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# Effect of Salt Stress on Germin Gene Expression in Barley Roots

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**Germin gene expression in barley (*Hordeum vulgare* L.) seedlings responds to developmental and environmental cues. During seed germination, germin mRNA levels were maximal 2 d after the start of imbibition in control seedlings and declined to low levels by 6 d. When seeds were sown in the presence of 200 mM NaCl, germin mRNA levels were also maximal after 2 d, but NaCl treatment, which slowed seedling growth, prolonged germin gene expression for an additional 1 d. In 4-d-old seedlings, germin mRNA levels were highest in roots and higher in the vascular transition region than in shoots. In roots of 6-d-old seedlings, germin gene expression was regulated by salt shock and plant growth regulators. Induced germin mRNA levels were maximal 8 h after treatment with NaCl, salicylate, methyl salicylate, or methyl jasmonate and 4 h after treatment with abscisic acid and indoleacetic acid. Like germin mRNA, dehydrin mRNA levels were maximal 8 h after NaCl treatment. In contrast, peroxidase mRNA levels declined to less than control levels within 30 min of treatment. Germin gene expression is regulated developmentally by salt stress and by treatments with plant hormones. Since germin is an oxalate oxidase, these results imply that oxalate has important roles in plant development and homeostasis.**

When barley (*Hordeum vulgare* L. cv CM 72) seedlings are grown in the presence of 200 mM NaCl, the pattern of protein synthesis in the roots is altered (Hurkman and Tanaka, 1987). The most significant changes involve two 26-kD and two 27-kD polypeptides with pIs of 6.3 and 6.5 (Hurkman and Tanaka, 1987, 1988; Hurkman et al., 1989). The synthesis of these polypeptides, as measured by in vivo labeling with [$^{35}$S]Met, increases in roots of seedlings grown in the presence of NaCl (Hurkman and Tanaka, 1987; Hurkman et al., 1989) or in roots of seedlings shocked with NaCl (Robinson et al., 1990) and decreases when salt-treated plants are returned to control conditions (Hurkman and Tanaka, 1987). Increased synthesis of the 26-kD, pI 6.3 and 6.5, polypeptides is specific to salt stress and does not occur in response to desiccation or treatments with PEG or mannitol (Hurkman and Tanaka, 1988). The 26-kD, pI 6.3 and 6.5, polypeptides also accumulate, as quantified by PAGE, in roots of plants grown in the presence of NaCl (Hurkman et al., 1991).

The N-terminal amino acid sequences reveal that the 26-kD, pI 6.3 and 6.5, polypeptides share strong sequence homology to germin (Hurkman et al., 1991). Germin is an approximately 130-kD homopentameric protein comprising subunits of approximately 26 kD (McCubbin et al., 1987) that increases significantly in the embryos of cereal seeds during germination (Grzelczak and Lane, 1984). Germin has been identified independently by Dumas et al. (1995) and Lane et al. (1993) as an oxalate oxidase.

To examine the effect of salt stress on the expression of germin mRNA, a full-length cDNA encoding barley germin was isolated from a λgt11 library (Hurkman et al., 1994). In this paper we report germin mRNA levels in barley seedlings during development and in response to treatments with NaCl and various plant growth regulators. For comparison, expression of dehydrin and peroxidase genes, genes known to be regulated by salt stress (Gulick and Dvořák, 1992; Valpuesta et al., 1993), was examined.

## MATERIALS AND METHODS

### Plant Material and Treatments

Barley (*Hordeum vulgare* L. cv CM 72) and wheat (*Triticum aestivum* L. cv Cheyenne) seedlings were grown as described previously (Hurkman and Tanaka, 1987). Barley seedlings were grown in the absence or presence of 200 mM NaCl or treated with NaCl 6 d after seeds were sown. Treatments with plant growth regulators were also initiated 6 d after seeds were sown. To the nutrient solution, ABA or IAA to a final concentration of $10^{-5}$ M, MeJA to 45 μM, and MeSA or SA (free acid) to 250 μM were added. Plant organs were collected following the treatment times indicated in "Results," frozen in liquid nitrogen, and stored at −70°C until use.

### Cloning and Expression of Germin cDNA in *Escherichia coli*

A germin cDNA, isolated previously from a λgt11 library constructed using poly(A)$^+$ RNA isolated from roots of 6-d-old CM 72 seedlings grown in the presence of 200 mM NaCl and screened with a cDNA encoding wheat germin gf-2.8 (Hurkman et al., 1994), was cloned into *E. coli*. Recombinant proteins were expressed using the pET

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Abbreviations: MeJA, methyl jasmonate; MeSA, methyl salicylate; SA, salicylate.

System[1] (Novagen, Inc., Madison, WI) according to the manufacturer's instructions. The full-length germin cDNA was digested with *Bgl*I, and then *Eco*RI linkers were ligated to the cDNA. An *Eco*RI/*Not*I digest resulted in an approximately 900-bp fragment containing the mature coding region and 3′ untranslated region of the germin cDNA. The *Eco*RI/*Not*I fragment was subcloned into a pET-22b(+) vector and transformed into expression host BL21(DE3)pLysS containing a chromosomal copy of the T7 RNA polymerase gene under *lac*UV5 control. Expression of recombinant proteins was induced by the addition of isopropylthio-β-galactoside. Proteins were separated by PAGE and immunoblots probed with antibodies to germin as described previously (Hurkman et al., 1991).

### RNA Isolation

Total RNA was isolated from frozen plant samples based on a method described by Hurkman et al. (1989). Plant material, 200 to 600 mg fresh weight, was ground to a powder in liquid nitrogen with a precooled mortar and pestle. Extraction reagent (Hurkman et al., 1989) was added in a tissue:reagent ratio of 1:4 (fresh weight/v) for roots and 1:10 for other plant organs. The powder was ground until a liquid homogenate was obtained. Following centrifugation at 10,000*g* and 4°C for 15 min, the aqueous phase was re-extracted with an equal volume of phenol: chloroform:isoamyl alcohol (25:24:1, v/v) and then with an equal volume of chloroform:isoamyl alcohol (24:1, v/v). Total nucleic acids were precipitated by addition of 0.1 volume of 2 M sodium acetate and 2.5 volumes of cold (−20°C) 100% ethanol. Following incubation overnight at −20°C, precipitated nucleic acids were collected by centrifugation at 12,000*g* and 4°C for 15 min. The precipitate was rinsed with cold (−20°C) 75% ethanol, and the pellet was dried and then solubilized in 300 μL of cold (4°C) sterile water. An equal volume of cold (4°C) 4 M lithium acetate was added. Following incubation on ice for 3 to 4 h, the solution was centrifuged at 12,000*g* and 4°C for 15 min. The RNA pellet was rinsed with cold 2 M lithium acetate and then with cold 75% ethanol. The dried pellet was solubilized in cold sterile water.

The RNA was purified further by one of two procedures. The RNA was reprecipitated with sodium acetate and ethanol as described above, extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1, v/v) and once with chloroform:isoamyl alcohol (24:1, v/v) and then precipitated with sodium acetate and ethanol. Alternatively, the RNA was purified using an RNeasy Total RNA spin column (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's instructions. The concentration of RNA was estimated spectrophotometrically, and after a final sodium acetate and ethanol precipitation, the pellet was solubilized with cold sterile water to a concentration of approximately 3 μg/μL. The final concentration of the RNA was determined spectrophotometrically.

[1] Mention of a specific product name by the U.S. Department of Agriculture does not constitute an endorsement and does not imply a recommendation over other suitable products.

### Synthesis of Probes

All probes were labeled with digoxigenin according to protocols supplied with the Genius System (Boehringer Mannheim). The pET vector containing the approximately 900-bp germin *Eco*RI/*Not*I cDNA fragment was digested with *Eco*RI/*Hin*dIII resulting in an approximately 700-bp germin fragment, which was subcloned into pSPT 19. The plasmid was linearized by digestion with *Eco*RI and transcribed with T7 polymerase to generate a digoxigenin-labeled RNA probe. The cDNA encoding DHN4 dehydrin from barley seedlings was a generous gift from Dr. Peter Chandler (Commonwealth Scientific and Industrial Research Organization, Canberra, Australia). The *Bam*HI/*Eco*RI approximately 800-bp fragment of the dehydrin cDNA was subcloned into pSPT 18. The plasmid was linearized with *Bam*HI and transcribed with T7 polymerase to generate a digoxigenin-labeled RNA probe. The cDNA encoding P8.5 peroxidase from barley leaves was a generous gift from Dr. Shauna Somerville (Carnegie Institution of Washington, Stanford, CA). The *Eco*RI/*Hin*dIII approximately 900-bp fragment of the peroxidase cDNA was labeled with digoxigenin using random priming.

### Northern Analysis

RNA (13–15 μg/lane) was separated in 1.2 or 1.5% agarose/0.66 M formaldehyde gels and blotted by capillary transfer using 20× SSC onto a positively charged nylon membrane (Boehringer Mannheim). The transferred RNA was UV linked to the membrane using a Stratalinker (Stratagene). Blots were hybridized with digoxigenin-labeled probes and visualized using the Genius System (Boehringer Mannheim) according to the manufacturer's instructions. Following hybridization at 62°C with the germin or dehydrin RNA probe, blots were washed twice with 2× SSC/0.1% SDS at room temperature for 5 min and twice with 0.1× SSC/0.1% SDS at 65°C for 15 min. Following hybridization at 42°C with the peroxidase DNA probe, blots were washed in 2× SSC/0.1% SDS, twice at room temperature for 5 min and twice at 45°C for 15 min.

## RESULTS

### Expression of Germin in *E. coli*

To verify that the cDNA for germin encoded a germin-like protein, recombinant proteins synthesized in *E. coli* were separated by PAGE and a blot was probed with antibodies produced previously to the 26-kD subunit of barley germin (Hurkman et al., 1991). The antibodies did not react with any of the proteins synthesized in *E. coli* that were transformed with vector alone (Fig. 1, lane A). In contrast, the antibodies reacted strongly with a polypeptide of approximately 26-kD that was synthesized in *E. coli* transformed with vector containing the germin insert (Fig. 1, lane B).


**Figure 1.** Analysis of recombinant proteins expressed in *E. coli*. Lane A, Proteins synthesized in *E. coli* transformed with vector that did not contain the germin insert. Lane B, Proteins synthesized in *E. coli* transformed with vector containing the germin insert. Proteins were separated by SDS-PAGE, and a protein blot was probed with antibodies to a 26-kD subunit of germin. Based on positions of markers, the molecular mass of the polypeptide is approximately 26 kD.

### Effect of Salt Stress on Expression of Germin mRNA in Roots of Salt-Grown Barley Seedlings

The effect of NaCl on germin gene expression was analyzed initially in roots of 6-d-old barley seedlings, the developmental stage used previously in studies of the effects of salt stress on patterns of protein synthesis (Hurkman and Tanaka, 1987, 1988; Hurkman et al., 1989, 1991; Robinson et al., 1990). Preliminary comparisons of germin mRNA levels in roots of seedlings grown in the absence or presence of 200 mM NaCl for 6 d revealed that transcripts were 1 kb in size and that levels were the same in control and salt-grown seedlings (Fig. 2). To determine whether NaCl had an effect earlier in the treatment period, germin mRNA levels were also compared in roots of 3-, 4-, 5-, and 6-d-old seedlings grown in the absence or presence of NaCl. In both control and salt-grown seedlings, germin mRNA levels were maximal by 3 d and then decreased through d 6 (Fig. 2). A comparison of germin mRNA levels in control and salt-grown seedlings during the time course revealed a striking increase in germin mRNA levels in roots of seedlings grown in the presence of NaCl for 3 d. These findings demonstrate that germin gene expression in roots is developmentally regulated and that NaCl causes an increase in germin mRNA levels at a specific stage of development.

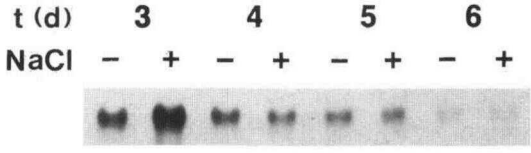


**Figure 2.** Effect of NaCl treatment on germin mRNA levels in roots of salt-grown barley seedlings. Plants were germinated and grown in the absence (−) or presence (+) of 200 mM NaCl for 3, 4, 5, or 6 d (t). Based on positions of markers, the germin transcripts are approximately 1.0 kb in size.

### Effect of Salt Stress on Expression of Germin mRNA in Germinating Barley Seeds

Because germin and its mRNA increase uniquely and conspicuously during germination of wheat seeds (Thompson and Lane, 1980; Grzelczak et al., 1985), germin gene expression was examined in intact barley seedlings following germination for 4 d in the absence or presence of 200 mM NaCl (Fig. 3A). When seeds were sown in the absence of NaCl, germin mRNA levels increased sharply and were maximal 2 d after the start of imbibition and then declined to much lower levels by d 3 and 4 (Fig. 3A). When seeds were sown in the presence of 200 mM NaCl, germin mRNA levels were lower than those of the control at 1 d and, like the control seedlings, increased sharply by d 2 (Fig. 3A). Unlike the control seedlings, germin mRNA remained at high levels 3 d after the start of imbibition and then declined to much lower levels by d 4 (Fig. 3A).

In control seedlings, comparison of germin gene expression (Fig. 3A) with fresh weight (Fig. 3B) revealed that germin mRNA levels were maximal in seedlings that weighed approximately 25 mg. When seeds were germinated in the presence of NaCl, seedling growth, measured as fresh weight, was inhibited significantly (13.7% after 1 d, 54.0% after 2 d, 67.5% after 3 d, and 68.2% after 4 d) and germin mRNA levels were maximal in seedlings that had

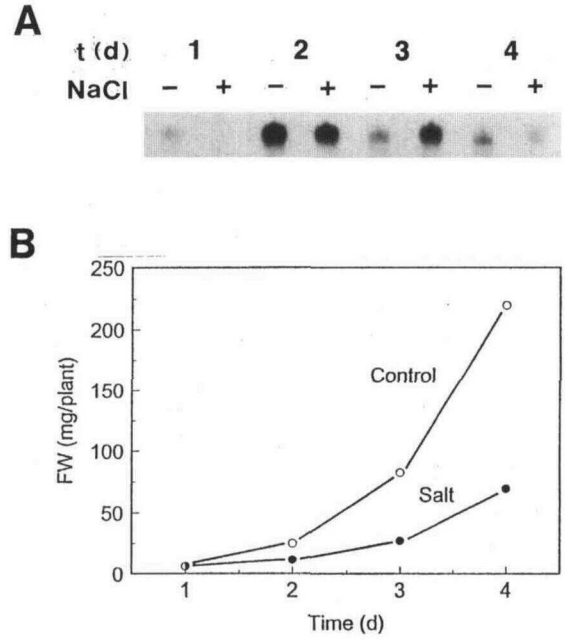


**Figure 3.** Effect of NaCl treatment on germin mRNA levels in developing barley seedlings. A, Seeds were sown in the absence (−) or presence (+) of 200 mM NaCl, and total RNA was isolated from intact seedlings after 1, 2, 3, and 4 d (t). B, Plot of fresh weight (FW) of seedlings analyzed in A as a function of time after initiation of imbibition.

fresh weights of approximately 12 to 25 mg (Fig. 3B). In both control and salt-grown seedlings, the decline in germin mRNA levels occurred in seedlings that weighed more than approximately 25 mg. Thus, NaCl treatment, which slows seedling growth, prolongs germin gene expression for an additional 1 d.

### Differential Expression of Germin mRNA in Barley and Wheat Seedlings

The distribution of germin mRNA among organs of 4-d-old seedlings was examined. A comparison of roots, the vascular transition region (tissues remaining when roots and shoots are excised near the scutellum; Esau, 1965), and shoots revealed that germin gene expression was regulated in a tissue-specific manner. Germin transcripts were expressed in highest amount in roots and levels were higher in the vascular transition region than in shoots (Fig. 4). In the roots and the vascular transition region, germin transcripts were approximately 1 kb. In the shoots, an additional germin transcript of approximately 0.8 kb was expressed, although at lower levels than the 1-kb transcript. When shoots were separated into leaves and coleoptiles, this transcript was specifically associated with the leaves (W.J. Hurkman and C.K. Tanaka, unpublished results).

Because germin was reported previously to be in highest amount in the vascular transition region ("stem") of wheat seedlings (Grzelczak et al., 1985), germin gene expression was also examined in 4-d-old wheat seedlings. Unlike in barley, germin transcripts were expressed in highest amount in the vascular transition region and levels were higher in the shoots than in the roots (Fig. 4).

### Effect of Salt Shock and Plant Growth Regulators on Expression of Germin mRNA in Barley Roots

To determine the effect of salt shock on germin gene expression, NaCl was added to the nutrient solution of 6-d-old seedlings to a final concentration of 200 mM. Previous studies demonstrated that salt shock induces germin synthesis in roots of seedlings at this stage of development (Hurkman and Tanaka, 1987; Robinson et al., 1990). In addition, we wanted to determine whether expression of germin mRNA could be induced in roots at a developmental stage when transcript levels, as demonstrated in Figure 2, are relatively low. Germin mRNA levels were examined in roots of control seedlings at 0, 8, and 24 h and in roots of

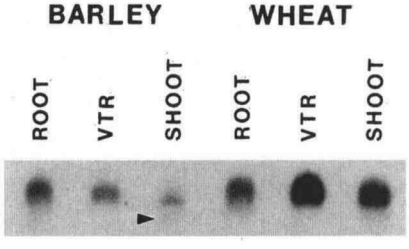


**Figure 4.** Spatial distribution of germin mRNA in 4-d-old barley and wheat seedlings. VTR, Vascular transition region. The arrowhead indicates an additional 0.8-kb transcript expressed in barley shoots.

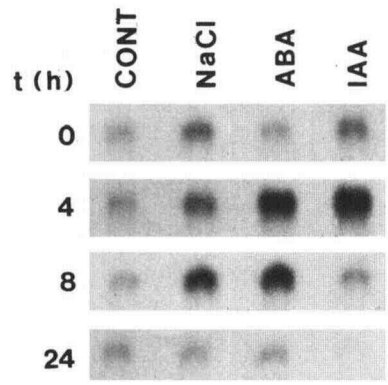


**Figure 5.** Effect of NaCl, ABA, and IAA on germin mRNA levels in barley roots. Six-day-old seedlings were treated as described in "Materials and Methods." Total RNA was isolated from samples collected during a 24-h time course. CONT, Control; t, time.

treated seedlings at 0 h, immediately before additions were made. Although there was some variation in germin mRNA levels among these controls, there were greater changes in mRNA levels following treatment with NaCl, ABA, or IAA (Fig. 5). During the salt-shock time course, germin mRNA levels increased and were maximal by 8 h; mRNA levels then decreased to control levels by 24 h (Fig. 5).

ABA regulates expression of genes that are responsive to osmotic stress (Mundy and Chua, 1988), a component of salt stress (Yeo, 1983). Following treatment of roots with ABA, germin mRNA levels were maximal by 4 h, remained high at 8 h, and returned to control levels by 24 h (Fig. 5). Lane (1994) reported that IAA regulates germin gene expression in wheat seedlings. Likewise, IAA treatment of barley seedlings altered germin gene expression. During IAA treatment, germin mRNA levels increased in roots by 4 h and returned to approximately control levels by 8 h (Fig. 5).

Since germin may have a role in plant defense (Lane et al., 1986; Lane, 1994; Dumas et al., 1995; Zhang et al., 1995), barley seedlings were treated with SA, MeSA, or MeJA, compounds that activate pathogenesis-related gene expression. Again, there was some variation in germin mRNA levels in the control samples, but there were greater changes in mRNA levels when roots were treated with SA, MeSA, or MeJA (Fig. 6). With all three treatments, germin mRNA levels were maximal by 8 h and declined by 24 h (Fig. 6). The increase in germin transcript levels was much higher following MeSA treatment, indicating that roots are more permeable to MeSA than to SA.

### Effect of Salt Shock on Expression of Dehydrin and Peroxidase mRNA in Barley Roots

Since dehydrin expression is regulated by salt stress (Gulick and Dvořák, 1992) as well as water deficit (Close and Chandler, 1990), the effect of salt shock on dehydrin mRNA levels was examined in roots of 6-d-old seedlings during an 8-h time course (Fig. 7A). The dehydrin probe hybridized with several mRNA size classes; the most

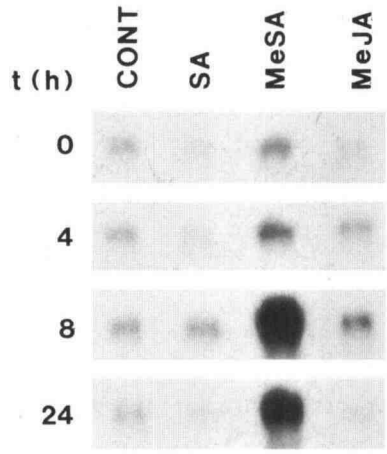


**Figure 6.** Effect of SA, MeSA, and MeJA on germin mRNA levels in barley roots. Six-day-old seedlings were treated as described in "Materials and Methods." Total RNA was isolated from samples collected during a 24-h time course. CONT, Control; t, time.

prominent classes had sizes of 2.2, 1.2, and 0.9 kb. Following salt shock, the 2.2-kb transcript levels were maximal at 3 h and remained at high levels thereafter. In contrast, the 1.2-kb transcript levels were also maximal at 3 h but declined thereafter. The 0.9-kb transcripts increased gradually to high levels during the 8-h time course.

Since germin is an oxalate oxidase (Dumas et al., 1995; Lane et al., 1993), an enzyme that catalyzes the conversion of oxalate to $CO_2$ and $H_2O_2$, and Valpuesta et al. (1993) reported that peroxidase transcripts increase in tomato roots during salt stress, the effect of salt shock on peroxidase mRNA levels was examined. Peroxidase transcripts, 1.4 kb in size, decreased rapidly and were almost undetectable after 2 h of salt shock (Fig. 7B).

## DISCUSSION

Germin gene expression in barley seedlings is responsive to a variety of developmental and environmental cues. Germin mRNA levels are regulated in a tissue-specific manner. Germin mRNAs were present in greatest quantity in the roots, in an intermediate amount in the vascular transition region, and in smallest amount in the shoots. In agreement with the mRNA distribution, the protein is present, on a per organ basis, in highest amount in the roots and at lower levels in the coleoptiles but is not detectable in leaves (Hurkman et al., 1991). It is interesting that germin and its mRNA have different spatial distributions in wheat. Grzelczak et al. (1985) reported that germin and its translatable mRNA are present in much higher proportion in the vascular transition region than in any other part of the wheat seedling, either on a fresh mass basis or a per organ basis. We also observed in this study that germin mRNA in wheat seedlings is present in highest amount in the vascular transition region.

In barley seedlings, germin gene expression is regulated developmentally and, in turn, developmental timing is influenced by salt stress. In control seedlings, germin mRNA levels were maximal 2 d after the start of imbibition and declined thereafter. In seedlings grown in the presence of 200 mM NaCl, germin mRNA levels were also maximal 2 d after the start of imbibition but remained at these levels for an additional 1 d before declining. In both control and salt-grown seedlings, the decline in germin mRNA levels occurred when seedlings attained a fresh weight of approximately 25 mg, a fresh weight attained 2 d after imbibition in control seedlings, and 3 d after imbibition in salt-grown seedlings. These findings suggest that germin gene expression is up-regulated at a specific stage in early seedling development and that salt treatment, which slows seedling growth, prolongs this developmental stage.

The regulation of germin gene expression in roots of seedlings subjected to salt stress is complex. In roots of salt-grown seedlings, germin mRNA levels increase substantially above developmental levels only 3 d after the start of imbibition. In roots of 6-d-old barley seedlings shocked with 200 mM NaCl, germin mRNAs increase transiently; levels are maximal by 8 h and decrease to control levels by 24 h. These responses of germin mRNA levels in roots of salt-grown and salt-shocked seedlings indicate that germin gene expression is regulated at the transcriptional level. Previously, we observed that germin synthesis, as measured by in vivo labeling of seedlings with [$^{35}$S]Met, also increases transiently in response to salt shock (Robinson et al., 1990). Germin synthesis is greater in roots of 6-d-old salt-grown seedlings than in control seedlings (Hurkman and Tanaka, 1987) and germin accumulates, as quantified by PAGE and western blot analysis (Hurkman et al., 1991). The increased synthesis of germin in roots of salt-grown seedlings when the corresponding mRNAs are at low levels suggests that germin mRNA is translated preferentially. The resultant accumulation of germin in roots of salt-grown seedlings can be explained by the remarkable stability of the germin oligomer (Lane, 1994).

Germin gene expression is modulated in roots of 6-d-old seedlings by IAA, ABA, SA, MeSA, and MeJA. Lane

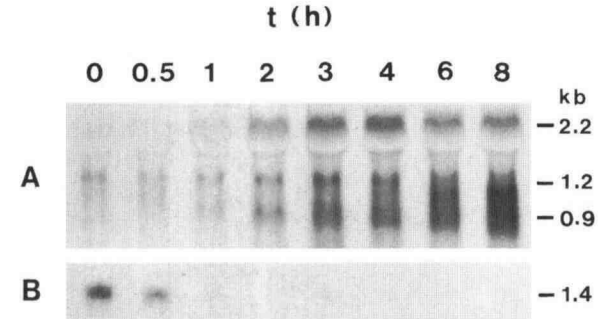


**Figure 7.** Effect of salt shock on dehydrin and peroxidase mRNA levels in barley roots. Six-day-old seedlings were treated with 200 mM NaCl and total RNA isolated from samples collected during an 8-h time course. A, Gel blot analysis of dehydrin mRNAs. B, Gel blot analysis of peroxidase mRNAs. t, Time.

(1994) reported previously that IAA regulates germin gene expression in wheat seedlings. Genes that respond to water deficit, a component of salt stress (Yeo, 1983), often respond to ABA. Among these genes are the dehydrins, which not only respond to water deficit (Close and Chandler, 1990) and ABA treatment (Morris et al., 1991) but are also regulated by salt stress in wheat roots (Gulick and Dvořák, 1992) and barley roots, perhaps in response to the decrease in osmotic potential that accompanies salt stress. Valpuesta et al. (1993) reported that peroxidase transcripts increase in tomato roots in response to salt stress. In barley roots we found, by using a probe that encodes a barley leaf peroxidase, that expression of peroxidase transcripts decreased during salt stress. However, a number of peroxidase isozymes are present in plants, and it is possible that a different, yet to be discovered, transcript of this gene family increases in barley roots during salt stress. The response of germin gene expression in roots to salicylate and jasmonate, compounds that are known to act as endogenous signals that activate pathogenesis-related gene expression and at least some components of systemic acquired resistance (Raskin, 1992; Reinbothe et al., 1994), suggests that germin, like osmotin (Xu et al., 1994), responds to pathogen invasion as well as a number of hormonal and environmental signals. Dumas et al. (1995) and Zhang et al. (1995) provided support for this view in reports demonstrating that germin increases in barley leaves during infection by powdery mildew, *Erysiphę graminis* f. sp. *hordei*.

In this study we demonstrated that germin gene expression in barley seedlings is developmentally regulated in a tissue-specific manner, and that it is modulated by treatments with NaCl and various plant hormones. The functional role for germin in these responses is not known. Barley and wheat germin have strong sequence similarity to oxalate oxidase and also have oxalate oxidase activity (Lane et al., 1993; Hurkman et al., 1994; Dumas et al., 1995; Zhang et al., 1995). Oxalate oxidase catalyzes the conversion of oxalate to $CO_2$ and $H_2O_2$. The modulation of germin gene expression by an array of treatments provides support for the view that oxalate and $H_2O_2$ have important roles in plant development and homeostasis (Lane, 1994). $H_2O_2$ is involved in the oxidative cross-linking of cell-wall polymers (Olson and Varner, 1993) and germin may, as Lane (1994) suggested, provide an important substrate for cell-wall biosynthesis during cereal seed germination and plant development. Recent studies have demonstrated that $H_2O_2$ has a role in strengthening the plant cell wall against pathogen attack through the oxidative cross-linking of cell-wall structural proteins (Levine et al., 1994). $H_2O_2$ also serves as a signal for the induction of genes involved in disease resistance responses (Levine et al., 1994; Mehdy, 1994). Because salt stress slows growth, perhaps the $H_2O_2$ generated by germin during salt stress serves as a signal or generates other signals that induce genes encoding proteins involved in mechanisms that protect barley against salt stress. Rapid progress in understanding the roles of germin should be possible with the knowledge that germin gene expression is modulated by a number of cues and that oxalate and, subsequently, $H_2O_2$ thus appear to have important roles in a variety of plant processes.

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