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Rapid Upregulation of *Dehyrin3* and *Dehydrin4* in Response to Dehydration Is a Characteristic of Drought-Tolerant Genotypes in Barley

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The identification of molecular markers and marker-aided selection are essential to the efficient breeding of drought-tolerant plants. However, because that characteristic is controlled by many quantitative trait loci, such markers that can screen and trace desirable barley genotypes in a segregating population or germplasm have not yet been determined. Relative water content has been used to estimate drought tolerance in plants because it is highly correlated with the drought index of yield. To develop reliable gene-specific markers for identifying tolerant versus susceptible genotypes, we performed suppression subtractive hybridization to identify candidate genes. We used two domestic barley cultivars, one having the highest RWC (drought-tolerant 'Chalbori') and the other having the lowest (drought-susceptible 'Daebaekbori'). In response to dehydration at the early seedling stage, rapid upregulation of *Dehydrin3* (*Dhn3*) and *Dhn4* occurred in the drought-tolerant genotypes, but not in the susceptible ones. Similar results were obtained with mature plants growing under frequent drought stress in the greenhouse. In addition, *Dhn3* and *Dhn4* conferred higher drought tolerance when they were over-expressed in transgenic *Arabidopsis*. Thus, in addition to using assessments of RWC, we propose that *Dhn3* and *Dhn4* expressions can serve as drought-induced gene-specific markers to determine drought-tolerant barley genotypes at the seedling stage.

Keywords: barley, *Dhn3*, *Dhn4*, drought tolerance, molecular marker, suppression subtractive hybridization

Soil water deficit is one of the most severe environmental stresses limiting stable crop production worldwide (Boyer, 1982). It negatively affects nearly all aspects of metabolism during stages of growth and development, causing depreciated crop yields when plants are unable to produce adequate dry matter. Conventional breeding efforts to improve tolerance would be aided if researchers could identify efficient biochemical and/or molecular markers associated with improved field performance under drought conditions.

To cope with unfavorable environments, higher plants as sessile organisms can respond immediately to stresses through signaling processes and changes in their gene expression. A particular set of genes is rapidly induced in response to osmotic stresses caused by drought or salinity, as well as low-temperature stress (Bray, 1993; Iuchi et al., 1996; Cellier et al., 1998; Seki et al., 2001; Chen et al., 2002). Drought-inducible genes can be divided into two groups encoding functional or regulatory proteins (Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997). Functional proteins play important roles in protecting cells against dehydrative damage, via water channels in cell membranes, osmolytes, chaperones, LEA proteins, proteinases, and detoxifying enzymes. Regulatory proteins are involved in the regulation of signal transduction, which induces a set of genes encoding functional proteins for transcription factors, protein kinases, and enzymes for phosphoinoside metabolism.

Among these drought-inducible proteins, the late-embryogenesis-abundant proteins were first described through studies of genes expressed abundantly during the maturation phase of seeds, when embryos are being dehydrated prior to entering dormancy (Baker et al., 1988; Dure, 1993). *LEA* genes generally belong to a small multigene family; the LEA proteins often contain sequence motifs that are repeated several times. Different members of an LEA family can vary in the number of those repeats (Campbell and Close, 1997). In addition, even within the same gene family, the expression of different members is not tightly coordinated because some *LEA* genes are responsive not only to developmental cues but also to environmental factors. For example, dehydrin genes (*Dhn*, LEA D11 family) are expressed not only in seeds during late embryogenesis but also in seedlings or more mature tissues in response to drought, low temperature, high salt, or ABA applications (Close et al., 1989; Close, 1997; Choi et al., 1999; Choi and Close, 2000). The amino acid sequences of dehydrin proteins (DHNs) are very similar to one another and to previously identified rice proteins, e.g., RAB, which are induced by either ABA or high salt (Robertson and Chandler, 1992). Campbell and Close (1997) have postulated that DHNs are directly associated with traits necessary for adaptation to winter hardiness as well as to the capability for yield retention under drought stress. Moreover, DHNs may be directly involved in the stability of the plasma membrane during seed desiccation and dormancy (Houde et al., 1995; Thomashow et al., 1996). However, the biological functioning of each DHN has not yet been determined, especially in response to drought stress.

Relative water content is an appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. RWC, through its relationship to cell

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volume, may more closely reflect the balance between water supplies to leaves and water loss from transpiration under drought stress (Schonfeld et al., 1988). Thus, RWC has long been used for estimating relative drought tolerance among crop plants, having first been termed as 'relative turgidity' (Barrs and Weatherley, 1968).

Although several physiological and genetic mechanisms of drought tolerance have been investigated in many species, an efficient method for selecting drought-tolerant genotypes has not yet been established, possibly due to its experimental difficulty and reliability. Furthermore, the field performance of drought-tolerant crop plants is mainly controlled by quantitative trait loci. Thus, it would be helpful to plant breeders if researchers could identify reliable, gene-specific markers that are induced only in drought-tolerant genotypes in response to water deficit. Here, we investigated the correlation among RWC, drought index of yield, and *Dhn3* and *Dhn4* expressions in tolerant genotypes of barley. We also examined the degree of tolerance in transgenic *Arabidopsis* plants over-expressing *Dhn3* or *Dhn4*. Our objective was to determine whether upregulation of *Dhn3* and *Dhn4* in dehydrated seedlings could serve as a practical molecular marker for screening and tracing tolerant genotypes in conventional barley breeding programs.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

Based on our previous results (Fu et al., 2003a, b), we selected the following cultivars of barley (*Hordeum vulgare* L.): Korean drought-tolerant 'Samdobori', 'Chalbori', and 'Dongbori-1'; Korean drought-susceptible 'Suwonmac-360', 'Wolsung87-31', and 'Daebaekbori'; and US drought-tolerant 'Dicktoo'. All plants were reared in a growth chamber, where they were placed in soil-filled plastic pots under cool white fluorescent light (150 mol m^{-2} s^{-1}; 16-h photoperiod) at 20°C. For the dehydration treatment, the leaf blades of seedlings at 5 d post-emergence were detached and placed on dry filter paper in plastic boxes, which were then sealed and held at room temperature (25°C) for 2 or 12 h. To determine a drought index of yield in the greenhouse before grain harvest, one-month-old plants were subjected to frequent water deficit by irrigating only when the soil was nearly dry and most plants were stressed by dehydration. Control plants were grown under continuous, water-sufficient soil conditions.

Relative Water Content

Discs (1.5 cm diam.) were cut, with a metal borer, from the mid-leaf sections on mature plants that had been frequently stressed in the greenhouse. To measure RWC, the fresh weight (FW) of each disc was recorded immediately. Afterward, the discs were floated on distilled water for 4 h in a covered box, rapidly blotted with a paper towel, and measured for their turgid weight. Finally, they were wrapped in 3MM filter paper and oven-dried at 60°C for 24 h to obtain dry weights. RWC was calculated using the formula of Barrs and Weatherley (1968):

$$RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100$$

where, FW = fresh weight, TW = turgid weight, and DW = dry weight.

Suppression Subtractive Hybridization and Differential Screening

Total RNA was extracted with a Nucleospin RNA Plant kit (Macherey-Nagel, Germany), and the poly(A)-enriched RNA was purified by an mRNA purification kit (Ambion, USA). The cDNAs were synthesized using M-MLV reverse transcriptase (Promega, USA), according to the manufacturer's protocol. Suppression subtractive hybridization was performed between the drought-tolerant 'Chalbori' as tester and the drought-susceptible 'Daebaekbori' as driver, using a PCR-Select cDNA Subtraction Kit (Clontech, USA) according to the manufacturer's manual. A subtractive cDNA library was established using secondary PCR products from the forward subtraction, cloned by the T/A cloning system (Promega, USA), and transformed into *E. coli* strain JM109. These were arrayed on a nylon membrane using the Micro-Grid (Genomic Solution, USA). Bacterial colonies were grown on this membrane, then denatured by the NaOH method for dot blotting. A forward-subtracted probe was the secondary PCR product of the forward-subtracted cDNA, while the unsubtracted tester probe was the tester cDNA. Each [α-^{32}P] dCTP-labeled probe was separately hybridized to determine possible gene expression in 'Chalbori' and 'Daebaekbori'.

One-Step Semi-Quantitative RT-PCR

Total RNA was isolated from leaf tissues with a Nucleospin RNA Plant kit (Macherey-Nagel, Germany). To evaluate the expression levels of *Dhn* genes, one-step semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed, using 2 μg of total RNA and gene-specific primer sets (50 pmole per primer). These were added to 50 μL of AccuPower RT/PCR PreMix (Bioneer, Korea). RT was conducted at 42°C for 50 min before 25 cycles of PCR amplification were carried out, with 60°C set as the annealing temperature.

Arabidopsis Transformation and Drought Stress Conditions

Using linker-attached, gene-specific primer sets, we amplified the full-length ORFs of *Dhn3* and *Dhn4* via RT-PCR, with total RNA of 'Chalbori' and the T/A cloning kit (Promega). The *35S:Dhn3* and *35S:Dhn4* transgenes in the recombinant pMBP-1 binary vector were transformed into *Arabidopsis thaliana* (Columbia ecotype) by a simplified flip dipping method with *Agrobacterium tumefaciens* strain GV3101 (Clough and Bent, 1998). Transgenic *Arabidopsis* plants were selected on a 0.5x GM medium containing 100 μM kanamycin, and then 6 *Dhn3* and 13 *Dhn4* transformants were finally selected by RT-PCR. Four-day-old seedlings were grown on a standard GM medium and transplanted onto a GM medium containing 500 mM mannitol in order to examine their drought tolerance. In addition, both wild-type and transgenic plants were reared for 4 weeks in soil in a growth chamber at 22°C, with 60%

humidity, cool white fluorescent light (100 mol m^{-2} s^{-1}) and a 16-h photoperiod. To induce frequent drought stress conditions, WT and transgenic plants were grown in 2 cm of soil, with sufficient water supplied for one week followed by only minimal irrigation when the soil was nearly dried and the plants appeared to be severely stressed by dehydration. Fresh and dry weights of the WT and transgenic plants were measured at 4 weeks after germination.

RESULTS

RWC and Drought Index of Yield

Using the controlled environment of a greenhouse, we investigated the relationship between relative water content and the drought index of yield for six domestic barley cultivars (Fig. 1). Under frequent water deficit, RWC varied among cultivars, ranging from 69% in drought-susceptible 'Daebaekbori' to 94% for the drought-tolerant 'Chalbori' and 'Dongbori-1'. Drought indices of yield were higher when RWC values also were elevated, demonstrating significant correlation (r^2=0.84) between these two parameters, as had been previously described with 36 barley varieties (Fu et al., 2003b).

Identification of Candidate Genes by SSH and Differential Screening

The molecular technique of SSH has been used to isolate differentially expressed genes from two independent RNA populations (Diatchenko et al., 1996, 1999). Using seedlings stressed by dehydration for 12 h, we subtracted the drought-susceptible 'Daebaekbori' cDNAs from the drought-tolerant 'Chalbori' cDNAs. After SSH and differential screening, we initially isolated 3072 bacterial colonies, which were placed on duplicated nylon membranes and then hybridized with a forward-subtracted probe or an unsubtracted tester probe

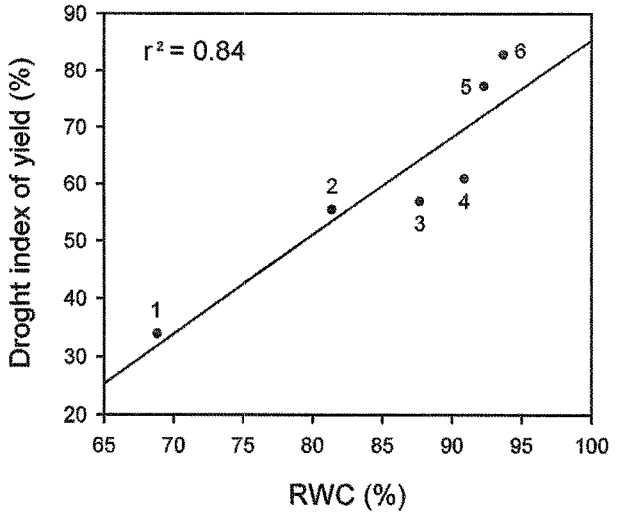

Figure 1. Correlation between RWC and drought index of yield among six domestic barley cultivars. Drought index of yield (%) = Drought yield × 100/Control yield: 1, 'Daebaekbori'; 2, 'Suwonmac-360'; 3, 'Wolsung87-31'; 4, 'Samdobori'; 5, 'Dongbori-1'; 6, 'Chalbori'.

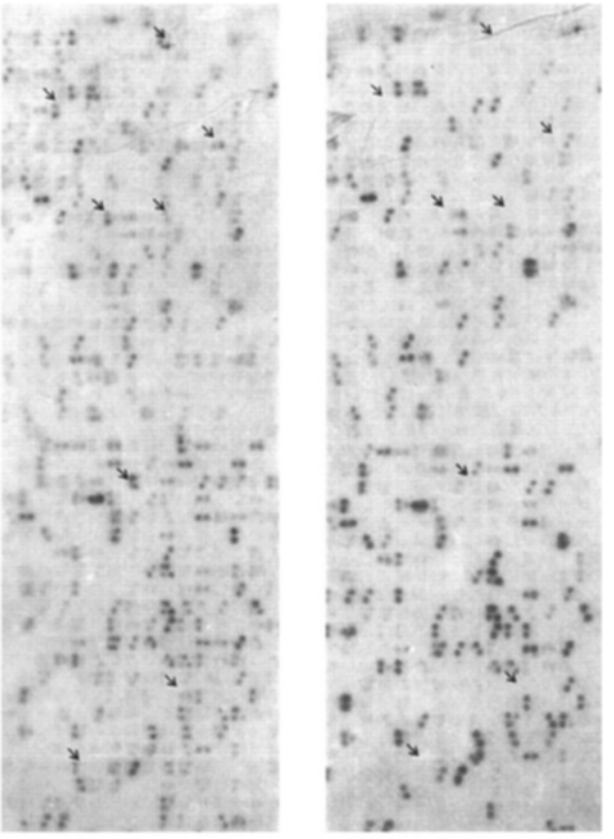

Figure 2. Differential screening to identify candidate genes highly expressed in drought-tolerant barley. Left panel, hybridization with forward-subtracted probe; right panel, with unsubtracted tester probe. Arrows indicate genes differentially expressed between tolerant 'Chalbori' and susceptible 'Daebaekbori' after 12 h of drought stress at early seedling stage.

(Fig. 2). Based on the presence or absence of a hybridization signal on the duplicated blots, we finally selected 6 out of 32 candidate genes by DNA sequencing, and repeated the RNA dot blot analyses (data not shown). These included *DnaJ*, *RbcL*, *RbcS*, *Lipid transfer protein* (*Ltp*), *Cab*, and *Dhn3*, of which *RbcL*, *RbcS*, *DnaJ*, and *Cab* are not specifically drought-inducible genes. Instead, it is more likely that, under severe drought stress at the seedling stage, their expression was repressed in the drought-susceptible 'Daebaekbori' rather than being highly induced in the drought-tolerant 'Chalbori'.

To verify their differential expression by drought stress, we performed northern blot analyses with total RNA isolated from the dehydrated seedlings (Fig. 3), and determined that expression levels of *RbcS*, *Ltp*, and *Dhn3* were significantly higher in the drought-tolerant 'Chalbori' than in the drought-susceptible 'Daebaekbori'. *Dhn3* expression in particular was highly induced by dehydration in the tolerant cultivars, but not in the susceptible ones, indicating its association with drought tolerance (Fig. 4). However, *Ltp* also was rapidly induced in other drought-susceptible cultivars, suggesting no correlation between its expression and tolerance at the seedling stage (data not shown).

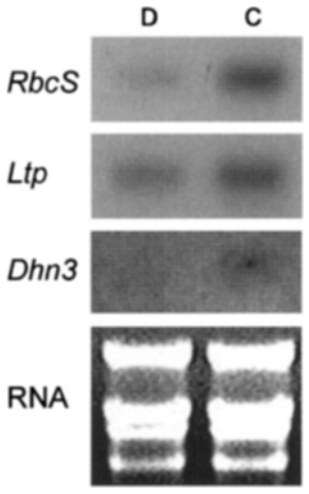

Figure 3. Northern blot analysis of three candidate genes identified by differential screening. Ethidium bromide staining of total RNA is shown as loading control (lower panel). D, 'Daebaekbori'; C, 'Chalbori'.

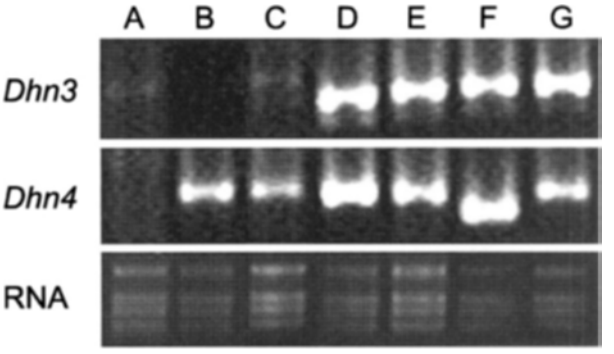

Figure 5. Expression levels of *Dhn3* and *Dhn4* under water deficit conditions, determined by semi-quantitative RT-PCR. One-month-old plants grown at 25°C were subjected to frequent drought stress in greenhouse. A, 'Daebaekbori'; B, 'Suwonmac-360'; C, 'Wolsung87-31'; D, 'Samdobori'; E, 'Dongbori-1'; F, 'Chalbori'; G, 'Dicktoo'.

Dhn3 and *Dhn4* Gene Expression Is Related to Drought Tolerance in Barley

The *Dhn* multigene family in barley is highly induced under dehydration, high salt, low temperature, or the application of ABA, but is not expressed under normal conditions (Choi et al., 1999; Choi and Close, 2000). Here, we used semi-quantitative RT-PCR to investigate transcript levels of 12 *Dhn* genes in the dehydrated seedlings of drought-tolerant and -susceptible cultivars. As reported previously, none of those genes were expressed in our unstressed seedlings (Choi et al., 1999; Choi and Close, 2000). Furthermore, after 2 or 12 h of dehydration, expression of *Dhn3* and *Dhn4* was very low or undetectable in the susceptible 'Daebaekbori', 'Suwonmac-360', and 'Wolsung87-31' (Fig. 4, lanes A-C). In contrast, both genes were rapidly and highly expressed in tolerant 'Samdobori', 'Chalbori', 'Dongbori-1', and 'Dicktoo' (Fig. 4, lanes D-G). However, the other *Dhn* genes (*Dhn1*, *2*, and *5* through *12*) were not especially expressed in the tolerant cultivars, with some being highly induced in all seven cultivars within 2 and/or 12 h of drought treatment while others were highly expressed in both the susceptible 'Daebaekbori' and the tolerant 'Dicktoo' (data not shown). This indicated that their expressions were not entirely relevant in drought-tolerant barley genotypes.

In mature plants that were frequently drought-stressed in the greenhouse, expression of *Dhn3* was very low or undetectable in the susceptible 'Daebaekbori', 'Suwonmac-360', and 'Wolsung87-31' (Fig. 5, lanes A-C), while both *Dhn3* and *Dhn4* were highly expressed in the tolerant 'Samdobori', 'Dongbori-1', 'Chalbori', and 'Dicktoo' (Fig. 5, lanes D-G). These results demonstrated that the expression patterns for *Dhn3* and *Dhn4* at the mature leaf stage, as induced by this frequent stress, were similar to those detected at the early seedling stage, when plants were undergoing dehydrative stress in the growth chamber. Therefore, we might conclude that both these induced expressions are closely associated with drought tolerance in barley genotypes.

Barley *Dhn3* and *Dhn 4* Overexpression Confers Drought Tolerance in Transgenic *Arabidopsis*

To confirm the role of DHN3 and DHN4 proteins *in vivo* under drought conditions, and to evaluate possible applica-

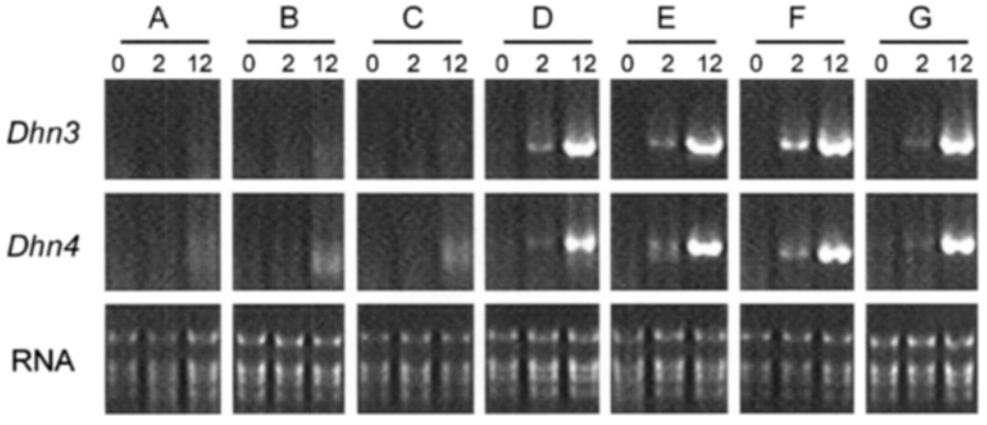

Figure 4. Expression levels of *Dhn3* and *Dhn4* under drought stress, determined by semi-quantitative RT-PCR. Five-day-old barley seedlings were dehydrated for indicated hours. A, 'Daebaekbori'; B, 'Suwonmac-360'; C, 'Wolsung87-31'; D, 'Samdobori'; E, 'Dongbori-1'; F, 'Chalbori'; G, 'Dicktoo'.

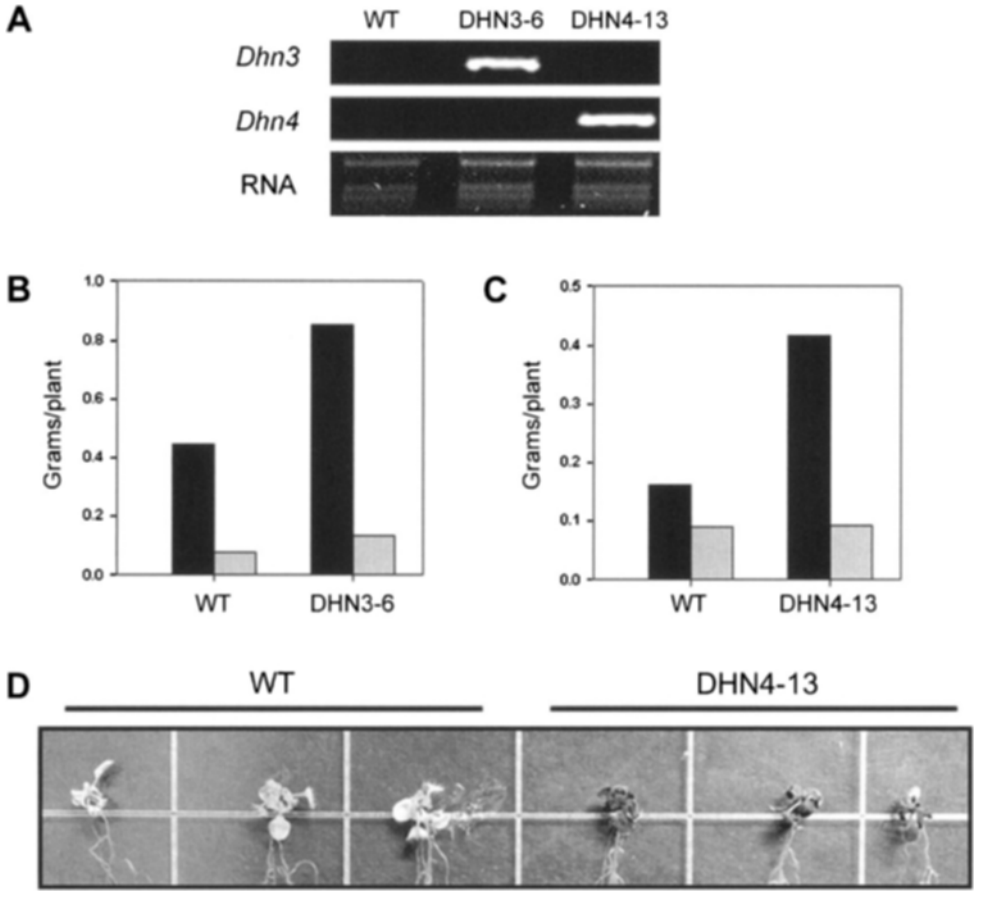

Figure 6. Overexpression effect of *Dhn3* and *Dhn4* genes on drought tolerance in transgenic *Arabidopsis*, as confirmed by one-step semi-quantitative RT-PCR. Two transgenic lines, DHN3-6 and DHN4-13, showing the highest expression of *Dhn3* and *Dhn4*, respectively, were chosen for analysis (**A**). Wild-type and transgenic plants were grown in same soil, and subjected to frequent drought stress in growth chamber for 4 weeks before fresh (black bars) and dry (grey bars) weights were measured (**B**, **C**). Four-day-old wild-type and transgenic DHN4-13 plants were transferred to GM medium containing 500 mM mannitol, and grown for 3 weeks (**D**).

tions for them in other higher plants, we generated transgenic *Arabidopsis* plants that over-expressed them under the control of CaMV 35S promoter, and then selected 6 and 16 transgenic plants that stably expressed *Dhn3* and *Dhn4*, respectively (data not shown). To test their drought tolerance, we used semi-quantitative RT-PCR to select transgenic DHN3-6 and DHN4-13 plants with relatively higher expressions of *Dhn3* and *Dhn4* among their respective transformants (Fig. 6A).

In one experiment, plants were grown in soil, under frequent drought stress, while in the other, the plants were held on GM media containing 500 mM mannitol. In the first test, with soil and pots, increases in fresh and dry weights were much greater for both transgenic lines than for the wild-type plants (Fig. 6B, C). In the second trial, using GM media and mannitol, all the WT plants turned yellow and died at approximately four weeks after germination, whereas the transgenic DHN3-6 plants remained pale green and alive despite their growth apparently being arrested by severe osmotic stress (data not shown). Similarly, the transgenic DHN4-13 plants maintained their green leaves (Fig. 6D) in the GM media, with some recovering their normal development when transferred to new, standard (mannitol-less) GM media. All of these results imply that the accumulation of barley DHN3 and DHN4 transcripts contributes to increased drought tolerance in transgenic *Arabidopsis*.

DISCUSSION

Improving the status of drought tolerance is the first priority in barley breeding strategies because the major portion of reduced grain yields results from frequent water deficit in the field. Barley breeders have tried to develop drought-tolerant varieties that display improved field performance on semidry lands. However, efficient marker systems for screening and tracing these tolerant genotypes in segregating populations and variable germplasms have not yet been established because drought tolerance is mainly regulated by several quantitative trait loci. Thus, researchers must develop reliable molecular markers in order to compare tolerant genotypes with those that are susceptible.

Relative water content, considered a reliable measure of water status in plant tissues, is used to estimate physiological drought tolerance. Here, the tolerant 'Samdobori', 'Chalbori', and 'Dongbori-1' maintained higher RWC values compared with susceptible 'Daebaekbori', 'Suwonmac-360', and 'Wolsung87-31', which had the lowest RWC (Fig. 1), as

previously reported (Fu et al., 2003b). Furthermore, we reconfirmed that RWC was highly correlated (r^2=0.84) with the drought index of yield in barley (Fig. 1). However, measurements of RWC are not practical for identifying drought-tolerant genotypes in the field, so its usage is not an important component of breeding strategies for barley or other crop plants.

Most drought-inducible genes are thought to be identifiable by contrasting the levels of gene expression between dehydration-treated and -untreated plants. However, no specific correlation is generally found when studies are based on field performance under drought. Therefore, our preliminary data demonstrating a correlation between RWC and drought index of yield (Fig. 1) (Fu et al., 2003a), led us to apply the SSH protocol in order to find highly reliable gene-specific markers whose expression, under dehydration stress, would be upregulated specifically in tolerant cultivars but not in susceptible ones (Fig. 2). Our differential screening produced six candidate genes that were identified by DNA sequencing: *DnaJ*, *RbcL*, *RbcS*, *Ltp*, *Cab*, and *Dhn3*. Northern blot analysis indicated that *Dhn3* expression typically was much more highly induced in the tolerant 'Chalbori' than in the susceptible 'Daebaekbori' (Fig. 3). Although *Dhn* genes (*Dhn1* through *Dhn12*) in barley are upregulated by low temperature, ABA, high salt, or dehydration (Close et al., 1989; Choi et al., 1999; Choi and Close, 2000), little is known about any correlation between the degree of drought tolerance and the expression level of each *Dhn* gene. Moreover, although both the upregulation of *Dhn* orthologs and the high accumulation of DHN proteins have been observed in many other species when those plants are exposed to low temperature, high salt, or drought, the relationship between each *Dhn* expression and drought tolerance has not been carefully examined, and no correlation has yet been reported between expression and tolerance (Iturriaga et al., 1992; Lang et al., 1993; Kaye et al., 1998; Ha et al., 2006). The only exception has been regarding tolerance to freezing stress in transgenic *Arabidopsis* over-expressing multiple *Dhn* genes (Puhakainen et al., 2004).

Among the 12 *Dhn* genes studied via semi-quantitative RT-PCR, transcripts of *Dhn3* were weak or absent in 'Daebaekbori', 'Suwonmac-360', and 'Wolsung87-31', whereas, in 'Samdobori', 'Chalbori', 'Dongbori-1', and 'Dicktoo', expression levels during induced drought stress were strong or present not only at the early seedling stage in our growth chamber tests but also at the mature stage in the greenhouse trials (Fig. 4, 5). This suggests a significant correlation between rapid *Dhn3* expression and drought-tolerance in barley. Furthermore, although *Dhn4* expression was very similar to that of *Dhn3*, no exact match was seen between the former and RWC in any cultivar at the mature leaf stage (Fig. 5). Moreover, *Dhn3* and *Dhn4* were induced rapidly and highly in several drought-tolerant lines in ICARDA when they were exposed to water deficit at the early seedling stage. This may have been because those lines are bred for cultivation in semidry regions (data not shown). With regard to the expression of other dehydrin genes, rapid upregulation of *Dhn1*, *2*, *6*, *7*, and *9* through *11* occurred randomly in both tolerant and susceptible cultivars within 2 and/or 12 h of drought stress (data not shown), indicating their irrelevance to the properties of drought-tolerant genotypes. *Dhn5* was highly expressed in both the susceptible 'Wolsung87-31' and the tolerant 'Dongbori-1' and 'Dicktoo', while *Dhn8* was upregulated specifically in the susceptible 'Daebaekbori' and the tolerant 'Dicktoo'. Interestingly, two domestic cultivars that show stress-induced expression of *Dhn5* and other *Dhn* genes are used as freezing-tolerant winter varieties in Korea. However, *Dhn12* expression in those two cultivars is weak, whether they are reared in the growth chamber or greenhouse, presumably because their expressions are embryo-specific (Choi and Close, 2000). Nevertheless, based on their expression patterns under drought stress, we can conclude that the two dehydrin genes examined here, i.e., *Dhn3* and *Dhn4*, are closely correlated with genotypic properties of drought tolerance in barley.

A strong correlation has been reported between chlorophyll degradation and drought stress (Schurr et al., 2000; Yan et al., 2004). In the current study, whereas our wild-type *Arabidopsis* plants turned yellow and died on the mannitol-containing GM media, the transgenic plants over-expressing *Dhn3* (DHN3-6) and *Dhn4* (DHN4-13) maintained their green leaf color, and were viable when again transferred to a GM medium (Fig. 6). This indicates that the overexpression of barley *Dhn3* or *Dhn4* contributes to increase drought tolerance in *Arabidopsis*.

In barley, a cluster of *Dhn* loci (*Dhn1*, *Dhn2*, and *Dhn4*a) overlaps the major QTL on Chromosome 7(5H) for winter hardiness in a winter ('Dicktoo') by spring ('Morex') dihaploid mapping population (Pan et al., 1994). Therefore, we speculated that, among our 12 *Dhn* genes, rapid and high expression of *Dhn3* and *Dhn4* might be specifically related to drought tolerance because those other *Dhn* genes were either highly expressed in all genotypes or less expressed in the tolerant genotypes under stress conditions (data not shown). This suggested that expression of the other *Dhn* genes might not be related to tolerance, as had been implied with the susceptible genotypes. Because of their physical properties, DHNs may function in stabilizing membranes and macromolecules in the cytoplasm (Campbell and Close, 1997). An interaction between dehydrin proteins and cell membranes also has been suggested by *in vitro* studies of freezing tolerance in *Arabidopsis* (Houde et al., 1995; Thomashow et al., 1996). In addition, Danyluk et al. (1998) have provided direct evidence for the accumulation of the WCOR410 dehydrin protein near the plasma membrane during cold acclimation in wheat, and have suggested a protective role by the plasma membrane in plants subjected to low-temperature stress. Thus, we might also use our current transgenics results to hypothesize about a possible contribution by the barley dehydrin proteins, DHN3 and DHN4, in protecting cells against dehydration, possibly by binding to cell membranes.

In conclusion, we have found strong positive correlations among the expressions of *Dhn3* and *Dhn4*, RWC values, and drought indices of yield. Therefore, we propose that rapid upregulation of two dehydrin genes, *Dhn3* and *Dhn4*, in response to dehydration can serve as reliable molecular markers for screening and tracing drought-tolerant barley

genotypes at the early seedling stage. However, we must still determine the biochemical roles of DHN3 and DHN4 in that germplasm.

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LITERATURE CITED

Baker J, Steele C, Dure LIII (1988) Sequence and characterization of 6 Lea proteins and their genes from cotton. Plant Mol Biol 11: 277-291

Barrs HD, Weatherley PE (1968) A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust J Biol Sci 15: 413-428

Boyer JS (1982) Plant productivity and environment. Science 218: 443-448

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

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

Cellier F, Conejero G, Breitler JC, Casse F (1998) Molecular and physiological responses to water deficit in drought-tolerant and drought-sensitive lines of sunflower. Plant Physiol 116: 319-328

Chen W, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T, Mauch F, Luan S, Zou G, Whitham SA (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 14: 559-574

Choi DW, Close TJ (2000) A newly identified barley gene, *Dhn12*, encoding a YSK_2 DHN, is located on chromosome 6H and has embryo-specific expression. Theor Appl Genet 100: 1274-1278

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

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

Close TJ, Kortt AA, Chandler PM (1989) A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol Biol 13: 95-108

Clough SJ, Bent AF (1998) Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16: 735-743

Danyluk J, Perron A, Houde M, Limin A, Fowler B, Benhamou N, Sarhan F (1998) Accumulation of an acidic dehydrin in the vicinity of the plasmamembrane during cold acclimation of wheat. Plant Cell 10: 623-638

Diatchenko L, Lau YFC, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov S, Lukyanov S, Lukyanov K, Gurskata N, Sverdlov E, Siebert PD (1996) Suppression subtractive hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci USA 93: 6025-6030

Diatchenko L, Lukyanov S, Lau YF, Siebert PD (1999) Suppression subtractive hybridization: A versatile method for identifying differentially expressed genes. Methods Enzymol 303: 349-380

Dure LIII (1993) A repeating 11 mer amino acid motif and plant desiccation. Plant J 3: 363-369

Fu JD, Paek NC, Kim JG, Lee BW (2003a) Changes in physiological characteristics of barley genotypes under drought stress. Kor J Crop Sci 48: 506-515

Fu JD, Paek NC, Kim JG, Lee BW (2003b) Relationship between drought-tolerance and physiological parameters in Korean barley genotypes. Kor J Crop Sci 48: 516-526

Ha YI, Lim JM, Ko SM, Liu JR, Choi DW (2006) Sequence variability and expression characteristics of the ginseng (*Panax ginseng* C.A. Meyer) dehydrin gene family. J Plant Biol 49: 205-211

Houde M, Daniel C, Lachapelle M, Allard F, Laliberte S, Sarhan F (1995) Immunolocalization of freezing-tolerance-associated proteins in the cytoplasm and nucleoplasm of wheat crown tissues. Plant J 8: 583-593

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

Iturriaga G, Schneider K, Salamini F, Bartels D (1992) Expression of desiccation-related proteins from the resurrection plant *Craterostigma plantagineum* in transgenic tobacco. Plant Mol Biol 20: 555-558

Iuchi S, Yamaguchi-Shinozaki K, Urao T, Terao T, Shinozaki K (1996) Novel drought-inducible genes in the highly drought-tolerant cowpea: Cloning of cDNAs and analysis of the expression of the corresponding genes. Plant Cell Physiol 37: 1073-1082

Kaye C, Neven L, Hofig A, Li QB, Haskell D, Guy C (1998) Characterization of a gene for spinach CAP160 and expression of two spinach cold-acclimation proteins in tobacco. Plant Physiol 116: 1367-1377

Lang V, Heino P, Palva ET (1989) Low-temperature acclimation and treatment with exogenous abscisic-acid induce common polypeptides in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 77: 729-734

Pan A, Hayes PM, Chen F, Chen THH, Blake T, Wright S, Karsai I, Bedo Z (1994) Genetic analysis of the components of winter hardiness in barley (*Hordeum vulgare* L.). Theor Appl Genet 89: 900-910

Puhakainen T, Hess MW, Makela P, Svensson J, Heino P, Palva ET (2004) Overexpression of multiple dehydrin genes enhances tolerance to freezing stress in *Arabidopsis*. Plant Mol Biol 54: 743-753

Robertson M, Chandler PM (1992) Pea dehydrins: Identification, characterization and expression. Plant Mol Biol 19: 1031-1044

Schonfeld MA, Johnson RC, Carver BF, Mornhinweg DW (1988) Water relations in winter wheat as drought resistance indicators. Crop Sci 28: 526-531

Schurr U, Heckenberger U, Herdel K, Walter A, Feil R (2000) Leaf development in *Ricinus communis* during drought stress: Dynamics of growth processes, of cellular structure and of sink-source transition. J Exp Bot 51: 1515-1529

Seki M, Narusaka M, Abe H, Kasuga M, Yamaguchi-Shinozaki K, Carnici 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 (1997) Gene expression and signal transduction in water-stress response. Plant Physiol 115:

327-334

Thomashow MF, Artus N, Gilmour S, Stockinger E, Wilhelm K, Zarka D, Joseph RA, Uemura M, Steponkus PJ (1996) Function and regulation of *Arabidopsis thaliana* Cor (cold-regulated) genes. Plant Physiol 111: 29-37

Yan J, He C, Wang J, Mao Z, Holaday SA, Allen RD, Zhang H (2004) Overexpression of the *Arabidopsis* 14-3-3 protein GF14 lambda in cotton leads to a "stay-green" phenotype and improves stress tolerance under moderate drought conditions. Plant Cell Physiol 45: 1007-1014