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Accumulation of dehydrin transcripts and proteins in response to abiotic stresses in *Deschampsia antarctica*

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Abstract: *Deschampsia antarctica* Desv. is one of two vascular plants from the Maritime Antarctic. It is usually exposed to cold, salt, and desiccating winds. We hypothesize that *D. antarctica* has genes that encode dehydrin proteins and their expression is regulated by low temperature, salt or osmotic stress. To test this hypothesis a fragment of a dehydrin gene from *D. antarctica* was identified and used as a probe to study dehydrin expression under low temperature, salt, and osmotic stress, and exogenous ABA (abscisic acid) treatments. An anti-dehydrin antibody was also used to study dehydrin protein accumulation under the same treatments. Southern analysis of genomic DNA treated with different endonucleases showed more than four bands recognized by the probe, suggesting that *D. antarctica* has several dehydrin genes. Northern analysis showed two putative dehydrin transcripts of 1.0 kb accumulated only under exogenous ABA and 1.6 kb under osmotic and salt treatments, suggesting that *D. antarctica* would have ABA-dependent and -independent pathways for regulation of dehydrin expression. Western analysis showed seven dehydrin proteins (58, 57, 55, 53, 48, 30 and 27 kDa) under the different stress treatments. Cold-accumulated dehydrin proteins were immunolocalized, showing that they are associated with vascular and epidermal tissue, which are preferential ice nucleation zones.

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Introduction

Plants have to deal with various environmental stresses during their life cycle. A common element in response to many environmental stresses is cellular dehydration. Insufficient soil moisture, low relative humidity, low and elevated temperatures are among the conditions that can affect tissue water potential in plants and cause water deficiency symptoms (Shinozaki & Yamaguchi-Shinozaki 1996, Bray 1997). Plants have evolved physiological responses to defend themselves against environmental stress. Among these responses is the expression of dehydrins, which are also known as late-embryogenesis-abundant protein Group II (LEA) (Ingram & Bartels 1996). They have been studied in several plants, yet we still have an incomplete knowledge of their fundamental role in the cell. They are evolutionarily conserved among photosynthetic organisms including angiosperms, gymnosperms, ferns, mosses, liverworts, algae and cyanobacteria, as well as in some non-photosynthetic organisms such as yeast and nematodes (Close & Lammers 1993, Mtwisha *et al*. 1998, Browne *et al*. 2002). Many dehydrins have been identified in plants in response to low temperature, salt, drought and exogenous ABA (abscisic acid) (Close & Lammers 1993, Labhilili *et al*. 1995, Close 1996, Pelah *et al*. 1997, Nylander *et al*. 2001). Dehydrins are characterized by conserved amino acid motifs, including a DEYGNP motif and a lysine rich block (EKKGIMDKIKEKLPG). This block is also called K-segment and is present in one or more copies and string of serine residues, which is also a common feature of many dehydrins (Close & Lammers 1983, Dure *et al*. 1989, Close 1997). Other features, such as glycine-rich repeats, can also be present in the dehydrin sequence (reviewed in Close 1996, 1997). Dehydrins are hydrophilic, thermostable and have a high amount of charged amino acids (Close 1997). It has been hypothesized that dehydrins function by stabilizing large-scale hydrophobic interactions, such as membrane structures or hydrophobic patches of proteins (Tytler *et al*. 1993, Koag *et al*. 2003). Highly conserved polar regions of dehydrins have been suggested to form hydrogen bonds with polar regions of macromolecules, acting essentially as a surfactant, to prevent coagulation under conditions of cellular dehydration or low temperature (Campbell & Close 1997). Immunoelectron microscopy analyses of the dehydrin Wcor410 from wheat have revealed that these proteins accumulate in the vicinity of the plasma membranes of the cells where freeze-induced dehydration is likely to be more detrimental (Danyluk *et al*. 1998). These results support the hypothesis of a stabilizing function for dehydrins. Most dehydrins have been found in the cytoplasm and nucleus (Svensson *et al*. 2002). Most dehydrin studies have been done in crop plants (Barley,

Wheat, Peach) or *Arabidopsis thaliana*. Few studies have been made in wild species from dry or cold regions (Sauter *et al*. 1999, Ndima *et al*. 2001).

Deschampsia antarctica Desv. (Poacea) is one of two vascular plants that have naturally colonized the maritime Antarctic (Edwards & Smith 1988, Casaretto *et al*. 1994). The native Antarctic vegetation must have various mechanisms that allow the maintenance of metabolism at low temperature during the Antarctic summer (growing season) and survival during winter (Bravo *et al*. 2001). Nonetheless, no special features that allow only them to survive in the Antarctic have been identified so far (Smith 2003). *Deschampsia antarctica* does not have unusual contents of total polar lipids or a high degree of unsaturation of fatty acids compared with other Poaceae (Zúñiga *et al*. 1994). High amounts of sucrose and fructans are found in this plant mainly towards the end of summer under field conditions (Zúñiga *et al*. 1996). This plant also has high sucrose phosphate synthase activity (Zúñiga-Feest *et al*. 2003). This is consistent with the findings that *D. antarctica* has relatively high net photosynthetic rates on cool days (Xiong *et al*. 1999). At 0ºC, this plant maintains about 30% of the photosynthetic rate found at the optimum temperature (Edwards & Smith 1988). Since *D. antarctica* is usually exposed to cold, salt, and desiccant winds in the field, we hypothesize that *D. antarctica* has genes that encode dehydrin proteins and that their accumulation is likely to be affected by low temperature, salt or osmotic stress. These conditions are common during the growing season. This paper reports the characterization of a partial sequence of a *D. antarctica* dehydrin gene and the effect of low temperature, salt, osmotic stress and ABA treatments on dehydrin mRNA level and protein accumulation.

Materials and methods

Plant material, growth conditions and stress treatments

Deschampsia antarctica was collected in the Coppermine Peninsula on Robert Island, South Shetland Islands (62°22'S, 59°43'W). A description of the environmental conditions of the habitat has been published elsewhere (Zúñiga *et al*. 1996, Alberdi *et al*. 2002). Plants were reproduced vegetatively in pots with a rich organic soil:peat mixture (3:1) and maintained at 15°C in a growth chamber, with light intensity of 150 μmol m^{-2} s^{-1} and a photoperiod of 21/3 L/D (Long Day). Plants were watered daily and supplemented with Phostrogen®, using 0.12 g l^{-1} once every two weeks. There were four basic treatments: low temperature, salinity, osmotic stress and ABA. Low temperature treatment was performed at 4 ± 2ºC day/night. NaCl (salt stress), polyethylene glycol 15000–20000 (osmotic stress) and ABA, were added separately to the growth medium to reach final concentrations of 500 m*M*, 24% (w/w) and 100 μ*M*, respectively. All treatments were for 24 h, except for low temperature in which long term treatments were included (5 h, 10 h, 2 d, 4 d, 10 d, 14 d, 18 d and 21 d). Two samples of 0.1 g fresh leaf tissue were taken at 24 h for Western analysis. All the assays were performed in duplicate with the same light intensity and photoperiod as described above.

RNA blot analysis and probe

Total RNA was isolated from control and treated plants using a phenol-guanidine hydrochloride procedure. RNA was separated onto a 1.2% agarose gel containing formaldehyde. Gels were stained with ethidium bromide to visualize rRNA bands for verification of equal loading, then photographed and transferred onto a nylon membrane by capillary blotting with 20X SSC (1X SSC, 0.15 *M* NaCl, 0.015 *M* sodium citrate), fixed with UV light (UVP) and by baking at 80°C. Blots were prehybridized overnight at 45°C in 5X SSC, 0.1% (w/v) SDS, 50% (w/v) Formamide, 5X Denhart, 100 μ*g* ml^{-1} salmon sperm DNA. Blots were

a.

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GTCCGTTGGC GGGCATGGCG GCATGGGAGG AGCACACGCT  40
GCTCCCGGCA CCGGTGGGCA GTTCCAGCCC AGCAGGGAGC  80
AGCACAAGAC CGGCGGCATA CTGCATCGCT CCGGCAGCTC 120
CAGCTCCAGC TCCGTAAGTG TCTCTGTCCG CTCGGATATA 160
TATGGTTATG ATCGTGCGCT GCGGATACGT TTAGTTTGCG 200
TGCGTTTTTA CAGTCTTCCG ACGAGGACGA CGGCATGGGC 240
GGGAGGAGGA AGAAAGGCAT GAAAGATAAG ATCAAGGAGA 280
AGATTCCTGG TGGCCACAAA GACAACCAGC AGCACATGGC 320
CACGGGGACG GGCGGCACGG GGGCGGGAAT TGATACCTAC 360
GGGCAGCAAG GGCACGAAGG AATGACCGGC ACCGGCACCG 400
GCACAGGCAC AGGCGAGAAG AAAGGACTCA TGGACAAGAT 440
AAGGAGAAGC TCCC
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b.

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SVGGHGGMGGAHAAPGTGGQFQPSREQHKTGGILHRSGSSSSSS≈SSDEDD

GMGGRRKKGMKDKIKEKIPGGHKDNQQHMATGTGGTGAGIDTYGQQGHEG

MTGTGTGTGTGEKKGLMDKIRRS
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Fig. 1. Nucleotide and deduced amino acid sequence of a genomic fragment of a dehydrin gene obtained by PCR. **a.** Nucleotide sequence of 454 nucleotide, from nucleotide 135 to 213 correspond to intron (underlined). **b.** Amino acid deduced sequence from the genomic fragment. Amino acid repeats typical for proteins belonging to the DHN/LEA/RAB proteins are underlined: dark line = the ϕ-segments, dotted line = the K-segments, bold = S-segment, ≈ corresponds to intron. (NCBI, accession: AY266052).

hybridized with a dehydrin specific probe consisting of a 454 bp PCR–amplified genomic fragment from *D. antarctica*. This probe was obtained by using the following degenerate primers: 5`-GAYGARTAYGGIAAYCC-3´ that corresponds to DEYGNP segment and 5`-GGIARYTTYTCYTTIATYTT-3´ that corresponds to KIKEKLP segment. The DNA probe was radioactively labelled with [α-^{32}P]dCTP by PCR for Southern and random primer (Rad Prime DNA Labelling System; Invitrogen) for Northern analysis. Filters were washed three times with 0.1X SSC, 0.1% (w/v) SDS at 65°C and then exposed to a Kodak X-Omat AR film (Eastman Kodak, Rochester, NY, USA) for one day at -80°C. The sequence of the probe was obtained by automatic sequencing (Kit BigDye® Terminator v1.1; ABI PRISM 310 Genetic Analyzer). This experiment was repeated twice.

DNA blot analysis

Deschampsia antarctica genomic DNA was extracted from fresh young leaves, as described by Dellaporta *et al*. (1983). Genomic DNA (15 μg) was dissolved in water and digested with restriction enzymes (EcoR I, BamH I, Xba I, Xho I, Nde I and Hind III), fractionated by agarose gel electrophoresis (0.7% w/v) and transferred onto a nylon membrane by capillary blotting with 20X SSC (1X SSC, 0.15 *M* NaCl, 0.015 *M* sodium citrate), fixed with UV light and by baking at 80°C. Hybridization conditions were as previously mentioned for RNA hybridization. This experiment was repeated three times.

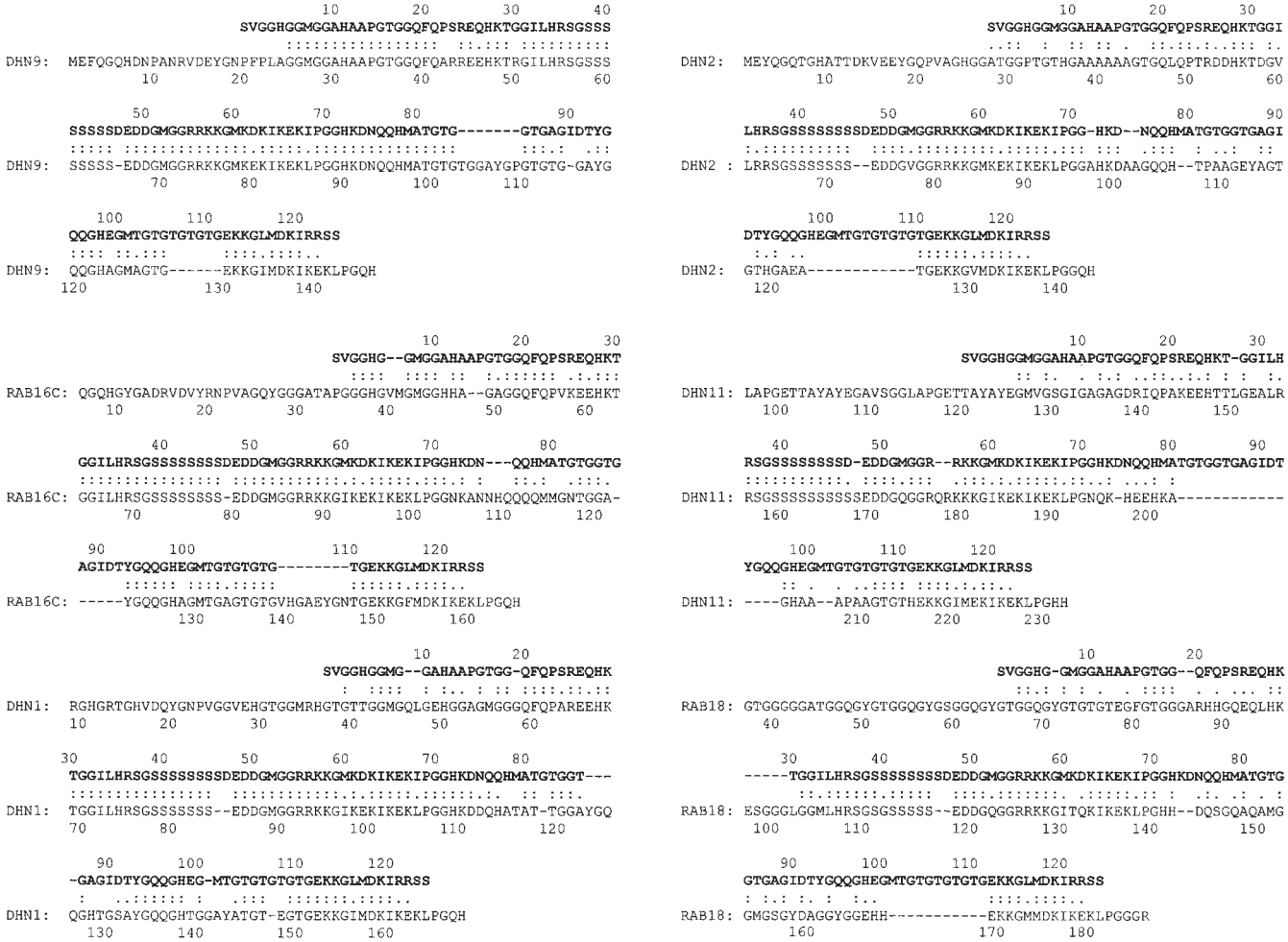

Fig. 2. Amino acid alignment of a genomic fragment from *D. antarctica* (Fig. 1b) with DHN9, RAB16C, DHN1, DHN11, RAB18 and DHN2. ":" and "." corresponds to identical and similar amino acids, respectively. The identities with DHN9 (*Hordeum vulgare* cv. Morex), RAB16C (*Oryza sativa* cv. Indica-IR-36), DHN1(*Zea mays* cv. AC1507) DHN2 (*Hordeum vulgare* cv. Himalaya), DHN11 (*Hordeum vulgare* cv. Dicktoo) and RAB18 (*Arabidopsis thaliana* cv. Columbia) are 72%, 69%, 67%, 56%, 48% and 47% respectively. The identities were obtained from FASTA3 program. The alignment was performed in Clustal W (1.74) with default parameters (Altschul *et al*. 1997). Bold letters indicate portion of dehydrin like-protein from *D. antarctica*.

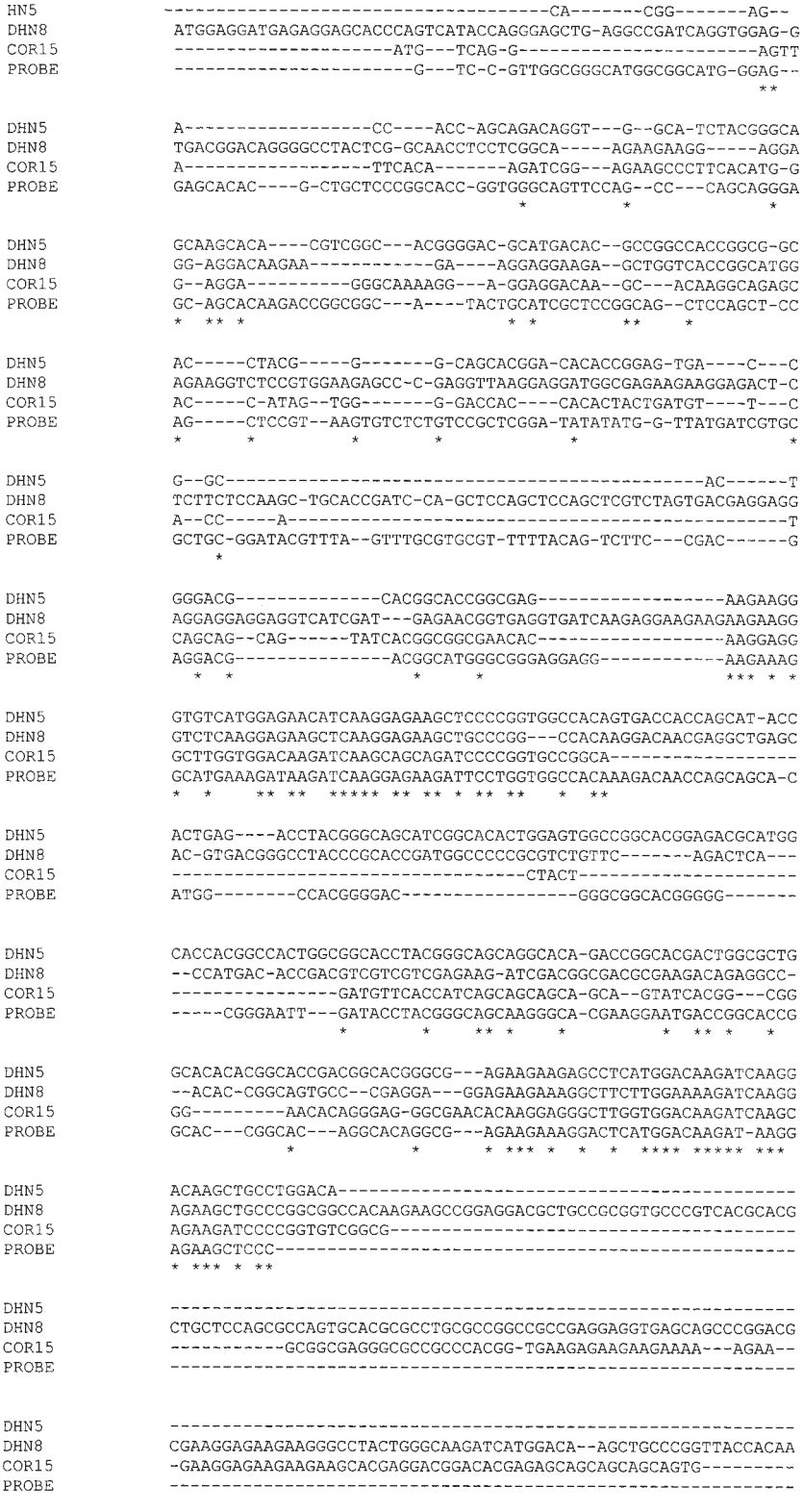

Fig. 3. Nucleotide alignment of the probe without intron (375 bp) with *dhn5* (*Hordeum vulgare* cv. Dicktoo), *dhn8* (*Hordeum vulgare* cv. Dicktoo) and *cor15* (*Citrus paradisi*). * corresponds to identical nucleotide, and – corresponds to gaps. The identity of the probe with *dhn5*, *dhn8* and *co15* was 24.4%. The alignment was performed in Clustal W (1.74) with default parameters (Altschul *et al*. 1997).

Protein extraction and immunoblot analysis

Leaf material from control and treated plants was frozen in liquid nitrogen, and powdered using a mortar and pestle. The frozen powder was transferred directly to the extraction buffer, 60 m*M* Tris-HCl, pH 6.8, 100 m*M* NaCl and boiled for 5 min, then chilled on ice and centrifuged. The supernatant was transferred to a fresh tube and stored at -20ºC. Total protein content was determined by the

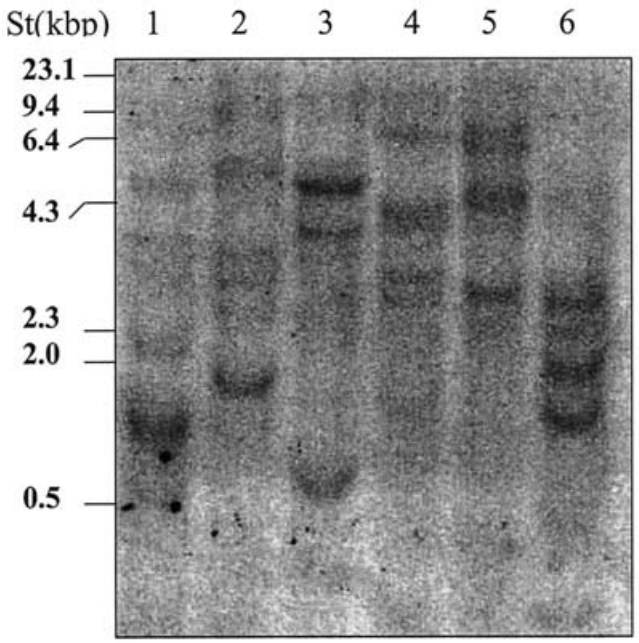

Fig. 4. Southern analysis for dehydrin genes in *D. antarctica*. Genomic DNA (15μg) from *D. antarctica* leaves was digested with (1) *Eco* RI, (2) *Bam* HI, (3) *Xba* I, (4) *Xho* I, (5) *Nde* I and (6) *Hind* III and hybridized with *Dadhn454*. DNA fragments of λ DNA digested with *Hind* III were used as molecular mass markers (indicated at the left).

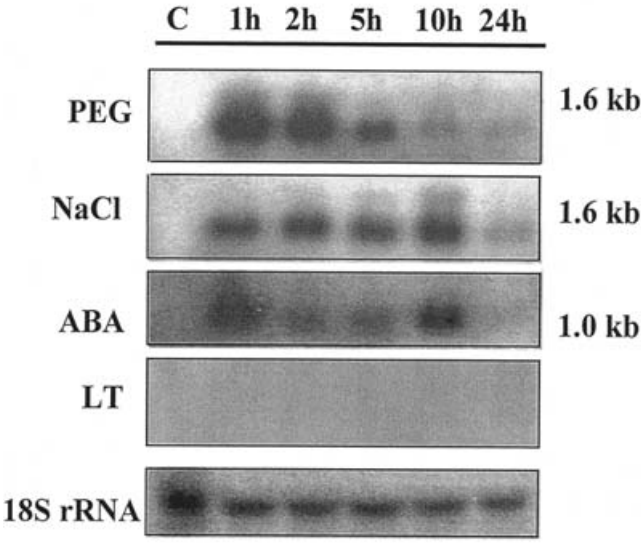

Fig. 5. Expression of dehydrin genes in *D. antarctica* under exogenous ABA, low temperature, salt and osmotic stress. Northern analysis of total RNA (20 μg) was performed using homologue probe. Controls (C) are tissues collected before treatments. The samples were taken at 1, 2, 5, 10 and 24 hours after each treatment. Treatments are indicated at the left and the size of the transcripts at the right. A loading control showing the hybridization of 18S rRNA with a specific probe is also included (18S rRNA).

Bradford assay (Bradford 1976). Ten micrograms of protein from each sample were separated by SDS-PAGE in 10% gel and stained with colloidal Coomassie brilliant blue G-250 (Neuhoff *et al*. 1988). The separated polypeptides were transferred on to a nitrocellulose membrane for 30 min at 450 m*A*. After transfer, the membrane was blocked with 10% (w/v) dry non-fat milk in Tween Tris-buffered saline (TTBS) (0.1% (w/v) Tween-20 in TBS; 20 m*M* Tris-HCl, pH 7.5; 500 m*M* NaCl) for 1 h. After blocking, the membrane was incubated overnight at 4ºC with the first antibody, a polyclonal antiserum raised against the dehydrin consensus peptide, also called K segment (EKKGIMDKIKEKLPG) at a dilution of 1:1000 in TBS (Close & Lammers 1993). After three consecutive washes of 20 min each in TTBS, the membrane was incubated for 1 h at 4ºC with the secondary antibody, anti-rabbit IgG raised in goat and conjugated to alkaline phosphatase at a dilution of 1:1000 TBS. After three washes in TTBS, the membrane was developed by incubating it for 5 min in 0.4 m*M* *nitroblue* tetrazolium chloride (NBT) and 0.4 m*M* 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in developing buffer (100 m*M* Tris, pH 9.0; 100 m*M* NaCl; 5 m*M* $MgCl_2$). All western blots were repeated twice with plant extracts from independent experiments.

Immunolocalization in fresh sections

Fresh cross sections of leaves (10 μm thick) from control and cold-treated plants (21 days) were prepared using a hand microtome (Carl Zeiss). Tissue sections were washed briefly in distilled water prior to incubation with the anti-dehydrin antiserum at a dilution of 1:2000 for 4 h or with the antidehydrin serum blocked with the K synthetic peptide. After three washes of 20 min each with TBS, sections were incubated for 1 h in the secondary antibody, anti-rabbit IgG-AP. After another cycle of washes in TBS, sections were incubated in developing buffer with NBT and BCIP added to detect alkaline phosphatase activity. Tissue sections were mounted on a glass slide and photographed under a light microscope (Nikon).

Results

Identification of an homologous dehydrin gene fragment

A dehydrin fragment with a size of 454 bp was generated by PCR from genomic DNA of *D. antarctica* (Fig. 1). This fragment contained a 79 bp intron between nucleotides 134 and 213 and two portions of exon each 134 and 241 bp, respectively. The sequence without the intron was similar to dehydrin genes from various plant species and possessed similar characteristics shared by several dehydrins. Alignment of the amino acid deduced sequence from both

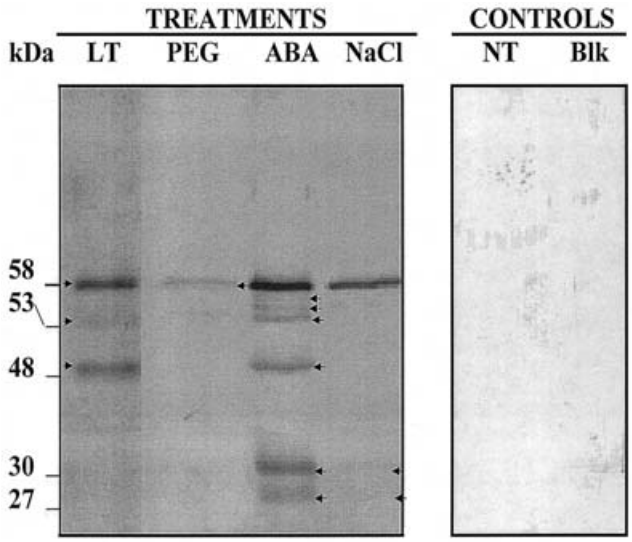

Fig. 6. Dehydrin proteins in *D. antarctica* under different stress treatments. Total protein was extracted 24 hours after salt stress (500 m*M* NaCl), osmotic stress (24% w/w PEG), exogenous ABA (100μ*M*) and LT: low temperature (4ºC). Proteins were separated by SDS-PAGE and immunoblots were performed using antidehydrin antibody.
NT = untreated control. Blk (blocked) = immune serum was pre-incubated with the K-segment synthetic peptide. The arrow heads indicate proteins recognized by antiDHN-antiserum. Band molecular mass is indicated at the left.

exons with amino acid sequences present in the Swall protein database showed that it had high identities to ABA and dehydration induced dehydrins, DHN9 (77%), RAB 16C (69%), DHN1 (67%) among others (Fig. 2). Alignment analysis also revealed that the mayor differences are in the C-terminal portion between the amino acids 70–110. Alignment analysis of the nucleotide sequence of the probe (454 bp) with DHN9, RAB 16C and DHN1 showed similarities from 60 to 74% (data not shown) while cold-induced dehydrin genes showed lower similarities (about 24%) (Fig. 3).

Southern and Northern analysis

Southern analysis revealed that the homologue probe recognized different size fragments between 0.5 and 6.5 kbp (Fig. 4). Therefore, it is likely that there are several genes in *D. antarctica* genomic DNA that have homology to probe. This result was expected because the sequence of the probe had the dehydrin conserved domains (Fig. 1) that could hybridize with several members of the dehydrin gene family in *D. antarctica*. RNA blot analysis performed with the same probe, but random primer labelled, showed two transcripts for 1.6 kb and 1.0 kb putative dehydrins, (Fig. 5). There was no detection of transcripts in plants exposed for one day to low temperature (Fig. 5). NaCl and PEG treatment induced a 1.6 kb transcript for a putative

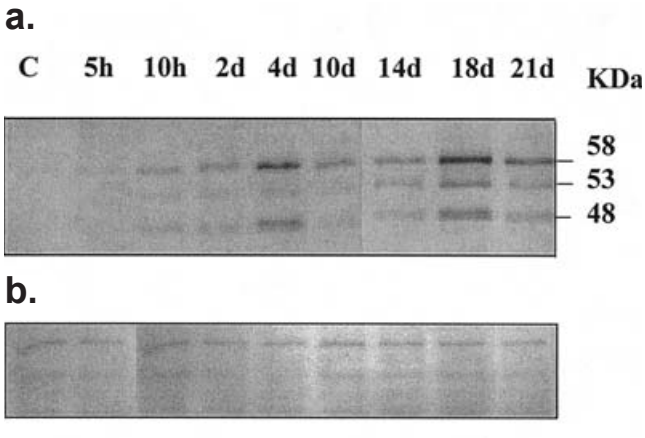

Fig. 7. Accumulation of dehydrins in *D. antarctica* leaves. Plants subjected to 21 days of cold-acclimation at 4ºC and nonacclimated controls at 15ºC. **a.** Western blot that shows the accumulation of dehydrin proteins during low temperature treatment is placed at the bottom. Protein samples were taken from 5 h until 21 d of treatment. Molecular mass is indicated at the right. **b.** Coomassie blue stained gel, showing total protein (10 μg).

dehydrin. This transcript was induced within 1 h after exposure to NaCl treatment, with a maximum at 10 h, decreasing after 24 h. The same transcript was strongly induced within 1 h after PEG treatment (Fig. 5). After 5 h the expression was weak, but was detectable for at least 24 h. This transcript was induced faster in plants under PEG than under NaCl treatment. Additionally, a 1.0 kb transcript was observed in plants treated with ABA. This 1.0 kb transcript was not detected in plants under the other treatments; therefore, this transcript accumulated specifically in response to ABA. There was no detection of dehydrin genes in untreated controls. Therefore, there was no constitutive expression detected by the probe (Fig. 5).

Western analysis

Seven polypeptide bands were recognized by the antidehydrin antibody in the leaves of *D. antarctica*. NaCl, PEG, low temperature and exogenous ABA treatments led to accumulation of different patterns of dehydrin proteins (dlp) in the leaves of *D. antarctica* (Fig. 6). A dlp with 58 kDa M_r (relative migration) accumulated in all treatments. However, its accumulation was lower under PEG treatment and higher under exogenous ABA. Two dlp with M_r 30 and 27 kDa were detected at 24 h of ABA treatment and slightly detected under salt stress. Two dlp with Mr 53 and 48 kDa were accumulated by low temperature stress and exogenous ABA (Fig. 6). There were two additional inconspicuous bands of 57 and 55 kDa that were observed only under exogenous ABA treatment. A kinetic study of dlp accumulation under low temperature revealed that dlps with 58, 53 and 48 kD Mr were weakly detected within 5 h after low temperature exposure. However, the accumulation of

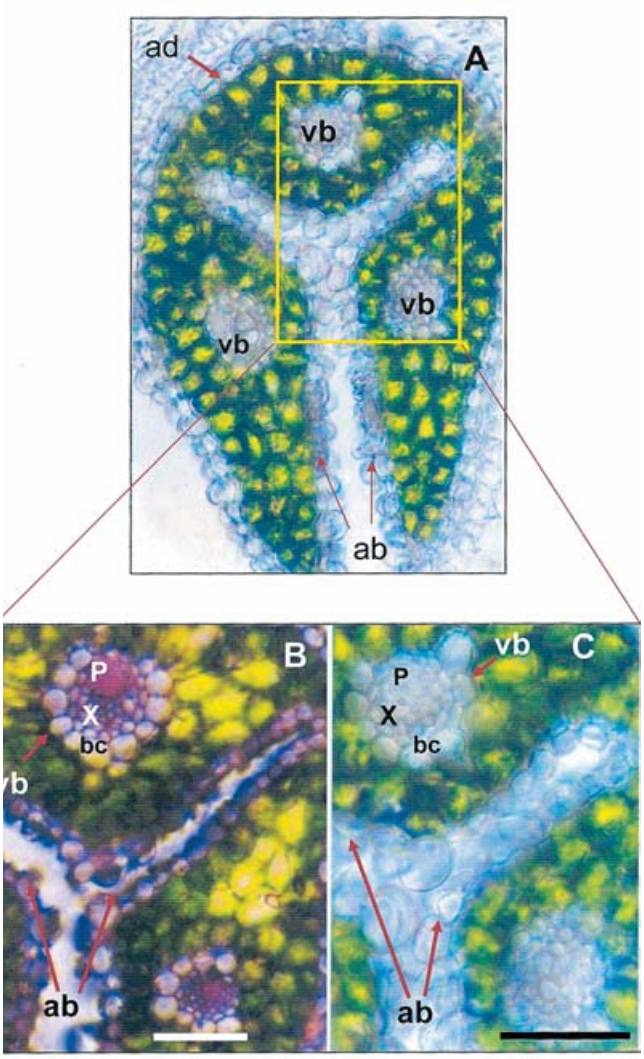

Fig. 8. Tissue localization of dehydrins in *D. antarctica* leaves. Fresh cross sections were prepared from plants subjected to 21 days of cold-acclimation and nonacclimated controls. **a.** Fresh section of a nonacclimated *D. antarctica* leaf. **b.** Localization of dehydrins in central vascular bundles of fresh sections of cold-acclimated, and **c.** nonacclimated *D. antarctica* leaves. The rectangle in photograph a indicates a similar area shown with higher magnification in b & c. Purple colouration indicates recognition by the anti-dehydrin antibody. Abaxial epidermis (ab), adaxial epidermis (ad), bundle sheath cells (bc), phloem (P), vascular bundles (vb), xylem (X). Scale bar = 50 μm. The antibody blocked with K-segment synthetic peptide did not show signal (data not shown).

these proteins was clearly detectable from 10 h to 21 days of cold acclimation in the leaves of *D. antarctica* (Fig. 7a). Dlps were not detected in untreated controls. Recognition of all detected dlp was blocked when the immune serum was pre-incubated with the K-segment synthetic peptide. These results confirm that all polypeptides observed contain this dehydrin consensus peptide.

Immunolocalization of cold acclimation-induced dehydrins

Cold-induced dehydrins were localized in fresh tissue sections from 21 day cold-acclimated *D. antarctica* leaves and non-acclimated controls using anti-DHN serum. A strong antibody recognition was observed in sections from cold-acclimated plants incubated with the anti-DHN-antiserum. Reactivity was observed in the vascular cylinder around both xylem and phloem and was also associated with the epidermis and periphery of the bundle cells (Fig. 8a). Leaf sections from non-acclimated plants treated with anti-DHN serum showed only a faint signal (Fig. 8b).

Discussion

Identification of a dehydrin gene fragment in D. antarctica.

In order to demonstrate that *D. antarctica* has genes for dehydrins, we used a fragment of 454 bp which was obtained from genomic DNA of *D. antarctica* by PCR. The analysis of this sequence without the intron showed that it had high identity with dehydrin genes. Major homology to dehydrins would be in the N-terminal region. The C-terminal portion showed differences with the members of LEA II group on the amino acids 70 to 110 (Fig. 2).

Southern analysis allowed the identification of different DNA fragments generated by restriction nuclease treatment of genomic DNA from *D. antarctica* using a homologue probe. Southern restriction patterns revealed that *D. antarctica* would have different genes encoding dehydrins and that they could possibly be part of a gene family. This is consistent with the knowledge that a multigene family encodes the LEA D-11 proteins, and that there are several other distinct families of dehydration-related proteins (Hughes & Galau 1989, Jarvis *et al*. 1996, Mtwisha *et al*. 1998, Choi *et al*. 1999, Richard *et al*. 2000).

Northern analysis

In spite of the presence of an intron, the probe detected more than one transcript, possibly reflecting the presence of several conserved dehydrin domains within the probe. Recognition was observed only in extracts of stress treated plants, but not in the untreated control and low temperature treated plants. This was expected because the nucleotide sequence of the probe showed identity with dehydrins induced by water stress, salinity, and ABA (Fig. 2), but not with those induced by low temperature as *dhn5*, *dhn8* and cor15, where the identity was only 24% with a high number of gaps (Fig. 3). The transcript with Mr 1.6 kb was common to NaCl and PEG treatments. The transcript level in osmotic stress was higher than in salt stress, suggesting that ionic effect of salt may reduce its expression. Salt stress induces osmotic adjustment of the cytoplasm by balancing the increased osmotic potential (lowered water potential)

caused by sodium sequestration inside the vacuole, where it can accumulate to concentrations that have marked effect on the osmotic balance of the plant cell (Adams *et al*. 1998), suggesting that salt and osmotic stress have a similar signal transduction pathway. Therefore, it is reasonable to find that the expression of 1.6 kb transcript is regulated by salt and osmotic stress. Examples of these include osmolyte biosynthesis genes, LEA/dehydrins-type genes, chaperones, etc. (Zhu 2002). The other transcript (1.0 kb) was induced only by ABA. Other examples of dehydrin genes induced by ABA are Aba3 in barley, Rab21 in rice or Le4 in tomato (Close 1997). This hormone has broad functions in plant growth and development. Its main function is to regulate plant water balance and osmotic stress tolerance (Zhu 2002). ABA has two major roles in drought and salt stress: water balance and cellular dehydration tolerance. Whereas the role in water balance is mainly through guard cell regulation, the latter role deals with induction of genes that encode dehydration tolerance proteins in the cells (Zhu 2002). Other studies showed that some osmotic stress responsive genes are induced independently of ABA, some are fully dependent on ABA, and others are only partially ABA dependent (Shinozaki & Yamaguchi-Shinozaki 1997). Our results suggest that dehydrin gene expression has ABA-dependent and ABA-independent signalling in *D*. *antarctica*. We did not detect transcripts in plants under low temperature with the probe, but we observed accumulation of dehydrin proteins by low temperature treatment. The level of transcripts was probably too low to be detected by northern blot. In other cases, dehydrin protein content and their corresponding mRNA are not always commensurate with each other (Parmentier-Line *et al*. 2002).

Western analysis

The dehydrin presence in response to NaCl, PEG, low temperature and ABA treatment was studied using an antiDHN-antiserum. Different patterns of dehydrin proteins were observed in the different treatments, confirming the presence of a family of dehydrins in *D*. *antarctica* (Fig. 6).

The presence of 58 kDa protein in all treatments is not surprising because all these stresses produce cell dehydration and osmotic adjustment (Zhu 2001). This protein could act by protecting dehydration sensitive structures in the leaves of *D*. *antarctica*. The accumulation of this protein, however, was lower in the PEG intermediate treatment under low temperature and NaCl treatments and highest under exogenous ABA, suggesting that the osmotic effect of PEG is insufficient to induce high amounts of 58 kDa protein and its accumulation may be mediated by ABA. This is reasonable because salt, osmotic, and low temperature stresses enhance ABA accumulation in plants and there are several ABA up-regulated dehydrins that contain ABA responsive elements in their promoter region (Shinozaki & Yamaguchi Shinozaki 1997).

Two inconspicuous dlp of 57 and 55 kDa were only observed under exogenous ABA treatment. Considering that the exogenously applied ABA concentration was two orders of magnitude higher than the expected endogenous concentration in plants, it is probable that during the other treatments, endogenous ABA was not sufficiently high to induce the same accumulation. This speculation is consistent with the higher number of dlps observed in ABA treated plants and with the higher intensity of 30 and 27 kDa proteins observed in ABA treatment, compared to NaCl treatment.

The kinetic study of dlp accumulation by low temperature in *D*. *antarctica* revealed that low temperature induced two peaks of dlps accumulation in *D*. *antarctica* (4 and 18 day after treatment). This response would be an adaptive condition of *D*. *antarctica* to low temperature, where the turnover of dehydrins would be very important. These results complement those reported by Bravo *et al*. (2001). The authors found that around 4 and 18 days after cold treatment, a decrease in LT_{50} and an increase in sucrose and fructans levels are produced. We think that in cold climates the accumulation of dehydrins, along with sucrose and fructans, would be strongly related to cold resistance in *D*. *antarctica*.

Localization of dehydrin in plants treated by low temperature

Cold-induced dehydrins were present in the epidermis and in the vascular cylinder around xylem and phloem in *D*. *antarctica*. Localization of dehydrins near the vascular tissue is in agreement with previous reports on dehydrins localization in other plant species. LTI30 was present in the vascular tissue in *A*. *thaliana* treated by low temperature, while only a weak staining of mesophyll and stomatal guard cells was found (Nylander *et al*. 2001); P-80, a dehydrin from barley, accumulates in vascular cylinders and epidermis in response to low temperature (Bravo *et al*. 1999); PCA60, a dehydrin from peach, was accumulated in epidermal, cortical, phloem, and xylem tissue (Wisniewski *et al*. 1999). Vascular tissue and the epidermis have been reported to be the primary sites for ice nucleation (Pearce 1988, Pearce & Ashworth 1992). Early ice nucleation in vascular cylinder and epidermis could induce a severe dehydration in adjacent cells. Therefore, the presence of cold-induced dehydrins in these tissues could be related to preventing protoplasmic dehydration stress associated with freezing and thus increasing freezing tolerance (Houde *et al*. 1995, Danyluk *et al.* 1998, Bravo *et al*. 1999). In cells surrounding the xylem vessels dehydrins might function as water attractants during the transport of water from xylem vessels to sink tissues. The vascular system, together with meristematic tissue, is crucial for plant growth and survival and needs extra protection from environmental stress.

Accumulation of dehydrins at low non-freezing temperatures in these sensitive tissues could constitute an adaptative response that could help the plant to cope with further stresses imposed by freezing. The general production of cellular dehydration would simply reflect the need for all cells to keep the water balance with the environment when water availability is hampered. Dehydrin location is extensive. Immuno-electron microscopy studies have also identified dehydrins in the cytosol of desiccated leaf cells of *Craterostigma plantagiineum* Hochst (Schneider *et al*. 1993) and the euchromatin, nucleolus and cytosol of various cells in tomato seedling and mature plant tissues (TAS14) (Godoy *et al*. 1994). In *A*. *thaliana*, tissue and cell type specific accumulation of dehydrins has been reported as ERD14 in vascular tissue, LTI29 in roots and stems, RAB18 provascular tissue of the embryo (Nylander *et al*. 2001).

This is a first study on dehydrins in Antarctic plants. Our results demonstrate that *D*. *antarctica* has a dehydrin gene family. Some of these dehydrins respond differentially to different factors that may cause dehydration (salt, osmotic stress or exposure to low temperature) and ABA. It is known that freezing tolerance is a result of several cryoprotective mechanisms operating concurrently (Sakai & Larcher 1987). *Deschampsia antarctica* has high antifreeze activity (Doucet *et al*. 2000), but there is no evidence on the structural properties of the antifreeze proteins. It has been reported that low temperature induced dehydrins could have cryoprotective or antifreeze activity (Wisniewski 1999, Bravo *et al*. 2003), but this remains to be shown in Antarctic plants. *Deschampsia antarctica* is exposed to cold, salt (sea spray) and desiccant winds during the Antarctic summer. Therefore, we suggest that the survival of *D*. *antarctica* in the field could be partially explained by the presence of dehydrin genes and accumulation of dehydrin protein in its leaves.

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