J Plant Growth Regul (2001) 20:78–86
DOI: 10.1007/s003440010003

J P G R
Journal of Plant Growth Regulation

Contributed Articles

Li^+ Induces Hypertrophy and Down Regulation of *Myo*-Inositol Monophosphatase in Tomato

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Abstract

Lithium (Li^+) is known to disrupt the development of several different organisms, presumably through the inhibition of Li^+-sensitive enzymes such as the *myo*-inositol monophosphatase (IMP) enzyme. In animal cells, decreases in IMP activity result in down regulation of phosphatidylinositol signaling, which can alter development. We characterized the effects of Li^+ on germinating tomato seedlings. It was found that an increase in cell size, termed hypertrophy, results from application of LiCl but not other tested salts. Hypertrophic growth was found to consist of greatly enlarged cells that originate from within the cortex of the seedling stem. Measurements of 44 elements within the hypertrophic tissue indicated that only Li^+ accumulated in a pattern consistent with the observed alterations in stem growth. We found that in response to Li^+, the IMP activity and levels of the three isoforms of IMP were down regulated. These results suggest that down regulation of IMP via Li^+ is involved in the process of hypertrophic growth.

Key words: Hypertrophy; Cell enlargement; Inositol monophosphatase; Lithium; Tomato; Signaling

Introduction

Lithium (Li^+) is a Group IA alkali metal used in many medical and industrial applications. It is normally found associated with sedimentary deposits such as clay and shale. In areas adjacent to Li^+ sources, Li^+ deposition may be greater than the amount of Li^+ leached from the soil, thus Li^+ can build up naturally in soil over a period of time. This phenomenon has occurred in the Central Valley of California where irrigation water containing Li^+ has gradually resulted in injurious levels of Li^+ in citrus orchards (Aldrich and others 1974). Normal plant growth does not require Li^+, and it is well documented that long-term exposure of soil-grown plants to Li^+ causes several effects, including necrosis and phytotoxicity (Anderson 1990). With the increasing use of Li^+ in such devices as batteries and the subsequent disposal of these items into landfills, Li^+ contamination of soils may become a significant

Received 14 June 2000; accepted 28 November 2000; online publication 7 June 2001
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issue in the future. To develop strategies to protect plants from the injurious effects of Li^+ pollution, we must first understand the physiological targets of its actions at the molecular level.

Studies have shown that Li^+ produces developmental alterations in many organisms, including *Xenopus laevis* (Nieuwkoop 1973), sea urchin (Livingston and Wilt 1990), and *Dictyostelium discoideum* (Peters and others 1989). In these organisms, Li^+ affects early events involved in embryo pattern formation, and one model suggests that Li^+ reduces signal transduction through the phosphatidylinositol (PI) signaling pathway via inhibition of the enzyme *myo*-inositol (inositol) monophosphatase (IMP) (for review see Parthasarathy and others 1994). IMP catalyzes both the last step in *de novo* inositol synthesis and in inositol 1,4,5-trisphosphate (IP_3) breakdown, thus it plays a critical role in maintaining cellular inositol levels (Chen and Charalampous 1966; Eisenberg 1967; Hallcher and Sherman 1980). This model is supported by results from experiments in which the effects of Li^+ are prevented by co-introduction of inositol (Busa and Gimlich 1989). In humans, Li^+ is used in the therapeutic treatment of psychiatric disorders where its action is thought to reduce synthesis of inositol from inositol monophosphates in brain cells, thus depleting the inositol pool available for IP_3 signaling (Berridge and others 1989).

To determine if the effects of Li^+ on soil-grown plants are a result of IMP enzyme inhibition and/or PI signaling alterations, we characterized the effects of LiCl on the growth, development, and IMP levels of tomato (*Lycopersicon esculentum*) seedlings. Tomato was chosen as a model system for these studies because we had previously cloned three IMP genes from tomato that encode Li^+-sensitive IMPs (Gillaspy and others 1995). Here we describe a unique effect of Li^+ on developing tomato seedlings. We show that Li^+, but not any other tested ions, results in a hypertrophic growth (cell enlargement in the absence of cell division) along the stem of seedlings. Li^+ accumulates within this tissue and we also show that IMP activity and protein levels are reduced in Li^+-treated seedlings as compared with untreated seedlings. These results imply that Li^+ can affect cellular processes related to growth regulation in plants, and one potential target for this altered growth is down regulation of the plant IMP.

Materials and Methods

Plant Materials

Lycopersicon esculentum cv. VFNT Cherry LA 1221 seeds were surface sterilized with 20% household bleach and were germinated in magenta boxes on 0.5x Murishiage and Skoog Salts solution containing 20 g/L sucrose and 1.0% agar. The RBCS-β-glucuronidase (GUS) transgenic tomato plants have been described previously (Meir and others 1995). Transgenic tobacco plants ectopically expressing LeIMP1 were constructed by transferring the complete coding region of the LeIMP1 gene into a shuttle vector (pBS316) which contains the 35S cauliflower mosaic virus (35S CaMV) promoter and nopaline synthase gene (nos) 3′ sequences. The promoter-LeIMP1-nos fragment was subcloned into the binary vector pSL7292 (Jones and others 1992) and transferred into *Agrobacterium tumefaciens* strain LBA4404 by triparental mating (Hoekema and others 1983). This strain was used to transform *Nicotiana tabacum* cv SR1 leaf discs as described by Horsch and others (1985).

Inositol Monophosphatase Activity

Activity was determined in tomato stem sections as described by Ragan and others (1988). Plant tissues were homogenized in 150 mM KCl, 50 mM Tris, pH 8.5, 0.1 mM EGTA, 0.5 mM EDTA. Equal amounts of protein (300 µg) were incubated in reactions at room temperature in a final volume of 0.3 ml containing 50 µCi of L-[^{14}C]Inositol-1-phosphate (300 Ci/mmole; American Radiolabeled Chemicals Inc., St. Louis, MO). A competitor substrate (10 mM glucose-1-phosphate, Sigma) was included in the buffer of test reactions. Reactions were terminated after 1 h by the addition of 0.05 ml of 10% (w/v) trichloroacetic acid. The mixture was diluted with 1 ml water and applied to a Dowex column AG1 X8 (formate form, Biorad). The column was washed with 3 ml water, and the radioactivity of the eluate was determined by scintillation counting. Three to five determinations were made for each sample. Mean and standard deviation are presented. For determination of relative specific activity, the IMP activity was divided by the normalized amount of IMP protein quantitated by Western blot (see below).

Li^+-Treatment of Plants

Tomato seeds were germinated in magenta boxes on 0.5x Murishiage and Skoog Salts solution containing 20 g/L sucrose, 1.0% agar, and 1 µM, 10 µM, 100 µM, 1 mM, 10 mM, and 1 M LiCl, KCl, CsCl, NaCl or choline chloride (Sigma).

Li^+ accumulation was measured in plants that were untreated or grown in the presence of 0.1 mM or 1 mM LiCl. Dry plant tissue was heated with nitric acid at 85° C overnight. Distilled water was added

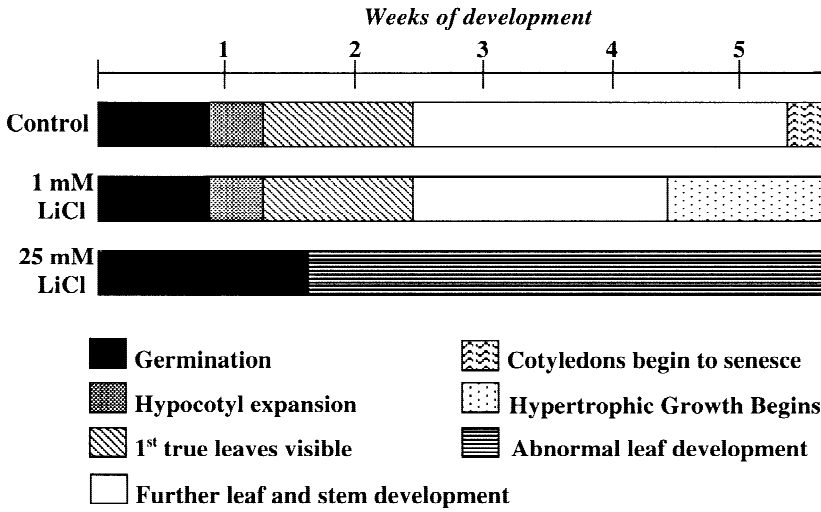

Figure 1. The effects of LiCl on tomato seedling development. Seeds were germinated on MS-agar alone (Control) or in the presence of 1 mM or 25 mM LiCl. Development was monitored over the course of 7 weeks. Relevant developmental stages are depicted by the patterned bars.

and samples were placed in an ice bath. Samples were analyzed with a Jarell-Ash Atomcomp 800 Series simultaneous multi-element spectrometer using inductively coupled plasma optical emission spectrometry according to the manufacturers instructions. A table of spectrum lines and other instrumental parameters are found elsewhere (Bakhtar and others 1989; Bradford and Bakhtar 1991).

For histological examination, plant material was fixed in 4% glutaraldehyde, dehydrated, and embedded in LR White resin according to the manufacturers instructions. Sections (10 μm) were made with a Zeiss microtome and applied to glass slides with water. They were stained for 1 minute with an aqueous 0.1% toluidine blue solution, dehydrated, and mounted in Permount mounting media (Fisher, Pittsburgh, PA). Sections were observed under a Zeiss Axioscope compound microscope and photographed with an attached 35 mm camera. Photographs of plant sections were analyzed as follows: 10 cells from each side of the stem were examined. The measurements of the lengths and widths of cells were converted to actual values using a scale bar. Hand sectioned material was stained with saffranin/fast green and photographed under a Nikon dissecting microscope.

Western Blot Analysis

The isolation of crude protein extracts and Western blot conditions were performed according to Schuster and Gruissem (1991). A digital camera and software package (AlphaInnotech, San Leandro, CA) were used to image and quantitate resulting data. The anti-LeIMP1 antibody has been described in detail elsewhere (Gillaspy and others 1995). Briefly, a rabbit was injected with a glutathione-S-transferase (GST)-LeIMP1 fusion protein in RiBi adjuvant. The antisera was subtracted over *E. coli* antigen and GST columns, followed by purification over a GST-LeIMP1 affinity column. The resulting antisera was specific for IMP and contained no immunoreactivity towards GST or *E. coli* antigens.

Results

To investigate the effects of Li^+ on plant development we germinated tomato (cv. VFNT cherry) seeds on Murishiage and Skoog-agar containing LiCl or control salts (NaCl, KCl, CsCl, choline chloride) at concentrations ranging from 1 μM to 1 M. In general, LiCl was toxic at lower levels than the other salts. For example, LiCl was toxic at concentrations greater than 25 mM, whereas tomato seeds germinated and developed normally on 100 mM NaCl. Tomato seedlings that developed on Murishiage and Skoog-agar containing 25 mM LiCl were delayed in their germination, stunted, and rarely developed leaves (Figure 1). Tomato seedlings that developed on Murishiage and Skoog-agar containing 1–10 mM LiCl, but not any of the other tested salts, germinated normally but developed hypertrophic growths along the stems and occasionally on petioles 4–6 weeks post-germination (see Figure 2A).

The origin of the hypertrophic growth was determined by examining Li^+-treated stems containing various amounts of hypertrophic growths. We first staged stem material according to the amount of hypertrophic growth. Figure 2A indicates the approximate position of each staged stem sample. Stage 1 stems were from control seedlings and contained no hypertrophic growth. Stages 2–4 stems were taken from Li^+-treated stems and contained increasing

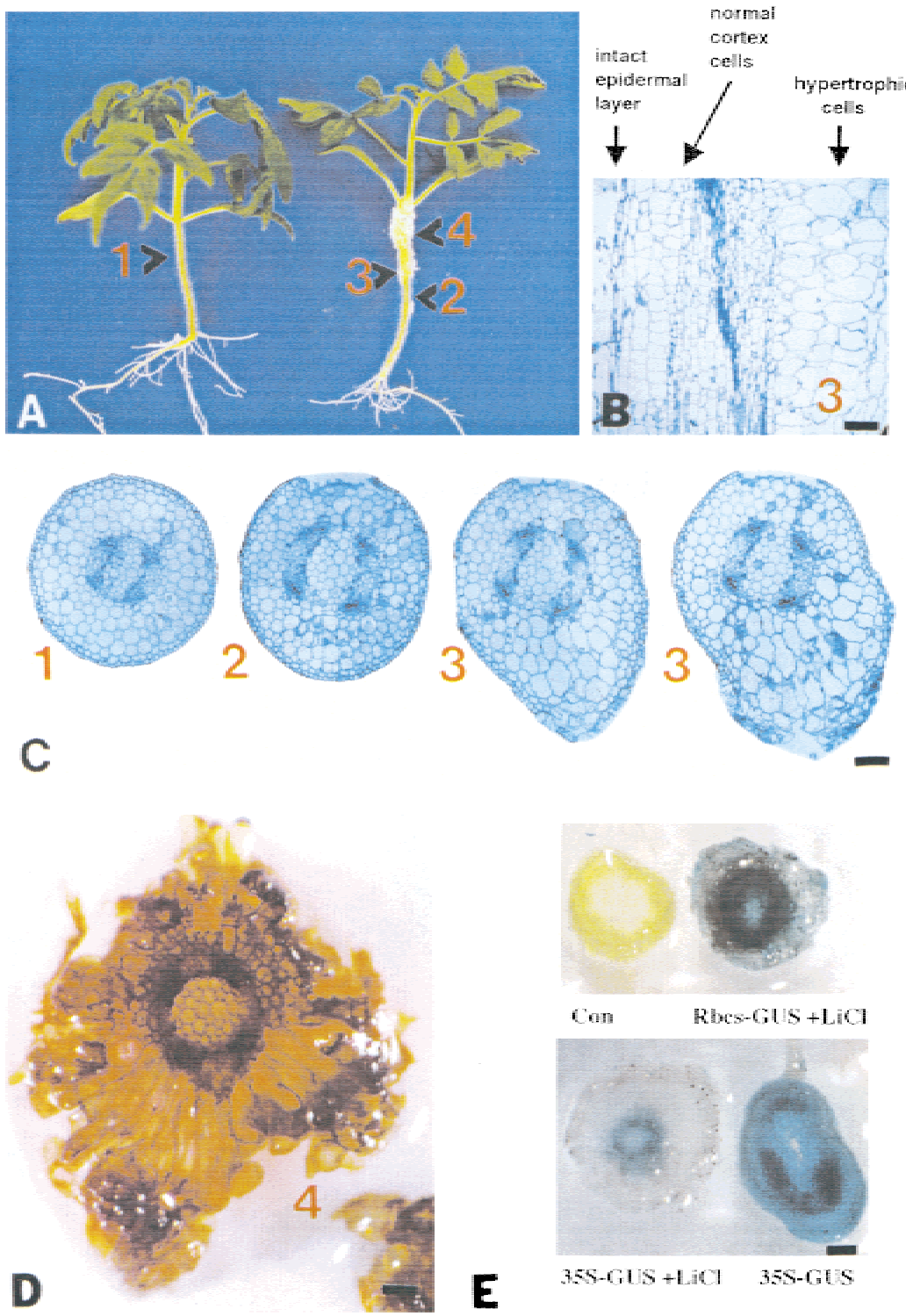

Figure 2. Li^+ effects on tomato seedlings. Seeds were germinated on MS-agar alone (Control) or in the presence of 1 mM LiCl in magenta boxes. Development was allowed to proceed for 5 weeks when whole seedlings or tissue from the stems of seedlings were examined and staged according to the development of hypertrophic growth. Stage 1 stem sections were isolated from untreated seedlings. Stage 2, 3, and 4 stem sections were isolated from seedlings germinated on 1 mM LiCl. **(A)** Left: untreated 5-week-old seedling; right: 5-week-old seedling germinated on 1 mM LiCl. Approximate positions of stages of stem sections are indicated by numbers. **(B)** Longitudinal section of stage 3 hypertrophic growth showing asymetrical hypertrophic growth development. Arrows indicate the normal epidermis (left) and cortex cells (middle) on the left side of the stem, and hypertrophic cortex cells (right arrow) on the right side of the stem section. **(C)** Plastic sections (10 µm) from a stage 1 stem section (left) and selected serial sections from hypertrophic growth-containing stage 2 and 3 stem sections. **(D)** Handsection through a stage 4 hypertrophic growth-containing stem section. **(E)** GUS activity present in control untransformed tomato stem sections (Con), and in transgenic tomato stem sections grown without LiCl (35S-GUS) or with LiCl (+LiCl). Note that the hypertrophic growth on both 35S- and Rbcs-GUS transgenic stem sections does not contain GUS activity. B, C, D scale bar = 250 µM; E, scale bar = 100 µM.

amounts of hypertrophic growth (Figure 2 A, B, C, and D). Stage 4 stem samples (Figure 2D) contained such advanced hypertrophy that cells were easily dislodged from the stem and thus were not embedded in plastic before sectioning of the material. Microscopic examination of stages 1–3 plastic-embedded stem sections indicated that the hypertrophic tissue originated from cells in the stem cortex (Figure 2B and C). This analysis also indicated that the early development of hypertrophic growth was asymmetrical, that is, cells on one side of the stem only gave rise to the new tissue (Figure 2B and C). When the average dimensions of the cortex cells on the normal side of the Li^+-treated stem (143±39 µm length by 120 ± 24 µm width) were compared to those of cells on the hypertrophic growth side (165 ± 36 µm by 275 ± 62 µm), it was found that most of the increase in growth occurs via radial expansion. Cell length was increased only slightly (132%), whereas cell width, indicative of radial expansion, was increased 229%.

In advanced stages of hypertrophy (Figure 2D), the hypertrophic growth covered the circumference of the stem. Hand sections through stage 4 stem samples indicated that hypertrophic cells were unusually expanded and appeared to have thinner cell walls (Figure 2D). This is similar to what is seen in plant cells grown in tissue culture. The cells were viable and could be maintained in tissue culture under an appropriate hormone regime. Advanced hypertrophy resulted in a seedling-lethal phenotype, most likely as a result of water loss through the hypertrophic tissue and impairment or disruption of vascular bundles. Induction of hypertrophy under less humid conditions (that is, in magenta boxes allowing increased air circulation) caused necrosis of the stems and seedling death. Li^+-induced hypertrophy was observed in different cultivars of tomato we tested (VFNT, Ailsa Craig, and T5), indicating that hypertrophic growth is not cultivar-dependent. Hypertrophy was induced at the shoot base in maize seedlings. In contrast, no hypertrophy was induced in tobacco or *Arabidopsis* seedlings at any LiCl concentration.

To determine the differentiation state of hypertrophic tissue, we examined cells in hypertrophic growths for the presence of chloroplasts. Cells within the region of hypertrophy did not contain visible chloroplasts nor did they accumulate chlorophyll. The expression of a specific promoter normally upregulated in photosynthetically active tissues was examined by inducing hypertrophic growth in transgenic plants carrying a chimeric *RBCS-3B* promoter-*GUS* fusion gene (Meier and others 1995). Non-transformed stem sections from seedlings grown in the absence of Li^+ yielded no detectable GUS activity when incubated with GUS substrate (Figure 2E- Con). In contrast, transgenic plants containing either a 35S CaMV promoter or RBCS-3B promoter fused to GUS, expressed high levels of GUS product in all cells of control stems (data shown for 35S-GUS construct, Figure 2E). Hypertrophic growth-containing transgenic plants showed high levels of GUS activity in the normal, interior part of the stem (data shown for 35S-GUS and RBCS-GUS, Figure 2E). From this data, we conclude that the alterations occurring in hypertrophic growths down regulate both of these promoters. This down regulation is indicative of a change in differentiation state. As the CaMV 35S promoter was also down regulated in the hypertrophic tissue, the alteration in gene expression was not limited to photosynthetically active genes.

Dose response experiments indicated that tomato seeds required continuous application of 1 mM LiCl during seed germination for hypertrophic growth formation. The presence of Li^+ during this developmental phase was apparently critical because addition of LiCl post-germination did not result in the appearance of hypertrophic growth. We used plasma optical emission spectroscopy of 44 elements to determine if Li^+ was taken up from the MS-agar during development and accumulated in seedlings in the presence of 1 mM or a 0.1 mM of LiCl (Table 1). Stem tissue from 6-week-old seedlings grown in the presence of 1 mM LiCl (including the hypertrophic growth) had accumulated 284 mg Li^+/kg dry plant tissue, corresponding to an intracellular concentration of 6 mM Li^+. A higher amount of Li^+ (374 mg Li^+/kg) was detected in leaves from seedlings grown in the presence of 1 mM LiCl. At a concentration of 0.1 mM LiCl (suboptimal for induction of hypertrophy) seedlings accumulated high levels of Li^+ in stem and leaf tissues (191 and 350 mg Li^+/kg, respectively). The level of Li^+ in seedlings grown on MS-agar alone was below the detection limit.

The intracellular concentration of 6 mM Li^+ measured in stem sections of seedlings grown on 1 mM LiCl exceeds the concentration required for half-maximal inhibition (0.1 mM) of the tomato IMP enzymes *in vitro* (Gillaspy and others 1995). To measure IMP activity levels in these plants, we utilized an IMP activity assay that measures the ability of an extract to remove a phosphate from a ^{14}C-labeled inositol phosphate (IP) substrate (Figure 3A and Methods). This assay has been used to measure the activity of recombinant IMPs and endogenous IMPs in crude extracts (Joseph and others 1989; Atack and others 1993; Gillaspy and others 1995). To determine the specificity of this assay, a competitor

Table 1. Accumulation of Elements in Li^+-Treated Tomato Seedlings

	No treatment	0.1 mM LiCl		1 mML LiCl	
	Stem	Stem	Leaves	Hypertrophic growth	Leaves
Al	0.0000	90.1000	155.0000	660.0000	37.9000
Ca	5320.0000	6860.0000	9610.0000	5550.0000	4800.0000
Cu	0.0351	2.9100	9.0100	1.7700	6.4200
K	92,800.0000	91,000.0000	47,000.0000	70,800.0000	31,800.0000
Li	0.0000	191.0000	350.0000	284.0000	374.0000
Na	9540.0000	6540.0000	5440.0000	6840.0000	5790.0000
Ni	0.9410	0.1860	1.6400	0.7670	9.8300
Fe	114.0000	240.0000	457.0000	81.0000	224.0000
Mg	1650.0000	1880.0000	2200.0000	2450.0000	2340.0000
Mn	163.0000	217.0000	449.0000	232.0000	159.0000
P	6990.0000	5480.0000	3980.0000	2240.0000	5040.0000
Si	1.8900	16.1000	95.7000	101.0000	0.0000
Zn	196.0000	234.0000	116.0000	169.0000	75.0000

Tissues were pooled from 6-week-old tomato seedlings grown without LiCl (no treatment), or grown in 0.1 mM LiCl or 1 mM LiCl. After drying, tissues were analyzed by mass spectrometry as described in Methods. Values are given in mg/kg dry plant tissue.

substrate (10 mM glucose-1-phosphate) was used. The inclusion of glucose-1-phosphate reduced IMP activity by 23%, indicating that most of IMP activity measured in our assays results from IMP and not nonspecific phosphatases. This assay was also used to measure IMP activity in transgenic tobacco that ectopically expressed LeIMP1 under control of the 35S CaMV promoter. LeIMP1-transformed tobacco leaves expressed higher levels of IMP as compared with control tobacco plants (Figure 3B). These LeIMP1-transformed tobacco plants contained threefold more IMP activity than control plants (data not shown). This is in agreement with the increased LeIMP1 protein production in these plants.

To test if Li^+ accumulation in seedlings with hypertrophy correlated with a reduction of IMP activity *in vivo*, we measured IMP levels in stems with hypertrophic tissue and in sections from the corresponding region of the stem of control seedlings (Figure 3A). LeIMP activity was reduced approximately fourfold in protein extracts prepared from stem sections with hypertrophic growth as compared with control stem sections and stem sections from seedlings grown on 0.1 mM LiCl. It is interesting to note that the increase in LeIMP activity in seedlings grown on 0.1 mM LiCl was reproducible, suggesting that suboptimal levels of Li^+ may induce a compensating mechanism to maintain IMP enzyme activity. These results indicate that LeIMP activity is reduced when conditions are appropriate for Li^+-induction of hypertrophic growth. Immunoblot analysis of these protein extracts using an anti-LeIMP antibody indicated that LeIMP protein levels are reduced approximately threefold in stem tissues that accumulate Li^+ (Figure 3B). The effect of increasing Cl^- was examined by testing whether NaCl affected IMP protein levels (Figure 3B). NaCl also reduced the amount of IMP proteins approximately twofold. Therefore, part, but not all of the down regulation of IMP proteins by LiCl may be due to the general effects of Cl^- ions. NaCl treatment reduced the levels of IMPs, but it did not change the relative ratio of LeIMP1:LeIMP2:LeIMP3. In contrast, LiCl treatment did alter this ratio and had a greater affect on LeIMP2 expression than on LeIMP1 and LeIMP3. Together, these results confirm that, under the appropriate conditions, Li^+ administration to germinating and developing seedlings can reduce both LeIMP protein and activity levels in tomato.

To determine whether the primary effect of Li^+ is on IMP protein production or IMP enzyme activity, we calculated the relative specific activity of total IMP in control stems and in stem sections containing hypertrophic growth grown on 1 mM LiCl. The relative specific activity of the Li^+-treated sample was 70% of the control sample (Figure 3C). Considering the error intrinsic to these measurements, we conclude that most of the effect of LiCl on hypertrophic growth development in tomato arises from the down regulation of IMP protein production.

Discussion

We have shown that Li^+ treatment of tomato seedlings results in hypertrophy, an altered form of cell

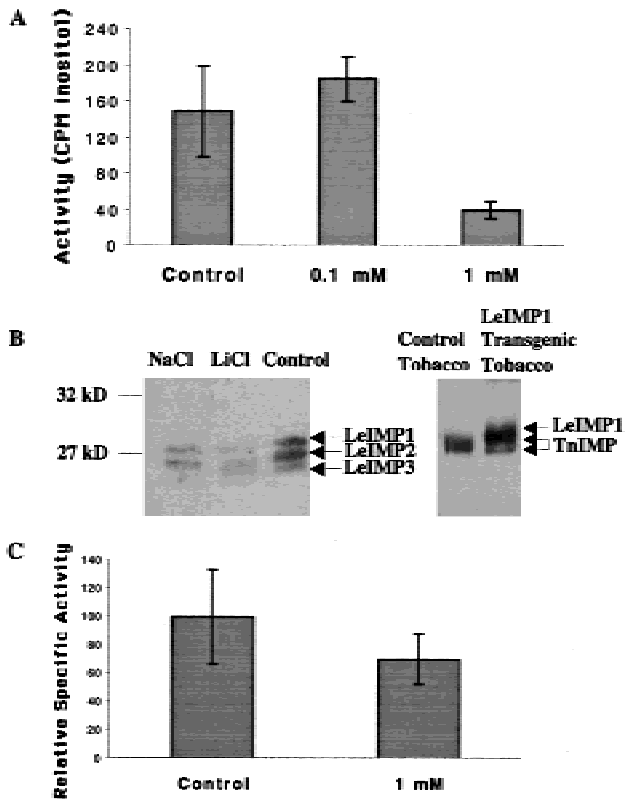

Figure 3. Down regulation of LeIMP in Li^+-induced hypertrophic tissue. Seeds were germinated in magenta boxes on MS-agar alone (Control) or in the presence of a suboptimal dose of LiCl (0.1 mM) or an optimal dose of LiCL (1 mM LiCl). Development was allowed to proceed for 5 weeks. The section of stem below the cotyledons and above the roots was dissected from both Control and LiCl-grown seedlings. Crude protein extracts were made as described in Methods and were used to measure IMP activity **(A)** and IMP protein levels **(B),** respectively. **(A)** Reduction in IMP activity. Crude protein extract (50 µg) from control stem tissue (Control), 0.01 mM LiCl-grown stems 1 mM LiCl-grown stems containing hypertrophic growth was incubated with ^{14}C-labeled IP. After separation of product inositol and substrate (IP), product was quantitated by scintillation counting. Background (82 cpm) was subtracted from each value. Three to five determinations were made for each sample. Mean and standard deviation are presented. **(B)** Alterations in LeIMP protein levels. Left: Crude protein extracts were made from stem tissues of control seedlings (Control), of plants grown on 100 mM NaCl (NaCl), and of plants grown on 1 mM LiCl (1 mM), and were analyzed by Western blotting, as described in Materials and Methods. Equal amounts of protein (10 µg) were loaded as determined by Ponceau S staining of the nitrocellulose filter. Right: crude protein extracts from control tobacco and LeIMP1 transgenic tobacco were similarly analyzed. Arrowheads on both panels indicate the positions of IMP migration as deduced from prior work. Positions of molecular weight markers are shown on the left. **(C)** Relative specific activity. IMP activity and protein levels were used to calculate a relative specific activity as described in Materials and Methods.

growth. Hypertrophy on stems and petioles forming as a result of intumescence injury caused by mechanical stress or red light has been reported for tomato (Morrow and Tibbitts 1988; Lang and others 1983), therefore it is possible that multiple signals can lead to this type of morphological change. Our results indicate that the initial event in Li^+-induced hypertrophy is expansion of cortical cells within the stem (Figure 2C). We have shown that the affected plants accumulate Li^+, and that levels of other elements are not greatly affected in Li^+-treated seedlings (Table 1). We did observe decreased levels of K^+ in both Li^+-treated stem and leaves and decreased levels of Ca^{2+}, Mn^{2+}, and Zn^{2+} in Li^+-treated leaves. These changes in Li^+-treated leaves were not correlated with phenotypic changes. It is interesting to note that more Li^+ accumulated in leaves than stems, but the hypertrophy was always observed on stems.

Li^+ is not an essential plant micronutrient. It has been shown to accumulate in many plant species when present at elevated concentrations in the soil or tissue culture medium (for review see Anderson 1990). Accumulation of Li^+ in plant tissues may occur through nonspecific ion channels; at present, no Li^+-specific channels have been reported. Application of 2 ppm Li^+ to tomato in hydroponic solutions or soil causes accumulation of Li^+ to levels between 80 ppm (approximately 2 mM) to 765 ppm in leaves (Okhrimenko and Kuz'menko 1975; Wallihan and others 1978). These concentrations are similar to those measured in our studies (Table 1). Long-term exposure to Li^+ has been reported to have pleiotropic effects on plants, including phytotoxicity and changes in biomass (Anderson 1990). Li^+ toxicity has been a significant agricultural problem in citrus orchards of the southwestern United States where use of Li^+-contaminated water for irrigation causes Li^+-buildup in the soils (Hilgenan and others 1970; Aldrich and others 1974). Thus, it is desirable to determine the regulatory systems affected by Li^+ in plants.

Induction of hypertrophy in tomato seedlings is a significant developmental alteration induced by long-term Li^+ application during germination and subsequent growth. We were interested in determining whether a known Li^+-sensitive protein, IMP, was involved in the process of inducing this alteration. Our results show that when seedling stems were exposed to high levels of Li^+ (1 mM vs. 0.1 mM treatment), IMP activity and protein levels were significantly reduced compared with untreated stem tissue. This Li^+ concentration (1 mM) is the same required to induce hypertrophy. Therefore, there is a correlation between cellular IMP levels and induction of hypertrophy. We conclude that most, if not

all, of the effect of Li^+ in tomato is on IMP expression levels (Figure 3C). It is interesting to note that recent studies in yeast have documented that the yeast IMP homologue contains a Li^+-sensitive promoter that downregulates expression in the presence of Li^+ (Murray and Greenburg 2000).

We cannot conclude that there is a direct causal link among Li^+ accumulation, reduced IMP levels, and hypertrophy, yet the correlation among these events is interesting in view of the reported effects of Li^+ in animals. We have not measured changes in inositol levels during tomato seedling development in the presence of Li^+, but addition of inositol to the MS-agar could not reverse the hypertrophic effect of Li^+ during seedling development. However, it is not known at present how imported inositol is distributed within the cellular inositol pool. The possibility that Li^+-inhibition of IMP could result in depletion of inositol must be reconciled with the reported abundance of free inositol in plant cells (for review see Loewus and Loewus 1983; Drøbak 1993). If inositol is sequestered as separate pools (for example, free inositol, the PI pool, the phytic acid pool) in plant cells, then one pool alone may become limiting without affecting other pools. For example, bean embryos store high concentrations of inositol as phytic acid, but *de novo* synthesis of inositol is induced during germination before the phytic acid pool is utilized (Sasaki and Taylor 1986). The existence of multiple plant IMPs (Gillaspy and others 1995) may allow the cell to regulate such hypothetical inositol pools independently.

Li^+-induced reduction of IMP levels in the hypertrophic tissue could have several consequences. If the inositol level is reduced in this tissue then any pathway requiring inositol could be adversely affected, including auxin conjugation, phytic acid synthesis, cell wall biosynthesis, PI synthesis, and the PI signaling pathway (Loewus and Loewus 1983). Auxin is an important signal for stem cell elongation. Failure to regulate auxin levels via inositol conjugation in an appropriate developmental and/or cellular context could result in unregulated cell enlargement, as could deregulation of PI signaling.

Only the cells in the stem give rise to hypertrophic growths. This suggests that cells within the stem are physiologically different with respect to Li^+ metabolism and/or storage. Interestingly, immunolocalization experiments have shown that cells associated with vascular bundles (possibly phloem parenchyma cells) express more IMP than other cell types (Gillaspy and others 1995). Thus, these cells may be especially sensitive to alterations in IMP levels.

ACKNOWLEDGMENTS

We thank Gordon Bradford and Heather Wright for their analysis of element accumulation in tomato seedlings, and Bhadra Gunesekara for critical reading of the manuscript. This research was supported by the Fralin Biotechnology Center, a Jeffress Trust award to G.G., and a Department of Energy award (No. 85ER13375) to W.G.

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