The Plant Cell, Vol. 10, 1151–1161, July 1998, www.plantcell.org © 1998 American Society of Plant Physiologists

HOS1, a Genetic Locus Involved in Cold-Responsive Gene Expression in Arabidopsis

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Low-temperature stress induces the expression of a variety of genes in plants. However, the signal transduction pathway(s) that activates gene expression under cold stress is poorly understood. Mutants defective in cold signaling should facilitate molecular analysis of plant responses to low temperature and eventually lead to the identification and cloning of a cold stress receptor(s) and intracellular signaling components. In this study, we characterize a plant mutant affected in its response to low temperatures. The Arabidopsis *hos1-1* mutation identified by luciferase imaging causes superinduction of cold-responsive genes, such as *RD29A*, *COR47*, *COR15A*, *KIN1*, and *ADH.* Although these genes are also induced by abscisic acid, high salt, or polyethylene glycol in addition to cold, the *hos1-1* mutation only enhances their expression under cold stress. Genetic analysis revealed that *hos1-1* is a single recessive mutation in a nuclear gene. Our studies using the firefly luciferase reporter gene under the control of the cold-responsive *RD29A* promoter have indicated that cold-responsive genes can be induced by temperatures as high as 19°C in *hos1-1* plants. In contrast, wild-type plants do not express the luciferase reporter at 10°C or higher. Compared with the wild type, *hos1-1* plants are less cold hardy. Nonetheless, after 2 days of cold acclimation, *hos1-1* plants acquired the same degree of freezing tolerance as did the wild type. The *hos1-1* plants flowered earlier than did the wild-type plants and appeared constitutively vernalized. Taken together, our findings show that the *HOS1* locus is an important negative regulator of cold signal transduction in plant cells and that it plays critical roles in controlling gene expression under cold stress, freezing tolerance, and flowering time.

INTRODUCTION

Low temperature is one of the most important environmental factors limiting the geographic distribution of land plants (Levitt, 1980). Plants from temperate regions can increase in freezing tolerance by being exposed to low nonfreezing temperatures. This process is known as cold acclimation (Guy, 1990). Changes in gene expression (Thomashow, 1994), membrane composition and cryobehavior (Lynch and Steponkus, 1987), enzyme activities, and accumulation of cryoprotectants, such as sugars and polyamines, have been associated with cold acclimation (Levitt, 1980). However, the underlying mechanisms by which plant cells perceive and transduce the cold signal to activate these cellular responses are unclear.

Transient increases in cytosolic calcium appear to be an early step in cold signaling (M.R. Knight et al., 1991; Monroy and Dhindsa, 1995; H. Knight et al., 1996). The phytohormone abscisic acid (ABA) is known to play an important role in cold acclimation. Treatment with ABA at normal growth temperatures increases the freezing tolerance of a wide range of plants (Chen et al., 1983; Mohapatra et al., 1988). Furthermore, exposure to low temperature transiently increases ABA levels in a number of plant species, including Arabidopsis (Chen et al., 1983; Guy and Haskell, 1988; Lång, et al., 1994). Recently, the activity of a MAP kinase has been shown to increase in alfalfa when cold shocked (Jonak et al., 1996). However, this increase is not specific to cold because drought stress can also activate the kinase (Jonak et al., 1996).

The expression of a number of genes in plants is regulated by low temperature (Dhindsa and Monroy, 1994; Guy et al., 1994; Palva et al., 1994; Thomashow, 1994). Most of these genes maintain high levels of expression throughout cold treatment, but their expression decreases rapidly upon return from cold to normal growth temperatures (Thomashow, 1994). The cold-responsive genes encode a diverse array of proteins, such as enzymes involved in respiration and metabolism of carbohydrates, lipids, phenylpropanoids and antioxidants, molecular chaperones, antifreezing proteins, and many others without a known function (Guy et al., 1994).

Arabidopsis cold-regulated genes identified to date can also be induced by ABA or osmotic stress treatment (Hajela et al., 1990; Kurkela and Borg-Franck, 1992; Nordin et al., 1993). However, the cold induction of these genes may not

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necessarily depend on endogenous ABA. For example, although the *RD29A* gene (also known as *COR78* or *LTI78*) can be induced by either cold or ABA treatment, its cold regulation is ABA independent (Gilmour and Thomashow, 1991; Nordin et al., 1993; Yamaguchi-Shinozaki and Shinozaki, 1994). Cold induction of *RD29A* is not impaired by the ABA-deficient *aba1* mutation or the ABA- insensitive *abi1* mutation (Gilmour and Thomashow, 1991; Nordin et al., 1993). Yamaguchi-Shinozaki and Shinozaki (1994) and Stockinger et al. (1997) have identified a DRE/C repeat element from the promoters of *RD29A* and *COR15A* genes. The DRE/C repeat mediates ABA-independent regulation of gene expression by cold or osmotic stress. A transcriptional factor that binds to the DRE/C repeat has been cloned from Arabidopsis (Stockinger et al., 1997). Other components in the ABA-independent cold signal transduction pathway remain to be identified.

We are interested in the mechanism of ABA-independent regulation of gene expression by cold. Our group has taken a genetic approach by identifying Arabidopsis mutants with altered regulation of gene expression in response to low temperature (Ishitani et al., 1997). We present here a detailed characterization of a plant mutant, *hos1-1*, that shows enhanced gene expression in response to low-temperature treatments. This mutant defines a genetic locus important not only for cold-regulated gene transcription but also for freezing tolerance and the control of flowering time.

RESULTS

Identification of the *HOS1* Locus

To facilitate genetic analysis of stress responses, we transformed Arabidopsis plants with the chimeric *RD29A–LUC* gene consisting of the firefly luciferase coding sequence under control of the *RD29A* promoter (−650 to −1 fragment; Ishitani et al., 1997). The *RD29A–LUC* plants emit bioluminescence in response to cold, osmotic stress, or ABA treatment. Homozygous plants with a single *RD29A–LUC* transgene, referred to as the wild type in this study, were mutagenized with ethyl methanesulfonate, and mutants with altered bioluminescence responses to cold, osmotic stress, or ABA were selected as described in Ishitani et al. (1997). Preliminary analysis identified several mutants exhibiting an enhanced luminescence response to cold stress but showing normal responses to ABA and osmotic stress treatments. One of the mutants, designated *hos1-1*, was chosen for detailed characterization.

Figure 1 shows *RD29A–LUC* expression in *hos1-1* seedlings when no treatment was applied or when treated with low temperature, ABA, or high salt. Similar to the wild type, *hos1-1* did not show any luciferase expression in the absence of stress treatments (Figure 1). Significant luciferase expression was induced in the mutant as well as in the wild type when treated with either cold, ABA, or high salt. The *hos1-1* mutant, however, showed a much higher level of expression than did the wild type under cold treatment. The increased level of luciferase expression in *hos1-1* plants was specific to cold stress because induction by ABA or NaCl in the mutant was not substantially different from that of the wild type. Figure 2 shows the luminescence images of *hos1-1* and the wild-type seedlings when treated with cold (Figure 2B), ABA (Figure 2D), or NaCl (Figure 2F). *hos1-1* seedlings displayed a substantially brighter luminescence phenotype than did the wild type specifically under cold treatment.

The *hos1-1* mutant was backcrossed with the wild type. The resulting F_1 plants all exhibited a wild-type phenotype (Table 1). The F_2 progeny of the selfed F_1 segregated ~3:1 for wild type/mutant (Table 1). These results indicate that the *hos1-1* mutant is caused by a recessive mutation in a single nuclear gene. Allelism tests showed that the *hos1-1* mutant complemented all other mutants (i.e., mutant lines 1609, 1400, 970, 738, 737, 734, 470, and 56) that show specifically enhanced bioluminescence responses to cold treatment only (L. Xiong and J.-K. Zhu, unpublished data).

Cold-Regulated Gene Expression in *hos1-1* Plants

To determine whether the *hos1-1* mutation affects endogenous *RD29A* gene expression, we extracted total RNA from *hos1-1* and wild-type seedlings that were treated with ABA, NaCl, or polyethlylene glycol (PEG) or by using different periods of low temperature. Figure 3 shows that the steady state levels of *RD29A* message were higher in *hos1-1* than

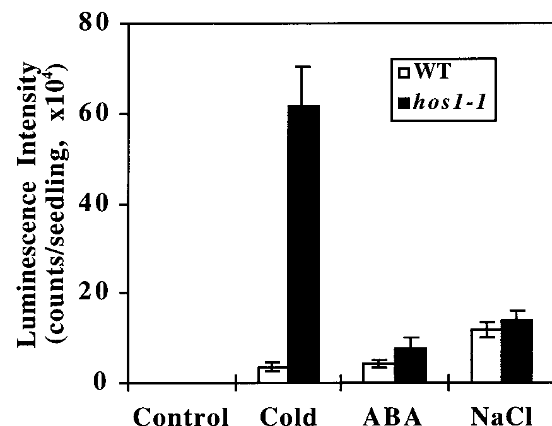

Figure 1. *RD29A–LUC* Expression (Luminescence Intensity) in *hos1-1* and Wild-Type Plants.

Data represent the average of 20 individual seedlings. Error bars are standard deviation. Control, room temperature without treatment; Cold, 0°C for 24 hr; ABA, 100 μM ABA for 3 hr; NaCl, 300 mM NaCl for 5 hr; WT, wild type.

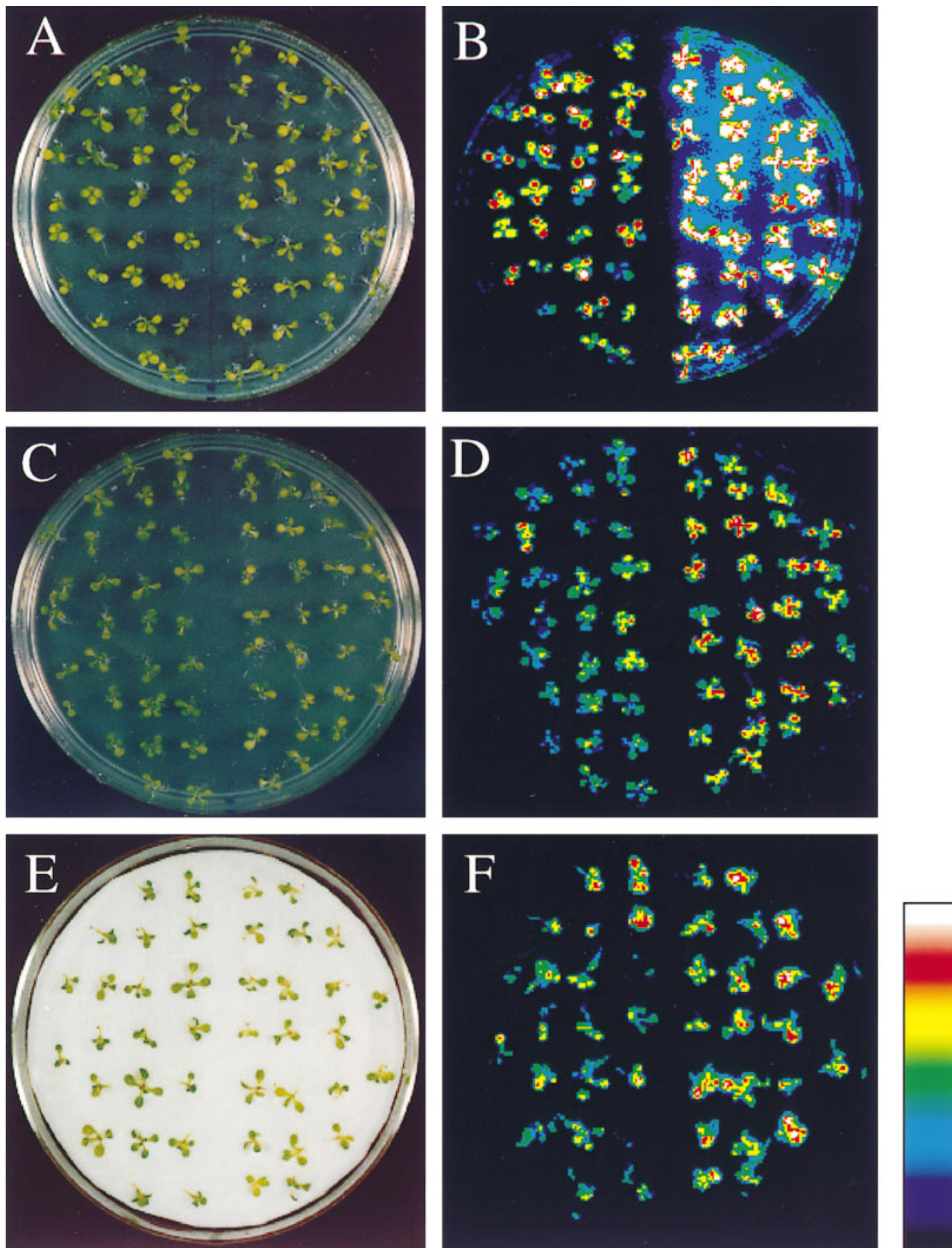

Figure 2. Luminescence Images of *hos1-1* and Wild-Type Plants.

The color scale at right shows the luminescence intensity from dark blue (lowest) to white (highest).
(A) Wild-type (left) and *hos1-1* (right) seedlings.
(B) Luminescence after low-temperature treatment at 0°C for 24 hr.
(C) Wild-type (left) and *hos1-1* (right) seedlings.
(D) Luminescence after treatment with 100 μM ABA for 3 hr.
(E) Wild-type (left) and *hos1-1* (right) seedlings on filter paper saturated with 300 mM NaCl.
(F) Luminescence after treatment with 300 mM NaCl for 5 hr.

Table 1. Genetic Analysis of *hos1-1* Mutants (Wild Type × *hos1-1* Cross)[a]

Generation	Seedlings Tested	Mutant	Wild Type	χ^2
F_1	12	0	12	
F_2	480	119	361	0.011

[a] Female × male.

in the wild type when treated by cold stress. This enhanced *RD29A* induction can be seen at all of the time points for cold treatment, although the difference is especially great at the 6- and 12-hr time points (Figure 3). Figure 4 shows the quantification of the endogenous *RD29A* expression level in *hos1-1* and wild-type plants under cold treatment. As expected, *RD29A* message levels in *hos1-1* were not different from those of the wild type when treated with ABA or NaCl (Figure 3). However, the *hos1-1* mutation appears to reduce the level of *RD29A* induction by PEG (Figure 3).

In addition to *RD29A*, cold-induced expression of several other genes examined was also higher in *hos1-1* than in the wild type (Figure 3). *COR47* (Gilmour et al., 1992) induction was higher in the *hos1-1* mutant throughout the cold treatment. Like *RD29A*, *COR47* induction in the wild-type plants also peaked at 24 hr of cold treatment. In contrast, *hos1-1* plants showed a peak level of *COR47* message after only 12 hr of cold exposure (Figure 3). Induction of *COR15A* (Lin and Thomashow, 1992) transcripts by cold was detectable by 6 hr and peaked at 48 hr in the wild type. In *hos1-1* plants, *COR15A* expression peaked at 24 hr of cold treatment (Figure 3). Cold-induced *COR15A* expression was higher in the *hos1-1* mutant at 6, 12, and 24 hr but not at the 48-hr time point (Figure 3). *KIN1* (Kurkela and Franck, 1990) expression was higher in the *hos1-1* mutant than in the wild type throughout cold treatment (Figure 3). Cold induction of the alcohol dehydrogenase (*ADH*; Jarillo et al., 1993) gene in the

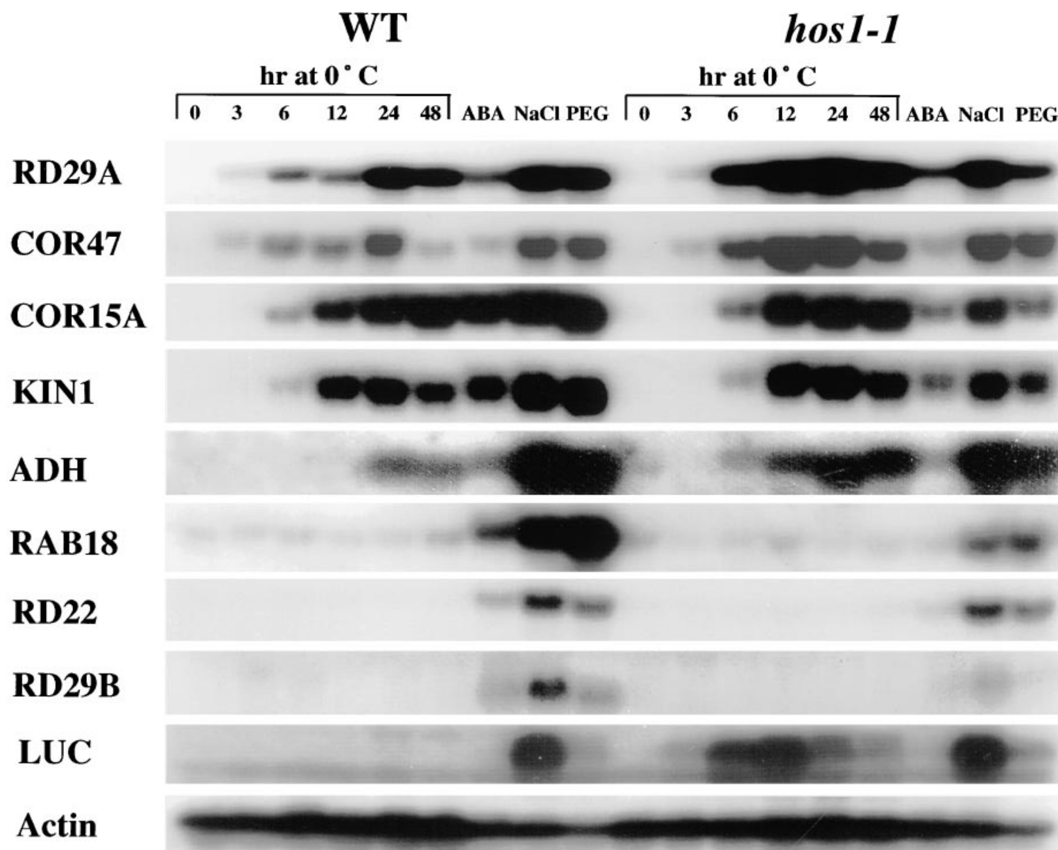

Figure 3. Expression of the Endogenous *RD29A* Gene and Other Stress-Responsive Genes in *hos1-1* and Wild-Type Plants.

Plants were subjected to low temperature (0°C) for the indicated times. ABA, 100 μM ABA for 3 hr; NaCl, 300 mM NaCl for 5 hr; PEG, 30% PEG (average molecular weight, 6000) for 5 hr; WT, wild type.

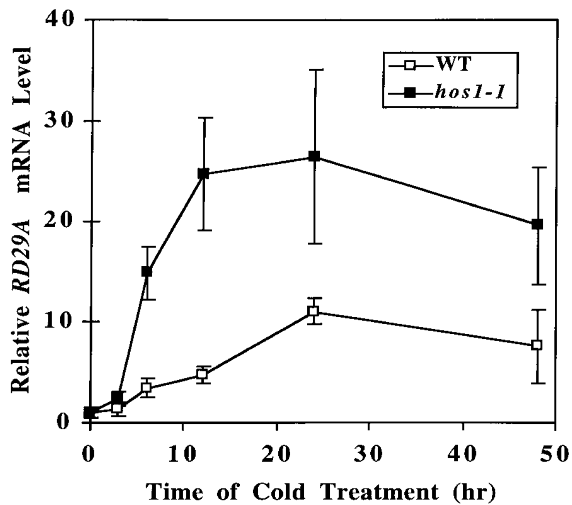

Figure 4. Quantification of Steady State *RD29A* mRNA Levels in *hos1-1* and Wild-Type Plants Treated at 0°C for Different Time Periods.

Results are the averages of three independent experiments. Error bars represent standard deviation. WT, wild type.

wild type was detectable after 24 hr of cold treatment (Figure 3). In contrast, the *ADH* transcript was detected in the *hos1-1* mutant after only 6 hr of cold treatment. *ADH* expression was substantially higher in *hos1-1* plants throughout cold treatment (Figure 3).

As shown in Figure 3, the expression of *COR47*, *COR15A*, *KIN1*, and *ADH* was not higher in the *hos1-1* mutant when plants were treated with ABA, NaCl, or PEG. In fact, the induction of *COR47*, *COR15A*, and *KIN1* by NaCl or PEG was considerably lower in the mutant than in the wild type (Figure 3). ABA induction of *COR15A* and *KIN1* also was inhibited by the *hos1-1* mutation (Figure 3).

To further examine the effect of *hos1-1* mutation on osmotic stress–regulated genes, we analyzed the expression of *RAB18* (Lång and Palva, 1992), *RD22* (Yamaguchi-Shinozaki et al., 1992), and *RD29B* (Yamaguchi-Shinozaki and Shinozaki, 1993) (Figure 3). The expression of these genes was upregulated by osmotic stress and to some extent by ABA as well, but they showed little response to cold stress (Figure 3). Although the expression of *RAB18* and *RD29B* was inhibited in the *hos1-1* mutant when plants were treated with NaCl, PEG, or ABA, the expression of *RD22* was not altered substantially in the mutant. As for the wild type, no expression of either *RAB18*, *RD22*, or *RD29B* was detected in the mutant under cold treatment (Figure 3).

As controls, the transcript levels of the *LUC* transgene and the actin gene were determined. Figure 3 shows that the *LUC* transcript was induced by cold to a much greater extent in the *hos1-1* mutant than in the wild type. However, its induction by ABA, NaCl, or PEG was not changed in the *hos1-1* mutant (Figure 3). The expression of the actin gene was not affected by the *hos1-1* mutation when plants were not treated or were treated with cold, ABA, NaCl, or PEG (Figure 3).

RD29A–LUC Expression during Cold Acclimation and Deacclimation

Figure 5A shows the time course of *RD29A–LUC* expression when plants were subjected to cold treatment. At all time points after cold treatment, *RD29A–LUC* expression was significantly higher in the mutant (Figure 5). In both the wild type and *hos1-1* mutant, maximum levels of *RD29A–LUC* expression were reached after 2 days of cold treatment. The enhanced *RD29A–LUC* expression in *hos1-1* persisted throughout the cold treatment for as long as 7 days (data not shown). Time-course analysis as well as dosage experiments on *RD29A–LUC* expression under ABA or high-salt treatment were also performed but failed to detect any substantial differences between *hos1-1* and wild-type plants (Figures 5B and 5C).

To determine whether the *hos1-1* mutation affects *RD29A–LUC* expression during recovery from cold (i.e., deacclimation), we treated seedlings at 0°C for 24 hr and then incubated them at 22°C for various time periods. Figure 6 shows that although *RD29A–LUC* expression was higher at all time points during the deacclimation (Figure 6A), the rate of decline in *RD29A–LUC* expression is the same in *hos1-1* and wild-type plants (Figure 6B). Cold-induced luciferase activity decayed rapidly during the first few hours after plants were removed from the cold and was reduced to preacclimation levels by 48 hr after transfer to room temperature.

RD29A–LUC Expression Can Be Induced at Higher Temperatures in *hos1-1* Plants

Wild-type and *hos1-1* plants were treated for 24 hr at different temperatures ranging from −10°C to 22°C. Figures 7A and 7B show that maximal *RD29A–LUC* expression in both *hos1-1* and the wild-type plants was achieved at 0°C. At temperatures >0°C, *RD29A–LUC* expression declined sharply. The wild-type plants did not exhibit any expression at 10°C or higher. Although *RD29A–LUC* expression was also much lower in *hos1-1* plants at temperatures >0°C, expression was detectable at temperatures as high as 19°C. At 22°C, *hos1-1* plants also failed to show *RD29A–LUC* expression. These results suggest that cold-responsive genes can be induced at higher temperatures in the mutant.

hos1-1 Mutation Decreases Freezing Tolerance

To evaluate the effect of the *hos1-1* mutation on plant freezing tolerance, an electrolyte leakage test (Sukumaran and Weiser, 1972; Ristic and Ashworth, 1993) was conducted

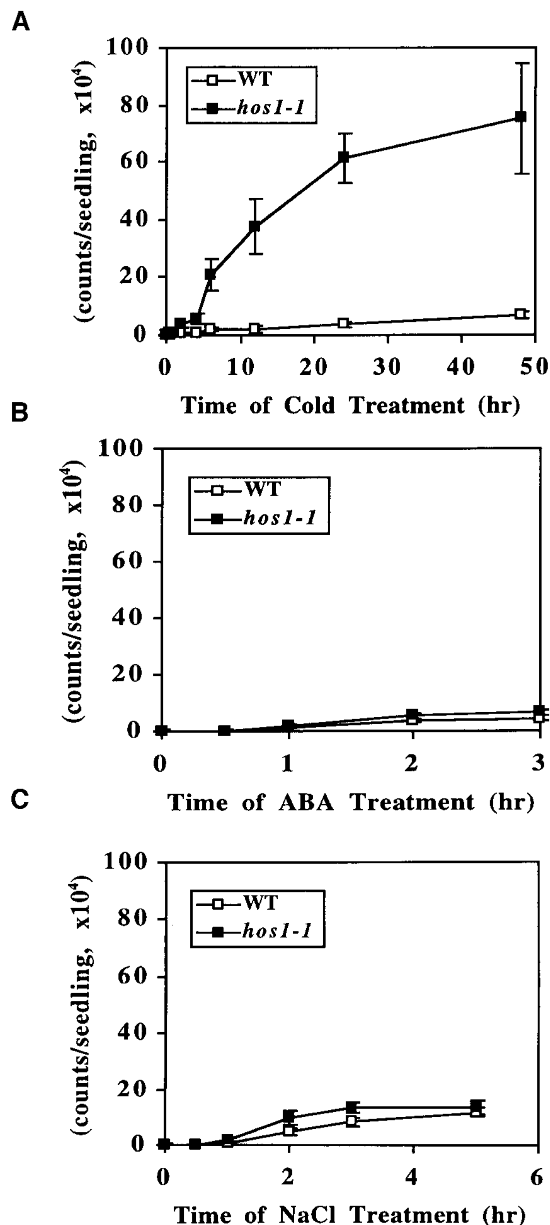

Figure 5. Time Course of *RD29A–LUC* Expression in *hos1-1* and Wild-Type Plants in Response to Low Temperature, ABA, or NaCl Treatment.

(A) Low-temperature treatment. One-week-old *hos1-1* and wild-type seedlings grown on the same plate were placed in an incubator at 0°C. Plates were removed from the incubator at different time points; luminescence images were taken immediately, and the intensities were determined.

(B) ABA treatment. *hos1-1* and wild-type seedlings grown on the same plate were sprayed with 100 μM ABA and incubated under light for the indicated time periods before luciferase imaging.

(C) NaCl treatment. Plants were transferred on filter paper saturated with a solution of MS salts plus 300 mM NaCl under light for the indicated time periods before luciferase imaging.

Error bars represent standard deviation ($n = 20$). WT, wild type.

with *hos1-1* and wild-type plants. The *hos1-1* mutation appeared to decrease freezing tolerance of nonacclimated plants; however, little difference was observed between the mutant and the wild type when both were pretreated for 48 hr at 4°C (Figure 8). Temperatures at 50% ion leakage (LT_{50}) were estimated to be −3.0 and −4.1°C for nonacclimated *hos1-1* and wild-type plants and −5.6 and −5.8°C for cold-acclimated *hos1-1* and cold-acclimated wild-type plants, respectively (Figure 8). The ion leakage experiments were repeated independently three times by using different batches of plants, and similar results were obtained. Based on these results, we conclude that without acclimation, *hos1-1* plants are less cold hardy than are the wild-type plants.

hos1-1 Mutant Flowers Early

Under the growth conditions used in this study (16 hr of light at 22°C; 8 hr of dark at 18°C), *hos1-1* plants flowered ∼2 weeks after imbibition, compared with 3 weeks for wild-type plants. We selected from >100 *hos1-1* plants from a segregating F_2 population (selfed from a cross between the wild type and *hos1-1*); thus far, none has been an exception to this early-flowering phenotype. Because flowering time in Arabidopsis can be influenced by periods of low-temperature treatment (i.e., vernalization), we determined the vernalization responses of *hos1-1* and wild-type plants. To accurately determine the flowering phenotype of *hos1-1* plants, we measured the total leaf number (*LN*) at flowering. As shown in Figure 9, the *LN* value of *hos1-1* is smaller than that for wild-type plants, without vernalization or with various periods of vernalization. Both *hos1-1* and wild-type plants responded to vernalization. Without vernalization, the *LN* values for *hos1-1* and wild-type plants were 8.0 ± 0.9 and 13.2 ± 1.9, respectively. After 8 weeks of vernalization, the *LN* value for *hos1-1* plants decreased to 5.0 ± 0.4, whereas the *LN* value for the wild type dropped to 7.7 ± 0.9. It is interesting that the *LN* value for nonvernalized *hos1-1* was nearly the same as the *LN* value for wild-type plants after 8 weeks of vernalization.

Both *hos1-1* and wild-type seeds germinated during the long periods of vernalization. Because the vernalization was performed with dim influorescent light in a cold room, the seedlings were etiolated. When the seedlings were moved to a growth chamber after 8 weeks of vernalization, all wild-type plants deetiolated rapidly and resumed normal growth. In contrast, *hos1-1* seedlings deetiolated slowly, and only 30% survived. The *hos1-1* seedlings appeared to have sustained damage during the long period of vernalization, and most withered and died before deetiolation.

The appearance of *hos1-1* plants is shown in Figure 10. In general, *hos1-1* plants are smaller and appear less vigorous than wild-type plants. Whereas the wild-type plants typically reach a height of 25 to 30 cm, *hos1-1* plants usually do not grow taller than 18 cm. Mature *hos1-1* plants possess fewer leaves than do wild-type plants. The leaves on *hos1-1* plants

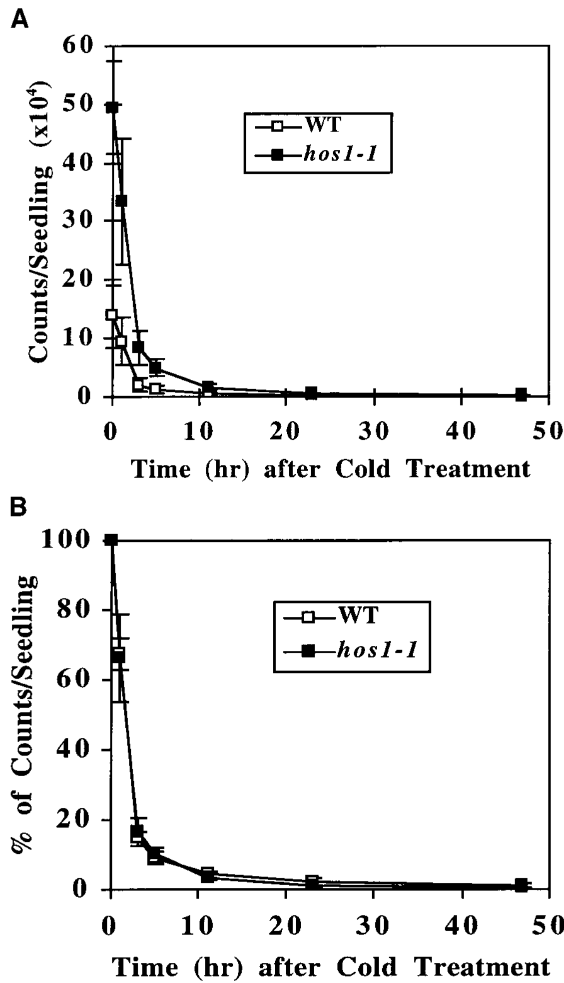

Figure 6. *RD29A–LUC* Expression of *hos1-1* and Wild-Type Plants during Deacclimation.

hos1-1 and wild-type (WT) plants on the same agar plate were placed at room temperature under cool-white light after they were treated at 0°C for 24 hr. Luminescence intensities were determined at different time points. Error bars represent standard deviation (n = 20).

(A) Decrease in luminescence intensities with time after removal from cold.

(B) Percentage of decrease in luminescence intensity relative to nondeacclimated plants.

do not appear as green as those of the wild type. Siliques on *hos1-1* are typically less than two-thirds the length of wild-type siliques.

DISCUSSION

hos1-1 is a plant mutant whose gene regulation is altered under low-temperature conditions. This recessive mutation enhances cold induction of stress genes. Gene regulation by osmotic stress or ABA is not affected significantly in this mutant. The recessive *hos1-1* mutation thus reveals an important negative regulator of low-temperature signal transduction in plants. Our results indicate that the *HOS1* locus not only plays a significant role in cold regulation of gene transcription but also influences other cold-regulated processes, such as freezing tolerance and time to flower.

It is clear that not all of the cold-responsive genes are affected to the same degree by the *hos1-1* mutation (Figure 3). The difference between *hos1-1* and the wild type is less dramatic for *COR15A* and *KIN1*, although the expression of

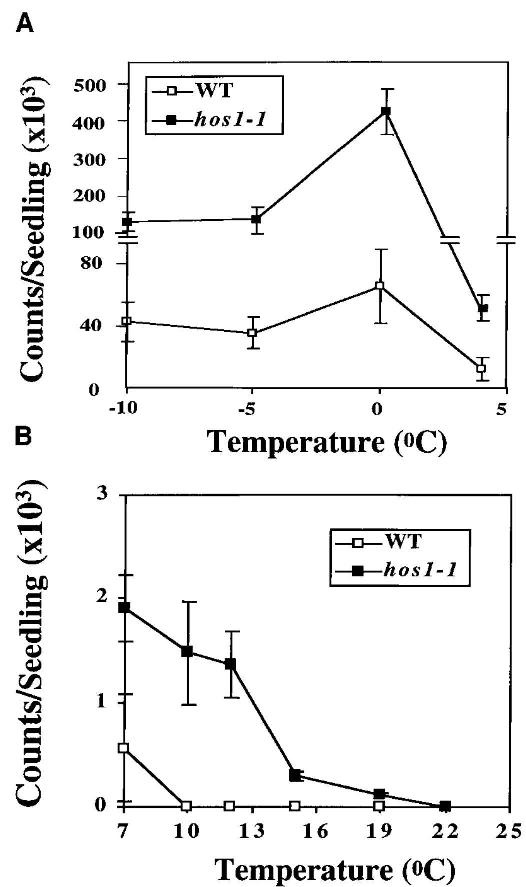

Figure 7. *RD29A–LUC* Expression in *hos1-1* and Wild-Type Plants under Different Temperature Treatments.

hos1-1 and wild-type (WT) plants were planted on the same agar plates and allowed to grow for 1 week under constant lighting at room temperature (22 ± 2°C). The plates were then treated at the indicated temperatures (±0.1°C) for either 3 hr (−5 or −10°C treatment) or 24 hr (all other temperature treatments), and luminescence images were taken (see Methods for details). Error bars represent standard deviation (n = 20).

(A) Lower temperature range.

(B) Higher temperature range.

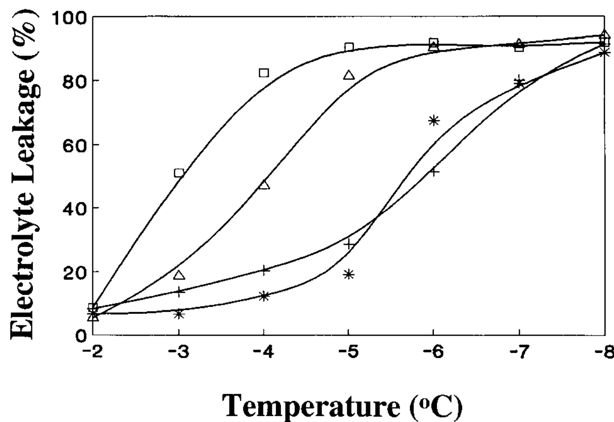

Figure 8. Leakage of Electrolytes in *hos1-1* and Wild-Type Plants When Treated at Temperatures below Freezing.

For cold acclimation treatment, plants were incubated at 4°C for 48 hr under white fluorescent light. Triangles, nonacclimated wild type; squares, nonacclimated *hos1-1*; (+), acclimated wild type; asterisks, acclimated *hos1-1*.

these two genes was still reproducibly higher in *hos1-1* plants. The reason for this differential effect is not clear. It is possible that these two genes may use signaling pathways that overlap with but differ from the ones used by *RD29A*, *COR47*, and *ADH*, whose expression is more dramatically affected by the *hos1-1* mutation. Alternatively, *COR15A* and *KIN1* transcripts could be degraded more rapidly in *hos1-1* plants, thereby preventing their overaccumulation.

Expression of the *RD29A* gene is induced by cold, ABA, and osmotic stress. Cold and osmotic signaling pathways converge to activate gene transcription through the *cis*-DNA element DRE/C repeat (Yamaguchi-Shinozaki and Shinozaki, 1994; Stockinger et al., 1997). Because only cold regulation is altered in *hos1-1* plants, HOS1 is likely in a step before the cold and osmotic signaling pathways converge. Figure 11 presents a model of HOS1 function in the activation of stress gene transcription. The model depicts HOS1 as a negative regulator for a cold-specific pathway. In addition, results presented in Figure 3 indicate that HOS1 plays a positive role in the upregulation of *RAB18*, *RD29B*, *COR15A*, and *KIN1* by osmotic stress or ABA.

Little is known about the initial perception of cold in living organisms. In the cyanobacterium *Synechocystis* sp PCC6803, changes in membrane fluidity have been suggested as the basis for low-temperature perception (Vigh et al., 1993). It was demonstrated that reduction of cyanobacterial cell membrane fluidity by hydrogenation at room temperature could trigger the expression of the *desA* gene, as does low temperature (Vigh et al., 1993). The importance of membrane lipid fluidity for plant chilling tolerance is well known (Murata et al., 1992; Miquel et al., 1993). However, it is unclear whether there exists a connection between membrane fluidity and gene regulation by low temperature in plants. In plant cells, an increase in cytosolic calcium level has been suggested to be important in cold acclimation (Knight et al., 1996) and cold induction of gene expression (Monroy and Dhindsa, 1995). The *HOS1* locus could play a role in either the initial perception of cold stress or its subsequent intracellular signaling.

It is intriguing that *hos1-1* plants are less tolerant to freezing stress (Figure 8). The results may suggest membrane alterations in the mutant. For example, certain lipids in the plasma membrane of *hos1-1* cells may have a slightly higher degree of saturation, which may result in increased membrane leakage during freezing stress. By analogy to the cyanobacterium system, an elevated level of membrane lipid saturation also might cause expression of cold-responsive genes at higher temperatures. We have observed that *RD29A* gene transcription can be induced by temperatures as high as 19°C (Figure 7). We also have observed that *hos1-1* plants had a much reduced survival rate after long periods of cold treatment (i.e., vernalization). This latter observation indicates that *hos1-1* plants are chilling sensitive, unlike wild-type plants, which are chilling tolerant. The level of membrane lipid saturation is known to correlate with plant chilling sensitivity (Miquel et al., 1993).

hos1-1 plants show a clear early-flowering phenotype. The early-flowering phenotype always cosegregated with the high *RD29A–LUC* expression observed after cold treatment. Because the total leaf number of nonvernalized *hos1-1* plants is approximately the same as that of wild-type plants after 8 weeks of vernalization, the mutant may be considered as constitutively vernalized. Induction of *RD29A* by temperatures near 20°C (Figure 7B) indicates that "cold"

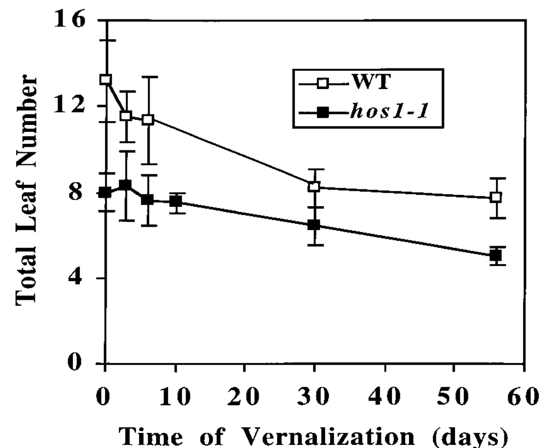

Figure 9. *hos1-1* Plants Flower Earlier than Wild-Type Plants.

Seeds of *hos1-1* and the wild-type (WT) plants were sown in pot media and placed in a cold room (4°C) for the indicated time periods before moving to a growth chamber. When their first flowers were at anthesis, 10 to 20 plants were counted for total leaf numbers.

Figure 10. Morphology of *hos1-1* (right) Compared with a Wild-Type *RD29A–LUC* Plant (left).

The mutant and the wild-type plants were planted at the same time and allowed to grow in a growth chamber for 5 weeks before being photographed. Note that the *hos1-1* plant is smaller and flowered earlier; its leaves are not as green as those of the wild-type plant. Some leaves on the *hos1-1* plant were already dead and not visible when photographed. The bar insert at right represents 5 cm.

signaling operates in the mutant under normal growth conditions (i.e., 18°C nighttime temperature). Therefore, the mutant may experience a vernalization effect under normal growth conditions. It is clear from the results shown in Figure 9 that *hos1-1* plants can be further vernalized by exposure to low temperature, resulting in flowering at an *LN* of only 5. Recently, Chandler et al. (1996) identified several Arabidopsis mutants with reduced vernalization responses. Expression of cold-induced transcripts was not altered in these mutants (Chandler et al., 1996). Based on these results, the authors suggested that vernalization may be mediated through a pathway that is different from those for cold acclimation and gene regulation, and the perception of low temperature may be different for vernalization and acclimation (Chandler et al., 1996). Our results with *hos1-1* plants suggest, however, that cold acclimation and vernalization may share some common mechanisms.

METHODS

Plant Materials and Growth Conditions

Plants (*Arabidopsis thaliana* ecotype C24) were transformed with the chimeric gene *RD29A*–luciferase (*LUC*), and the seeds from selfed progenies of the resulting transgenic plants containing homozygous transgene (referred to as wild type) were mutagenized with ethyl methanesulfonate (Ishitani et al., 1997). M_2 seedlings grown on 0.8% agar plates containing a Murashige and Skoog (MS) salt base (JRH Biosciences, Lenexa, KS) were screened for altered *RD29A–LUC* expression in response to low temperature, exogenous abscisic acid (ABA), or osmotic stress by using a video-imaging system, as described by Ishitani et al. (1997). Plants for imaging analysis were usually 1-week-old seedlings grown on MS agar plates under constant white fluorescent light at room temperature. Plants for genetic analysis, vernalization, or freezing tolerance assays were grown in pot media (Metro-Mix 350; Scott-Sierra Horticultural Products Co., Marysville, OH) in growth chambers with 16 hr of light at 22°C and 8 hr of dark at 18°C and 70% relative humidity.

Cold Acclimation, Vernalization, and Other Stress Treatments

For mutant screening and primary characterization, low-temperature treatment was conducted at 0°C for 48 hr in the dark. For detailed characterization of *RD29A–LUC* expression in response to low-temperature treatment, we placed agar plates with 1-week-old *hos1-1* and wild-type seedlings in an incubator set at the designated temperature (±0.1°C) for 24 hr, as stated in Results. At the end of the treatment, plates were removed from the incubator, and luminescence images were taken as described in Ishitani et al. (1997). Because longer treatment at −5 or −10°C results in freezing of the agar plates, these treatments only lasted for 3 hr. After treatment, the plates were placed at room temperature for 2 hr to thaw before taking luminescence images. For ABA treatment, 100 μM ABA in water (mixed isomers) was sprayed on leaves of the seedlings, and luminescence images were taken 3 hr after treatment. NaCl treatment was conducted on filter paper saturated with an MS salt solution that was supplemented with 300 mM NaCl, and luminescence images were taken 5 hr after the treatments.

To determine vernalization effect, seeds of the mutant and wild-type plants were sown in pot media, and the pots were kept at 4°C for different times (days), as indicated in Results. After vernalization, the pots were placed in a growth chamber until flowering occurred.

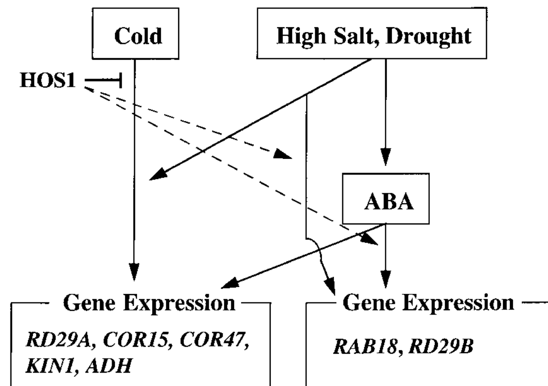

Figure 11. Proposed Model of HOS1 Function.

HOS1 negatively regulates low-temperature–induced expression of many stress-responsive genes. It is also a positive factor for specific gene regulation by osmotic stress or ABA.

At the emergence of the first flower, the rosette and cauline leaves were counted.

Freezing Tolerance

For the freezing-tolerance assay, seeds of the *hos1-1* and wild-type plants were sown in pot media. For cold-acclimation treatment, plants at the rosette stage were placed in a cold room at 4°C under white fluorescent light for 48 hr before we sampled the leaves for the freezing-tolerance assay.

Fully developed rosette leaves were used to determine freezing-caused electrolyte leakage, essentially as described by Sukumaran and Weiser (1972) and Ristic and Ashworth (1993). Briefly, for each treatment, one excised leaflet was placed in a test tube containing 100 μL of deionized H_2O, and the tube was placed in a refrigerated circulator (freezing bath) (model 1187; VWR Scientific, San Francisco, CA) with the temperature set at 0°C. There were three replicates for each temperature treatment. The temperature of the bath was programmed to decrease to −12°C, with 1°C decrement after 30 min. The tubes were removed from the bath when the designated temperature was reached, and they were placed immediately on ice to allow gradual thawing. The leaflets then were transferred carefully to another tube containing 25 mL of deionized water and shaken overnight, and the conductivity of the solution was measured. The tubes with the leaflets were then autoclaved. After cooling down to room temperature, conductivities of the solutions were measured again. The percentage of electrolyte leakage was calculated as the percentage of the conductivity before autoclaving over that after autoclaving.

RNA Analysis

Ten-day-old seedlings grown on MS agar plates were subjected to low temperature, ABA, or polyethylene glycol (PEG). Respective treatment conditions were as stated in Results. Total RNA from control or stressed plants was extracted as described by Liu and Zhu (1997). The *RD29A* gene–specific probe was from the 3′ noncoding region (Liu and Zhu, 1997). *COR15* and *COR47* cDNAs (Gilmour et al., 1992; Lin and Thomashow, 1992) were kindly provided by M.F. Thomashow (Michigan State University, East Lansing, MI). DNA probes for *RD22* and *RD29B* (Yamaguchi-Shinozaki et al., 1992) were cloned from genomic DNA of wild-type Columbia plants by polymerase chain reaction by using the following primer pairs: 5′-AACGCACCGATGCAGAAGTACA-3′ and 5′-CCGGATCCGCAACACAAGATACAGAC-3′; and 5′-CCCGGATCCTTGCTGTTACACGTA-3′ and 5′-ATGCTCGAGCTGACGCTATGATTTTG-3′, respectively. The probe for *KIN1* (Kurkela and Franck, 1990) was a 0.4-kb EcoRI fragment of the Arabidopsis expressed sequence tag (EST) clone YAP368T7. The probe for *RAB18* (Lång and Palva, 1992) was a 0.8-kb SalI-NotI fragment of the Arabidopsis EST clone 246K10T7. The probe for *ADH* (Jarillo et al., 1993) was a 1.6-kb SalI-NotI fragment of the Arabidopsis EST clone 199P20T7.

ACKNOWLEDGMENTS

We thank Dr. Michael F. Thomashow for kindly providing *COR15a* and *COR47* cDNA probes. We also thank Drs. Robert T. Leonard and Frans Tax for critical reading of the manuscript. This work was supported by grants from the National Science Foundation Integrative Plant Biology Program and the United States Department of Agriculture National Research Initiative Competitive Grants Program to J.-K.Z.

Received January 7, 1998; accepted May 5, 1998.

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