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Cloning, Characterization, and Functional Analysis of Maize *DEHYDRIN2*

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ABSTRACT: Dehydrins (LEA D11 proteins) are one of the typical families of plant proteins that accumulate in response to dehydration, cold stress, abscisic acid, or during seed maturation. A 1.3-kb cDNA was cloned from a cDNA expression library of 5-day-old germinating maize scutellums under drought stress. The deduced protein sequence indicated a dehydrin gene encoding SK_3 LEA protein typically expressed during cold acclimation, but not by drought stress in barley and wheat. Thus, it was named maize *DEHYDRIN2* (*ZmDhn2*). It accumulates rapidly and highly in drought-stressed scutellum and leaf tissues at any stage, but not under cold stress. *ZmDhn2* gene was transformed into *Arabidopsis thaliana* for functional analysis under drought condition. From electrolyte leakage test, no significant difference showed between wild type and transformants under normal growth condition, but the leakage level of electrolyte in wild type plants was about 3 times as high as that in the transformed plants under drought stress. It suggests that ZmDHN2 play a role in increasing drought tolerance.

Keywords **:** maize, dehydrin, *ZmDhn2*, drought tolerance, electrolyte leakage, transformation.

Environmental stresses such as drought, salinity, flood, and low temperature extremes, place major limits on plant productivity (Boyer, 1982). To overcome these limitations and improve production efficiency in the face of a burgeoning world population, more stress-tolerant crops must be developed in the near future.

A number of water-deficit-induced gene products are predicted to protect cellular structures from the effects of water loss. The prediction is derived from the deduced amino acid sequences and the characteristics of their expression. Above of all, dehydrin proteins are well known to the prediction. Dehydrins (DHNs) are an immunologically distinct protein family (LEA D11) and typically accumulate during the maturation phase of seed development or in response to low temperature, drought, salinity or ABA application (Close, 1996; Campbell and Close, 1997). Dehydrins are characterized by the highly conserved Lys-rich 15 amino acid consensus K segment (EKKGIMDKIKEKLPG), S segment (Ser residues), Y segment ((V/T)DEYGNP), and repeated units that are rich in polar amino acids (Campbell and Close, 1997; Choi *et al.*, 1999). All data so far are consistent with the formation of an amphipathic α-helix by the K segment. One role of the dehydrin K segment may be hydrophobic interaction with partially denatured proteins or membranes (Julia *et al.*, 2000). The S segment can be phosphorylated, and it has been proposed that phosphrylation is related to the binding of nuclear localization signal peptides and, therefore, to nuclear transport (Goday *et al.*, 1994). The Y segment has significant amino acid sequence relatedness to a portion of the nucleotide binding site of chaperones of plants and bacteria (Martin *et al.*, 1993), but there has as yet been no report of nucleotide binding by dehydrins that contain a Y segment. Distinct subclasses of dehydrins have been noted (Houde *et al.*, 1995), and a YSK nomenclature scheme within the dehydrin family has been developed (Close, 1997). And dehydrins can be further distinguished by the composition of the less conserved amino acid sequences within the N-terminal portion of the protein and between the K segments (Dure, 1993). Using the YSK shorthand, there are five distinct types of dehydrins, which are Y_nSK_2, K_nS, SK_n, Y_2K_n, and K_n types.

Evidences of several lines are consistent with a role of dehydrins in membrane interactions, including immunolocalization data that imply an endomembrane association of a basic YSK_2 dehydrin in the cytoplasm (Egerton-Warburton *et al.*, 1997). Dehydrins can also be present in nuclei (Asghar *et al.*, 1994), which may require phosphorylation (Jensen *et al.*, 1998): The majority of the *LEA* gene products are predominantly hydrophilic, biased in amino acid composition, and lacking in Cys and Trp and are proposed to be located in the cytoplasm (Close, 1996). Immunolocalization studies have shown that dehydrins can be present in the nucleus or cytoplasm. A recent study of wheat acidic-SK_3-type dehydrin, WCOR410, showed that the gene products are located in the vicinity of the plasma membrane (Danyluk *et al.*, 1998). It has been suggested that dehydrins act as stabilizers of membranes under freezing or water-stress conditions. Genetic studies have also been consistent with *Dhn* genes being associated with environmental stress tolerance.

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LEA (Late-embryo-abundant) genes generally belong to small multigene family. They often contain sequence-motifs repeated several times and the different members of a gene family may differ in the number of repeats they possess. In addition, in the same gene family, the expression of different members is usually not tightly coordinated. Moreover, some *LEA* genes are responsive not only to developmental cues but also to environmental factors. One way to understand these complex patterns of expression in *LEA* or *LEA*-related genes is to identify the various members of already identified gene families as well as new gene families, and to analyze their expression in correlation with promoter studies. Although a large number of drought-regulated genes have already been identified in a wide range of plant species, cellular and molecular responses involved in drought tolerance remain largely unknown.

To get a better understanding of the role of a dehydrin gene cloned from germinating maize scutellums, named *DEHYDRIN2* (*ZmDhn2*), we characterized the expression patterns under drought and cold stresses, and performed the functional analysis by transforming *ZmDhn2* into *Arabidopsis*.

MATERIALS AND METHODS

Plant material and stress treatments to seedling

Maize (cv. Suwon19) kernels were obtained from the National Crop Experiment Station, RDA, Korea. After surface sterilization and 1-day imbibition in water, seeds were planted in the moistened vermiculite. Plants were grown in a growth chamber (16-hr light/day, 25°C, 60 to 80% humidity).

For drought treatment, seedlings were pulled out of vermiculite and placed on filter papers and air-dried for different periods. For cold treatment, seedlings were placed in a growth chamber at 4°C for different periods. Each sample was frozen with liquid nitrogen and stored in deep freezer until further analysis.

cDNA cloning of maize *DEHYDRIN2*

Several germination-specific proteins were isolated by comparing a protein subunits of 6-day-old geminating maize scutellums with 30-day-old developing ones (Paek, 1994). Among the proteins, a 63-kD protein was isolated and used to develop polyclonal antibody from a rabbit. To clone the full-length cDNA coding the 63-kD protein, 5-day-old geminating scutellums were used for mRNA preparation for the construction of cDNA expression library (Stratagene, USA). Using antiserum, the approximately 1.3 kb cDNA fragment was isolated and subcloned in pBluescript phagemid. The cDNA were sequenced from both directions using the Prism Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer, USA) and an ABI 377 automated DNA sequencer (Applied Biosystems, USA). Sequences analysis and database searches were carried out with BLAST programs (http://www.ncbi.nlm.nih.gov/ BLAST).

Total RNA extraction, RT-PCR and Northern analysis

Total RNA was extracted using RNcleospin RNA Plant kit (Macherey-Nagel, Germany). The quantification of total RNA was conducted with RiboGreen RNA Quantification Reagent kit (Molecular Probes, USA) using F-4500 fluorescence spectrophotometer (Hitachi, USA). For RT-PCR, the primer sequences of *ZmDhn2* are as following: Forward: 5'-ATG GAG GAT GAG AGG AAC AC-3', and Reverse: 5'-TTA GGA GCT GGT CTT GTG -3'. PCR reactions were performed for 35 cycles, each consisting of 95°C for 30 s, 60°C for 30 s, 72 for 1 min, and terminated at 72°C for 10 min. PCR products were separated on 1.2% agarose gels and stained with ethidium bromide for photography. For Northern analysis, 5 μg of total RNA were separated in denaturing formaldehyde agarose gels and transferred to nylon membranes (Hybond-N, Amersham). Membranes were prehybridized at 63°C in 5x SSPE, 5x Denhardt's solution, 0.2% SDS and 0.1 mg/ml denatured salmon sperm DNA. *ZmDhn2* cDNA was labeled with α-[^{32}P]-dCTP using the Prime-A-Gene labeling kit (Promega, USA). Hybridization was performed at 63°C for more than 16 h. Membranes were washed at 37°C with 2× SSPE/0.5% SDS twice, at 63°C with 1× SSPE/0.5% SDS, at 63°C with 0.1× SSPE/0.5% SDS for 15 min with each solution. Filters were exposed to X-ray film with an intensifying screen at -80°C.

Growth condition of *Arabidopsis*

A few seeds of *Arabidopsis thaliana* ecotype Columbia were placed on approximately 8 cm square pots filled with soil. The plants were grown at 22°C for three weeks under long day condition (16-h light/day) and used for *ZmDhn2* transformation.

Vector construction for transformation

For the transformation of *ZmDhn2* gene into *Arabidopsis*, the gene was amplified with forward primer, 5'-ATT CTA GAA TGG AGG ATG AGA GGA AC-3', and reverse primer, 5'-TAG GAT CCT TAG GAG CTG GTC TTG TG-3', because *Xba*I/*Bam*HI restriction site must be incorpo-

rated in the end sequence of cDNA. The *Agrobacterium tumefaciens* strain GV3101 carrying the binary vector pBI121 (Clonetech, USA) was used for transformation. This vector is carrying the neomycin phosphotransferase II (NPTII) selectable marker and β-glucuronidase (GUS) screening marker genes. For the selection of *Agrobacterium* carrying the appropriate plasmids, the Luria-Bertani (LB) medium was supplemented with 100 μg/ml kanamycin.

Preparation of *Agrobacterium* for vacuum infiltration

A liquid culture of *Agrobacterium* was grown at 28°C in YEP medium (Bacto-peptone 10 g, yeast extract 10 g, NaCl 5 g) containing the kanamycin antibiotics for the selection, to an OD_{600} of 1.6-1.8. The cells were pelleted by 10,000 ×g centrifugation for 5 min at room temperature, and resuspended in vacuum infiltration media (0.5× MS salts, 1× Gamborg's vitamins, 5% sucrose, 0.04 μM BAP, 0.01% silwet L-77, adjusted to pH 5.7) to reach at OD_{600} of 0.8.

Transformation into *Arabidopsis*

To infiltrate the plants, the pots were inverted and the above-ground portion of the plant submerged in a container filled with a suspension of *Agrobacterium* in vacuum infiltration medium. The pot was placed in a vacuum chamber, and a vacuum was drawn for 10 min at a pressure close to 0.05 bar and released very rapidly. The pots were transferred in a cool-white light growth chamber, and covered the whole box with vinyl-wrap for 1 day. Transformed plants (T_0) were grown in normal growing condition and allowed setting seeds. Harvested seeds (T_1, T_2 and T_3) were surface-sterilized and plants were grown *in vitro* on MS medium supplemented with 2% sucrose and 100 μg/ml kanamycin for selection. The growth condition was at 22°C under 16-h light/day. Homozygous transformants were selected in the subsequent generations.

Electrolyte leakage test

Electrolyte leakage tests were conducted as described previously (Jaglo-Ottosen *et al.*, 1998). The detached leaves of non-drought- and drought-conditioning plants were punched about 1 cm diameter, immediately placed in distilled water and washed for 5 min. After samples dried briefly on the sheets of paper towel, they were floated on 5 ml distilled water for 24 h. Electrolyte leakage from leaves was measured with a conductivity meter (Jenway, Germany).

RESULTS

Sequence Analysis of maize *DEHYDRIN2*

Fig. 1 shows the open reading frame (ORF) of 1.3 kb cDNA sequence (GenBank accession No. L35913) and deduced amino-acid sequence. Database searches using deduced

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   1 ctggtctgtt tagttggaaa gccatctgta ctgtacgttt ttcgccgatc atggaggatg
  61 agaggaacac ccagcagcac cagggcggtg agcaggccca ggaccaggag aacgaggtga
 121 aggacagggg actcctggac agccttctcg gcaggaacaa gcacgacgac caggagaaga
 181 agaaccagca ggaggaggag gagctcgcga ccggcatgga gaaggtcacg gtggctgagc
 241 ccgaccacaa ggaggaggga cacgaggccg ccgagaagaa ggacagcctt ctcgccaagc
 301 tgcaccgcac cagctccagt tccagctcgt cgagcgacga cgaggaagag gaggtgatcg
 361 atgagaacgg cgaaattgtc aagaggaaga agaagggcct taaggagaag gtcaaggaga
 421 agtcggcggc ccacaaggcc cacgatgagg gcgaccacca ccagccgggc gtacctgccc
 481 cggcgcccgc accgcccgtg gcggtggaca cgcatgctca ccaccaggag ggagagcaca
 541 agccgcactt ccccggcgccg gcgcctcccc cgcacgtgga gacgcaccac cccgtcgtcg
 601 tccacaagat cgaggacgac gacacgaaga ctcagacccc accacaggca ccggaggagg
 661 agaagaaagg cctgctggac aagatcaagg agaagctacc cggtggccac aagaagccgg
 721 aagacgctgc tgccgccgcc gccgcgccgg ccgtccacgc gccaccgccg ccggcgcccc
 781 acgccgaggt cgacgtcagc agcccggatg gcaagaaggg cttgctgggc aagatcatgg
 841 acaagatacc cggctaccac aagagctcgg gtgaagaaga ccgcaaggac gccgccggcg
 901 agcacaagac cagctcctaa ggtcgcagcg tgtgcgtgtc cgtcgtacgt tctggccggc
 961 cgggccttgg gcgcgcgatc agaagcgttg cgttggcgtg tgtgtgcttc tggtttgctt
1021 taattttacc aagtttgttt caaggtggat cgcgtggtca aggcccgtgt gctttaaaga
1081 cccaccggca ctcgcagtga gtgttgctgc ttgtgtaggc tttggtacgt atgggcttta
1141 tttgcttctg gatgttgtgt actacttggg tttgttgaat tattatgagc agttgcgtat
1201 tgtaattcag ctgggctacc tggacattgt tatgtattaa taaatgcttt gctttcttct
1261 aaagatcttt aagtgct 1277

   1 MEDERNTQQH QGGEQAQDQE NEVKDRGLLD SLLGRNKHDD QEKKNQQEEE ELATGMEKVT
  61 VAEPDHKEEG HEAAEKKDSL LAKLHRTSSS SSSSSDDEEE EVIDENGEIV KRKKKGLKEK
 121 VKEKSAAHKA HDEGDHHQPG VPAPAPAPPV AVDTHAHHQE GEHKPHFPAP APPPHVETHH
 181 PVVVHKIEDD DTKTQTPPQA PEEEKKGLLD KIKEKLPGGH KKPEDAAAAA AAPAVHAPPP
 241 PAPHAEVDVS SPDGKKGLLG KIMDKIPGYH KSSGEEDRKD AAGEHKTSS 289
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Fig. 1. The cloned cDNA sequence and open reading frame (51-920) of *ZmDhn2* (GenBank accession No. L35913).

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Maize-Dhn2      MEDERNTQQHQGGEQAQDQEN--EVKDRGLLDSLLGRNKHDDQEKKNQQEEEELATGMEKVTVAEPDHKE----EGHEAAEKKDSLLAKL
Wheat-Cor410    MEDERSTQSYQGG----EAAEQVEVTDRGLLGNLLGKK--KAEEDKEK--EEELVTGMEKVSVEEPEVKK----EEHEDGEKKETLFSKL
Wheat-Wcor410c  MEDERSTQSYQGG----EAAEQVEVTDRGLLGNLLGKK--KAEEDKEK--QEELVTGMEKVSVEEPEVKK----EEHEDGEKKETLFSKL
Wheat-Wcor410b  MEDERSTQSYQGG----EAAEQVEVTDRGLLGNLLGKK--KAEEDKEKK-EEELVTGMEKVSVEEPEVKK----EEHVDGEKKETLFSKL
Barley-Dhn8     MEDERSTQSYQG-----AEADQVEVTDRGLLGNLLGKK--KEEEDKKK--EEELVTGMEKVSVEEPEVK--------EDGEKKETLFSKL
Maize-Dhn1      MEYGQQGQHGHGATG---HVDQYGNPVGGVEHGTGGMR-----------HGTGTGGMGQLGEHGGAGMG---GGQFQPAREEHKTGGIL

Maize-Dhn2      HRTSSSSSSSSDDEEEE-VIDENGEIVKRKKK-GLKEKVKEKSAAHKAHDEGDHHQPGVPAPAPAPPVAVDTHAHHQEGEHKPHFPAPAP
Wheat-Cor410    HRSSSSSSSSSDEEEEE-VIDDNGEVIKRKKKKGLKEKLQGKLPGHKDTE----GEHVTGLPAPAAPASVQTHGGHH------------
Wheat-Wcor410c  HRSSSSSSSSSDEEEEE-VIDDNGEVIKRKKKKGLKEKLKEKLPGHKDTE----GEHVTGLPAPAAPASVQTH--H------------
Wheat-Wcor410b  HRSSSSSSSSSDEEEEE-VIDDNGEVIKRKKKKGLKEKLQEKLPGHKDTE----GEHVTALPAPAAPASVQTH--H------------
Barley-Dhn8     HRSSSSSSSSSDEEEEEEVIDENGEVIKRKKKKGLKEKLKEKLPGHKDNE----AEHVTGLPAPMAPASVQTH---H------------
Maize-Dhn1      HRSGSSSSSSSE--------DDGMG--GRRKK-GIKEKIKEKLPGGHKDD-----QHATATTGGAYGQQGHTG----------------

Maize-Dhn2      PPHVETHHPVVVHKIEDDDTKTQTPPQAPEEEKKGLLDKIKEKLPGGHKK-P--------EDAAAAAA-APAVHAPPPPAPHAEVDVSSP
Wheat-Cor410    -------DTDVVVEKIDGDVKTEAAPAVPEEEKKGFLEKIKEKLPGGHKKPE--------DAAAVPVTHAAPAPVHAPVPAPEEVSSPDA
Wheat-Wcor410c  -------DTDVVVEKIDGDVKTEAAPAVPEEEKKGFLEKIKEKLPGGHKKPE--------DAAPVPVTHAAPAPVHAPAPAAEEVSSPDA
Wheat-Wcor410b  -------DTDVVVEKIDGDVKTEATPAVPEEEKKGFLEKIKEKLPGGHKKPEDAAAVPVTHAAPAPVTHAAPAPVHAPAPAAEEVSSPDA
Barley-Dhn8     -------DTDVVVEKIDGDAKTEATPAVPEEEKKGFLEKIKEKLPGGHKKPE--------DAAAVPVTHAAPAPVHAPAPAAEEVSSPDA
Maize-Dhn1      ----------SAYGQQGHTGGAYATGTEGTGEKKGIMDKIKEKLPGQH-----------------------------------------

Maize-Dhn2      DGKKGLLGKIMDKIPGYHKSSGEED--RKDAAGEHKTSS   289
Wheat-Cor410    KEKKGLLGKIMDKLPGYHKTGEEDK--AAAATGEHKPSA   262
Wheat-Wcor410c  KEKKGLLGKIMDKLPGYHKTGEEDK--AAAAAGEHKPSA   259
Wheat-Wcor410b  KEKKGLLGKIMDKLPGYHKTGEEDK--AAAATGEHKPSA   268
Barley-Dhn8     KEKKGLLGKIMDKLPGYHKTGEEDK--AAAPSGEHKPRA   255
Maize-Dhn1      ----------------------------------------   167 (partial cDNA sequence)
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Fig. 2. The comparison of amino acid sequences between *ZmDhn2* and the homologous genes from barley and wheat using PIMA (Multiple sequence alignment: http://dot.imgen.bcm.tcm.edu:9331).

Table 1. The comparison of amino acid composition between *ZmDhn2* and other homologous dehyrin genes. Unit: %

	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu
M-Dhn2	0.1000	0.0000	0.0724	0.1107	0.0000	0.0759	0.0829	0.0207	0.0310	0.0552
M-Dhn1	0.0539	0.0000	0.0359	0.0659	0.0060	0.5390	0.0778	0.0299	0.7780	0.0240
B-Dhn8	0.0941	0.0000	0.0627	0.1686	0.0078	0.0941	0.0392	0.0196	0.1490	0.0627
W-cor410	0.0916	0.0000	0.0611	0.1641	0.0076	0.9160	0.0458	0.0191	0.1412	0.0611
W-wcor410b	0.1082	0.0000	0.0597	0.1604	0.0075	0.1082	0.0448	0.0187	0.1418	0.0597
W-wcor410c	0.1004	0.0000	0.0618	0.1660	0.0077	0.1004	0.0425	0.0193	0.1467	0.0618
	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
M-Dhn2	0.0138	0.0138	0.0931	0.0414	0.0207	0.0587	0.0379	0.0586	0.0000	0.0034
M-Dhn1	0.0359	0.0060	0.0240	0.0778	0.0299	0.0539	0.0778	0.0180	0.0000	0.0299
B-Dhn8	0.0157	0.0118	0.0706	0.0157	0.0196	0.0667	0.0431	0.0706	0.0000	0.0078
W-cor410	0.0150	0.0076	0.0687	0.0191	0.0153	0.0649	0.0458	0.0763	0.0000	0.0078
W-wcor410b	0.0112	0.0075	0.0709	0.0187	0.0149	0.0634	0.0522	0.0784	0.0000	0.0075
W-wcor410c	0.0116	0.0077	0.0695	0.0193	0.0154	0.0656	0.0425	0.0734	0.0000	0.0077

M: maize, B: barley, W: wheat

amino-acid sequences indicated in every case the highest homology to *DHN/COR/RAB* and other LEA proteins (Fig. 2). The cloned cDNA has a SK_3 type among dehydrins, which is homologous to *WCOR410* in wheat (Danyluk *et al.*, 1998), *Dhn8* and *Paf93* in barley (Choi *et al.*, 1999; Grossi *et al.*, 1995), *ci7* in potato (Kirch *et al.*, 1997). As described by Close (1996) that the most of the LEA proteins are lacking in Cys and Trp, the protein is lack of Cys and Trp in the same manner (Table 1). As a result, we concluded that the cloned cDNA is a dehydrin and thus named *DEHYDRIN2* (*ZmDhn2*).

Expression patterns of *ZmDhn2* gene

To determine the expression pattern of *ZmDhn2* gene, the shoots and roots of maize seedlings collected from the conditions of non-stress (control), dehydration, or low temperature were analyzed by northern blot experiments. The transcripts was gradually accumulated and peaked at 5 days after germination (DAG) and gradually decreased thereafter (Fig. 3A). When maize seedlings were stressed by dehydration, *ZmDhn2* transcripts were accumulated rapidly and highly (Fig. 3B). However, *ZmDhn2* gene was not induced in cold-stressed condition within a few days (Fig. 3C). It suggests that *ZmDhn2* is expressed at a detectable basal level under normal growth condition and up-regulated by drought condition at Leaf and scutellum tissues. The results indicate that expression of *ZmDhn2* gene is differentially regulated under dehydration and cold condition.

Transformation into *Arabidopsis*

To confirm whether *ZmDhn2* gene product has a function of

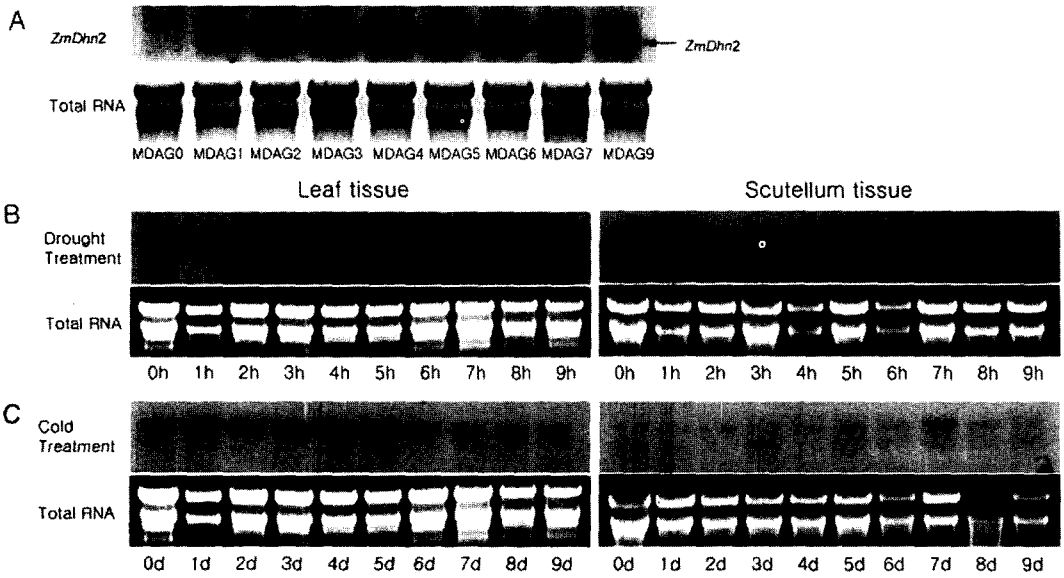

Fig. 3. Expression patterns of *ZmDhn2* gene in maize leaf and scutellum tissues under non-stressed, drought, and cold conditions. A: Expression of *ZmDhn2* in scutellum during normal germination. B and C: Effect of drought and cold treatments on the expression of *Dhn2* gene. Five-day-old seedlings were submitted to drought treatment (RH 90%, air-dried) during each time (B) and cold treatment (4°C) during each day (C).

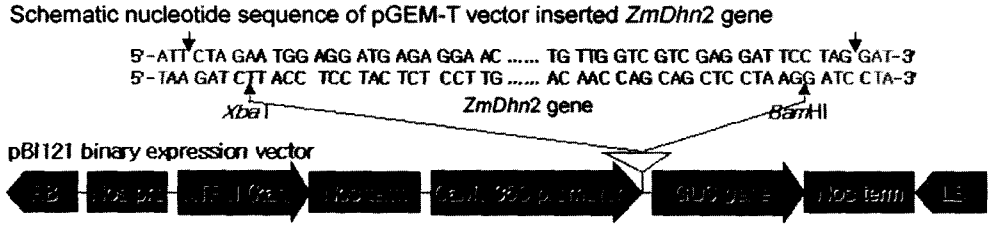

Fig. 4. Vector construction of *ZmDhn2* insertion into pBI121 for *Agrobacterium* transformation.

drought tolerance, *ZmDhn2* gene was transformed into *Arabidopsis* and analyzed phenotypes under drought condition. Vector construction was performed as Fig. 4. When *Arabidopsis* had flower buds and a few open flowers, they were submerged in a suspension of *Agrobacterium* and the bacteria were vacuum-infiltrated into the plant. Seeds (T_1) were obtained from five transformed plants and tested by kanamycin media. Kanamycin-resistant plants were selected and the T_2 seeds were harvested. All of T_2 plants show 3 kanamycin-resistant: 1 kanamycin-susceptible segregations at each transformants. When T_3 seeds were tested with kanamycin for selection, homozygous *ZmDhn2* transformed lines appeared and, for further confirmation, PCR tests are conducted with *ZmDhn2* specific primers (data not shown). From these experiments, we obtained eleven transformants with homozygous *ZmDhn2*.

Drought response of transformants with ZmDhn2 gene

The selected transformants were tested whether they are

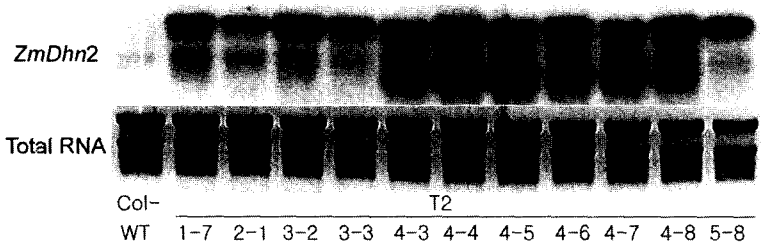

Fig. 5. Confirmation of *ZmDhn2* in eleven transformed lines of *Arabidopsis*.

tolerant to drought stress. To examine the levels of *ZmDhn2* transcript, northern blot analysis was conducted. *ZmDhn2* gene was not expressed in the non-transformed wild-type plants, but accumulated at different level in each transformed line (Fig. 5).

When the wild type and eleven lines of transformed plants that had been grown for 3 weeks were transplanted to dry soil under RH 80%, only T3-1-7 transformed plants were the most tolerant in response to drought stress (Fig. 6A). To determine whether the *ZmDhn2* gene also affects drought tolerance physiologically, electrolyte leakage test was compared between the wild type and T3-1-7 lines. There is no significant difference between the wild type and T3-1-7 transformants under non-drought condition, but the leakage level of electrolyte in the wild type plants was about 3 times as high as T3-1-7 line under drought stress (Fig. 6B).

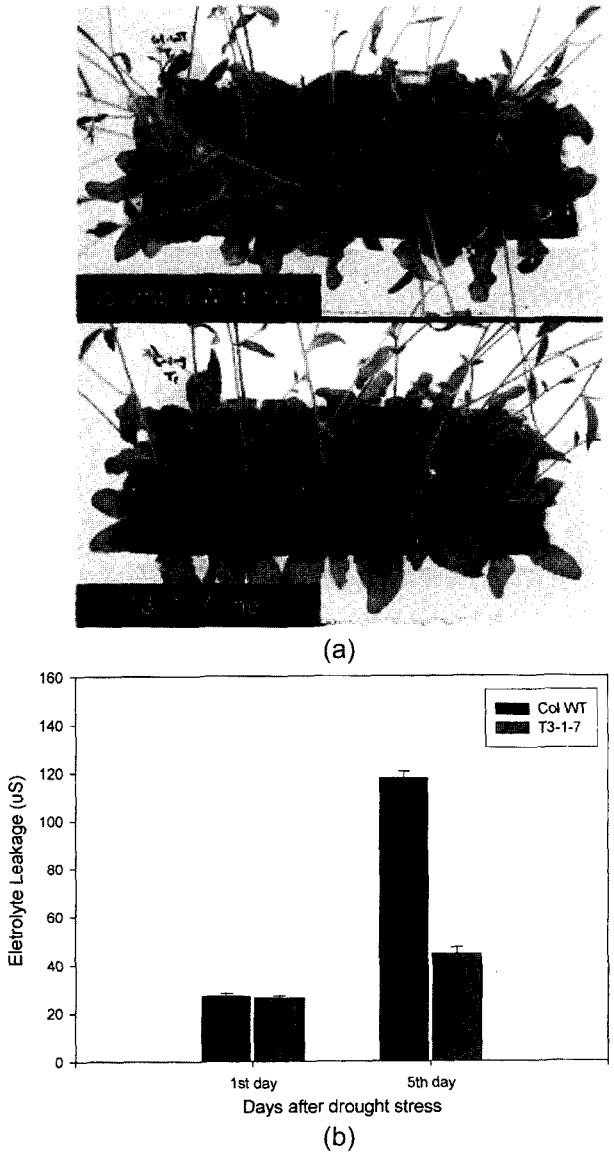

Fig. 6. The phenotypic and physiological effects of *ZmDhn2* gene on drought stress. A: Phenotypic difference between non-transformed plants and *ZmDhn2* tranformed plants (T_3-1-7) under drought stress for 5 days. B: Electrolyte leakage test between the wild type and *ZmDhn2* transformants under drought treatment for 1 and 5 days.

DISCUSSION

Here we cloned a full-length cDNA whose protein sequence indicated the highest homology to DHN/COR/RAB and other LEA proteins by comparison to the known genes from GenBank database search engine. Especially, the cloned cDNA was SK_3-type dehydrin lacking of Cys and Trp, which is homologous to *WCOR410* in wheat (Danyluk *et al.*, 1998), *Dhn8* and *Paf93* in barley (Choi *et al.*, 1999; Grossi *et al.*, 1995), *ci7* in potato (Kirch *et al.*, 1997). Thus, we designated the newly cloned gene from maize *DEHYDRIN2* (*ZmDhn2*). From northern analysis, *ZmDhn2* transcript accumulated in dehydration-stressed scutellum and leaf tissues. Whereas the levels of dehydrin transcript continued to decrease as seedling grows in other plants, *ZmDhn2* transcript was first observed at 3 DAG, peaked at 5 DAG, and decreased thereafter. In addition, the expression of *ZmDhn2* differed from the similar genes of wheat (*WCOR*410) and barley (*HvDhn*8) in that *ZmDhn2* transcript was not accumulated under cold-stressed condition (Fig. 3C). Kirch *et al.* (1997) suggested that the stability of *ci*7 transcripts increased at low temperature, and that the turnover of CI7 protein is also controlled in a temperature-dependent manner. In order to avoid the cellular damage, dehydrin proteins might help minimize, and the post-transcriptional control may be an important means of rapidly producing these proteins when plants are exposed to stress conditions.

The *ZmDhn2* gene was transformed into *Arabidopsis thaliana* for analysis of drought tolerant response, and 11 independent homozygous transformed-lines were established. We examined the levels of expression and selected the drought tolerant line under drought condition. Cell membranes are one of the first target of plant stressed (Levitt, 1980). Alteration of membrane structure under water stress was first observed by Iljin (1957). These alterations are caused by a modification of cellular compartmentation (Vieira da Silva, 1976) linked to an increase in the activity of hydrolytic enzyme (Stocker, 1961). One of the consequences of the loss of membrane integrity after water stress is an increase in the cell permeability (Senaratna and Mc Kersie, 1983). This leads to an increase in ion efflux (Levitt, 1980). When a selected transformed plant was analyzed by electrolyte leakage test, there is no significant difference from the wild-type plant under non-drought condition, but

the leakage amount of electrolyte in wild type plants was about 3 times as high as that in the transformed plant under drought stress. This result suggests that *ZmDhn2* gene in maize product act on membrane stability under drought condition while in wheat and barley under cold condition. Although *ZmDhn2* has no known enzymatic function, the induction of *ZmDhn2* expression under drought stress suggests that they may have an adaptive role in plant survival (Close, 1996). As yet, this role is unclear, but our current evidence suggests that they act as stabilizers for cellular macromolecules under low water condition. It is remained to determine what are the up-regulatory element and function at protein level.

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