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Communication

Group 3 LEA Gene *HVA1* Regulation by Cold Acclimation and Deacclimation in Two Barley Cultivars with Varying Freeze Resistance[1]

Fedora Sutton*, Xiang Ding, and Donald G. Kenefick

Plant Science Department (F.S., X.D., D.G.K.) and Department of Biology/Microbiology (F.S.), South Dakota State University, Brookings, South Dakota 57007

ABSTRACT

The level of expression of the group 3 late embryogenesis abundant abscisic acid-regulated gene (HVA1) to cold treatment has been studied in winter barley (*Hordeum vulgare*) seedling tissue. The cDNA clone (pHVA1) encoding this late embryogenesis abundant protein was used as a hybridization probe to detect the corresponding mRNA. Expression of the HVA1 gene was determined after the tissue had been subjected to a regimen of 2°C exposure (cold acclimation), followed by a return to 25°C growth conditions (deacclimation). Accumulation of HVA1 mRNA occurred upon cold acclimation of the tissue and disappeared as early as 2 hours after exposure to deacclimation conditions. A comparison of the response to cold acclimation and deacclimation was made between seedling tissue of a freeze-resistant and less freeze-resistant cultivar. In both cultivars, the HVA1 gene was expressed and modulated by cold treatment. Within 2 hours of deacclimation HVA1 mRNA was no longer detectable in either cultivar independently of freeze resistance. The level of expression of HVA1 appeared to be greater in the less freeze-resistant cultivar (Winter Malt).

A number of LEA[2] cDNA clones have been isolated from different species. The RAB (responsive to ABA) gene from rice (17) and maize (20) have been shown to be modulated by a number of environmental stresses including cold. These RAB genes are expressed during the desiccation phase of embryogenesis and are thus LEA genes. It is necessary for freeze-resistant plants to receive a period of exposure to low nonfreezing temperatures if they are to withstand freezing temperatures (14, 19). This period of cold acclimation has been shown to result in a number of changes, including changes in gene expression (2, 10, 15, 16). It has also been shown that the ability to withstand freezing temperatures as a result of some of the changes in plants during the cold acclimation period can be mimicked by ABA treatment of the tissue in the absence of cold acclimation (3, 4).

Hong *et al.* (11) isolated from a barley aleurone layer cDNA library a clone corresponding to an mRNA that was rapidly induced by ABA. This cDNA was designated pHVA1 and is 91% homologous at the nucleotide level with the cDNA clone (pMA2005) isolated from wheat (6). Both of these cDNA clones have been shown to encode group 3 LEA proteins based on their sequence (7), and both have been induced by ABA treatment and dehydration of tissue. The effect of cold treatment on expression of the group 3 LEA transcripts encoded by pHVA1 or pMA2005 has not yet been reported. Because sequence comparisons between the LEA proteins reveal common amino acid domains that may play a role in preventing plant tissue from dehydration during freezing (7), it was of interest to us to determine whether the level of expression of the LEA gene HVA1 was modulated by cold acclimation and whether any discernable difference could be observed in expression of this gene between cultivars differing in degree of freeze resistance.

Freeze resistance of winter cereals is differentially attained, depending on duration of cold acclimation and cultivar (1). A relationship between tissue RNase I activity and freeze resistance potential has been reported (12). We report here that the group 3 LEA protein gene *HVA1* is induced in barley shoots of both a freeze-resistant and a less freeze-resistant cultivar that are cold treated at 2°C. However, *HVA1* expression was abruptly terminated within 2 h when seedlings were returned to 25°C.

MATERIALS AND METHODS

Plant Material

The source of tissue was winter barley (*Hordeum vulgare*). The cultivars Dicktoo (freeze-resistant) and Winter Malt (less freeze-resistant) were utilized. The survival rates of these cultivars and thus their degree of freeze resistance were described by Reid (18). The tissue used in these experiments consisted of primary leaf and coleoptile (shoots) excised after the seedlings had been exposed to various growth conditions. Nonacclimated tissue was obtained by growing both resistant and less resistant cultivars for 6 d at 25°C in the dark. Cold acclimation was accomplished by transferring nonacclimated tissue to 2°C in the dark for periods of 2, 4, or 6 d. Deacclimation was accomplished by transferring 6-d cold-acclimated

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[2] Abbreviation: LEA, late embryogenesis abundant.

tissue back to 25°C for 2, 4, or 6 h. All tissue was harvested in the dark. Samples maintained at 2°C were harvested at 2°C, and the samples that had been returned to 25°C for deacclimation were harvested at 25°C. All tissue was ground in liquid nitrogen before homogenizing in 4 M guanidinium thyocyanate buffer.

RNA Preparation and Gel Blot Analysis

To determine the effect of cold treatment and deacclimation on the expression of the *HVA1* gene, northern blots were performed on total RNA isolated from barley shoots grown under one of the following three conditions: (a) growth for 6 d at 25°C; (b) growth for 6 d at 25°C followed by 2, 4, or 6 d at 2°C (cold acclimated); (c) cold acclimated followed by 2, 4, or 6 h at 25°C (deacclimation). In all cases, RNA was isolated from shoots by a modification of the procedure described by Chirgwin *et al.* (5). Total RNA was then fractionated on denaturing formaldehyde gels (13) and transferred to Zeta probe membrane (Bio-Rad), according to manufacturer's specification. A ^{32}P-labeled probe was made from the gel-purified cDNA insert of pHVA1 using random oligonucleotide primers (9). Prehybridization and hybridization were performed as described by Hong *et al.* (11). Hybridization was detected by autoradiography. Equivalent loading and transfer was determined by stripping and reprobing the blot with ^{32}P-labeled 18S rRNA gene (8).

RESULTS

Effects of Cold Acclimation

Incubation of the barley shoots for periods of 0, 2, 4, and 6 d at 2°C resulted in an accumulation of the 1.1-kilobase *HVA1* mRNA in both cultivars (Fig. 1A). The signals obtained from stripping and reprobing the filters with the ^{32}P-labeled 18S rRNA gene showed that there was differential loading and transfer of RNA to the filter (Fig. 1B). Densitometry scanning of both the signals obtained with the pHVA1 and the ribosomal probes were performed and the values were used to correct for the differences in loading. The results were then plotted as a percentage of the maximum value obtained (Fig. 1C). From the values obtained, it was clear that accumulation of *HVA1* mRNA is greater for the less resistant cultivar (Winter Malt) than the more resistant cultivar (Dicktoo). Expression of the *HVA1* gene also peaks at about 4 d in both cultivars. Further incubation at 2°C results in a decrease in the accumulation of *HVA1* mRNA (Fig. 1C).

Effects of Deacclimation

Figure 2A shows the results of deacclimation studies. Comparison of RNA extracted from tissue maintained for 6 d at 25°C (lane 0) with that of RNA from 4-d cold-acclimated tissue (lane 4d) shows that in both Dicktoo and Winter Malt cultivars there is an accumulation of the 1.1-kilobase *HVA1* mRNA as observed in Figure 1. The autoradiographs also reveal that in both cultivars the level of *HVA1* mRNA observed after 4 d of cold acclimation is almost undetectable if the tissue is removed from 2°C and placed at 25°C for as short a period as 2 h (lane 2h). To show that RNA was loaded in the lanes corresponding to the periods of deacclimation (Fig. 2a, lanes 0, 2h, 4h, or 6h), the filter was stripped and reprobed with the 18S rRNA gene (Fig. 2B).

DISCUSSION

The role of the *HVA1* gene product is still unknown. It is clear that it is induced by environmental stress such as dehydration. We have shown that this group 3 LEA gene, which is induced in aleuron tissue by ABA treatment, is also induced in barley shoots that have been cold treated. We have also determined that 6 h of cold acclimation is not enough time to allow detection of the *HVA1* mRNA in these cultivars (data not shown) and that, even though the cold-stress condition is maintained up to 6 d, the levels of *HVA1* mRNA do

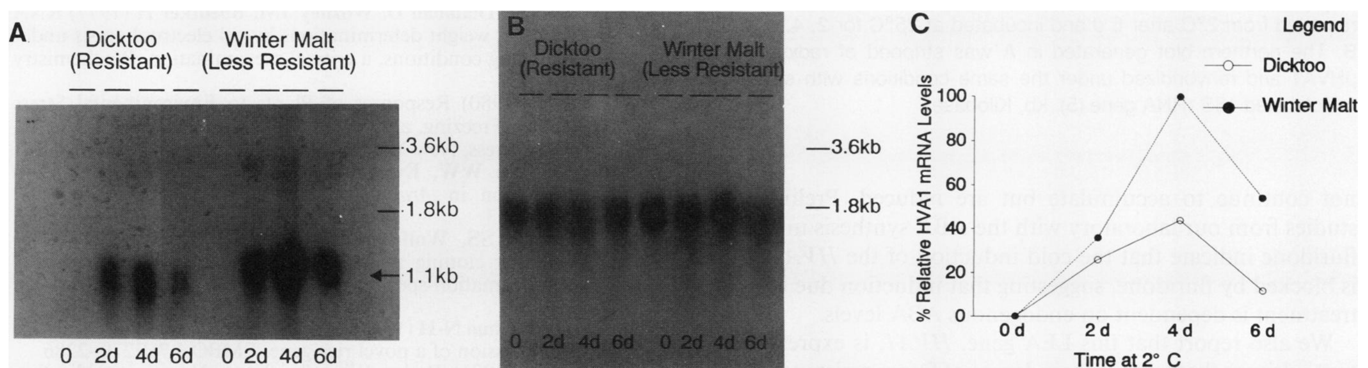

Figure 1. A, Effect of varying periods of cold acclimation on pHVA1 mRNA accumulation in Dicktoo and Winter Malt. Total RNA (20 μg/lane) was separated by agarose gel electrophoresis, transferred to nylon filters, and probed with the ^{32}P-labeled cDNA clone pHVA1 as described by Hong *et al.* (11). Lane 0, RNA from tissue grown at 25°C for 6 d in the dark (nonacclimated); lane 2d, 4d, or 6d, RNA from tissue that was first grown at 25°C for 6 d and then transferred to 2°C for periods of 2, 4, and 6 d. Autoradiographs were obtained after 16 h at −70°C. B, The northern blot generated in a was stripped of radiolabeled pHVA1 and rehybridized under the same conditions with a soybean radiolabeled 18S rRNA gene (8). C, Maximum areas for the *HVA1* and 18S rRNA mRNA levels were obtained from densitometry scanning, and the pHVA1 values were normalized for equivalent loading with the 18S rRNA values. The percentage of the maximum *HVA1* value obtained was then plotted for each time point. kb, Kilobase.

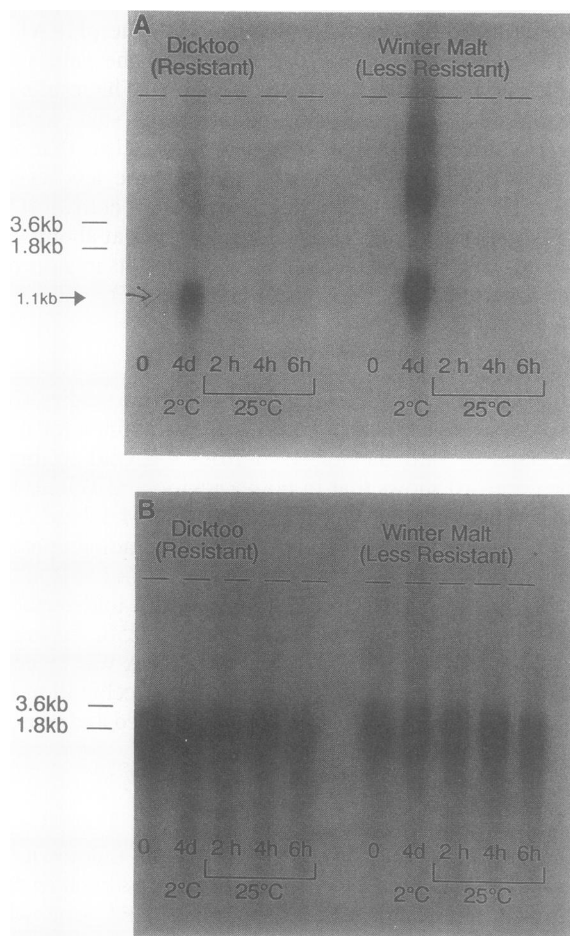

Figure 2. A, Effect of deacclimation on the accumulation of pHVA1 mRNA in Dicktoo and Winter Malt. Total RNA (20 μg/lane) was separated by agarose gel electrophoresis, and northern blot hybridization analysis was performed with the ^{32}P-labeled cDNA clone pHVA1 as described by Hong *et al.* (11). Lane 4d, RNA from barley tissue incubated at 2°C for 4 d in the dark after growth for 6 d at 25°C in the dark; lane 0, RNA from tissue that was grown for 6 d at 25°C; lanes 2h, 4h, or 6h, RNA isolated from tissues that were removed from 2°C after 6 d and incubated at 25°C for 2, 4, or 6 h. B, The northern blot generated in A was stripped of radiolabeled pHVA1 and rehybridized under the same conditions with soybean radiolabeled 18S rRNA gene (5). kb, Kilobase.

not continue to accumulate but are reduced. Preliminary studies from our laboratory with the ABA synthesis inhibitor fluridone indicate that the cold induction of the *HVA1* gene is blocked by fluridone, suggesting that induction due to cold treatment is dependent on endogenous ABA levels.

We also report that this LEA gene, *HVA1*, is expressed in two cultivars that vary in their degree of freeze resistance. The level of expression appears to be greater in the less freeze-resistant (Winter Malt) than in the more freeze-resistant (Dicktoo) cultivar. Although suggestive, there is no evidence to indicate that the differential regulation of this gene in the two cultivars during the cold acclimation period would actually determine their degree of freeze resistance. In fact, the difference in expression of the *HVA1* gene may just reflect differences in the levels of endogenous ABA or ABA precursors already present in the cultivars at the beginning of the study. Work is in progress to compare the endogenous levels of ABA in these cultivars with and without cold treatment.

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