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The Wheat Abscisic Acid-Responsive Protein Kinase mRNA, PKABA1, Is Up-Regulated by Dehydration, Cold Temperature, and Osmotic Stress[1]

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The effects of dehydration, cold-temperature treatment, and osmotic and salt stress on the expression of an abscisic acid-responsive protein kinase mRNA (PKABA1) were determined in wheat (*Triticum aestivum* L.) seedlings. The PKABA1 transcript was detectable at basal levels in tissues of nonstressed plants and accumulated to higher levels in shoot, scutellar, and root tissues of stressed plants. PKABA1 transcript accumulated rapidly within 2 h following dehydration and within 24 h following other treatments (cold, osmotic stress, and high salt). The accumulation of PKABA1 mRNA could not be separated temporally from that of a wheat group 3 late embryogenesis abundant mRNA during dehydration and cold treatment. High PKABA1 mRNA levels were observed in field-grown plants growing under cold winter conditions but not under warmer summer conditions. A recent GenBank data base search indicated that other plant protein kinases with similar acidic amino acid stretches as in PKABA1 have been identified, and some of these kinases are responsive to environmental signals. These results suggest that PKABA1 may be part of general environmental stress responses in wheat.

Protein kinases are critical components in signal transduction pathways leading to cellular adjustments in response to changes in extracellular conditions. Protein kinases and their phosphorylated intermediates modulate many plant physiological processes, including the perception of external signals such as phytohormones, environmental changes, and light (reviewed by Bowler and Chua, 1994; Gilroy and Trewavas, 1994). For example, protein kinases have roles in phytohormone responses as indicated by ethylene-mediated responses. Arabidopsis mutants that fail to respond to ethylene or that constitutively display the ethylene response have been found to have protein kinase gene mutations (Chang et al., 1993; Kieber et al., 1993), and ethylene responses in wild-type Arabidopsis are blocked by protein kinase inhibitors (Raz and Fluhr, 1993). Protein kinases also have roles in changing environmental conditions. For example, osmotic stress results in the phosphorylation of unique sites on Suc phosphate synthase in spinach leaves (Huber et al., 1992), and low temperature and salt stress result in the accumulation of phosphorylated proteins (Garbarino et al., 1991; Monroy et al., 1993).

The plant hormone ABA is a signal common to the initiation of many environmental stress responses and there is increasing evidence that protein kinases have a role in ABA-mediated responses. ABA affects protein phosphorylation of *Lemna* chromatin proteins (Chapman et al., 1975) and carrot embryonic proteins (Koontz and Choi, 1993). Additionally, an Arabidopsis mutant with a defective protein phosphatase 2C is insensitive to ABA and has reduced ABA-mediated responses (Leung et al., 1994; Meyer et al., 1994).

In wheat (*Triticum aestivum* L.) we have identified a novel protein kinase mRNA, PKABA1, that accumulates in embryos treated with ABA and in severely dehydrated shoots (Anderberg and Walker-Simmons, 1992). The deduced amino acid sequence of PKABA1 has extensive homology to all 12 conserved catalytic subdomains characteristic of Ser/Thr protein kinases but has a unique stretch of Asp residues near the carboxyl terminus. The up-regulation of *PKABA1* at the transcriptional level is unusual because protein kinases are usually regulated at the post-translational level. The accumulation of PKABA1 mRNA may be part of the initial stress response to environmental signals mediated by ABA that ultimately result in the accumulation of osmoprotectants.

ABA increases in plants exposed to several types of environmental stress and acts as a signal, although certainly not the only signal, to promote tissue acclimation to stress (reviewed by Chandler and Robertson, 1994). Elevated ABA levels have been correlated with increased tolerance to dehydration (Bartels et al., 1990) and sometimes to cold (Machackova et al., 1989; Lee et al., 1993), osmotic, and salt (Singh et al., 1987) stress. ABA-deficient mutants provide genetic evidence for the role of ABA in the acclimation process to stress (reviewed by Reid, 1990). These mutants have a reduced capacity to survive dehydration but can recover from dehydration stress with exogenous

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Abbreviations: LEA, late embryogenesis abundant; RFW, relative fresh weight.

ABA treatments. Similarly, when ABA levels are decreased in Arabidopsis by mutations in the biosynthetic pathway or in *Brassica napus* by the ABA biosynthetic inhibitor, fluridone, induction of freezing tolerance is blocked and plants have reduced capacity to survive freezing temperatures (Heino et al., 1990; Johnson-Flanagan et al., 1991). Pretreatment with ABA prior to stress appears to enhance stress tolerance in desiccated *Craterostigma plantagineum* calli (Bartels et al., 1990), salt-stressed tobacco cells (Singh et al., 1987), cold-treated suspension cell cultures (reviewed by Guy, 1990), and wheat calli (Dallaire et al., 1994).

Seeds and young seedlings of winter wheat are very desiccation tolerant and provide a useful system for studying genes involved in stress tolerance. We have cloned several ABA-responsive mRNAs (reviewed by Curry and Walker-Simmons, 1993) in addition to PKABA1 protein kinase that accumulate in desiccating seeds and in dehydrated vegetative tissue of young seedlings. Some of these wheat clones represent mRNAs that encode LEA proteins that include the Em (group 1 LEA) protein, dehydrins (rab, group 2 LEA), a group 3 LEA protein (Curry et al., 1991), and a group 3 LEA (II) protein. Many ABA-responsive proteins including the LEA proteins are very hydrophilic and have proposed osmoprotectant roles in final cellular adjustments to the stress (reviewed by Chandler and Robertson, 1994). In wheat, desiccation tolerance of young seedlings has been demonstrated to correlate with increased ABA levels and group 3 LEA protein accumulation (Ried and Walker-Simmons, 1993).

In this study we have characterized the physiological responses in wheat subjected to adverse environmental conditions with respect to PKABA1. We compared the effects of slow dehydration and cold temperature on PKABA1 mRNA levels to changes in ABA. We also compared the rate of PKABA1 mRNA accumulation with that of wheat group 3 LEA, which encodes a proposed osmoprotectant. Responses in shoot, root, and scutellar tissues were assessed to determine whether the protein kinase is expressed in all of these tissues and because the scutellar region is more resilient than shoots or roots to extremely cold temperatures. Finally, we determined whether PKABA1 mRNA accumulates in field-grown wheat plants exposed to cold temperatures in the winter.

MATERIALS AND METHODS

Plant Materials and Application of Environmental Stresses

Seeds of winter wheat (*Triticum aestivum* L. cv Brevor) were obtained from field plots at Spillman Farm near Pullman, WA. Seeds were germinated in covered 100-mm Petri dishes containing 6 mL of water in a growth chamber in the dark at 22°C. At 2 d covers were removed from the Petri dishes and the seedlings were grown with a 16-h photoperiod (100 μE m^{-2} s^{-1}) at 100% RH.

For dehydration 30 shoots for each time were excised (4 cm from each tip) from 7-d-old seedlings, weighed (fresh weight), and placed in a beaker inside a chamber equilibrated with 25% (v/v) glycerol to 85% RH. After the appropriate dehydration period, shoots were weighed, quickly frozen in liquid nitrogen, and stored at −70°C. RFW was determined collectively for 30 shoots for each time using the following equation: RFW = (FW T_o − FW T_d)/FW T_o, where FW is fresh weight, T_o is time zero, and T_d is the time of dehydration.

For cold treatments intact 7-d-old seedlings were placed in a chamber at 2°C at 100% RH with a 16-h photoperiod (100 μE m^{-2} s^{-1}). For osmotic treatments intact 7-d-old seedlings were transferred to Petri dishes containing 250 mM NaCl or 7.8% mannitol with equivalent osmotic potentials (−1.06 MPa) for 24 h. For ABA treatments intact seedlings were transferred to 25 μM (+)-ABA in 5 mM Mes buffer (pH 5.7) for 24 h. (*S*)-(+)-ABA (supplied by Dr. S.R Abrams, National Research Council of Canada, Saskatoon, Saskatchewan, Canada) was solubilized in DMSO to a final concentration of 0.1 M and then diluted to 25 μM with 5 mM Mes, pH 5.7. In preliminary studies, diluted DMSO had no effect on wheat seedlings. For dehydration seedlings were dried for 24 h at 22°C and 35% RH. At the end of the treatments seedlings were sectioned quickly into shoot, scutellar, and root tissues. Sections were frozen immediately in liquid nitrogen and stored at −70°C.

ABA Analysis

Frozen samples were lyophilized and finely cut with scissors. ABA was extracted with 4 mL of extracting methanol per 15 mg of tissue in a 15-mL polypropylene tube (Ried and Walker-Simmons, 1993). Samples were stirred overnight in the dark at 4°C. ABA was quantified by immunoassay using a monoclonal antibody to (*S*)-(+)-ABA (Walker-Simmons and Abrams, 1991).

RNA Extraction and Analysis

Total RNA was extracted from frozen tissue using a phenol/cresol and chloroform procedure (Jepson et al., 1991). The tissue was pulverized to a fine powder in a mortar with liquid nitrogen and transferred to a 2-mL microfuge tube. The RNA homogenization buffer contained heparin (4 units/mL) and aurintricarboxylic acid (1 mM) to inhibit RNase activity. Fifteen micrograms of total RNA were denatured and fractionated by electrophoresis through 1.2% (w/v) agarose gels containing 1× Mops buffer and 2.2% (v/v) formaldehyde with a 1× Mops running buffer 0.22% formaldehyde (Ausubel et al., 1989). Total RNA was transferred to nylon membranes (Hybond N, Amersham)[2] overnight with 20× SSC at 22°C. After the transfer, membranes were UV cross-linked (Stratagene) and baked for 2 h at 80°C. Membranes were prehybridized overnight at 65°C in 7% (w/v) SDS, 250 mM NaH_2PO_4 (pH 7.4), 1 mM EDTA, pH 8. The next day, membranes were hybridized with ^{32}P-labeled probes synthesized by a random hexanucleotide-priming method. A PCR-derived product spanning 1154 bp of the PKABA1 cDNA was used as the kinase probe. Gel-isolated inserts from *Eco*RI restric-

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

tion digests of a group 3 LEA cDNA clone, pMA2005 (Curry et al., 1991), and a barley actin cDNA clone, Act8 (from R.W. Skadsen, U.S. Department of Agriculture-Agricultural Research Service, Madison, WI), were used for the other probes. The actin probe was used to assess RNA quality and quantity of RNA loaded.

RESULTS

The PKABA1 Transcript Is Up-Regulated by Dehydration

To determine the effects of dehydration on the accumulation of PKABA1 and LEA mRNA, shoots were excised from 7-d-old wheat seedlings and slowly dried in a controlled 85% RH chamber. Within 1 h of dehydration, a 3 to 5% decrease in fresh weight was accompanied by a 500-fold increase in ABA concentration (Fig. 1). ABA levels remained high through 24 h and then declined. Within 1 to 2 h of dehydration, the PKABA1 transcript was up-regulated from a basal level. The PKABA1 levels peaked at 12 to 24 h and then declined. By 2 h of dehydration high levels of group 3 LEA mRNA accumulated and remained elevated.

The PKABA1 Transcript Is Up-Regulated by Low Temperature

In preliminary cold-treatment experiments (data not shown) both PKABA1 and group 3 LEA mRNA levels increased after 1 or 2 weeks of low-temperature treatment. No changes in RFW or ABA levels were detected in the 1- or 2-week cold-treated plants compared to control plants. Based on these preliminary results, PKABA1 and group 3 LEA mRNA levels were compared with changes in ABA levels during the first 8 d of low-temperature treatment. Transient increases in ABA and transcript levels were observed in shoot, scutellar, and root tissues during the 1st d of cold treatment (Figs. 2, 3, and 4). ABA increases were first detected 10 h after the initiation of cold treatment and peaked during the first 24 h of cold treatment. Eight-fold, 3-fold, and 4-fold increases in ABA levels were observed for shoot, scutellar, and root tissues, respectively. ABA levels in the three tissues returned to control levels following 3 d of treatment. ABA levels in control scutellar tissue were consistently higher than in control shoot or root tissues.

The transient ABA increases in shoot, scutellum, and roots were accompanied by increases in both protein kinase and group 3 LEA mRNA. The PKABA1 transcript accumulated within 1 d of cold treatment, peaked slightly by 2 d of cold treatment, and then remained at low levels during extended cold treatment. The group 3 LEA transcript also peaked by 1 to 2 d of cold treatment but declined dramatically by d 3 in all three tissues. In a subsequent experiment responses in scutellar tissue at 1, 2, 4, 10, and 24 h of cold treatment were measured, and increases in ABA were detected at 10 h, but no detectable increases in PKABA1 and group 3 LEA mRNA were detected until 24 h of cold treatment (data not shown).

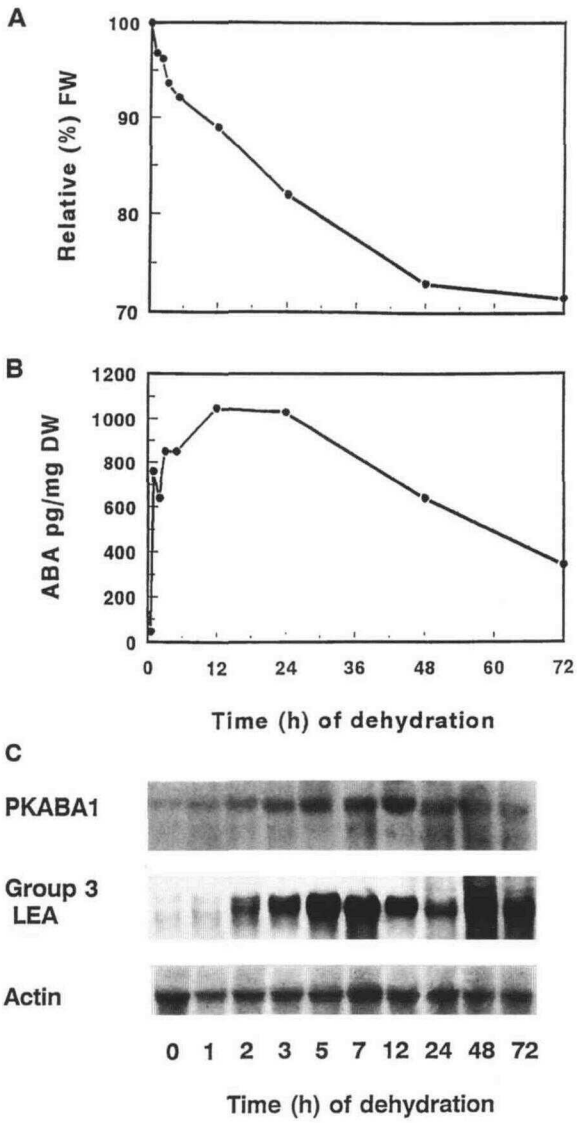

Figure 1. RFW changes, ABA levels, and transcript levels of PKABA1, the wheat group 3 LEA (pMA2005), and actin in dehydrated shoots. Thirty shoots were excised (approximately 4 cm from the tip) from 7-d-old wheat seedlings, dehydrated for the time indicated at 22°C in a controlled 85% RH environment, quickly frozen in liquid nitrogen, and stored at −20°C. A, RFW was determined collectively for 30 shoots. B, Shoot ABA was quantified by an indirect ELISA to (*S*)-(+)-ABA. C, Total RNA (15 μg) from shoot tissue, fractionated by electrophoresis, transferred to nylon membranes, and hybridized to ^{32}P-labeled probes synthesized from randomly primed inserts from the indicated cDNA clones. The barley actin cDNA clone (Act 8) was used as a control probe. Transcript sizes were 1.3, 1.0, and 1.4 kb for PKABA1, group 3 LEA, and actin, respectively. DW, Dry weight; FW, fresh weight.

The PKABA1 Transcript Is Up-Regulated by Salt and Osmoticum

The roots of intact seedlings (7 d) were bathed in mannitol or NaCl, and results were compared with ABA treatment or seedling dehydration. As shown in Figure 5 higher levels of PKABA1 mRNA accumulated in response to NaCl

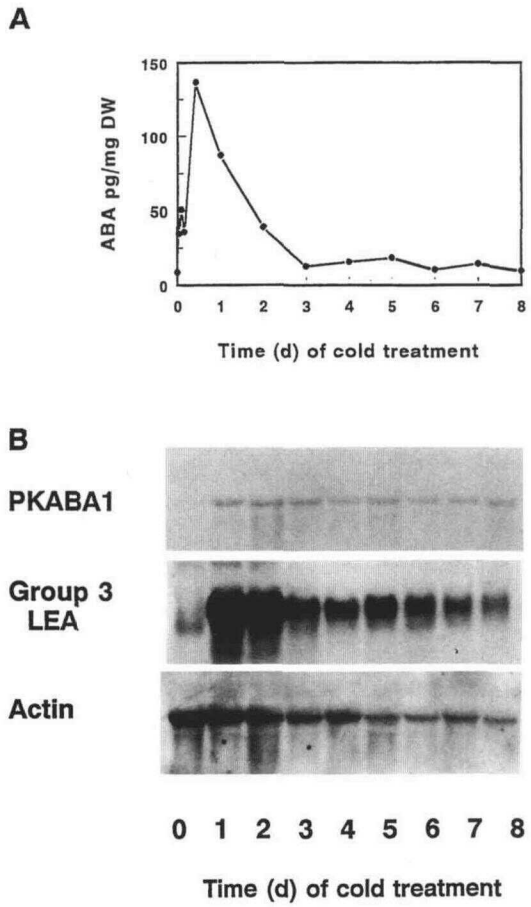

Figure 2. Relative changes in ABA (A) and transcript levels (B) of PKABA1, the wheat group 3 LEA (pMA2005), and actin in cold-treated shoot tissue. Intact 7-d-old wheat seedlings growing at 22°C in a controlled 100% RH environment were transferred to a 2°C, 100% RH environment for the number of days indicated. Shoots were sectioned from wheat seedlings, quickly frozen in liquid nitrogen, and stored at −20°C. Experimental methods were the same as described in Figure 1. Because seedlings grown at 22°C are more advanced developmentally than those grown at 2°C, those harvested prior to low-temperature treatments were considered controls. The barley actin cDNA clone (Act 8) was used as a control probe. DW, Dry weight.

than in response to mannitol. The group 3 LEA transcript also accumulated similarly in these tissues (data not shown). RFWs and ABA levels were not determined for these experiments.

The PKABA1 Transcript Is Present in Field-Grown Wheat Seedlings

To determine whether *PKABA1* is expressed in winter wheat plants exposed to cold temperatures under field conditions, PKABA1 transcript levels were compared in shoot tissue harvested from plants growing at cold temperatures in February and at warmer temperatures in June. As shown in Figure 6 both PKABA1 and group 3 LEA transcripts were highly abundant in wheat seedlings growing in the cold in February but not in plants growing under warmer conditions in June. PKABA1 and group 3 LEA transcripts in the February plant shoots declined to nondetectable levels when the excised shoots were incubated at a warmer temperature (22°C) for 24 h. Capability of the field-grown shoots to accumulate PKABA1 mRNA in response to dehydration was also measured. Dehydrated shoots from plants harvested in February and June had elevated transcript levels compared to controls (Fig. 6). These experiments also demonstrate that the transcript can accumulate in plants of two distinctly different ages. Wheat plants harvested in winter were at the three-leaf stage (approximately 5 cm tall, 2 on Feekes scale), whereas the plants harvested in June were at the late boot stage and were beginning to head (approximately 60 cm tall, 10.1 on Feekes scale).

DISCUSSION

Our results from winter wheat seedlings show that protein kinase PKABA1 mRNA is up-regulated within 1 to 2 h

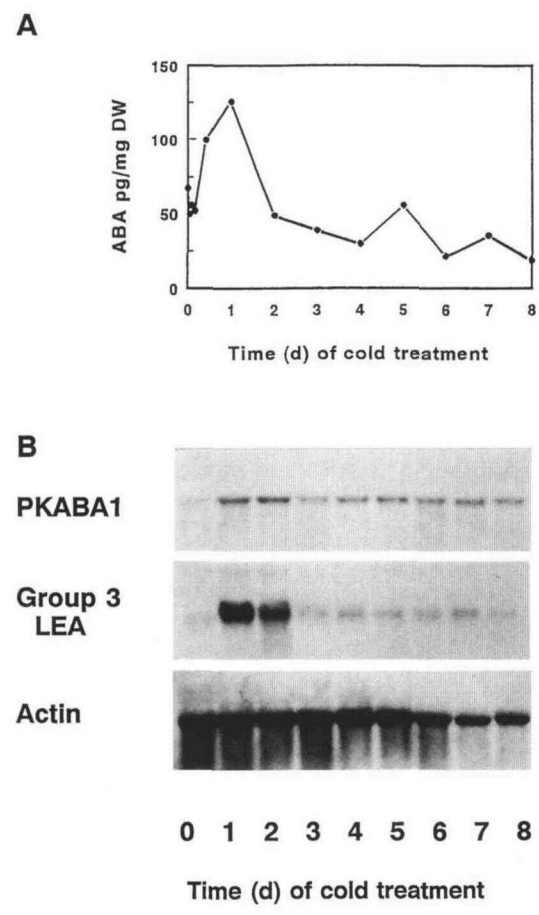

Figure 3. Relative changes in ABA (A) and transcript levels (B) of PKABA1, the wheat group 3 LEA (pMA2005), and actin in cold-treated scutellar tissue. Intact 7-d-old wheat seedlings growing at 22°C in a controlled 100% RH environment were transferred to a 2°C, 100% RH environment for the number of days indicated. Scutellar regions were sectioned from wheat seedlings, quickly frozen in liquid nitrogen, and stored at −20°C. Experimental methods were the same as described in Figures 1 and 2. The barley actin cDNA clone (Act 8) was used as a control probe. DW, Dry weight.

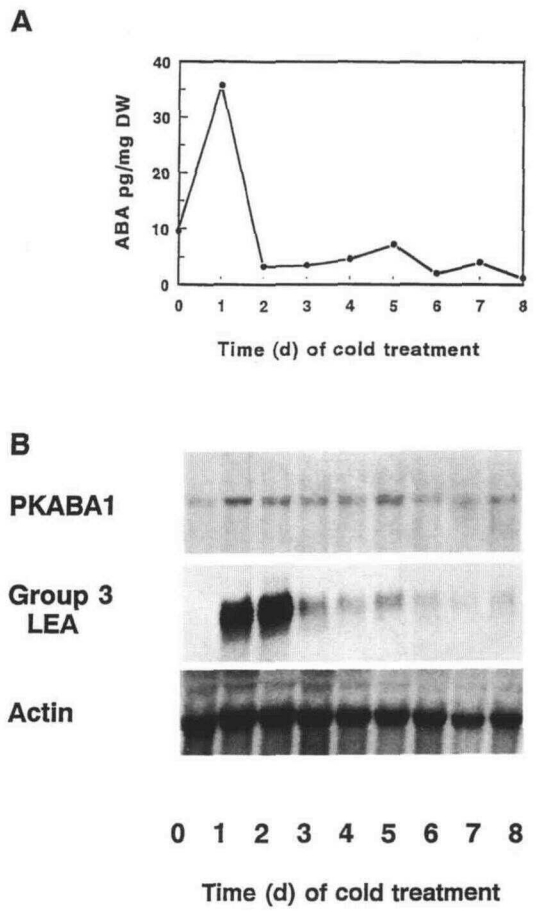

Figure 4. Relative changes in ABA (A) and transcript levels (B) of PKABA1, the wheat group 3 LEA (pMA2005), and actin in cold-treated root tissue. Intact 7-d-old wheat seedlings growing at 22°C in a controlled 100% RH environment were transferred to a 2°C, 100% RH environment for the number of days indicated. Roots were sectioned from wheat seedlings, quickly frozen in liquid nitrogen, and stored at −20°C. Experimental methods were the same as described in Figures 1 and 2. The barley actin cDNA clone (Act 8) was used as a control probe. DW, Dry weight.

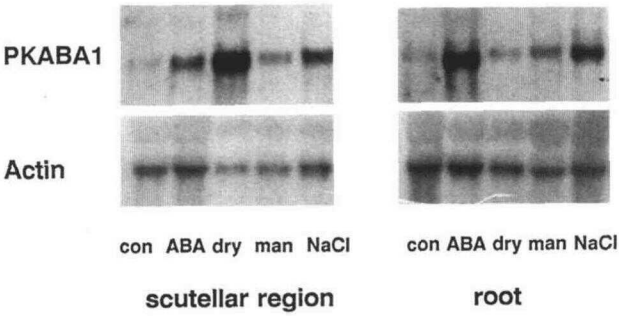

Figure 5. Expression of PKABA1 and actin mRNA in scutellar and root tissues from wheat seedlings subjected to various stresses. Intact 7-d-old wheat seedlings growing at 22°C in a controlled 100% RH environment were dehydrated (dry) or transferred to solutions containing 25 μM ABA (ABA), 7.8% mannitol (man), or 250 mM NaCl (NaCl) for 24 h. Scutellar regions and roots were excised, quickly frozen in liquid nitrogen, and stored at −20°C. Experimental methods were the same as described in Figure 1. The barley actin cDNA clone (Act 8) was used as a control probe.

of dehydration and also in response to cold-temperature treatment and osmotic and salt stress. In dehydrated and cold-treated tissue, increases in PKABA1 levels were detected at the same time or soon after increases in ABA levels. These results are consistent with our previous findings that PKABA1 mRNA accumulates in response to exogenously applied ABA and accumulates in severely dehydrated shoot tissue when ABA levels are high (Anderberg and Walker-Simmons, 1992). Our results here show that in response to environmental stress PKABA1 mRNA is up-regulated in all seedling tissues tested, including the shoot, root, and scutellar tissue. PKABA1 mRNA is not only up-regulated in young seedlings stressed under controlled laboratory conditions but is also expressed at high levels in field-grown plants subjected to cold winter temperatures. Results suggest that PKABA1 contributes to cold acclimation or to freezing tolerance required for survival of winter wheat. All of these results suggest that PKABA1 has a general role in environmental stress-response mechanisms.

Most protein kinases are regulated and activated by posttranslational modifications such as phosphorylation. Up-regulation of the PKABA1 transcript in response to environmental stress provides additional regulatory control points and suggests that the kinase has a specific role in ABA or stress signal transduction. Two Ca^{2+}-dependent protein kinases mRNAs that are induced by drought and

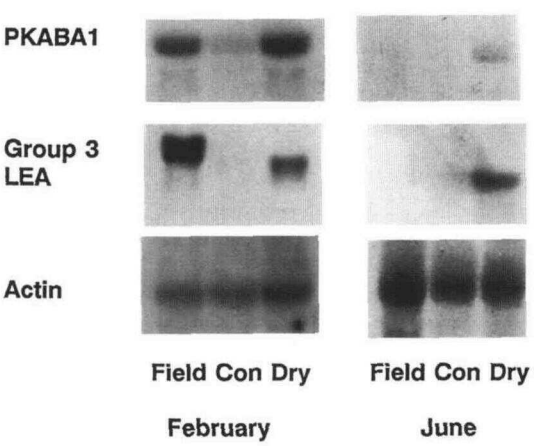

Figure 6. Expression of PKABA1, the wheat group 3 LEA (pMA2005), and actin mRNA in field-grown wheat shoots harvested in winter or late spring. The average minimum/maximum temperature on the day of collection: February, −6/2°C; June, 9/18°C. Thirty shoots (approximately 4 cm from the tip) were excised from field-grown wheat plants (Field) and quickly frozen in liquid nitrogen or placed in plastic bags and stored on ice until dehydrated in the laboratory under controlled environmental conditions. The time interval between the field and controlled dehydration was less than 15 min. Cut ends of shoots were placed in a beaker filled with water inside a 100% RH environment for the control (Con) or were slowly dried (Dry) in an 85% RH environment for 24 h at 22°C. Experimental methods were the same as described in Figure 1. The barley actin cDNA clone (Act 8) was used as a control probe.

Table I. *Comparison of deduced amino acid sequences of protein kinase genes with highest sequence similarity to PKABA1*

Pairwise alignments using the BESTFIT program (Wisconsin Computer Group, University of Wisconsin) were made between overall lengths of predicted polypeptides. The protein kinase sequences are from soybean (SPK1, SPK2, SPK3, CDPK_SOYBN), *B. napus* (BSK1, BSK2), *Arabidopsis thaliana* (ASK1, ASK2, ATPKa, ATPKb, AKIN10, raf CTR1, RLK), ice plant (ICE), tobacco (TOBK1), barley (BKIN12), rye (RKIN), yeast (SNF1_YEAST), pea (CDC2 P34), and rat (AMPK). GenBank accession numbers are provided for unavailable references.

Protein Kinase Group	Gene	Amino Acid Identity	Reference
PKABA1 subfamily protein kinases	ICE	75	Z26846
	SPK1	72	L01453
	ATPKb	71	L05562
	ATPKa	70	L05561
	SPK2	70	L19360
	SPK3	69	L19361
	ASK2	67	Park et al. (1993)
	BSK2	67	L12394
	ASK1	66	Park et al. (1993)
	BSK1	66	L12393
SNF1 subfamily protein kinases	TOBK1	41	Muranaka et al. (1994)
	RKIN	41	Alderson et al. (1991)
	AKIN10	40	LeGuen et al. (1992)
	SNF1—YEAST	38	Celenza and Carlson (1986)
	BKIN12	37	Halford et al. (1992)
Other plant protein kinases	CDPK—SOYBN	32	Harper et al. (1991)
	RLK	32	Walker (1993)
	CDC2 P34	31	Feiler and Jacobs (1990)
	raf CTR1	32	Kieber et al. (1993)
Mammalian protein kinase	AMPK	37	Carling et al. (1994)

high-salt stress but not ABA have also been recently reported (Urao et al., 1994), and in wheat a protein kinase mRNA (WPK4) up-regulated by cytokinin, nutrient stress, and light has been identified (Sano and Youssefian, 1994).

As shown in Figures 2 to 4 the level of the accumulated PKABA1 mRNA is still high at 8 d under cold conditions, even though the level of ABA transiently accumulated by d 1 and then decreased to basal levels by d 3. The prolonged expression of the PKABA1 mRNA levels suggests that other signals in addition to ABA, which are osmotically or cold responsive, can regulate PKABA1 expression.

Although both PKABA1 and group 3 LEA exhibit similar temporal responses to environmental stress, there are differences in the basal level of the transcripts in control, nonstressed tissue. PKABA1 mRNA, unlike group 3 LEA mRNA, was present at low levels in most of the tissues tested. The basal level of PKABA1 mRNA may indicate that minimal PKABA1 protein kinase levels are present under nonstressed conditions. If this is the case, a low level of kinase enzyme may be active during moderate stress or be available for rapid activation during severe stress.

One potential substrate for PKABA1 protein kinase is the LEA proteins, which accumulate in response to environmental stress and are known to be phosphorylated. In response to dehydration and cold stress, the accumulation of the PKABA1 mRNA could not be separated temporally from the accumulation of a group 3 LEA transcript. Up-regulation of the PKABA1 mRNA prior to that for group 3 LEA mRNA would have been anticipated if the PKABA1 protein kinase were involved in ABA signal transduction and the group 3 LEA protein functions in subsequent cellular adjustments to stress. Three explanations for the similar expression patterns exhibited by the two transcripts may explain these results. First, in stressed tissue it may be more technically difficult to detect initial levels of the relatively rare PKABA1 mRNA compared with the more abundant group 3 LEA mRNA. Second, both transcripts may be sensitive to similar stress response factors, but there may be differential control, possibly kinase mediated, at the translational or posttranslational levels. Third, the PKABA1 transcript may not be involved in the regulation of the expression of this particular LEA gene.

The phosphorylation target or substrate of PKABA1 protein kinase is not yet known, although low levels of autophosphoryating activity have been demonstrated in recombinant PKABA1 protein kinase (S. Verhey and M.K. Walker-Simmons, unpublished data). Further biochemical characterization of the recombinant PKABA1 kinase has been slowed by aggregation of the protein into insoluble inclusion bodies. Antibodies to the recombinant protein have been produced, and characterization of PKABA1 expression in stressed wheat tissue is in progress.

Since PKABA1 was submitted to GenBank in 1992, more plant protein kinases have been added to the data base, providing new information about its classification. A recent search of the GenBank data bases and pairwise BESTFIT analyses identified several plant protein kinase sequences with high sequence identity with the deduced

PKABA1 amino acid sequence (Table I). Plant protein kinases fall into three distinct groups with respect to the PKABA1 sequence. These three groups are the PKABA1 subfamily containing closely related kinase sequences with the characteristic acidic amino acid sequence at the carboxyl terminal end, the SNF1 (Suc nonfermenting) kinase subfamily, and other plant Ser/Thr protein kinases. Some of these kinases with similar sequences to PKABA1 also appear to be involved in responses to changes in environmental or nutrient conditions and to exhibit a degree of transcriptional regulation.

In the PKABA1 subfamily the most closely related protein kinase sequence with 75% sequence identity is a mRNA from the ice plant (*Mesembryanthemum crystallinum*), known for its desiccation-tolerant behavior. This protein kinase contains an Asp-rich stretch of amino acids like PKABA1. Two Arabidopsis mRNAs (ASK1 and ASK2) that have high deduced amino acid sequence identities (66 and 67%) with PKABA1 and multiple Glu residues near their carboxyl termini have been reported to be light inducible (Park et al., 1993). A few other protein kinases from Arabidopsis, rapeseed, and soybean with carboxyl-terminal acidic amino acid stretches have similar acidic carboxyl-terminal residues, but it is not yet known whether they respond to environmental signals. The wheat WPK4 protein kinase that is responsive to cytokinin lacks the acidic amino acid stretch of PKABA1 but does have some sequence identity with PKABA1 (37%) and with SNF1 protein kinases (Sano and Youssefian, 1994).

The similarity of the PKABA1 subfamily of kinases with the SNF1 subfamily (reviewed by Hardie et al., 1994) is especially interesting because yeast SNF1 protein kinases are responsive to nutrient stress. PKABA1 and the closely related kinases with an acidic amino acid stretch show closest sequence identities (37–41%) with the plant SNF1 protein kinase homologs (Table I). In yeast SNF1 kinases are involved in perceiving changes in carbon catabolism; they release Glc-repressible genes from repression (Celenza and Carlson, 1986). The function of the plant SNF1 kinase homologs is not yet known but a role in carbohydrate regulation has been proposed (Alderson et al., 1991; Muranaka et al., 1994).

Other classes of plant protein kinases also show some identity with PKABA1 (Table I). Representatives from these classes include the calcium-dependent protein kinases from soybean (Harper et al., 1991), the receptor-like RLK from maize (Walker 1993), the ethylene-responsive, raf-like CTR1 from Arabidopsis (Kieber et al., 1993), and a CDC2 kinase from pea (Feiler and Jacobs, 1990). The mammalian protein kinase with the highest sequence identity with PKABA1 is the rat AMP-activated protein kinase, which is activated in response to stresses that lower ATP levels (Hardie et al., 1994).

Even though a large number of ABA and stress-responsive genes have now been identified, their physiological role in the initial perception and transduction of stress signals is not well defined. Our results and those of other laboratories suggest that characterization of protein phosphorylation/dephosphorylation responses and investigation of stress-responsive protein kinases may be a useful focus toward identifying the critical regulatory steps in plant responses to environmental stress. Our future experiments will be directed at determining the structural features of the *PKABA1* gene that confer stress responsiveness and identifying the physiological substrate(s) of the PKABA1 protein kinase.

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