoplasmic contents of other cells (Table I). An expected large increase in sodium following stress was observed and, in addition, a 10-fold increase in *myo*-inositol and ononitol. That both compounds were present in the extracellular space indicated that *myo*-inositol might play a direct role in the facilitation of sodium uptake.

DISCUSSION

Growth of immature ice plants is severely inhibited by NaCl at concentrations much lower than those stimulating growth in mature plants (Adams et al., 1998). The results reported here describe a mechanism that might explain this behavior. Unlike mature plants with a fully developed photosynthetic system and the competence for increased *myo*-inositol synthesis following stress (Ishitani et al., 1996; Nelson et al., 1998a), immature plants do not increase *myo*-inositol and, at this stage, respond to stress like sodium excluders. However, uptake of externally provided *myo*-inositol also leads to sodium uptake and transport to the leaves (Figs. 3 and 4). In seedlings only the combination of *myo*-inositol and sodium will promote induction of the IMT1 methyltransferase leading to ononitol synthesis, an immediate reaction to salt stress in mature plants (Vernon and Bohnert, 1992; Ishitani et al., 1996). We suggest that phloem-translocated *myo*-inositol in mature plants indicates photosynthetic competence and acts as a leaf-to-root signal that leads to sodium uptake, transport in the xylem, and deposition in mesophyll cell vacuoles (Adams et al., 1992; Barkla and Pantoja, 1996; Nelson et al., 1998a). *Myo*-inositol itself could carry out this signal function. Alternatively, changes in pool size or flux could affect phosphoinositide metabolism, but it is unknown whether, or to what extent, *myo*-inositol fluctuations might contribute to changes in phosphoinositides and, hence, might affect signaling processes.

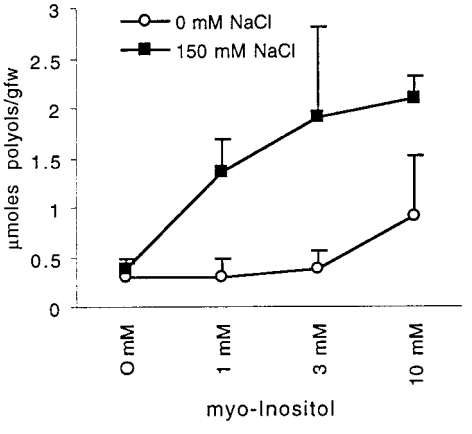

Figure 6. *Myo*-inositol stimulation of NaCl-induced polyol accumulation. Analysis of polyol (*myo*-inositol plus ononitol plus pinitol) accumulation in leaves of hydroponically grown 2-week-old seedlings treated for 3 d (n = 5 for each treatment). gfw, Grams fresh weight. Results are means ± SD.

Myo-Inositol Unloading, Extracellular Movement, and *Myo*-Inositol Uptake

Myo-inositol is mobile in the extracellular space, likely in all organisms (Garcia-Perez and Burg, 1991), but its movement seems regulated. In yeast, for example, membrane transport of ions is stimulated during the exponential phase and repressed in the stationary-phase cultures by the presence of *myo*-inositol. This regulation is independent of the amount of INPS (INO1 in yeast), which is not growth-phase dependent (Robinson et al., 1996). In animals uptake and efflux of osmolytes, including *myo*-inositol, occurs according to interstitial tonicity (Schmolke et al., 1996a, 1996b). The characteristics of inositol flux into and out of C6 cells, for example, are similar to those described for volume-regulatory sorbitol and taurine efflux in a number of cell types (Strange et al., 1993). In plants no *myo*-inositol efflux or unloading from cells has yet been reported.

Information about transporters of *myo*-inositol has become available. Two animal inositol transport systems have been identified. One of these is concentrative and Na^+ dependent, possibly mediated by ITR proteins (Kwon et al., 1992); the second is Na^+ independent (Russo et al., 1995). In yeast two inositol transport proteins, ITR1 and ITR2, both functioning as *myo*-inositol/proton symporters, are known to mediate inositol influx (Nikawa et al., 1991). Homologous coding regions have been detected in *Leishmania donovani* (Drew et al., 1995) and animals (Kwon et al., 1992), during sequencing of the Arabidopsis genome (accession no. F14007), and as plant expressed sequence tags from rice and alfalfa. All sequences show homology with Na^+/Glc cotransporters of the SGLT family (Turk and Wright, 1997). We have meanwhile obtained cDNAs for two different proteins whose deduced amino acid sequences show significant homology to the ITRs from yeast, *Leishmania,* and mammals (Y. Ran, F. Quigley, D.E. Nelson, and H.J. Bohnert, unpublished data).

Cultured cells may or may not require exogenous *myo*-inositol (Hrib et al., 1997) depending on their differentiation state. In cell systems in which *myo*-inositol is required, the specific requirement has not been identified. In plant cells, Biffen and Hanke (1990) used deoxyglucose, which inhibits INPS, to reduce intracellular *myo*-inositol concentrations. The reduction corresponded with reduced cell division rates, but the requirement for *myo*-inositol in cell division was not metabolic, i.e. not to sustain *myo*-inositol incorporation into cell wall components (Biffen and Hanke, 1991). For ononitol, evidence for movement throughout the plant has been provided (Wanek and Richter, 1997). Ricebean accumulates D-ononitol in a salt-stress-inducible manner in the leaves. However, the methyltransferase activity leading to ononitol increased only in stems, and accumulation of ononitol in leaves was linearly related to this methylation activity in the stem. In *Boekelovia hooglandii*, D-mannitol and a *myo*-inositol derivative accumulate with increasing salinity (Fujii and Hellebust, 1994). Flux through *myo*-inositol increased, whereas the amount remained constant.

Distinguishing Halophytes from Glycophytes

One aspect of the results is important in considering the mechanisms of sodium uptake in land plants. From physiological experiments, the only documented sodium-specific transport process is the Na^+/H^+ antiport system that confines sodium to vacuoles (Barkla and Pantoja, 1996; Tanner and Caspari, 1996). The components of the transport pathway from the root to mesophyll vacuoles are not known. Apoplastic entry through the root tip or a loading step at the boundary at the pericycle/endodermis followed by passive transport in the transpiration stream and apoplast has been assumed. However, gradients of ions in the phloem and xylem have been measured in NaCl-treated barley (Wolf et al., 1990). Their model implies sodium redistribution within the leaf during the life cycle, seemingly an adaptive response necessary in the absence of ion storage cells (e.g. epidermal bladder cells, such as those found in ice plants; Adams et al., 1998). Under salt stress, potassium is translocated back to the root when in excess of the plant's requirement (Jeschke and Pate, 1991). Desbiez et al. (1991) reported synergy between cation (K^+ or Ca^{2+}) accumulation and *myo*-inositol for breaking of the symmetry of bud growth.

Assuming a signaling role for inositol transport into roots in the ice plant, the difference between immature and adult ice plants may be relevant to sodium exclusion in glycophytes. If an inositol-coupled sodium uptake mechanism were a general plant feature, the decline of photosynthesis in glycophytes following stress could lead to a decrease in root *myo*-inositol followed by the cessation of sodium uptake. In fact, tobacco, a moderately salt-tolerant species when mature, increases inositol amounts in the leaves and phloem sap following stress (Sheveleva et al., 1997; E. Sheveleva and H.J. Bohnert, unpublished data). In contrast, we have seen no increase or only marginal increases of *myo*-inositol in the salt-sensitive Arabidopsis (Ishitani et al., 1996). Extending this view, our hypothesis is that the primary uptake of sodium into roots, possibly by inward-rectifying transporters for monovalent ions, may be similar in all plants, whereas the transport of sodium into the xylem, possibly depending on and coupled to *myo*-inositol presence and transport, might distinguish between glycophytes and halophytes. Whereas we have not proven a functional, direct relationship for a membrane-transport process involving *myo*-inositol and sodium, we present a correlation between the two. These initial data provide the foundation for the characterization and function of the presumptive ice plant ITR proteins. As an initial description, our data might provide a way for studying anew the observations made by Wood and Brown (1965) with present-day techniques.

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