Journal of Plant Biology, June 2006, 49(3) : 205-211

Sequence Variability and Expression Characteristics of the Ginseng (*Panax ginseng* C.A. Meyer) Dehydrin Gene Family

Young-Im Ha[1], Jong-Min Lim[1], Suck Min Ko[1], Jang Ryol Liu[2], and Dong-Woog Choi[3]*

[1]Eugentech Incorporated, Daejon 305-333, Korea
[2]Korea Research Institute of Biosciences and Biotechnology, Daejon 305-333, Korea
[3]Department of Biology Education, Chonnam National University, Kwangju 500-757, Korea

The dehydrins (DHN) are a family of late embryo abundant (LEA D-11) proteins, which accumulate during the late stage of seed development or under low temperature or water deficient conditions. They are believed to play a protective role in freezing and drought tolerance. The dehydrin genes exist as multi-gene families. Here, we have identified 9 unique dehydrin genes from Korean ginseng (*Panax ginseng* C.A. Meyer), a typical medicinal plant. Among these, *PgDhn1* and *PgDhn2* encode for YSK3- and KS-type dehydrins, respectively, and are very abundant. Gene expression analyses revealed that the majority of the *PgDhn* gene transcripts are detected under cold, as well as dehydration conditions. The exceptions are *PgDhn5* and *PgDhn9* – the former being unresponsive to cold treatment, and the latter exhibiting only seed-specific expression. We also identified an alternative transcript of the *PgDhn2* gene that harbors an intron in its 3'-untranslated region. Our results may prove useful in further studies of *Dhn* genes, including investigations into the mechanisms underlying gene expression, the nature of their variations, and their physiological functions.

Keywords: dehydrin, ginseng, *PgDhn*

Plants have defensive strategies against the many stresses that arise from frequent environmental fluctuations to which they are exposed. Among the most severe factors limiting growth and yield are drought and low temperatures. More than 100 genes have been shown to be responsive under such conditions (Seki et al., 2001; Bray, 2002). The proteins induced by stress include several believed to function either in the physical protection of cells from water deficiencies or temperature changes, or in the regulation of gene expression (Bray, 1993, 2002; Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 2000; Kim et al., 2005).

The dehydrins are the proteins most prominent in this adaptive process (Close, 1996, 1997). During the late stages of embryogenesis, they typically accumulate in various tissues in response to low temperature, drought, salinity, or ABA application (Close, 1996; Campbell and Close, 1997). The dehydrins belong to Group II of a larger family of proteins that are referred to as the late embryogenesis abundant or LEA D-11 proteins (Garay-Arroyo et al., 2000). The most distinctive feature of the dehydrins is their conserved lysine-rich 15-amino acids domain, which harbors a consensus EKKGIMDKIKEKLPG known as the K-segment. This segment is able to assume an amphipathic α-helix structure (Close, 1997). Other dehydrin domains include the S- and Y-segments – the former being a tract composed of serine residues while the latter, when present, being located proximally to the N-terminus. Based on the number and order of these conserved domains, dehydrins can be classified into five sub-classes or types: Y_nSK_n, SK_n, K_n, Y_nK_n, and K_nS (Campbell and Close, 1997). DNA sequence and immunological data have revealed that dehydrins are distributed throughout a wide range of photosynthetic organisms, including both higher and lower plants (Close, 1997).

A correlation is well established between dehydrins and cell dehydration, but their specific molecular functions remain somewhat unclear. On the basis of sequence features, the dehydrins are believed to function as stabilizers of membrane structures and proteins by exerting detergent and chaperone-like effects (Close, 1996). Protective interactions with membranes can occur via the K-segment, which, although intrinsically unstructured, assumes an amphipathic helical structure when bound to membranes (Koag et al., 2003).

The dehydrins may also act as water attractants in cells with low water potential, and may also play a role in regulating osmotic potential (Nylander et al., 2001). In addition, a phloem iron-transport protein (ITP) from the castor bean (*Ricinus communis*) has recently been identified as a KS-type dehydrin (Kruger et al., 2002). *Arabidopsis* ERD14, a SK_2 dehydrin, and celery VCaB45, a dehydrin-like vacuolar protein, bind calcium in a phosphorylation-dependent manner (Heyen et al., 2002; Alsheikh et al., 2003). These findings indicate that the dehydrin structural type may produce a specific effect, and that some of the dehydrins may function as ion-sequesters.

Ginseng root has traditionally been used as an herbal medicine that confers resistance to stress, disease, and exhaustion. *Panax ginseng* C.A. Meyer (Korean Ginseng) is a perennial herb that grows in northeastern

*Corresponding author; fax +82-62-530-2509
e-mail dwchoi63@chonnam.ac.kr

China, Korea, and far-eastern Siberia. As such, plants must endure arid conditions and freezing temperatures in the winter, and must have adaptive mechanisms for surviving in such a stressful environment. In this study, we identified dehydrin genes from the ginseng genome, and compared among their sequence variations and expression patterns.

MATERIALS AND METHODS

Plant Material

Korean ginseng (*P. ginseng*, C. A. Meyer) was used. For dehydration treatment experiments, whole seedlings were transferred to filter paper in 120-mm Petri dishes. When their fresh weights were reduced to 70%, the tissues were snap-frozen in liquid nitrogen. To determine the effects of cold stress, the plants were maintained in soil at 5°C in a growth chamber, and samples were obtained at various intervals. For the abscisic acid (ABA) treatments, seedlings were positioned for 1, 3, 6, or 10 h in Falcon tubes containing 100 mM ABA solution. Seedlings from these experiments were then harvested at the indicated time points, quickly frozen with liquid nitrogen, and stored at –80°C. Seeds are harvested from filed-grown plants.

Isolation of *Dhn* cDNA and Sequence Analysis

Previously, we generated nearly 20,000 ginseng ESTs from 7 ginseng cDNA libraries (Jung et al., 2003; Choi et al., 2005). These ESTs were then combined and analyzed for ginseng dehydrin gene resources. Clustering of individual ESTs for unique transcripts, as well as the homology searches, were conducted as described previously (Jung et al., 2003; Chung et al., 2004; Choi et al., 2005). The individual ginseng ESTs were searched against the GenBank nr database, using a BLASTX algorithm. Dehydrin cDNAs were identified via keyword searches with BLASTX results, and through domain searches of our ginseng EST database. A 15-amino acid sequence of the K-segment, EKKGIMDKIKEKLPG, was employed to identify the ginseng dehydrin cDNAs (Close, 1997). For multiple sequence alignment, sequence-editing and amino acid sequence predictions from the selected ESTs were conducted using the Sequencher program (Gene Code, USA). The putative molecular weights and PI values of the deduced polypeptides were predicted with the DNASIS Max program (MiraiBio, USA), while the deduced amino acid sequences were aligned through the CLUSTAL V program.

Gene-Specific RT-PCR

Total RNAs were prepared from plant tissues according to the hot-phenol procedure established by Verwoerd et al. (1989). First-strand cDNAs were constructed from 2 μg of total RNA via reverse-transcription in 20 μL reaction volumes, using the oligo$(dT)_{17}$ primer and Superscript II (Invitrogen, USA) reverse transcriptase, based on the manufacturer's instructions (BRL Life Technologies, USA). Reactions were conducted at 42°C for 60 min, followed by 5 min of heating at 70°C. The first-strand cDNA reaction was diluted by a factor of 5, after which 2 μL of diluted cDNA was applied to a 50 μL PCR-amplification reaction that contained 5 μL of 10x PCR buffer [200 mM Tris-HCl (pH 8.4) and 500 mM KCl], 1 μL of 10 mM dNTPs, 1 μL of each gene-specific primer (10 pmol μL^{-1}), and 2.5 units of ExTaq DNA polymerase (Takara, Japan). PCR reactions were conducted for 28 cycles, each consisting of 30 s at 95°C, 30 s at 58 to 64°C, and 90 s at 72°C; then 5 min of termination at 72°C. The ginseng *Dhn* gene-specific primers were as follows: 5′-CAAGATCATATATACCTC-CACCGG-3′ and 5-′CATCGGGGCTCTTATTTGAAG-GAA-3′ for *PgDhn1*; 5′-GGCAAAGTCAAGGATCAGA-TCCAA-3′ and 5′-GCAACAATCAGCATCCTAAATCCT-3′ for *PgDhn2*; 5′-AGAAGAAGGACAAGAAGAAGCACG-3′ and 5′-CACAACTGATTCCAGCAGGGTGT-3′ for *PgDhn3*; 5′-GAAAGTTCATGATTCGGAGACCGA-3′ and 5′-CAT-CATTAGTCTTGGAGTGGTACC-3′ for *PgDhn4*; 5′-GCA-CTCACGAACAATTCCATCTTC-3′ and 5′-CAGATAAG-TAGAGTAGGGTACGTA-3′for *PgDhn5*; 5′-GAGGAG-GAGGAGGATATGCTGCTG-3′ and 5′-CTCCACAGG-TGGTACTACTACATA-3′ for *PgDhn6*; 5′-CACCACG-CATGCAGTATGTAGTAC-3′ and 5′-CTTCTGACGTAC-CCGTACAGGAT-3′ for *PgDhn7*; 5′-GATTCCTGGGTA-CCACTCCAAGAC-3′ and 5′-CATATCACATCGAATGT-GCAAGGG-3′ for *PgDhn8*; and 5′-GATGGAGAAG-GTGGGAGAAGAAA-3′ and 5′-CCAATACCACCACAG-ATTTATTTA-3′ for *PgDhn9*. The annealing temperature for each *Dhn* gene-specific primer was adjusted for the optimal PCR reaction. Products were separated on 1% agarose gels, and stained with ethidium bromide for photography. Ginseng genomic DNA was isolated from green ginseng leaves, via the cetyltrimethyl-ammonium bromide (CTAB) method (Murray and Thompson, 1980) with slight modifications, and was then utilized as template for intron analysis. The 3′-UTR region of the *PgDhn2* gene was PCR-amplified using a primer set that flanked the putative intron region: 5′-CGACGGCGA-CAAGAAGAGCCA-3′ and 5′-TGCAACAATCAGCATC-CTAAATGGAA-3′.

RESULTS AND DISCUSSION

Isolation of Dehydrin cDNA

We previously generated 17,605 ESTs from 7 ginseng cDNA libraries (Jung et al., 2003; Choi et al., 2005). EST analysis via keyword and domain searches allowed

Table 1. *Dhns* identified from ginseng ESTs. Ginseng *Dhn* cDNAs were identified by keyword searching and domain searching against the ginseng EST database. A 15-amino acid sequence of the K-segment, EKKGIMDKIKEKLPG, was searched to find ginseng dehydrin.

	Cluster	No. sister ESTs	Representative EST	Organism	Description	Score	E-value
PgDhn1	cluster 1222	91	PG03013C02	*Pisum sativum*	dehydrin2	41.2	0.029
PgDhn2	cluster 401	80	PG03009E04	*Hordeum vulgare*	dehydrin 13	45.4	0.002
PgDhn3	cluster 875	34	PG03009H12	*Hordeum vulgare*	dehydrin 13	41.2	0.027
PgDhn4	cluster 1062	17	PG03022H02	*Phaseolus vulgaris*	dehydrin	73.2	2e-11
PgDhn5	cluster 1327	11	PG06015H12	*Helianthus annuus*	putative dehydrin	63.9	2e-09
PgDhn6	cluster 1195	4	PG03014F06	*Arabidopsis thaliana*	dehydrin RAB18-like protein	41.6	0.013
PgDhn7	-	1	PG03014F11	*Helianthus annuus*	putative dehydrin	47.4	0.001
PgDhn8	-	1	PG05012E04	*Solanum tuberosum*	dehydrin homolog CI7	70.1	2e-11
PgDhn9	-	1	PG06012G12	*Arabidopsis thaliana*	putative dehydrin	84.7	2e-16

Table 2. Ginseng dehydrin proteins deduced from cDNA.

	No. amino acid	Molecular weight (kDa)	Isoelectric point	DHN type	comment
PgDHN1	197	20.578	8.46	YSK3	
PgDHN2	101	10.774	7.12	KS	
PgDHN3	96	10.164	6.75	KS	
PgDHN4	218	24.505	4.80	SK3	
PgDHN5	174	18.151	9.58	YSK2	
PgDHN6	Na	Na	na	?SK3	5'-truncated
PgDHN7	196	20.385	6.67	YSK3	
PgDHN8	229	25.928	6.07	SK3	
PgDHN9	Na	Na	na	?SK2	5'-truncated

na, not available.

us to identify nine dehydrin (DHN) gene candidates. Results of the Blast X and redundancy analyses for each *Dhn* gene candidate are shown in Table 1. These candidates were named *PgDhn,* and each distinct gene was uniquely numbered, from 1 to 9. EST analysis data showed that *PgDhn1* and *PgDhn2* were highly redundant, harboring 70% of the total *PgDhn* gene transcripts. Candidates *PgDhn3* through *PgDhn6* were also identified as cluster range 34 to 4 sister ESTs, but the other three -- *PgDhn7*, *PgDhn8*, and *PgDhn9* -- were isolated as single ESTs. Our cluster analyses indicated that the majority of the sister ESTs of the *PgDhn1* and *PgDhn2* cluster originated from two cDNA libraries constructed from the roots and rhizomes of a four-year-old ginseng sample that had been harvested in November 2001 (Jung et al., 2003). These results revealed that PgDHN1 and PgDHN2 are major DHNs functioning in the roots under cold- and dehydration-stress conditions. Furthermore, PgDHN5 may be abundant in ginseng seeds because the majority of the sister ESTs originated from the seed cDNA library. The longest sister cDNA clone in a *PgDhn* cluster was selected as the representative EST for each cluster, and was used to determine the full sequences.

Sequence Analysis of Ginseng Dehydrins

The deduced polypeptides were characterized from our nine *PgDhn* cDNAs (Table 2).

Typical features of the dehydrins were consistent with the "YSK" shorthand described above. PgDHN1 encodes a 197-amino acid YSK3 polypeptide (20.6 kDa, pI 8.46) that is quite similar to PgDHN7, sharing 97% amino acid sequence identity (Fig. 1). The amino acid sequence analysis revealed that PgDHN6 also exhibits a significant degree of sequence homology with PgDHN1 and PgDHN7, although its 5'-terminal region is truncated. PgDHN2 encodes for a KS-type polypeptide of 101 amino acids (10.8 kDa, pI 7.12). Another KS-type DHN, PgDHN3, shows a high degree of sequence homology with PgDHN2. A 5-amino acid sequence (DKKSH), which is not present in PgDHN3, as well as two amino acid substitutions, account for the entirety of these differences. A consensus K-segment sequence detected in the angiosperm was not detected in these KS-type dehydrins, but it did harbor a Lys-rich sequence, DKKKKKDKKKHGEHG. The amino acid sequences deduced from *PgDhn4* and *PgDhn8* were extremely similar to one another, except for their C-terminal regions. PgDHN4 encodes for a 218-amino acid SK3

```
PgDHN1      MANHGERYGQEQGQGQQQRGTDAYGNPVGQTEQFGN-IIHQTGGGTMDGCGTGGAAGTEAYGSAGTGMHGTGAHDQSQLRRSGNSSSSEDDGMGGRKKKGI
PgDHN7      MANHGERYGQEQGQGQQQRGTDAYGNPVGQTEQFGN-PIHQTGGGTLDGCGTGGAAGTEAYGSAGTGMHGTGAHDQSQLRRSGSSSSSEDDGMGGRKKKGI
PgDHN6      ----------------------------QTEQLGNDPIYQTGGGTTGGCGSGGADGTEAYGSAGS-----GAHDQSQLSRSGSSSSSEDDGVGGRKKKGI
                                        ****:**  *:****** .***:*** *********:     ******** ***.********:********
PgDHN1      KGKIKEKLPGVGGHKKEGEQAIYSPVGGGYAAGEEQHHGHEKKGMMEKIKRHNKEGEQ-ATYSPAGGG-Y-AAGDEQHHVQEKKGMMEK-IKEKLPGGGRD
PgDHN7      KEKIKEKLPGVGGHK-EGEQAIYSPVGGGYAAGEEQHHGHEKKGMMEKIKGHNKEGEQ-ATYSPAGGG-Y-VAGDEQHHVQEKKGMMEK-IKEKLPGGGRD
PgDHN6      KGKIKEKLPGVGGHKQEGEQATYSPVGGGYAAGEEQHHGHEKKGMMEKIKGNNKEGEQAATYSPGGGGGYAAAGDEQHNVQEKIGMMEKIIKEKLPGGGRD
            * ************* ***** ************************** :****** *****.*** * .******:**** ***** ***********

PgDHN2      MANIIHKIEETLGMGGDKHKEGEQHKKPEGQHAGDHKEGGHGGGGIVGKVKDQIQGGHGGSSDDHHDGDKKSHDKKKKKDKKKHGEHGHDKHGDSSSSDSD
PgDHN3      MANIIHKIEETLGMGGDKHKEGDQHKKPEGQHGGDHKEGGHGGGGIVGKVKDQIQGGHGGSSEDHHDG-----DKKKKKDKKKHGEHGHDKHGDSSSSDSD
            **********************:*********.****************************:*****     ****************************

PgDHN4      MADEVVHHPLDKSSEVETTORGLFDFLGAKKDEGKSAPADYQQQEEIATEFEQKVHVSETEPKFEESKVEEEEEEKKPSLLDKLHRSGSSSSSSSDEEEV
PgDHN8      MADEVVHHPLDKSSEVETTORGLFDFLGAKKDEGKSAPADYQQQEEIATEFEQKVHVSETEPKFEESKVEEEEEEKKPSLLDKLHRSGSSSSSSSDEEEV
            ****************************************************************************************************
PgDHN4      EEGGEKKKKKKKSLKEKIHKEEDTSVPVEKIEEHVVEP-EEKKGFVDKIKEKLPGGGHKKAEDQEFPSPTPPPAPPAAVESYATVEGEPAKEEKGILGKDR
PgDHN8      EEGGEKKKKKKKSLKEKIHKEEDTSVPVEKIEETVVVEQEEKKGFLDKIKEKLPGGGHKKAEDQEFPS-----PAPPAAVESYATVEGEPAKEKKGILEKIK
            ********************************** **   ******:**********************    ********************:**** * :
PgDHN4      REDSWVPLQD----------------------
PgDHN8      EKIPGYHSKTSDEKKKRKKRKKKKKKTRGWS
            .:  .    :

PgDHN5      MADNYGYGYGQQHHGGRQTDAYGNPIPQTNAYGNPVRQTDDYGNPVHHTTTGAATMGDYSTAGTVGAYGTGTPTGGFGAPNVTGTGTHEQFHLRRSGSSSS
PgDHN9      ----------------------------------------------------------------------------------------------HRSTSSSS
                                                                                                            :** ****
PgDHN5      SSSEDDGMGRRRKKKGIKEKIKEKLPGGTGHKDTSTTTPGGYGYGSAAGGGEAHGHEKKGMMEKIKEKLPGHH-------
PgDHN9      SSSEDDGEGGRRKK-GLRAKIKEKLGGGK-HKNKDDQTHT----TGSGAPAGAYHEHEKKSMMEKIKDKLPGHHTTTTPP
            ******* * **** *:: ****** **. **:..  *      **.* .*  * ****.******:******
```

Figure 1. Comparison of amino acid sequences for ginseng dehydrins. Amino acid residues are designated via single-letter codes. Asterisk (*) and period (.) indicate identical and homologous amino acids, respectively. Dashes indicate places in which a sequence has been expanded to allow for optimal sequence alignment. Deduced amino acid sequences were aligned by CLUSTAL V program.

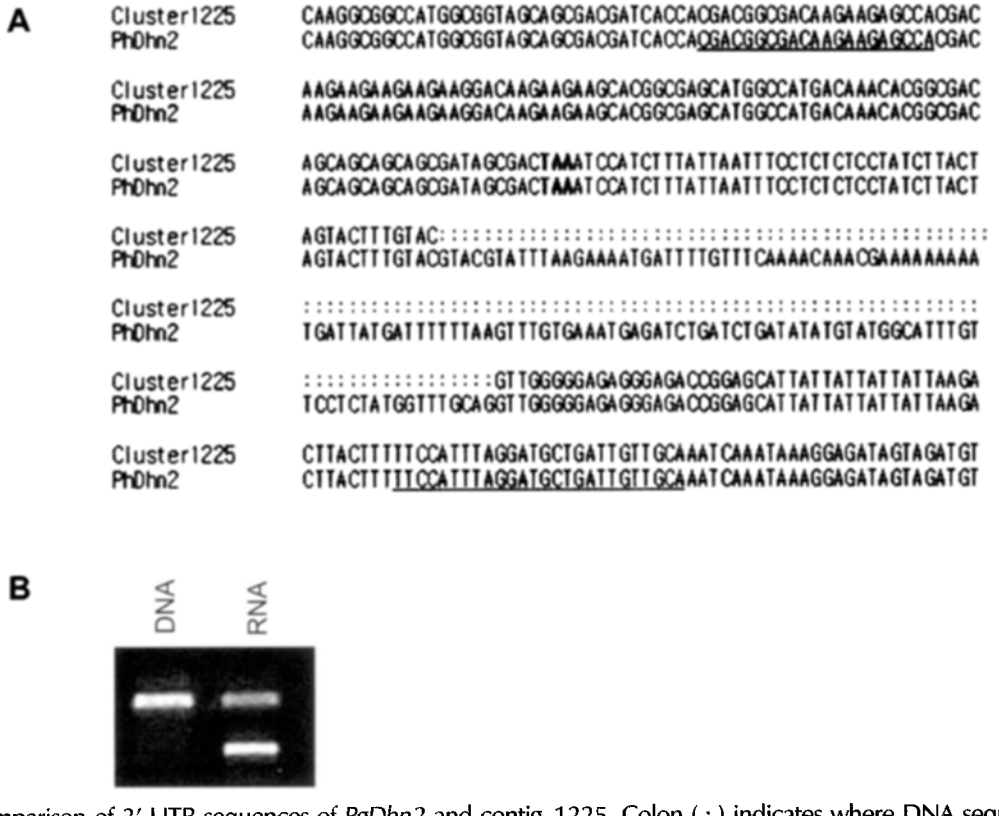

Figure 2. A, Comparison of 3′-UTR sequences of *PgDhn2* and contig_1225. Colon (:) indicates where DNA sequence has been deleted within cDNA, contig_1225. Stop codon is in boldface. cDNA sequences were aligned by CLUSTAL V program. Gene-specific primer sites are underlined. **B**, PCR analysis of 3′-UTR region of *PgDhn2* and contig_1225 from transcript and genomic DNA. PCR amplifications were conducted with *PgDhn2*-specific primer set flanking deletion region (Fig. 2A).

polypeptide (24.5 kDa, pI 4.8), while PgDHN8, which encodes for 229 amino acids (25.9 kDA, pI 6.07), exhibits three amino acid substitutions and an additional 11 amino acids (KKKKKKTRGWS) at its C-terminal region. These dehydrins have the most acidic pI values of all our PgDHNs. PgDHN5 encodes for an Y3SK2 polypeptide consisting of 174 amino acids (18.2 kDa, pI 9.58). Our amino acid sequence analyses indicated that *PgDhn9* is truncated at the 5′-terminal region, and that the deduced PgDHN9 is highly similar to PgDHN5.

Alignment of the amino acid sequences indicated that an EST cluster, contig_1225, encodes for a polypeptide identical to PgDHN2, but does not form the same cluster with the *PgDhn2* cDNA sequence. DNA sequence analyses demonstrated that the sequence of contig_1225 is identical to that of *PgDhn2*, except for the deletion of 127 bp in the 3′-untranslated region of contig_1225 (Fig. 2A). Therefore, to determine whether contig_1225 originated from the alternative splicing of the *PgDhn2* transcript or from a different dehydrin gene, we designed a primer set flanking the deletion region, and attempted PCR amplification from RNA-free genomic DNA and genomic DNA-free RNA templates. Our PCR results revealed that, although only a single gene exists within the genomic DNA, its two transcripts are of different lengths (Fig. 2B). Thus, we suggest that *PgDhn2* harbors an intron in its 3′-untranslated region (UTR) and that contig_1225 is an alternative splicing product from the *PgDhn2* transcript. Results of the genome analyses indicated that introns exist within the UTR region, as well as the coding sequence (CDS). The 3′-UTR evidences a much lower intron frequency than does the 5′-UTR, in the range of 1 to 11%, depending on the taxon (Mignone et al., 2002). Alternative 3′-UTRs can be formed by using different polyadenylation sites or splice acceptor sites. UTRs perform crucial functions in the post-transcriptional regulation of gene expression, including the modulation of mRNA transport out of the nucleus, subcellular localization, and stability (Mignone et al., 2002).

Expression of *PgDhn* Genes

To determine the expression pattern for each *PgDhn* gene, we amplified their transcripts using dehydrin gene-specific primers and RT-PCR. Total RNA was isolated from the shoots of control and treated ginseng seedlings. We selected RT-PCR because northern blot hybridization is fraught with ambiguities associated with the hybridization of various *Dhn* genes to each other, as has been observed with barley *Dhn* alleles (Lang et al. 1998; Choi et al., 1999).

Under our various treatment conditions (dehydration, cold temperature, exogenous ABA), expression of the *PgDhn* genes was regulated differentially (Fig. 3A). Except for the SK3-type dehydrin genes -- *PgDhn4* and *PgDhn8* -- expression for the majority of the *PgDhn* genes was not detected under normal (control) growth. However, most of these genes were up-regulated by dehydration, ABA treatment, and low temperature, although their individual expression patterns differed, especially under chilling. Transcripts of the *PgDhn* genes that encoded for the YSK3 type -- *PgDhn1*, *PgDhn6*,

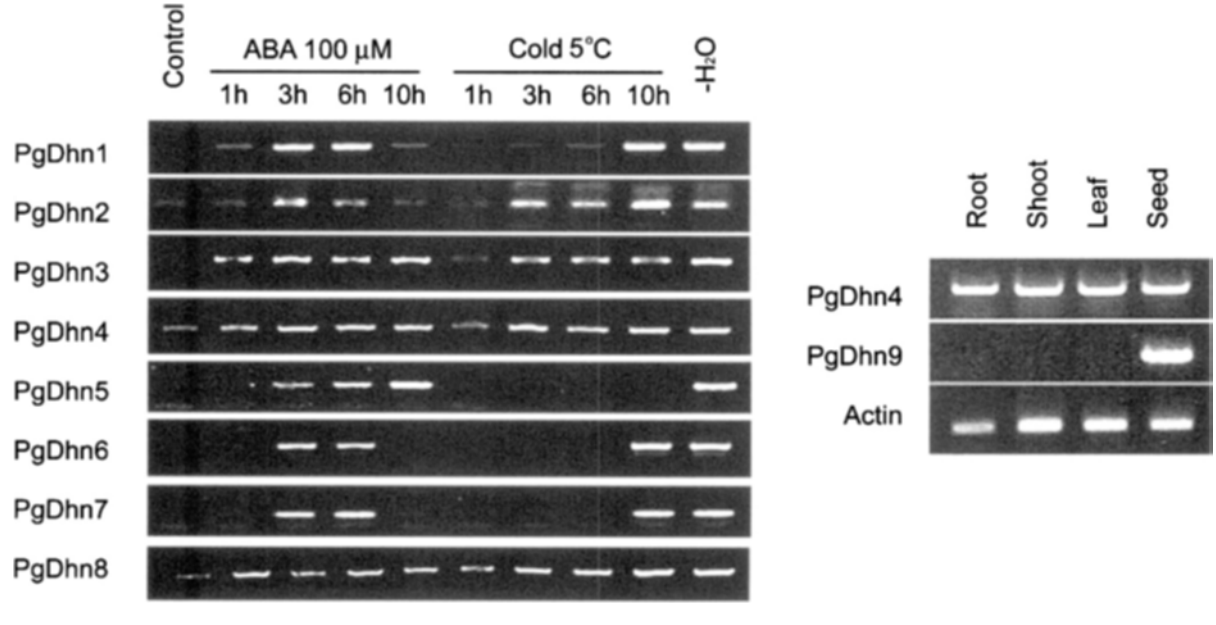

Figure 3. Expression of each *PgDhn* gene under various stress conditions. Soil-grown seedlings (control) were either dehydrated (-H_2O), cold-acclimated at 5°C for 10 h (Cold), or treated with 100 mM ABA for 10 h (ABA). Total RNA was isolated and reverse-transcribed using 3′-primer, then amplified with gene-specific primer sets. Annealing temperatures were adjusted for some primer sets to optimize PCR reactions. RT-PCR products were electrophoresed on 1.0% agarose gel.

and *PgDhn7* -- were detected 10 h after the administration of cold treatment, compared with just 3 h for transcripts of the KS-type genes. *PgDhn5*, specifically, was generally unresponsive to cold, suggesting that its expression is regulated via a pathway different from that of the other *PgDhn*s.

In contrast, transcript for *PgDhn9* was not detected under any of the treatment conditions, implying that its expression may be organ-specific. To determine whether this was true, total RNA samples were prepared from the roots, stems, and leaves of soil-grown seedlings, as well as from seeds produced by field-grown plants. RT-PCR trials revealed detection only in the seeds (Fig. 3B), thereby confirming that expression of *PgDhn9* is seed-specific, such that it cannot be induced in seedlings through the administration of dehydration, chilling, or ABA.

Dhn genes exist as multi-gene families in plants, and have been fairly well-characterized, particularly in barley. Thirteen *Dhn* genes have thus far been identified in that species, with evidence of different expression patterns under stress treatments and in various organs (Choi et al., 1999; Choi and Close, 2000; Rodríguez et al., 2005). All of those barley *Dhn* genes encoding for YSK_2 dehydrins have been shown to be up-regulated by both dehydration and ABA, but not by chilling, and all exhibit a basic isoelectric point (Choi et al., 1999). In contrast, *Dhns*, which is up-regulated by cold treatment, encodes for acidic DHN5 and DHN8, with predicted pIs of 6.68 and 5.10, respectively (Danyluck et al., 1998; Zhu et al., 2000). Moreover, a KS-type DHN, *Dhn13,* is constitutively expressed, while *Dhn12* exhibits seed-specific expression (Choi and Close, 2000; Rodríguez et al., 2005). In our study, the expression patterns of *PgDhn* differed slightly from those of barley, with the majority of their transcripts being detected under low-temperature conditions as well as ABA-treatment and dehydration. It is also particularly interesting that the KS-type DHN is one of the principal DHNs in ginseng. Such dehydrins have demonstrated ion-binding ability (Kruger et al., 2002). Therefore, the KS type may prove useful as an antioxidative, iron-sequestering agent under dehydration or low temperature, for cases in which harmful peroxides are likely to be generated near susceptible membranes (Hara et al., 2003; Rodríguez et al., 2005).

In summary, we have identified nine ginseng dehydrin genes and have classified them into four groups based on their amino acid sequence homology. The first and second groups encode for the YSK3- and KS-type DHN, respectively. Both of these types have been suggested as major ginseng DHNs, induced by abiotic stress. The third group is SK3-type acidic DHN, while the fourth is Y3SK2 DHN, with expression patterns that differ from those of other *PgDhn* genes. Here, we also identified an alternative transcript of the *PgDhn2* gene, which has been proposed as an alternative splicing product within the 3'-UTR, and has also been shown to harbor an intron. One of the questions arising from these findings is whether this intron functions as a post-transcriptional regulator in *PgDhn2* expression. Although that issue remains to be addressed, our current results provide a foundation that may facilitate further studies into new and lingering questions regarding the *Dhn* genes, including investigations into the mechanisms underlying gene expression, the nature and evolution of allelic variation, and the cause-and-effect relationships that occur among specific alleles and stress-tolerance traits.

ACKNOWLEDGEMENT

This study was financially supported by a research fund from the Chonnam National University, 2004.

Received February 27, 2006; accepted April 6, 2006.

LITERATURE CITED

Alsheikh MK, Heyen BJ, Randall SK (2003) Ion binding properties of the dehydrin ERD14 are dependent upon phosphorylation. J Biol Chem 278: 40882-40889

Bray EA (1993) Molecular response to water deficit. Plant Physiol 103: 1035-1040

Bray EA (2002) Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: An analysis using microarray and differential expression data. Ann Bot 89: 803-811

Campbell SA, Close TJ (1997) Dehydrins: Genes, proteins, and associations with phenotypic traits. New Phytol 137: 61-74

Choi DW, Close TJ (2000) A newly identified barley gene, *Dhn12* encoding a YSK2 DHN, is located on chromosome 6H and has embryo-specific expression. Theor Appl Genet 100: 1274-1278

Choi DW, Jung JD, Ha YI, Park HW, In DS, Chung HJ, Liu JR (2005) Analysis of transcripts in methyl jasmonate-treated ginseng hairy roots to identify genes involved in the biosynthesis of ginsenosides and other secondary metabolites. Plant Cell Rep 23: 557-566

Choi DW, Zhu B, Close TJ (1999) The barley (*Hordeum vulgare* L.) dehydrin multigene family: Sequences, allelic types, chromosome assignments, and expression characteristics of 11 *Dhn* genes of cv. Dicktoo. Theor Appl Genet 98: 1234-1247

Chung HJ, Park JY, Cho IS, Hur CG, Choi DW, Liu JR (2004) Isolation and characterization of *Panax ginseng* 14-3-3 gene family 47: 167-173

Close TJ (1996) Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plant 97: 795-803

Close TJ (1997) Dehydrins: A commonality in the response of plants to dehydration and low temperature. Physiol Plant 100: 291-296

Danyluk J, Perron A, Houde M, Limin A, Fowler B, Ben-

hamou N, Sarhan F (1998) Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation of wheat. Plant Cell 10: 623-638

Garay-Arroyo A, Colmenero-Flores JM, Garciarrubio A, Covarrubias AA (2000) Highly hydrophilic proteins in prokaryotes and eukaryotes are common during conditions of water deficit. J Biol Chem 275: 5668-5674

Hara M, Terashima S, Fukaya T, Kuboi T (2003) Enhancement of cold tolerance and inhibition of lipid peroxidation by citrus dehydrin in transgenic tobacco. Planta 217: 290-298

Heyen BJ, Alsheikh MK, Smith EA, Torvik CF, Seals DF, Randall SK (2002) The calcium binding activity of a vacuole-associated, dehydrin-like protein is regulated by phosphorylation. Plant Physiol 130: 675-687

Ingram J, Bartels D (1996) The molecular basis of dehydration tolerance in plants. Annu Rev Plant Physiol Plant Mol Biol 47: 377-403

Jung JD, Park HW, Hahn Y, Hur CG, In DS, Chung HJ, Liu JR, Choi DW (2003) Discovery of genes for ginsenoside biosynthesis by analysis of ginseng expressed sequence tags. Plant Cell Rep 22: 224-230

Kim SJ, Jeong DH, An G, Kim SR (2005) Characterization of a drought-responsive gene, *OsTPS1*, identified by the T-DNA gene-trap system in rice. J Plant Biol 48: 371-379

Koag MC, Fenton RD, Wilkens S, Close TJ (2003) The binding of maize DHN1 to lipid vesicles: Gain of structure and lipid specificity. Plant Physiol 131: 309-316

Kruger C, Berkowitz O, Stephan UW, Hell R (2002) A metal-binding member of the late embryogenesis abundant protein family transports iron in the phloem of *Ricinus communis* L. J Biol Chem 277: 25062-25069

Lang V, Robertson M, Chandler PM (1998) Allelic variation in the dehydrin gene family of 'Himalaya' barley (*Hordeum vulgare* L.). Theor Appl Genet 96: 1193-1199

Mignone F, Gissi C, Liuni S, Pesole G (2002) Untranslated regions of mRNAs. Genome Biol 3: 0004.1-0004.10

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucl Acids Res 8: 4321-4326

Nylander M, Svensson J, Palva ET, Welin BV (2001) Stress-induced accumulation and tissue-specific localization of dehydrins in *Arabidopsis thaliana*. Plant Mol Biol 45: 263-279

Rodríguez EM, Svensson J, Malatrasi M, Choi DW, Close TJ (2005) Barley *Dhn13* encodes a KS-type dehydrin with constitutive and stress responsive expression. Theor Appl Genet 110: 852-858

Seki M, Narusaka M, Abe H, Kasuga M, Yamaguchi-Shinozaki K, Carninci P, Hayashizaki Y, Shinozaki K (2001) Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell 13: 61-72

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular response to dehydration and low temperature: Difference and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 2: 217-223

Verwoerd TC, Dekker BM, Hoekema A (1989) A small-scale procedure for the rapid isolation of plant RNAs. Nucl Acids Res 17: 2362

Zhu B, Choi DW, Fenton R, Close TJ (2000) Expression of the barley dehydrin multigene family and the development of freezing tolerance. Mol Gen Genet 264: 145-153