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Accumulation of WCS120 and DHN5 proteins in differently frost-tolerant wheat and barley cultivars grown under a broad temperature scale

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Abstract

Proteins WCS120 and DHN5 are known as the major cold-inducible dehydrins in wheat and barley plants, respectively. WCS120 and DHN5 relative accumulation increased exponentially along with a growth temperature decline in the range from optimum to cold temperatures. Even at optimum growth temperatures, the most frost-tolerant wheat and barley cultivars can be distinguished from the remaining ones according to dehydrin relative accumulation. The highly tolerant wheat and barley cultivars started accumulating dehydrins at higher growth temperatures and reached higher dehydrin amounts than the less tolerant ones. Statistically significant correlations between lethal temperature for 50 % of the samples (LT50) and dehydrin relative accumulation have been found at all growth temperatures (5, 10, 15, and 20 °C) for WCS120 in wheats and at 5 and 10 °C for DHN5 in barleys. Analogous relationships between dehydrin relative accumulation at different growth temperatures and plant acquired frost tolerance have been proved for wheat WCS120 and barley DHN5.

Additional key words: cold acclimation, dehydrins, lethal temperature, *Hordeum vulgare, Triticum aestivum*.

Introduction

Dehydrins, highly hydrophilic late embryogenesis abundant (LEA) II proteins, accumulate not only in mature seeds, but also in whole plants under various stresses, *e.g.*, drought, salinity, wounding, and low temperature (cold and frost). Accumulation of hydrophilic cold- regulated COR/LEA proteins represents an important part of the complex process of plant cold acclimation (CA; Thomashow 1999). All dehydrins are characterised by the presence of at least one copy of conserved lysine-rich sequence, the so-called K-segment. Apart from this conserved motif, some dehydrins can also contain other conserved domains, *e.g.*, a tyrosine-rich Y-segment and a serine-rich S-segment which can be phosphorylated and can play an important role in dehydrin subcellular localization. According to the presence of conserved motifs, dehydrins can be categorized into five structural sub-groups: Kn, SKn, YxSKn, YxKn, and KnS (for review see Close 1997, Rorat 2006, Kosová *et al.* 2007). In common wheat, the major cold-inducible dehydrins belong to WCS120 protein family with at least five members – WCS200, WCS180, WCS66, WCS120, and WCS40 (reviewed in Sarhan *et al.* 1997) whereas in barley, the major cold-inducible dehydrin is DHN5 (Van Zee *et al.* 1995, Bravo *et al.* 1999, Choi *et al.* 1999, Zhu *et al.* 2000, Tommasini *et al.* 2008). The WCS120 proteins and DHN5 protein are orthologues; they all belong to Kn structural type, display cryoprotective activities and they are located on the long arm of group 6 homoeologous chromosomes (6AL, 6BL, and 6DL in common wheat and 6HL in barley; Houde *et al.* 1992,1995, Bravo *et al.* 1999,2003, Zhu *et al.* 2000).

Apart from dehydrins, a strong cold-inducible

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Abbreviations: ALP - alkaline phosphatase; CA - cold acclimation; CBF - C-repeat binding factor; COR - cold-regulated; DD - degree days (days of cultivation multiplied by growth temperature); DHN - dehydrin; FR - frost-resistance; FT - frost tolerance; GAR - goat anti-rabbit (secondary antibody); LEA - late embryogenesis abundant; LT50 - lethal temperature when 50 % of sample die; PAGE - polyacrylamide gel electrophoresis; SDS - sodium dodecyl sulfate; TBS - Tris-buffered saline; TTBS - *Tween-20* Tris-buffered saline; WCS - wheat cold-specific.

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expression has also been reported for chloroplast-located *Cor14b* gene in wheat and its barley orthologue which both belong to *Lea-III* gene family. An expression of *Cor14b* gene in barley plants, subjected not only to cold (4 °C) but also to higher growth temperatures (6, 8, and 10 °C), was first reported by Crosatti *et al.* in 1995. Since then, differential *Cor14b* gene expression has also been detected in common wheat (Vágújfalvi *et al.* 2000) and einkorn wheat (Vágújfalvi *et al.* 2003) grown under various temperature regimes. In all published papers, frost-tolerant wheat or barley cultivars can be distinguished from the frost-susceptible ones according to *Cor14b* expression not only under cold but also at higher growth temperatures (10 - 18 °C). At higher growth temperatures, *Cor14b* expression was detected usually only in the frost-tolerant genotypes while no *Cor14b* transcripts were detected in the frost-sensitive genotypes.

Recent work of Holková *et al.* (2009) has shown that transcripts of *Wcs120* and *Wdhn13* genes can be used as markers of maximum acquired frost tolerance (FT) in wheat plants grown at 17 °C. Analogous relationships have also been described for C-repeat binding factor (CBF) transcriptional activators which regulate the expression of *Cor* genes (Miller *et al.* 2006, Badawi *et al.* 2007, Stockinger *et al.* 2007, Knox *et al.* 2008, Campoli *et al.* 2009). The observed differences in *CBF* and *Cor* gene expression correspond well with the results of Fowler (2008) who studied acquired FT in a set of common wheat, barley, and rye genotypes. He found out that under the same growth temperature, tolerant genotypes reveal lower lethal temperature for 50 % of the samples (LT50) in frost tests than sensitive ones; moreover, the LT50 values started decreasing in frost-tolerant cultivars at significantly higher growth temperatures than in the frost-sensitive ones.

The aim of the present study was to investigate accumulation of the major cold-inducible dehydrins, WCS120 protein, which is the most abundant member of the WCS120 protein family in common wheat and DHN5 protein in barley, exposed to a broad scale of growth temperatures ranging from optimum (20 °C) to temperatures usually used for CA treatments (5 °C). In our previous work (Vítámvás *et al.* 2007, Kosová *et al.* 2008), a correlation between dehydrin relative accumulation in wheat and barley plants, respectively, and plant maximum acquired FT has been found when the plants were fully cold-acclimated. Furthermore, Vítámvás *et al.* (2010) have been able to distinguish differently frost-tolerant winter wheats in a set consisting of twenty winter wheat cultivars and one spring cultivar Sandra by evaluation of WCS120 protein relative accumulation when the plants were grown at 9 or 17 °C. However, no experiment has been carried out on dehydrin relative accumulation in barley exposed to a broad range of growth temperatures. Moreover, no comparison of WCS120 and DHN5 relative accumulation in a single experiment has been carried out until now.

Materials and methods

Plants and growth conditions: A set of eleven wheat and ten barley winter and spring cultivars with different frost tolerance was employed (Table 1). Seeds were obtained either from Genebank in Crop Research Institute, Prague (Dicktoo, Odesskij 31), breeding company *Selgen*, Stupice (Akcent, Amulet, Mironovskaya 808, Sandra, Zdar), or from Central Institute for Testing and Supervising in Agriculture, Brno, the Czech Republic (Amaretto, Bill, Igri, Ilias, Karolinum, Luran, Luxor, Mladka, Nela, Nelly, Šárka, Tiffany, Trend, Vilna). The seeds germinated on moist filter paper were planted into pots filled with soil. All plants were first grown in a growth chamber (*T-16/4*, *Tyler*, Budapest, Hungary) at 20 °C and 12-h photoperiod (350 μmol m^{-2} s^{-1}) for 7 d. After this period, plants were divided into four growth chambers with the same photoperiod and irradiance but different temperatures (5, 10, 15, and 20 °C). The plants were harvested after the same period of thermal time expressed in degree-days (DD; cultivation period multiplied by growth temperature) in order to obtain plant material in the same developmental stage (a three-leaf stage). Therefore, plants grown at 20 °C were harvested after additional 7 d of cultivation, plants grown at 15 °C were harvested after 10 d of cultivation while plants grown at 10 °C and the plants grown at 5 °C were harvested after 14 and 28 d of cold treatment, respectively.

Determination of frost tolerance: The cultivars were grown and hardened for 28 d under the same conditions as described above for plants hardened at 5 °C. Thereafter, the plants were removed from the soil, divided into six groups of 8 to 10 plants each, put on the tray, and exposed to -3 °C in the dark for 3 d. Then, each group was chilled for further 24 h at either -9, -11, -13, -15, -17 or -19 °C in freezing boxes (*MHM/52*, *Mirkoz*, Budapest, Hungary) after which they were repotted and left to recover at +20 °C for a further 21 d. Both the cooling and the thawing rates were 2 °C h^{-1}, following Prášil *et al.* (2007). At the end of the recovery period, the survival percentage was calculated and LT50 was obtained following Janáček and Prášil (1991).

Analysis of dehydrin protein relative accumulation: Plant leaf tissue from young but fully expanded second leaves was frozen in liquid nitrogen and stored at

-80 °C. Then the tissue (1 g) was homogenized with 5 cm^3 extraction buffer (100 mM Tris-HCl, pH 8.5, for wheat samples and pH 9.0 for barley samples, respectively, containing *Complete EDTA-free Protease Inhibitor Cocktail Tablets* (*Roche*, Basel, Switzerland) under liquid nitrogen. The mixture was centrifuged at 20 000 *g*, 4 °C for 20 min, then kept in boiling water for 15 min, cooled rapidly to 4 °C, and centrifuged again at 20 000 *g* (4 °C) for 20 min. Concentration of heat stable proteins was determined according to Bradford (1976). The supernatants were then precipitated by cold acetone with 1 % (v/v) 2-mercaptoethanol in 1:5 sample:acetone ratio (v/v). The pellet was then centrifuged at 20 000 *g* (4 °C) for 20 min and dried.

Dry samples from plants grown at 5 and 10 °C (200 mm^3) were resolved in 750 mm^3 SDS-sample buffer prepared according to the manual of *Biometra* (Göttingen, Germany). The samples from plants grown at 15 and 20 °C were 10 and 100 times less diluted, respectively. The samples were loaded on SDS-PAGE (*Biometra*; 10 % resolving gel; Laemmli 1970), 5 mm^3 of the sample per line. The SDS-PAGE was run at 10 mA per stacking gel and at 25 mA per resolving gel. For the estimation of the WCS120 and DHN5 bands, *Kaleidoscope* prestained standards (*Bio-Rad*, Hercules, CA, USA) were used.

Immunoblots were carried out on a semi-dry blotter (*Biometra*) at 1 mA cm^{-2} for 1.5 h using a nitrocellulose membrane (pore diameter 0.45 µm; *Bio-Rad*). Membranes were blocked in 3 % (m/v) gelatin in Tris buffered saline (TBS; 20 mM Tris-HCl, pH 7.5; 500 mM NaCl) for 2 h, washed in 0.1 % (v/v) *Tween-20* in TBS (TTBS) for 10 min, and incubated in anti-dehydrin antibody (Close *et al.* 1993) dissolved in 1 % (m/v) gelatin in TTBS (dilution 1:1000) overnight. After the wash in TTBS for 10 min, the membranes were incubated in goat anti-rabbit secondary antibody conjugated to alkaline phosphatase (*GAR-ALP*) in 1 % gelatin in TTBS (dilution 1:3000) for 2 h. After final washes in TTBS and TBS (each for 10 min), the membranes were developed in *ALP* conjugate substrate kit (*Bio-Rad*) until the reaction was completed. The length of development period varied significantly according to the growth temperature; dehydrin bands in samples from plants grown at 5 °C were clearly visible after just 10 - 15 min of development whereas samples from plants grown at 20 °C required 1 - 1.5 h of development. Both WCS120 protein and DHN5 protein were identified according to their relative positions on the immunoblots. The corresponding relative molecular masses (WCS120 *ca*. 50 kDa, DHN5 *ca.* 82 kDa) are unique for these dehydrin proteins (Sarhan *et al.* 1997, Choi *et al.* 1999, Bravo *et al.* 2003). Moreover, the WCS120 protein has already been identified in cold-acclimated winter wheat Mironovskaya 808 by mass spectrometry analysis by Vítámvás *et al.* (2007). The amount of accumulated WCS120 and DHN5 proteins on the immunoblots was analysed densitometrically using *Quantity One v. 4.6.2* software (*Bio-Rad*). One sample – a highly frost-tolerant winter wheat Mironovskaya 808 grown at 5 °C – loaded in two different concentrations (one concentration was the same as for other samples grown at 5 °C; the other was 10 times lower) was used as an internal standard for both wheat WCS120 and barley DHN5 in order to facilitate the comparison of the sample sets grown at different temperatures. For data presentation, the relative densities of dehydrin relative accumulation at 15 and 20 °C were recalculated to the same sample concentrations as for the samples grown at 5 and 10 °C.

Statistical analysis: For analysis of dehydrin relative accumulation, three biological replicates and two technical replicates were used and the results were compared using *ANOVA* analysis, multiple comparisons and Duncan´s multiple range test at 0.05 level (*Statistica v. 10* software, *StatSoft,* Tulsa, OK, USA). Acquired LT50 values were compared using $LSD_{0.05}$ test according to Janáček and Prášil (1991). For curve fitting and correlation analyses, applications in *SigmaPlot v. 11* (*Systat*, San Jose, CA, USA) were used.

Results

Frost tolerance expressed as LT50 values was determined in fully cold-acclimated plants (Table 1). From selected eleven wheat cultivars, Mironovskaya 808 and Šárka were the most hardy revealing LT50 values lower than -18 °C. Ilias, and Nela displayed LT50 values in the range from -17 to -15 °C, and Zdar, Karolinum, Mladka, Bill, and Trend in the range from -14 to -13 °C. The two spring cultivars (Sandra and Amaretto) revealed LT50 values around -11 °C. In barleys, the LT50 values ranged from -17.7 °C in Odesskij 31 *via* -15.5 to -14.5 °C in Dicktoo, Luxor, and Tiffany and -14 to -13 °C in Luran, Vilna, Nelly, and Igri to -11 and -10 °C in spring cvs. Akcent and Amulet.

From cold-inducible dehydrins, the most abundant wheat dehydrins were WCS120 and WCS66; the other members of the WCS120 dehydrin family were much less abundant. In barley, the most abundant cold-inducible dehydrin was DHN5 (Fig. 1). Therefore, WCS120 and DHN5 were chosen for further analyses on dehydrin relative accumulation upon different growth temperature treatments (Fig. 2). A comparison of WCS120 and DHN5 relative accumulation in wheat and barley plants grown at different temperatures revealed an exponential increase in WCS120 and DHN5 relative content with decreasing

Table 1. A list of wheat and barley cultivars used for determination of dehydrin relative accumulation and their maximum acquired LT50 values determined by a direct frost test after a 28-d CA treatment. Statistically significant differences ($LSD_{0.05}$) in LT50 values between individual cultivars are marked with different letters. Both wheat and barley cultivars have been evaluated as one group. In the brackets, a brief characteristic of each cultivar including a three-letter abbreviation used further in the figures, cultivar growth habit, frost tolerance, and row type of barley spike is given. Abbreviations used for growth habits: F - facultative cultivar, S - spring cultivar, W - winter cultivar, HT - highly tolerant, MeT - medium tolerant, MiT - mild tolerant.

Wheat cultivars	LT50 [°C]	Barley cultivars	LT50 [°C]
Mironovskaya 808 (Mir; W, HT)	-19.0a		
Šárka (Sar; W, HT)	-18.1ab	Odesskij31 (Ode; W, HT; six-row)	-17.7b
Ilias (Ili; W, MeT)	-16.8bc	Luxor (Lux; W, MeT; six-row)	-15.5c
Nela (Nea; W, MeT)	-15.3cd	Dicktoo (Dic; F, MeT; six-row)	-15.3cd
Zdar (Zda; W, MiT)	-14.3de	Tiffany (Tif; W, MeT; two-row)	-14.5d
Karolinum (Kar; W, MiT)	-14.3e	Luran (Lur; W, MiT; six-row)	-14.3de
Mladka (Mla; W, MiT)	-14.2e	Vilna (Vil; W, MiT; two-row)	-13.8e
Bill (Bil; W, MiT)	-14.0e	Nelly (Nel; W, MiT; six-row)	-13.5e
Trend (Tre; W, MiT)	-13.4e	Igri (Igr; W, MiT; two-row)	-13.4e
Sandra (San; S)	-11.3f	Akcent (Akc; S; two-row)	-11.0f
Amaretto (Ama; S)	-11.0f	Amulet (Amu; S; two-row)	-10.0f

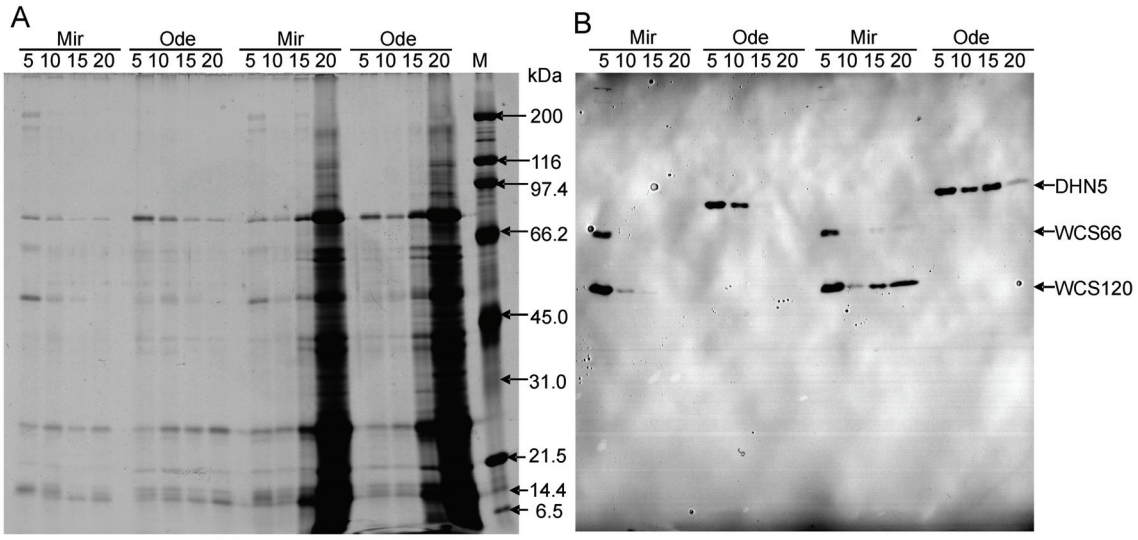

Fig. 1. Silver-stained 1-D SDS-PAGE gel (*A*) and a corresponding immunoblot (*B*) showing boiling-soluble protein separation (*A*) and dehydrin accumulation (*B*), respectively, in Mironovskaya 808 (Mir) and Odesskij 31 (Ode) grown at 5, 10, 15, and 20 °C after 280 degree-days. In the left half of both the gel and immunoblot, equal concentrations of all samples are loaded whereas in the right half, samples grown at 15 and 20 °C are 10 times and 100 times more concentrated, respectively, than samples grown at 5 and 10 °C. M - broad-range marker (*Bio-Rad*).

growth temperature from 20 to 5 °C (Fig. 3*A*,*B*). When WCS120 and DHN5 relative accumulation is expressed as a common logarithm (Fig. 3*C*,*D*), it could be well seen that the dependence of Log(WCS120) or Log(DHN5) on growth temperature is linear and the increase in WCS120 or DHN5 amount is around 10 000 times between 20 and 5 °C. Moreover, it became evident that in frost tolerant wheat and barley cultivars, WCS120 and DHN5, accumulation started rising at higher growth temperatures in comparison with the less tolerant ones (Fig. 3*A*,*B*). At lower growth temperatures (5 and 10 °C; Fig. 4*C*,*D*), dehydrin relative accumulation was significantly higher than at higher temperatures (15 and 20 °C; Fig. 4*A*,*B*), so the absolute differences in WCS120 and DHN5 relative contents among the individual wheat and barley cultivars, respectively, were larger at the lower temperatures than at the higher temperatures. Therefore, it can be concluded that the lower the growth temperature, the more groups revealing differences in dehydrin relative accumulation levels could be distinguished in both wheat and barley cultivar sets. Along with a growth temperature increase, the differences between the cultivars with different maximum acquired FT levels became smaller; however, the highly frost-tolerant wheat cvs. Mironovskaya 808 and Šárka, and barley cv. Odesskij 31 could be distinguished from the remaining less tolerant winter

cultivars and susceptible spring cultivars even at 20 °C (Fig. 4*A*).

The correlations between maximum acquired LT50 and WCS120 relative accumulation (Fig. 5*A*) were statistically significant at 0.05 level under all growth temperatures, however, for LT50 and DHN5, a statistically significant correlation was obtained only for 5 and 10 °C treatments (Fig. 5*B*).

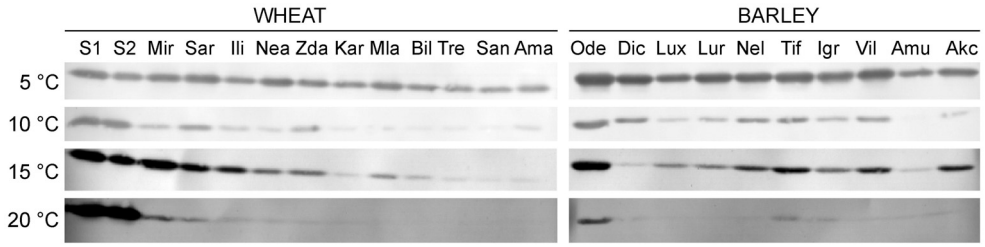

Fig. 2. Representative immunoblot sections showing accumulation of WCS120 in wheats and DHN5 in barleys grown at 5, 10, 15, and 20 °C after 280 degree-days. Both wheat and barley samples grown under the same temperature regimes were loaded on the same gel. Samples from the plants grown at 15 and 20 °C were 10 and 100 times more concentrated than samples from the plants grown at 5 and 10 °C. For comparison of band density on different 1-D gels, cv. Mir grown at 5 °C was used as an internal standard in two different sample concentrations (one sample revealing the same concentration as the samples grown at 5 °C and 10 °C - S1 and the other sample with a ten-times lower concentration - S2). For full names of wheat and barley cultivars, see Table 1.

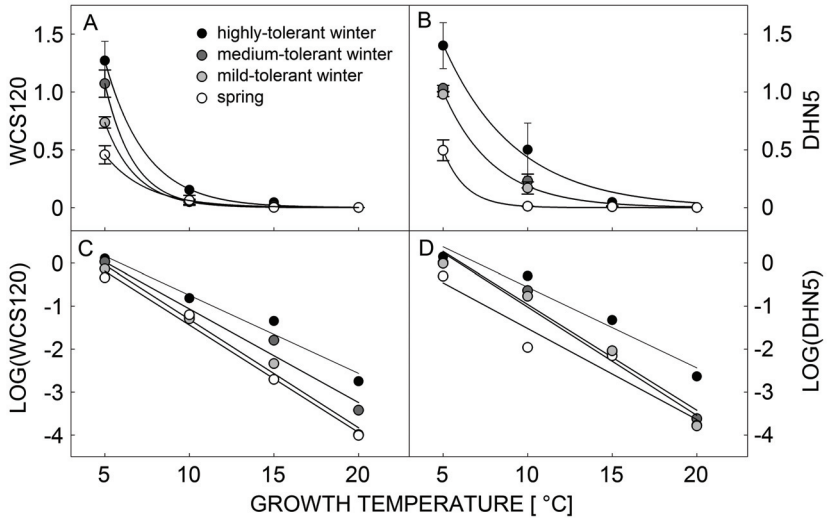

Fig. 3. WCS120 (*A*) and DHN5 (*B*) relative accumulation in wheat and barley cultivars with respect to growth temperature. For comparison of dehydrin relative accumulation, WCS120 accumulation in Mironovskaya 808 grown at 5 °C was taken as an internal standard equal 1. Dehydrin relative accumulation in the samples from 15 and 20 °C has been recalculated to the values corresponding to the concentrations of the samples grown at 5 and 10 °C. *Vertical bars* represent SE. In *C* and *D*, a logarithmic transformation of WCS120 and DHN5 relative accumulation is given (see Table 1 for details).

Discussion

Our data have provided a deeper insight into WCS120 and DHN5 protein relative accumulation along with a decreasing growth temperature. A cold-inducible increase in WCS120 and DHN5 protein relative accumulations was in accordance with the previous results (Fowler *et al.* 1999, 2001, Danyluk *et al.* 2003, Vítámvás *et al.* 2007, Ganeshan *et al.* 2008, Kosová *et al.* 2008, Vítámvás and Prášil 2008). However, in our experiment, four different temperature treatments (5, 10, 15, 20 °C) and eleven wheat and ten barley cultivars revealing a broad range of maximum acquired FT have been compared (Table 1). The use of 10 and 100 times less diluted protein samples from plants grown at 15 and 20 °C, respectively, for gel loading allowed us to detect WCS120 and DHN5 proteins not only in the plants grown at low temperatures but also at higher ones (Figs. 1, 2).

An exponential increase in WCS120 and DHN5 relative content with a decreasing growth temperature (Fig. 3*A*,*B*) was consistent with the hypothesis on threshold induction temperatures (Fowler 2008, Galiba

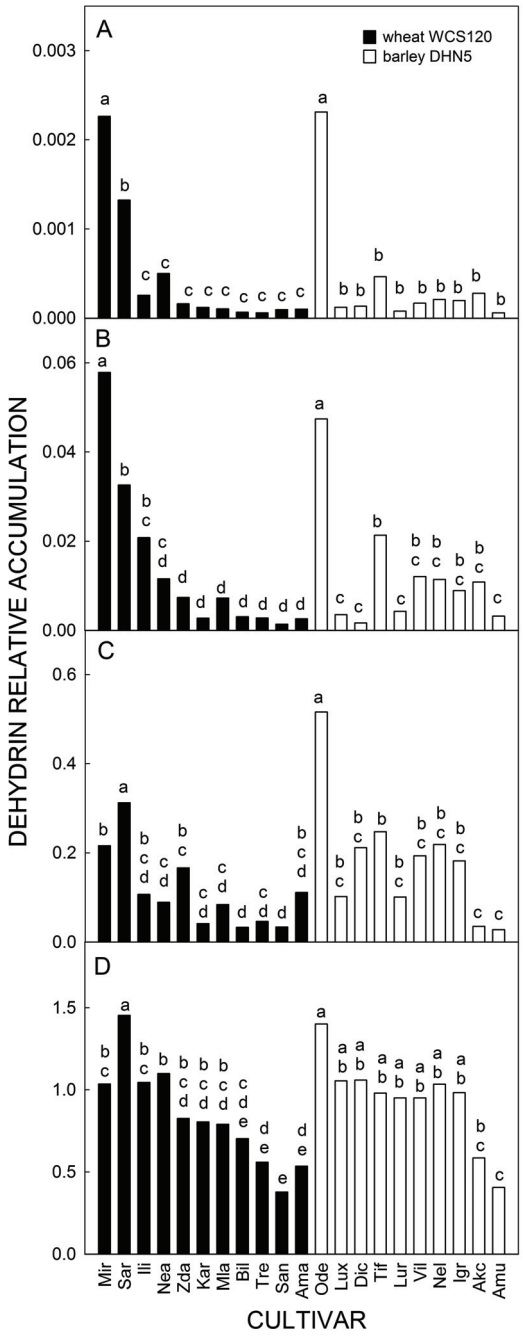

Fig. 4. Dehydrin relative accumulation in the individual wheat and barley cultivars grown at 20 °C (*A*), 15 °C (*B*), 10 °C (*C*), and 5 °C (*D*). For comparison of dehydrin relative accumulation, WCS120 relative accumulation in Mir grown at 5 °C was taken as an internal standard equal 1. Dehydrin relative accumulation in the samples from 15 and 20 °C has been recalculated to the values corresponding to the concentrations of the samples grown at 5 and 10 °C. Different letters represent statistically significant differences in dehydrin relative accumulation between the given cultivars (data from the different growth temperatures as well as data from wheat and barley cultivars at a given growth temperature have been evaluated separately) at $P \leq 0.05$. For full names of the cultivars, see Table 1.

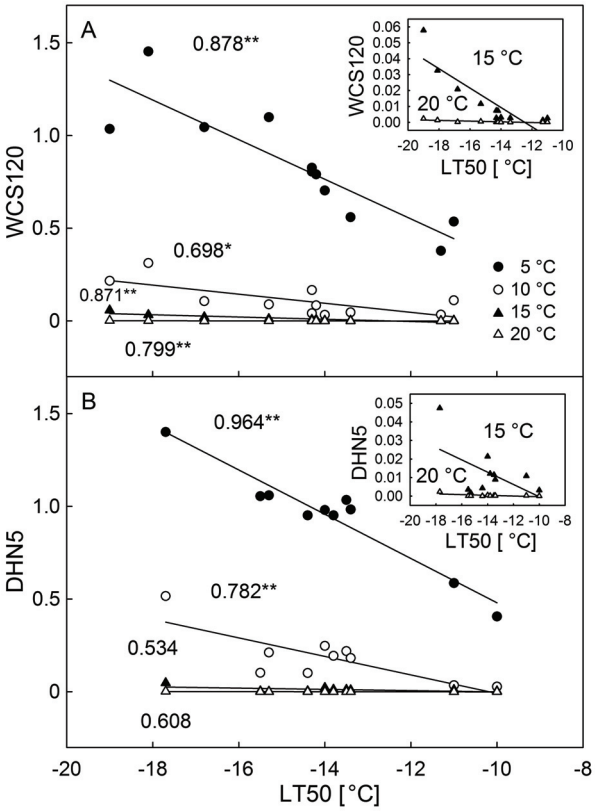

Fig. 5. Correlations between WCS120 (*A*) and DHN5 (*B*) relative accumulation in wheat and barley cultivars grown at 5, 10, 15, and 20 °C, and LT50. Dehydrin relative accumulation in the samples from 15 and 20 °C has been recalculated to the values corresponding to the concentrations of the samples grown at 5 and 10 °C. The values of correlation coefficients are shown for each sample set. *, ** - significant correlations at 0.05 and 0.01 levels, respectively.

et al. 2009, Tondelli *et al.* 2011). Quantitative differences in dehydrin relative accumulation between differently frost-tolerant wheat and barley cultivars were observed. The highly frost-tolerant cultivars start accumulating WCS120 and DHN5 at higher growth temperatures than less frost-tolerant winter and susceptible spring cultivars. This observation is in accordance with the previously published data on *Wcs120* and *Wdhn13* gene expression in wheat (Holková *et al.* 2009) and on WCS120 relative accumulation in differentially frost-tolerant winter wheats (Vítámvás *et al.* 2010). Differences between frost-tolerant winter genotypes and frost-susceptible spring genotypes of *Triticeae* have also been reported regarding the expression of *Cor14b* genes (Crosatti *et al.* 1995, Vágújfalvi *et al.* 2000, 2003) and the expression of *CBF* genes, important transcriptional activators of *Cor* gene expression (Miller *et al.* 2006, Badawi *et al.* 2007, Stockinger *et al.* 2007, Knox *et al.* 2008). However, no *Cor14b* transcripts have been detected in the susceptible spring genotypes at mild cold temperatures whereas some dehydrin relative protein accumulation can be detected even in the susceptible spring genotypes grown at 20 °C.

These results might indicate general protective functions of dehydrins in plant cells regardless of ambient conditions (Close 1997, Rorat 2006, Kosová *et al.* 2010). Our experiment showed an increasing differentiation of frost-tolerant and susceptible wheat and barley cultivars along with a temperature decrease. Nevertheless, our data have also shown that the highly frost-tolerant winter wheat (Mir, Sar) and barley (Ode) cultivars can be distinguished from the less tolerant winter and susceptible spring cultivars even at growth temperatures as high as 20 °C (Fig. 4*A*). This fact indicates that dehydrins could be used as reliable protein markers for selection of highly-tolerant *Triticeae* genotypes, similarly to genetic markers, without a need to undergo CA treatment.

One possible explanation of the differences in dehydrin relative accumulation can lie in differential regulation of *Cor* gene expression in tolerant and susceptible *Triticeae* genotypes. It has been found out recently that susceptible spring genotypes of *Triticeae* can contain either non-functional *CBF* genes (*e.g.*, mutation in CRT/DRE-binding AP2 domain in *TmCBF12* gene in spring line DV92 of *Triticum monococcum* causing the inability of this *TmCBF12* allele to activate expression of *Cor14b* gene) or a lower number of *CBF* gene paralogues at *Fr2* locus in comparison with tolerant winter genotypes (Knox *et al.* 2008, 2010). A higher copy number of cold-inducible *CBF* genes at *Fr2* locus in tolerant winter and facultative genotypes in comparison to the susceptible spring ones may determine higher expression levels of several *CBF* genes and *Cor* genes in the tolerant genotypes than in the susceptible ones (reviewed in Tondelli *et al.* 2011).

Statistically significant correlations between WCS120 relative accumulation and FT was found at all growth temperatures but for DHN5 only at 5 and 10 °C (Fig. 5*A*,*B*). This result is consistent with our previous papers dealing with WCS120 relative accumulation in cold-treated wheats (Vítámvás *et al.* 2007, 2010, Kosová *et al.* 2008). A correlation between dehydrin protein relative accumulation and plant acquired FT has also been observed in other economically important crop plants, for example in oilseed rape (Klíma *et al.* 2012). In wheats, relatively fine differences in cultivar maximum acquired FT level or even winter survival could be differentiated by WCS120 relative accumulation not only in fully cold-acclimated plants (Houde *et al.* 1992, Vítámvás *et al.* 2007) but also in plants grown at mild temperatures (Vítámvás *et al.* 2010). In contrast, when using DHN5 protein as a marker of maximum acquired FT in a broad range of barley cultivars, only the large differences between frost-tolerant winter and facultative cultivars and frost-susceptible spring ones could be distinguished (Bravo *et al.* 1999, Zhu *et al.* 2000, Kosová *et al.* 2008). In the present work, a higher DHN5 relative accumulation was found in a relatively low tolerant two-rowed winter barley cultivar Tiffany than in more tolerant six-rowed winter barley Luxor and facultative barley Dicktoo (Fig. 4*A*,*B*,*C*). Thus, a role of both WCS120 and DHN5 proteins as markers of wheat and barley maximum acquired FT levels has been confirmed for cold-treated plants, and, moreover, the role of WCS120 as a marker of wheat maximum acquired FT has been proven also for plants grown only under mild growth temperatures (15 and 20 °C). However, it should be kept in mind that acquired FT is a complex multigenic trait whose level is affected by a coordinated action of several stress-protective proteins (Thomashow 1999). Dehydrin relative accumulation thus represents only a part of a complex process of FT acquisition.

In conclusion, the relationships between WCS120 or DHN5 relative accumulation under a broad range of growth temperatures and plant acquired FT have been proved for both wheat and barley cultivars. It has thus become evident that wheat WCS120 and barley DHN5 reveal not only structural homologies but also similarities with respect to the protein relative accumulation under a broad range of growth temperatures.

References

Badawi, M., Danyluk, J., Boucho, B., Houde, M., Sarhan, F.: The *CBF* gene family in hexaploid wheat and its relationship to the phylogenetic complexity of cereal *CBFs*. - Mol. Genet. Genomics **277**: 533-554, 2007.

Bradford, M.M.: A rapid and sensitive method for the quantities of protein by an improved protein-dye binding assay. - Anal. Biochem. **72**: 248-254, 1976.

Bravo, L.A., Close, T.J., Corcuera, L.J., Guy, C.L.: Characterization of an 80-kDa dehydrin-like protein in barley responsive to cold acclimation. - Physiol. Plant. **106**: 177-183, 1999.

Bravo, L.A., Gallardo, J., Navarrete, A., Olave, N., Martínez, J., Alberdi, M., Close, T.J., Corcuera, L.J.: Cryoprotective activity of a cold-induced dehydrin purified from barley. - Physiol. Plant. **118**: 262-269, 2003.

Campoli, C., Matus-Cádiz, M.A., Pozniak, C.J., Cattivelli, L., Fowler, D.B.: Comparative expression of *Cbf* genes in the *Triticeae* under different acclimation induction temperatures. - Mol. Genet. Genomics **282**: 141-152, 2009.

Choi, D.W., Zhu, B., Close, T.J.: The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 *Dhn* genes of cv. Dicktoo. - Theor. appl. Genet. **98**: 1234-1247, 1999.

Close, T.J.: Dehydrins: A commonalty in the response of plants to dehydration and low temperature. - Physiol. Plant. **100**: 291-296, 1997.

Close, T.J., Fenton, R.D., Moonan, F.: A view of plant dehydrins using antibodies specific to the carboxy-terminal peptide. - Plant mol. Biol. **23**: 279-286, 1993.

Crosatti, C., Soncini, C., Stanca, A.M., Cattivelli, L.: The accumulation of a cold-regulated chloroplastic protein is light-dependent. - Planta **196**: 458-463, 1995.

Danyluk, J., Kane, N.A., Breton, G., Limin, A.E., Fowler, D.B., Sarhan, F.: *TaVRT-1*, a putative transcription factor associated with vegetative to reproductive transition in cereals. - Plant Physiol. **132**: 1849-1860, 2003.

Fowler, D.B.: Cold acclimation threshold induction temperatures in cereals. - Crop Sci. **48**: 1147-1154, 2008.

Fowler, D.B., Limin, A.E., Ritchie, J.T.: Low-temperature tolerance in cereals: model and genetic interpretation. - Crop Sci. **39**: 626-633, 1999.

Fowler, D.B., Breton, G., Limin, A.E., Mahfoozi, S., Sarhan, F.: Photoperiod and temperature interactions regulate low-temperature-induced gene expression in barley. - Plant Physiol. **127**: 1676-1681, 2001.

Galiba, G., Vágújfalvi, A., Li, C., Soltész, A., Dubcovsky, J.: Regulatory genes involved in the determination of frost tolerance in temperate cereals. - Plant Sci. **176**: 12-19, 2009.

Ganeshan, S., Vítámvás, P., Fowler, D.B., Chibbar, R.N.: Quantitative expression analysis of selected *COR* genes reveals their differential expression in leaf and crown tissues of wheat (*Triticum aestivum* L.) during an extended low temperature acclimation regimen. - J. exp. Bot. **59**: 2393-2402, 2008.

Holková, L., Prášil, I.T., Bradáčová, M., Vítámvás, P., Chloupek, O.: Screening for frost tolerance in wheat using the expression of dehydrin genes *Wcs120* and *Wdhn13* at 17 °C. - Plant Breed. **128**: 420-422, 2009.

Houde, M., Dhindsa, R.S., Sarhan, F.: A molecular marker to select for freezing tolerance in *Gramineae*. - Mol. gen. Genet. **234**: 43-48, 1992.

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

Janáček, J., Prášil, I.: Quantification of plant frost injury by nonlinear fitting of an S-shaped function. - Cryo-Letters **12**: 47-52, 1991.

Klíma, M., Vítámvás, P., Zelenková, S., Vyvadilová, M., Prášil, I.T.: Dehydrin and proline content in *Brassica napus* and *B. carinata* under cold stress at two irradiances. - Biol. Plant. **56**: 157-161, 2012.

Knox, A.K., Li, C., Vágújfalvi, A., Galiba, G., Stockinger, E.J., Dubcovsky, J.: Identification of candidate *CBF* genes for the frost tolerance locus *Fr-A^{m}2* in *Triticum monococcum*. - Plant mol. Biol. **67**: 257-270, 2008.

Knox, A.K., Dhillon, T., Cheng, H., Tondelli, A., Pecchioni N., Stockinger, E.J.: *CBF* gene copy number variation at *Frost-resistance-2* is associated with levels of freezing tolerance in temperate-climate cereals. - Theor. appl. Genet. **121**: 21-35, 2010.

Kosová, K., Vítámvás, P., Prášil, I.T.: The role of dehydrins in plant response to cold. - Biol. Plant. **51**: 601-617, 2007.

Kosová K., Holková, L., Prášil, I.T., Prášilová, P., Bradáčová, M., Vítámvás, P., Čapková, V.: Expression of dehydrin 5 during the development of frost tolerance in barley (*Hordeum vulgare*). - J. Plant Physiol. **165**: 1142-1151, 2008.

Kosová K., Prášil I.T., Vítámvás P.: Role of dehydrins in plant stress response. - In: Pessarakli M. (ed.): Handbook of Plant and Crop Stress. 3rd Ed. Pp. 239-285. CRC Press, Taylor & Francis, Boca Raton 2010.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. - Nature **277**: 680-685, 1970.

Miller, A.K., Galiba, G., Dubcovsky, J.: A cluster of 11 *CBF* transcription factors is located at the frost tolerance locus *Fr-A^{m}2* in *Triticum monococcum*. - Mol. Genet. Genomics **275**: 193-203, 2006.

Prášil, I.T., Prášilová, P., Mařík, P.: Comparative study of direct and indirect evaluations of frost tolerance in barley. - Field Crops Res. **102**: 1-8, 2007.

Rorat, T.: Plant dehydrins – tissue location, structure and functions. - Cell. mol. Biol. Lett. **11**: 536-556, 2006.

Sarhan, F., Ouellet, F., Vazquez-Tello, A.: The wheat *wcs120* gene family. A useful model to understand the molecular genetics of freezing tolerance in cereals. - Physiol. Plant. **101**: 439-445, 1997.

Stockinger, E.J., Skinner, J.S., Gardner, K.G., Francia, E., Pecchioni, N.: Expression levels of barley *Cbf* genes at the *Frost resistance-H2* locus are dependent upon alleles at *Fr-H1* and *Fr-H2*. - Plant J. **51**: 308-321, 2007.

Thomashow, M.F.: Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. - Annu. Rev. plant Physiol. plant mol. Biol. **50**: 571-599, 1999.

Tommasini, L., Svensson, J.T., Rodriguez, E.M., Wahid, A., Malatrasi, M., Kato, K., Wanamaker, S., Resnik, J., Close, T.J.: Dehydrin gene expression provides an indicator of low temperature and drought stress: transcriptome-based analysis of barley (*Hordeum vulgare* L.). - Funct. integr. Genomics **8**: 387-405, 2008.

Tondelli, A., Francia, E., Barabaschi, D., Pasquariello, M., Pecchioni, N.: Inside the *CBF* locus in *Poaceae*. - Plant Sci. **180**: 39-45, 2011.

Vágújfalvi, A., Crosatti, C., Galiba, G., Dubcovsky, J., Cattivelli, L.: Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the *cor14b* gene in frost-tolerant and frost-sensitive genotypes. - Mol. gen. Genet. **263**: 194-200, 2000.

Vágújfalvi, A., Galiba, G., Cattivelli, L., Dubcovsky, J.: 2003: The cold-regulated transcriptional activator *Cbf3* is linked to the frost-tolerance locus *Fr-A2* on wheat chromosome 5A. - Mol. gen. Genomics **269**: 60-67, 2003.

Van Zee, K., Chen, F.Q., Hayes, P.M., Close, T.J., Chen, T.H.H.: Cold-specific induction of a dehydrin gene family member in barley. - Plant Physiol. **108**: 1233-1239, 1995.

Vítámvás, P., Saalbach, G., Prášil, I.T., Čapková, V., Opatrná, J., Jahoor, A.: WCS120 protein family and proteins soluble upon boiling in cold-acclimated winter wheat. - J. Plant Physiol. **164**: 1197-1207, 2007.

Vítámvás, P., Prášil, I.T.: WCS120 protein family and frost tolerance during cold acclimation, deacclimation and reacclimation of winter wheat. - Plant Physiol. Biochem. **46**: 970-976, 2008.

Vítámvás, P., Kosová, K., Prášilová, P., Prášil, I.T.: Accumulation of WCS120 protein in wheat cultivars grown at 9°C or 17°C in relation to their winter survival. - Plant Breed. **129**: 611-616, 2010.

Zhu, B., Choi, D.-W., Fenton, R., Close, T.J.: Expression of the barley dehydrin multigene family and the development of freezing tolerance. - Mol. gen. Genet. **264**: 145-153, 2000.