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DIFFERENTIAL EXPRESSION OF WHEAT GENES DURING COLD ACCLIMATION

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Overwintering crops such as winter wheat display significant increase in freezing tolerance during a period of cold acclimation (CA). To gain better understanding of molecular mechanisms of CA, it is important to unravel functions and regulations of CA-associated genes. Differential screening of a cDNA library constructed from cold acclimated crown tissue of winter wheat identified three novel CA-associated cDNA clones. Nucleotide sequence analysis showed that the clones encode a high mobility globular protein (HMGB1), a glycine-rich RNA-binding protein (TaGRP2), and a LEA D-11 dehydrin (DHN14). Accumulation of the three mRNAs during 14 days of CA was differentially regulated. In response to drought, and ABA, DHN14 mRNA rapidly accumulated while HMGB1 and TaGRP2 mRNA levels remained unchanged. The possible functions of each of these genes in cold acclimation are discussed.

Introduction. Cold acclimation (CA) is the process that allows hardy plants to develop essential tolerance for winter survival through multiple levels of biochemical and cell biological changes [1, 2]. Dynamic alteration in gene expression occurs during CA process. Reports have identified CA-induced genes from many plant species including winter wheat [2]. Accumulation of some of the cold-induced genes shows positive correlation with the degree of freezing tolerance [3, 4]. Functions in cold tolerance have been suggested for a number of genes [5, 6] (see [7—10] for recent reviews). Previous research has shown that the cytosolic calcium concentration increases after plant exposure to low temperature [11, 12] and that Ca^{2+} modulates low temperature-induced nuclear gene expression [4, 13]. A small family of transcription factors (*CBF1-3*), identified in *Arabidopsis thaliana*, has been shown to activate multiple cold regulated (*Cor*) genes [14, 15]. The process of CA appears to require complex and coordinated expression of a whole battery of cold-induced genes. Thus, it is important to increase the number of identified CA-related genes/proteins with determined functions as well as to unravel regulatory mechanisms of CA.

In this paper, we report identification of three novel cDNA clones that accumulate during CA in wheat crown tissue. Analysis of gene expression during cold, drought and ABA treatments revealed that multiple regulatory pathways are involved in the regulation of CA-related genes.

Materials and Methods. *Plant materials and treatments.* Winter wheat plants (*Triticum aestivum* L. cv. Chihoku), used for cDNA library construction and differential screening, were grown under the following condition. Seeds were planted in commercial potting mix, irrigated with tap water, and grown in a growth chamber which is maintained under 22/18 °C (day/night) cycles for 28 d. Control plants were harvested at the end of this period. Cold acclimation was performed by subjecting the plant to 6/2 °C (day/night) cycles for 14 d. Crown tissue was harvested and frozen immediately in liquid nitrogen.

Plants used for stress treatments were grown hydroponically. Surface-sterilized seeds were imbibed in the dark for 12 h and planted on a plastic mesh grid supported by a container filled with tap water. The container was maintained in a growth chamber at 25 °C under continuous illumination. After growing for 7 d, wheat seedlings were sub-

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jected to stress treatments. Low temperature, ABA, and dehydration treatments were conducted by transferring the mesh grid to a container with tap water at 4 °C, 50 μM ABA solution, and without water, respectively.

Construction of cDNA library. Total RNA was isolated from cold-acclimated crown tissue, using TRIzol reagent (Invitrogen, CA, USA) and poly (A)⁺ RNA was purified from total RNA using Dynabeads Oligo(dT)₂₅ (Dynal A.S, Oslo, Norway). cDNA was synthesized with Zap Express cDNA Synthesis Kit and packaged into the phage particles with Gigapack Gold III (Stratagene, CA, USA). A primary cDNA library of 7·10⁶ plaques was amplified as suggested by the manufacturer's protocol and then used for differential screening.

Differential screening. Total RNA was isolated from non-acclimated (NA) and cold-acclimated (CA) wheat crown tissue, and poly(A)⁺ RNA was purified as described above. Double-stranded cDNA was synthesized from poly(A)⁺ RNA, using a oligo-dT primer (Amersham Pharmacia Biotech, NJ, USA). Both cDNAs from NA and CA were labeled with [α-³²P] dCTP, and used as probes for differential hybridization. Differential hybridization was performed according to the plaque hybridization method [16]. Two copies of plaque blots from the CA cDNA library, covering 5·10⁴ independent plaques, were hybridized with ³²P-labeled NA and CA cDNA probes. Hybridization was carried out at 65 °C with Rapid-Hyb buffer (Amersham Pharmacia Biotech). After overnight hybridization, membranes were washed once with 2×SSC and 0.1 % SDS for 15 min at 65 °C, twice with 0.1×SSC and 0.1 % SDS for 15 min at 65 °C. After three rounds of screening, several independent plaques were picked and subjected to *in vivo* excision using Exassist helper phage (Stratagene) according to the manufacturer's instructions. The excised plasmid DNA was isolated and purified by the alkaline lysis method [16].

Northern blot analysis. Total RNA (20 micrograms) was separated in 1.0 % formamide-agarose gel and then transferred onto Hybond-N⁺ membrane (Amersham Pharmacia Biotech) according to standard methods [16]. Rapid-Hyb buffer (Amersham Pharmacia Biotech) containing salmon sperm DNA (10 μg/ml) was used for both prehybridization and hybridization. RNA blots were hybridized with ³²P-labeled *wca142*, *wca212*,

or *wca341* full-length cDNA at 65 oC for 16 h. Blots were then washed with 2×SSC and 0.1 % SDS for 15 min and twice with 0.1×SSC and 0.1 % SDS for 20 min at 65 °C and then exposed to Kodak BioMax MR X-ray film (Kodak, New Haven, CT, USA) with an intensifying screen at −80 °C.

DNA sequencing analysis. The cloned DNA insert was sequenced by the dideoxy method using Thermo Sequence v2.0 kit (Amersham Pharmacia Biotech) with a DNA sequencer model 373A (Applied Biosystems, San Jose, CA, USA). The sequence databases were searched for sequence similarities at NCBI using the BLAST program. Sequence alignments of the DNA and deduced amino acid sequences were done by ClustalX [17] and visualized using Genedoc software [18].

Results. *Cloning of three cDNA clones that are accumulated in cold-acclimated crown.* A cDNA library constructed from cold acclimated crown tissue was screened with labeled cDNA from cold-acclimated and non-acclimated crown tissues. The differential hybridization identified several clones that are preferentially expressed in cold-acclimated crown. Subsequent Northern blot analysis revealed three cDNA clones, designated *wca142*, *wca212*, and *wca341*, that were induced in cold-acclimated crown (data not shown). The nucleotide sequencing analysis revealed that *wca142*, *wca212*, and *wca341* encoded a High Mobility Globular (HMG) protein, a glycine-rich RNA-binding protein, and a LEA D-11 dehydrin, respectively.

Structure and function of the encoded proteins. The *wca142* encodes a 161-amino-acid-polypeptide with the predicted molecular size of 17.2 kDa. Database searches revealed that the protein encoded by *wca142* showed striking homology with plant HMGB1 proteins. The identity to barley HvHHMG12 (CAA90679, [19]) was 92 %, followed by 86 % identity to a HMGB1 protein from maize (CAA41220; [20], 85 % identity to rice HMGB1 (AAP21609; [21]) and 54 % identity to *Arabidopsis* HMGB2 (At1g20693; [22]) (Fig. 1). Therefore we have designated the wheat HMG-box protein as HMGB1. The HMGB1 protein consists of the N-terminal domain, the central domain that contains basic DNA-binding regions (so-called HMG-box motifs) and the polyacidic C-terminal region, which is thought to interact with his-

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HMGB1       : MKGAKSKGAAK D KLAVKSKGAEKPA K G KGKAGKDPNKPKRAPSAFFVFM EFR EF : 60
BARLEY      : MKGAKSKGAAK D TKLAVKSKGAEKPA K G KGKAGKDPNKPKRAPSAFFVFM EFR EF : 60
RICE        : MKGAKSKGAAK PD KLAVKSKGAEKPA K G KGKAGKDPNKPKRAPSAFFVFM EFR KE  : 60
MAIZE       : MKGAKSKGAAK D AKLAVKSKGAEKPAK G KGKAGKDPNKPKRAPSAFFVFM E FRKE   : 59
A. THALIANA : MKGAKSK-TETRSSKI SVTKK PAKGAGRGKA---  AKDPNKPKR PASAFFVFM EDER TE : 56
              MKGAKSKgaak d KLaVksKgAekpa   g kgkAgKDPNKPKRapSAFFVFM eFR eF

HMGB1       : KQKNPKNKSVAAVGKAAG RWKSLSES KAPYVAKANKLKGEYNKAIAAYNKGE SAAAAA : 120
BARLEY      : KQKNPKNKSVAAVGKAAG RWKSLSES KAPY VAKANKLKGEYNKAIA SYNKGE STTAA : 119
RICE        : KEKNPKNKSVAAVGKAAG RWKSLT SADKAPYVAKANKLKAEYNKAIAAYNKGE ----ST : 116
MAIZE       : KEKNPKNKSVAAVGKAAG DRWKSLSESDKAPYVAKANKLKDEYNKAIAAYNKGE --STA : 116
A. THALIANA : KK NPKNKSVATVGKAAGDKWKSLSDS EKAPYVAK EKRKVEYERNIIK YNKKL------ : 110
              K kNPKNKSVAaVGKAAG rWKSLses KAPyVAKANKIK EYnKaIaaYNKge

HMGB1       : PKKAAKEVEEEDREEESDKSKSEINDDDDDEGS E EDDDE : 161
BARLEY      : PKKASSKEVEEEDEEESDKSKSEINDDDDDEGSDEAEDAA  : 160
RICE        : AKKAPAKEEEEDEEESDKSKSEVNDEDDDEGS E EDDDE  : 157
MAIZE       : AKKAPAKEEEEDEEESDKSKSEVNDEDDDEGS EE EDDDE : 157
A. THALIANA : ----EEGPKEDEESD---DVSEVNDEDDAE  SEEEEDDD  : 144
              kka  ke EeedeeesdKSkSE ND DD Egs E Edddde
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Fig. 1. Multiple alignment of wheat HMGB1 to plant HMG protein sequences. The conserved amino acid residues are shown as white letters on black background while those with partial conservation are shaded in grey. The aligned sequences are *Triticum aestivum* HMGB1 (AB272226; this study), *Hordeum vulgare* HVHHMG12 (CAA90679, [19]), *Zea mays* HMGB1 (CAA41220; [20]), *Oryza sativa* (indica) HMGB1 (AAP21609; [21]) and *Arabidopsis thaliana* HMVB2 (At1g20693; [22]). Consensus sequence was indicated in the bottom of the alignment (Cons.). Numbers on the right refer to amino acid position from the N-terminal methionine

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              <-RNP2->                              <-RNP2->
TaGRP2  : MA--DVEYRCFVGGLAWATDDQSLQNAFSKYGDVIDSK I DRETGRSRGFGFVTFASDE : 58
Blt801  : MA--DVEYRCFVGGL WATDDQSLQNAFSKYGDVIDSK I DRETGRSRGFGFVTFASDE : 58
whGRP1  : MA--ETEYRCFVGGLAWATD NNLQQAFSQYGEIIDAK  INDRETGRSRGFGFVTFGSEE : 58
Ma16    : MAAADVEYRCFVGGLAWATSNESLENAPASYGEIIDSKVID DRETGRSRGFGFVTFSSEN : 58
AtGRP7  : MASGDVEYRCFVGGLAWATDDRALETAFAQYGDVIDSK INDRETGRSRGFGFVTFKDEK : 58
nsRBP1b : MA--E EYSCFVGGLAWATDRTLADAFGTYGEVIDSK  INDRETGRSRGFGFVTFKDEK : 58
Cons.   : MA   vEYrCFVGGLaWAT d  L  AF  YG   DsKiI DRETGRSRGFGFVTF       : 58

              <------------------------- GR
TaGRP2  : AMRQAIEAMNGQDLDGRNITVNEAQSRRSGGGGGGGFGGGGGYG--GQRREGGGGGGYG : 116
Blt801  : AMRQAIEAMNGQDLDGRNITVNEAQSRRSDGGGC--FGGGGGYG--GQRREGGGGG-YG : 113
whGRP1  : SMRQAIE EMNGKELDGRNITVNEAQSRRSGGGGG-----GGGYG----GQRGGGGYGG : 109
Ma16    : SMLDAIE NMNGKELFGRNITVNCAQSRG--GGGC-----GGGYG------GGRGG--G : 103
AtGRP7  : AMKDAIEGMNGQDLDGR ITVNEAQ RCGGGGGC-HRGGGCGYRSGGGGGGYSGCGGSYG : 119
nsRBP1b : QMRDAIEGMNGQELDGR ITVNEAQ GYG----GGRREGGGGGGYG : 107
Cons.   : M AIE MNG LDGR ITVNeAQsR s GGGG       ggGYg       GgGG   G  : 58

              ------------------------- GR ------------------------->
TaGRP2  : GGGGGYRGGR--SGGGGGYGSR-DGGGYGGGGG----GG-YGGSRG ------SGGGN RE : 163
Blt801  : GGGGGYGGGR--SGGGGGYGSR-DGGGGGYGGG----GGGGGGSRG ------SGGGN RE : 161
whGRP1  : GGCYGGGGCGGYGGQSGGGGYGGQRGGGGCYGGGGGGYGGGGGGYGQRGCGGG--DSGCQ RN : 167
Ma16    : GYGGGRRDGG--YGGGGGYGGRREGGGGGYGGG----GGYGCRREGGGGGYGGGGG-CGGRD : 157
AtGRP7  : GGGCRREGGGGYSCGGGGYSSR-GGGGCSYGGGRREGGGGYGGGECGYGGSGGGGCGG-- : 176
nsRBP1b : GGGGGYGGCRREGGGGCYGGGRREGGGGCGYGGGGGYGGGGR----------------- : 148
Cons.   : GgGgg  gG  gGGGgyg r  GGGg   GGG     GG yg  gg      gg w       :
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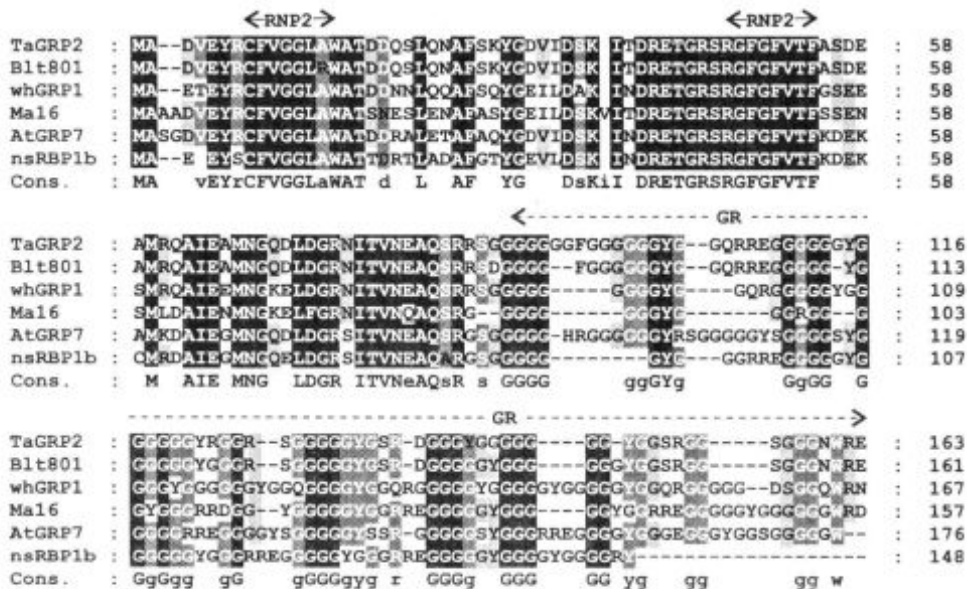
Fig. 2. Multiple alignment of TaGRP2 to plant glycine-rich RNA-binding protein sequences. The RNP1, RNP2 motifs and the Glycine Rich (GR) region are indicated above the sequence. The conserved amino acids are shown as white letters on black background while those with partial conservation are shaded in grey. The aligned sequences are *Triticum aestivum* TaGRP2 (AB272227; this study), *Hordeum vulgare* Blt801(AAB07749; Dunn et al., 1996), *Triticum aestivum* whGRP1 (AAA75104; [25]), *Zea mays* MA16 (CAA31077; [26]), *A. thaliana* AtGRP7 (At2g21660; [24]) and *N. sylvestris* NsRGP1b (BAA03742; [27]). Consensus sequence was indicated in the bottom of the alignment (Cons.). Numbers on the right refer to amino acid position from the N-terminal methionine

tones and may participate in modifying chromatin structure.

The *wca212* encodes a 163-amino-acid-polypeptide with the predicted molecular size of 16 kD

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DHN14      : MEHQGRGAGEKKGVVESITEKLPGGHGDHQQATGGTYGQQ-GHAGVTG--  :  47
WDHN13     : MEHQGRGAGEKKGVMESITEKLPRGHGDHQQATGGTYGQQ-GHTGVTGTG :  49
WCOR726    : MEHQGRGAGENKGVVESITEKLPGGHGDHQQTTGGTYGQQGHGAGVTG    :  50
Lopophyrum : MEHQGRGAGENKGVVESITEKLPGGHGDHQQTTGDTYGQQ-AHAGVTGTG :  49
Cons.      : MEHQG GAGE KGVvESITEKLPgGHGDHQQ TGgTYGQQ  haGVTGtg

DHN14      : ---------ENIKEKLPGGHGDHQQTTGMTGSETHATTATTDGNYGKSG   :  87
WDHN13     : TGTCEKKGVVENIKEKLPGGHGDHQHTTGMSGSKTHATTATTDGNYGKSG  :  99
WCOR726    : TGTSEKKGVIENIKEKLPGGHGGQHTTGMTGSETHATTATTDGNYGKSG   : 100
Lopophyrum : TGTCEKKGVIENIKEKLPGGHGDHQHTTGMTGSETHATTATTDGNYGKSG  :  99
Cons.      : tgt ekkgv ENIKEKLPGGHGGdhQTTGMtGSeTHATTATTDGNYGKSG

DHN14      : HTGTDGTGENKSIMDKIKDKLPGQH  : 112
WDHN13     : HTGTDSTGENKSVMDKIKDKLPGQH  : 124
WCOR726    : HTGTDGTGENKSIMDKIKDKLPGQH  : 125
Lopophyrum : HTGIDGTGENKSIMDKIKDKLPGQH  : 124
Cons.      : HTGtDgTGENKSiMDKIKDKLPGQH
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Fig. 3. Multiple alignment of DHN14 to plant dehydrin protein sequences. The conserved amino acids are shown as white letters on black background while those with partial conservation are shaded in grey. The K-segments are boxed. The aligned sequences are *Triticum aestivum* DHN14 (AB272228; this study), *Triticum aestivum* WDHN13 (BAC01112; [31]), *Triticum aestivum* WCOR726 (AAB18204; [29]), and *Lophopyrum elongatum* (AAC05924; [30]). Consensus sequence was indicated in the bottom of the alignment (Cons.). Numbers on the right refer to amino acid position from the N-terminal methionine
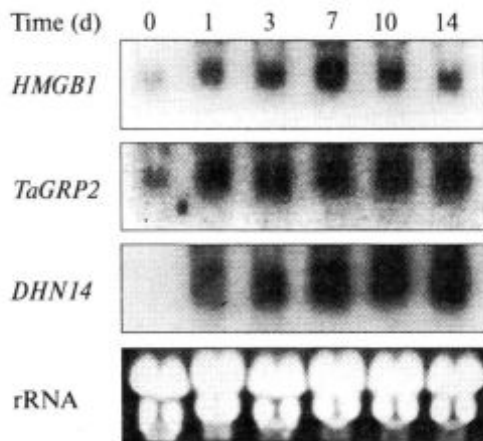
Fig. 4. Expression of HMGB1, TaGRP2 and DHN14 during the course of cold acclimation. 14-day-old plants were subjected to cold acclimation for designated time periods and total RNA (20 µg) from the crown tissue was analyzed by Northern blotting analysis. Ethidium bromide-stained gel is shown for a loading control

(TaGRP2) showing high sequence homology to glycine-rich RNA-binding proteins (GR-RBPs) from a number of other plant species. The protein encoded by *wca212* shared 94 % identity with the published barley Blt801(AAB07749; [23]). The identity to other published GR-RBPs was 68 % to *Arabidopsis* AtGRP7 (At2g21660; [24]), 67 % to another wheat GR-RBPs protein, whGRP1 (AAA75104, [25]), 63 % to maize MA16 (CAA31077; [26]) and 61 % to tobacco *N. sylvestris* NsRGP1b (BAA03742; [27]). Some of the GR-RBPs genes have been reported to increase expression levels in response to environmental stress. All these genes encode protein products with two definitive domains. The amino-terminal domain consists of 80—90 residues containing two consensus sequences, an octapeptide RNP1 and a hexapeptide RNP2 which are located about 30 amino acids apart (Fig. 2). These consensus sequences together with a number of other highly conserved residues comprise the consensus RNA-binding domain (RRM) found in many RNA-binding proteins localized in the nucleus, cytoplasm and cytoplasmic organelles in plant, animal and fungal cells [28]. The carboxy-terminal domain of plant GR-RBPs consists mainly of repeating glycine residues interspersed with tyrosine and arginine (Fig. 2).

Wca341 encodes an open reading frame of 112 amino acids with a predicted molecular mass of 11.5 kDA thought to be classified to Kn-type LEA D-11 dehydrin family, and consists of two K-segments and an incomplete K-segment. The encoded protein, designated as DHN14, shared 84 % identity with WCOR726 (AAB18204, [29]) and with dehydrin from *Lophopyrum elongatum* (AAC05924; [30]) while the identity to a recently published wheat dehydrin WDHN13 (BAC01112; [31]), was 83 % (Fig. 3). The DHN14 protein has a 12-amino-acid-deletion including a part of the second K segment. The presence of this deletion makes DHN14 shorter than WCOR726 reported to be the smallest member of WCS120 family [29].

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Differential accumulation of the HMGB1, TaGRP2 and DHN14 transcripts in response to low temperature. Since the cDNA clones were isolated by a differential screening between NA and CA plant tissues, the expression patterns of HMGB1, TaGRP2 and DHN14 during cold acclimation was determined (Fig. 4). Although an enchanced transcription of all cDNAs was detected within 1 day of CA each of them showed the distinct expression kinetics. In contrast to the other two clones, the mRNA expression of DHN14 could not be detected under the normal growth condition (Fig. 4). The accumulation of both HMGB1 and DHN14 transcripts reached their maximum at 7 days of CA. While expression of HMGB1 slowly declined afterwards up to 14 days of CA, DHN14 expression level remained unchanged. Transcript accumulation of TaGRP2 was almost unchanged after reaching its maximum in 1 day of CA (Fig. 4). To further characterize the cold-induced expression of CA-induced clones, a short time-course expression analysis was carried out using young seedlings. Northern blots showed that induction of TaGRP2 was detectable as soon as in 1 hour of cold treatment in shoots and in 6 hours in root tissues (Fig. 5). In contrast, the detectable expression of DHN14 was observed only in 6 hours of treatment in both root and shoot tissues but expression in roots was more pronounced. Induction of the HMGB1 was somewhat slower. It was detected in 12 hours of cold treatment in roots and in 48 hours in shoots (Fig. 5).

Expression of HMGB1, TaGRP2 and DHN14 in response to dehydration and ABA. To determine whether the functions of the wca genes are specifically associated with cold acclimation or more generally with both cold and dehydration stress responses, expression of *HMGB1, TaGRP2* and *DHN14* during drought stress was examined by Northern blot analyses with 7-day-old seedlings (Fig. 6). The HMGB1 and *TaGRP2* transcripts appeared to be slightly down-regulated by drought treatments in both root and shoot tissues. In contrast, induction of the *DHN14* mRNA was detectable in 6 h and highly accumulated in 24 h after initiation of the drought treatment (Fig. 6). Involvement of ABA in gene regulation was also examined. Strong induction of *DHN14* was detectable as early as in 1 h of ABA treatment in roots and in 6 h in shoots, while no induction of HMGB1, TaGRP2 was observed
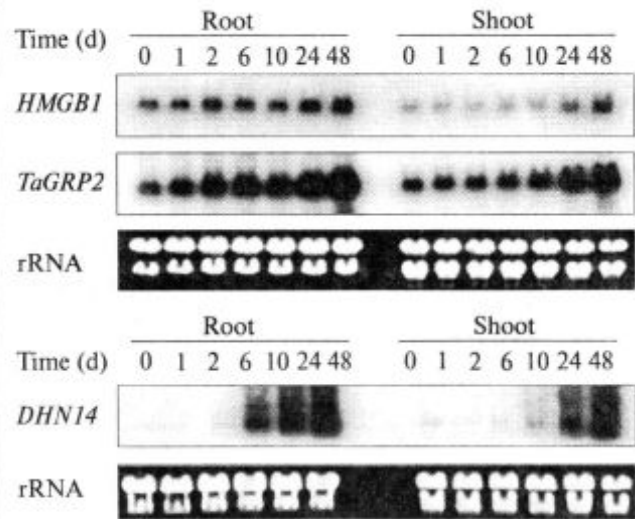
Fig. 5. Expression of HMGB1, TaGRP2 and DHN14 in response to short-term cold stress treatments. Hydroponically grown on plastic mesh grids 7-day-old seedlings were transferred together with the grid into plastic container with cold water (4 °C). At each time point total RNA (20 µg) was isolated from shoot and root tissues and analyzed by Northern blot analysis. Ethidium bromide-stained gel is shown for a loading control
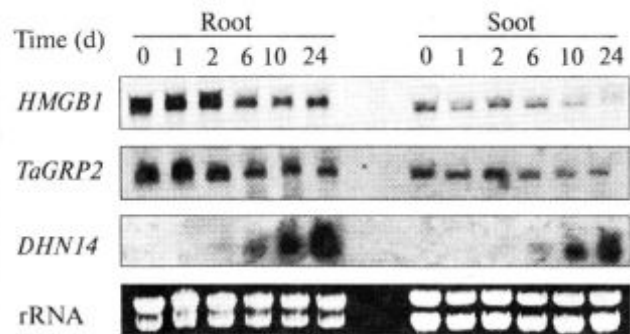

Fig. 6. Expression of HMGB1, TaGRP2 and DHN14 in response to short-term drought stress. Hydroponically grown on plastic mesh grids 7-day-old seedlings were transferred together with the grid into a new plastic container without water. At each time point total RNA (20 µg) was isolated from shoot and root tissues and analyzed by Northern blot analysis. Ethidium bromide-stained gel is shown for a loading control

in both root and shoot tissues (Fig. 7). These data suggest that *HMGB1* and *TaGRP2* may have specific functions during cold acclimation, and DHN14 may act in both cold and dehydration stress responses.

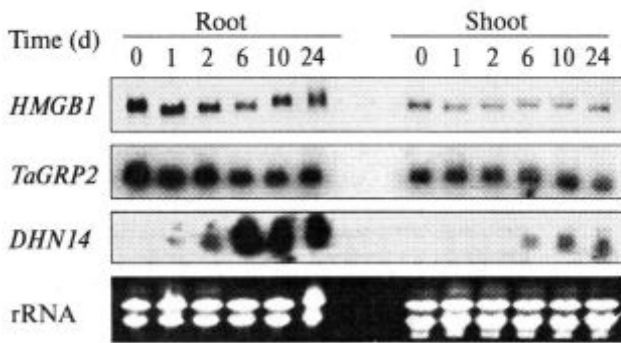**Discussion.** In this study we have identified three novel cDNA clones that are up-regulated during

Fig. 7. Expression of HMGB1, TaGRP2 and DHN14 in response to exogenous ABA treatment. Hydroponically grown on plastic mesh grids 7-day-old seedlings were transferred together with the grid into plastic container with water containing 50 µM ABA. At each time point total RNA (20 µg) was isolated from shoot and root tissues and analyzed by Northern blot analysis. Ethidium bromide-stained gel is shown for a loading control

cold acclimation. According to the sequencing analysis, two of the clones, wca142 and wca212, encode specific proteins thought to be involved in the regulation of gene expression.

The predicted protein encoded by wca142 had a three-domain structure, highly similar to plant HMGB1 proteins (Fig. 1). Members of the HMG 1 and 2 subgroups (HMGB according to the revised nomenclature [32]) have been variously implicated in DNA replication [33, 34], cellular differentiation [35], development [36] and gene expression [37, 38]. HMG1 directly stimulates in vitro transcription by RNA polymerases II and III [37], and overcomes the inhibition of transcription caused by histones [37, 39]. The in vitro binding of plant HMG proteins to A/T-rich regions of double-stranded DNA has also been observed [40—42]. Preferential binding of pea HMG proteins to a positive regulatory region of the plastocyanin gene promoter has been described [43]. Rice HMGB1 have been demonstrated to bind four-way DNA junctions and efficiently bend DNA [34], thus facilitating binding of transcriptional factors to their target regions. A wheat HMGB protein have been reported to stimulate the binding of the bZIP transcription factor EmBP-1 to the promoter of the ABA responsive Em gene [44]. For a recent review on structure and functions of plant HMG proteins see [45]. Based on Northern and Western blot analyses, the plant HMGB genes are considered to be expressed ubiquitously in the plant [21,

46—48]. On the other hand, there are several reports describing the HMG gene regulation in mammals. Changes in the levels of HMG transcripts are reported during the development in hatched chick [49], cancer-related increase in mRNA expression in human [50], and circadian rhythm in Pharbitis nil [51]. However this is the first to report that one of the cereal HMGB subgroup protein genes is up-regulated by low temperature.

Taken together, HMGB1 may play a significant role in controlling general aspects of gene expression by modifying chromatin structure, and as such, can be viewed as a higher order regulatory factor. It is possible that the wheat HMGB1 protein functions as a general regulator of gene expression during cold acclimation in wheat.

As there are no reports describing expression of plant HMGB proteins in response to abiotic treatments, we have used Expression Browser through the Botany Array Resource [52] to perform «electronic Northerns» of the Arabidopsis HMGB subgroup gene family. The results of eNortherns showed that three out of six Arabidopsis subgroup members, including AtHMGB1, AtHMGB2 and AtHMGB3, were induced by cold treatment and suggested similar functions of some dicot HMGB proteins in the cold regulation of gene expression.

TaGRP2 is a member of a small family representing plant genes encoding proteins with both RNA-binding and glycine-rich (GR) domains, which have been reported to be responsive to various stresses or external stimuli: low temperature and abscisic acid (ABA), Hordeum vulgare [23]; drought and ABA, Zea mays (Ludevid M.D. et al. 1992); wounding, Daucus carota [53]; heavy-metal stress, Zea mays [54]; low temperature and ABA, Brassica napus [55]; low temperature and drought, Arabidopsis thaliana [56]. In addition, transcripts of GR-RBPs from A. thaliana [56] and Sinapis alba [57] have been shown to exhibit a light entrained circadian rhythm, which persists for several cycles upon removal of the light stimulus. The expression patterns of GR-RBPs suggests that glycine-rich proteins represent a class of RNA-binding proteins involved in general molecular responses to environmental stress mediated by post-transcriptional regulatory mechanisms. Three plant GR-RBPs have been experimentally demonstrated to have RNA-binding properties. The

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maize GR protein MA16 [58], tobacco GRP-1b [27] were shown to have *in vitro* binding preference for homoribopolymers G and U, and barley BLT801 [23] has affinity for homoribopolymers G, A and U. Putative RNA-binding properties of all other plant GR-RBPs have been deduced by homology. The two-domain organization of plant GR-RBPs is similar to the A1 and A2/B1 groups of heterogeneous nuclear RNA-binding proteins (hnRNP) found in animals. Although each domain is therefore approximately twice the size of those found in plants. The hnRNPs are associated with nascent hnRNA and are not a part of the other complexes (e.g. snRNA). These proteins are located in the nucleus and have been implicated in pre-mRNA splicing [59]. It has been demonstrated that the GR-RBP from maize, MA16, interacts with a cold inducible DEAD box RNA helicase [60]. Very recently it was found that a DEAD box RNA helicase is essential for mRNA export and import and has important functions in stress responses [61]. The *Arabidopsis* GR-RBP AtGRP7 was reported to participate in splice site selection and autoregulation of the stability of its own transcript via alternative splicing [62].

The mRNA levels of a large number of genes change during the period of cold acclimation in overwintering cereals [2] and post-transcriptional processes may play crucial roles in the overall control and regulation of eukaryotic gene expression [63]. Therefore, the expression pattern and the sequence similarity of the TaGRP2 protein encoded by *wca212* strongly suggest its involvement in the control of steady-state mRNA levels of low temperature-responsive genes via one of the above mentioned mechanisms.

Expression patterns of *HMGB1* and *TaGRP2* transcripts were very similar especially at the early stages of cold acclimation suggesting that their transcription during cold acclimation might be triggered and regulated by a common mechanism.

In contrast to its closest homolog *wdhn13* [31], the expression of *wca341* was induced by ABA in addition to cold and dehydration treatments, clearly indicating that it is not alternative splicing variant of *wdhn13* but a novel member of the wheat dehydrin family named *DHN14* and showing distinct regulation mechanisms. The accumulation of *DHN14* transcripts in response to ABA treatment started most rapidly, compared to LT and drought stress treatment. This result would indicate that the plant hormone ABA is involved in the regulation of *DHN14* transcription caused by LT and drought stresses.

In this study three clones, though all of them were up-regulated by cold treatment, showed distinct expression patterns. The mechanisms of cold regulated gene expression in wheat might be more complicated than expected by the recent studies with the model plants. To better understand molecular events that occur during cold acclimation in cereals, it is important to increase the number of cold acclimation related genes and analyze their function and regulation. It is also important to examine interactions of each protein or signalling pathways involved in cold acclimation.

РЕЗЮМЕ. Зимующие растения, такие как озимая пшеница, проявляют значительное повышение зимостойкости в период холодовой акклиматизации. Чтобы лучше понять молекулярные механизмы холодовой акклиматизации, важно выявить функции и регуляцию соответствующих генов. Скрининг библиотеки кДНК тканей корончатых галлов озимой пшеницы, акклиматизированной к холоду, выявил три новых клона кДНК. Анализ нуклеотидной последовательности показал, что клоны кодируют высокомобильный глобулярный белок (HMGB1), богатый глицином РНК-связывающий белок (TaGRP2) и LEA D-11 дегидрин (DHN14). Накопление трех матричных РНК в течение 14 дней холодовой акклиматизации дифференциально регулировалось. В ответ на засуху и обработку абсцизовой кислотой быстро накапливалась мРНК DHN14, в то время как уровни мРНК HMGB1 и TaGRP2 не изменялись. Обсуждается возможная роль каждого из этих генов в холодовой акклиматизации.

РЕЗЮМЕ. Зимуючі рослини, такі як озима пшениця, проявляють значне підвищення зимостійкості в період холодової акклиматизації. Щоб краще зрозуміти молекулярні механізми холодової акклиматизації, важливо виявити функції і регуляцію відповідних генів. Скрінінг бібліотеки кДНК тканин корончатих галів озимої пшениці, акклиматизованої до холоду, виявив три нових клони кДНК. Аналіз нуклеотидної послідовності показав, що клони кодують високомобіль-

ний глобулярний білок (HMGB1), багатий гліцином РНК-зв'язувальний білок (TaGRP2) та LEA D-11 де-гідрин ((DHN14). Накопичення трьох матричних РНК протягом 14 днів холодової акліматизації диференційно регулювалося. У відповідь на засуху та обробку абсцизовою кислотою швидко накопичувалася мРНК DHN14, в той час як рівні мРНК HMGB1 та TaGRP2 не змінювалися. Обговорюється можлива роль кожного з цих генів в холодовій акліматизації.

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ABBREVIATIONS

CA	— cold acclimation
NA	— non-acclimation
DA	— de-acclimation
HMG protein	— high mobility globular protein
GR-RBP	— glycine-rich RNA binding protein
ABA	— abscisic acid.