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BRIEF COMMUNICATION

Changes in dehydrin composition in winter cereal crowns during winter survival

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Abstract

This study is focused on examination of crown dehydrin content during overwintering and spring dehardening periods in three *Poaceae* family winter plants: rye, wheat, and triticale. Frost resistances of seedlings in laboratory and field conditions were compared. Immunoblotting demonstrates that winter wheat and winter triticale differed from winter rye based on their dehydrin qualitative content. Unlike wheat and triticale, rye lacked a protein with a molecular mass of 55.3 kDa. Winter wheat contained a polypeptide with a molecular mass of 29 kDa in autumn but lacked it in winter compared with triticale. Comparison of dehydrin spectra from the three winter crops suggests a relationship between synthesis of dehydrins with molecular masses of 29 and 55.3 kDa and frost resistance of the plant species.

Additional key words: frost tolerance, LEA proteins.

A long-term winter stress tolerance in winter crops involves several components including resistance to dehydration, low negative temperatures, cycles of freezing and thawing, flooding, and other factors that affect plants individually or in an integrated manner. The most dangerous is the period when plants quit the state of winter dormancy and display reduced levels of stress resistance. The most unfavourable environmental factor in East Siberia is the impact of low negative temperatures, which are followed by thaws at the end of winter with a low or no snow cover (Dorofeev *et al.* 2004).

To withstand severe long-term winter conditions, the autumn growth and development period involves acclimation to low negative temperatures, which enhances frost resistance. Low temperature hardening triggers adaptive responses in plant cells including hydrophilic proteins such as dehydrins. They accumulate in intra-cell compartments and act as protectors (Kosová *et al*. 2007, 2008, 2013, Trunova 2007, Hara 2010, Romanenko *et al.* 2010, Tunnacliffe *et al.* 2010, Hanin *et al.* 2011, Takahashi *et al.* 2013). The ability of winter crops to withstand the negative effects of long-term stress depends on the viability of crowns in the vascular transition zone, which have a high dehydrin content in the cytoplasm and in the nucleus (Houde *et al.* 1995). Especially, large amounts of hydrophilic WCS120 and acidic WCOR410 dehydrins are synthesized due to low temperatures and accumulate in the crowns (Danyluk *et al.* 1994, Kosová *et al.* 2013).

The aim of the present study was to identify species-specific differences of dehydrin composition in the crowns of cereals, which may be responsible for various winter resistance of winter crops during overwintering and spring dehardening.

Winter wheat (*Triticum aestivum* L.) cv. Irkutskya, winter rye (*Secale cereale* L.) genotype No. 21, and winter triticale (×*Triticosecale hexaploidii* Kurk. *et* Filat.) genotype No.430-6002 were grown in field conditions on the experimental area of the SIPPB SB RAS, Irkutsk, Russia. All seeds were obtained from the SIPPB SB RAS. Field tests were performed in three biological replicates. The study was carried out between 2010 and 2013.

The relative frost tolerance of the seedlings was determined by the method of Samygin (1967) in a low temperature chamber. Seedlings with a coleoptile length between 5 and 8 mm were hardened at + 2 °C for 7 d

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Abbreviations: LEA - late embryogenesis abundant; LT_{50} - lethal temperature for 50 % of the samples.
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followed by a 3 d incubation at -4 °C. Then, temperature was gradually reduced every 24 h down to -7, -10, -12, -14, -16, -18, and -20 °C with seedling exposure to these temperatures for 24 h. Thereafter, the plants were thawed at 2 °C for 48 h. Then, the plants were transferred to temperatures ranging from 20 to 25 °C. Each experiment was performed in 15 replicates. The amount of survived seedlings was calculated as a percentage of the total number of seedlings in the sample. Upon checking the data acquired for the standard distribution, dispersion analysis was conducted to prove the significance of differences between the variants.

The monolith selection method was used to evaluate plant survivability under field conditions. During the winter time, four monoliths were collected from the crops of each variant. The monoliths were allowed to thaw at low positive temperatures (4 °C) and then transferred to a warm room for plant growth (18 - 20 °C). After 15 d, the number of live and dead plants was calculated (Tretyakov *et al.* 2003). All of the climatic data for this study were obtained from a general access meteorological data system (http://rp5.ru) (Table 1 Suppl.).

Thermostable proteins were extracted from winter wheat, rye, and triticale crowns as described by Lin *et al.* (1990). Protein content was determined according to Lowry *et al*. (1957). Electrophoresis was conducted according to Laemmli (1970) using a *Mini-PROTEAN III* electrophoresis unit (*Bio-Rad*, Hercules, USA). The proteins were then transferred onto nitrocellulose membranes (*GE Healthcare,* Buckinghamshire, UK) incubated with primary antibodies, washed, and incubated with secondary antibodies conjugated with alkaline phosphatase. We used antibodies common for plant dehydrin sequences (EKKGIMDKIKEKLPG) (kindly provided by professor J. Close, the University of California; Close *et al*. 1993) to identify dehydrins in the samples. Polypeptide molecular masses were determined using protein standards (*Sigma*, St. Louis, USA).

Statistical processing the experimental data was carried out using parametric methods. Normality of distribution was determined using the Kolmogorov-Smirnov and Shapiro-Wilk methods. Statistical analyses were done with one way analysis of variance (*ANOVA*) followed by Fisher's LSD multiple range test for independent samples (*SigmaPlot 12.5*).

Under the laboratory conditions, the winter rye and triticale seedlings demonstrated a high winter tolerance [lethal temperatures for 50 % of the samples (LT_{50}) -15.7 and -15.2 °C, respectively], whereas winter wheat had a lower value (LT_{50} -14.6 °C) (Fig. 1). It is noteworthy that winter triticale was less frost resistant than winter rye under all of the temperature modes. However, these differences were not statistically significant. To confirm the laboratory parameters for winter crop seedling resistance to freezing temperatures, we evaluated plant survival rates in field conditions. For the winter rye and winter triticale plants, the survival values were 95.8 and 88.5 %, respectively. The lowest survival of 50.8 % was registered in winter wheat (data not shown).

Accumulation of cold shock proteins including dehydrins from the late embryogenesis abundant (LEA) superfamily ensures a positive correlation between their expression and frost tolerance in crops (Vagujfalvi *et al.* 2000, Kobayashi *et al.* 2004, 2005). To determine differences in winter tolerance between the crops, we attempted to identify differences in crown dehydrin composition during the overwintering period and at the end of winter.

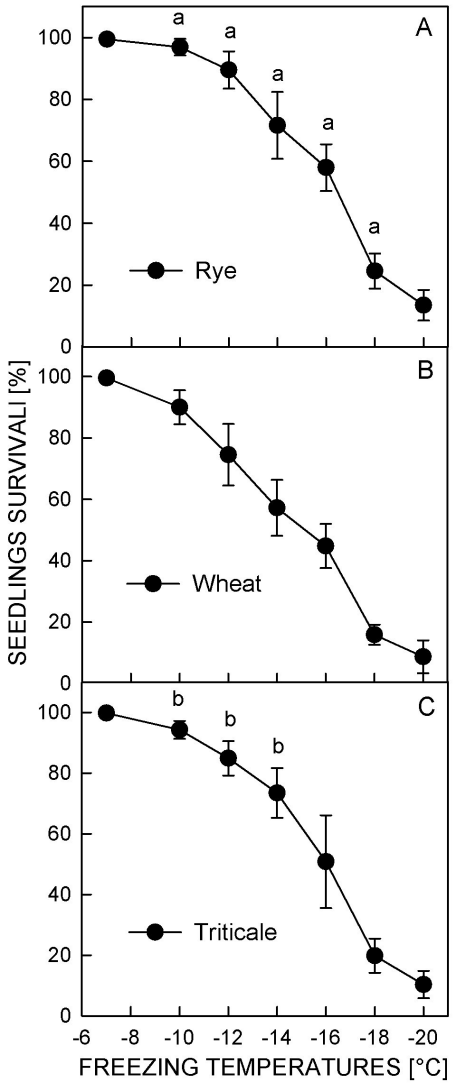

Fig. 1. Frost resistance of winter crop seedlings: *A* - rye, *B* - wheat, and *C* - triticale. Means of survival percentage ± SDs, n = 15. Within each set of experiments, different letters mark significant differences at $P < 0.05$ between rye and wheat (a) or between triticale and wheat (b).

Winter triticale and winter wheat demonstrated similar protein spectra of thermostable fractions, whereas winter rye lacked polypeptides with molecular masses 66 and 29 kDa. A polypeptide with a molecular mass of 66 kDa corresponds to protein WCS66 (Fig. 2*A*). Throughout the period of low-temperature adaptation,

wheat and triticale induced a dehydrin with a molecular mass of 29 kDa, which disappeared in the winter wheat proteins spectrum during overwintering and at the end of winter (Fig. 2*A*,*B*,*C*,*D*). Dehydration caused by a low temperature is known not only to induce expression of dehydrin genes but also to trigger biosynthesis of abscisic acid, which in turn activates synthesis of responsible to abscisic acid family proteins. These proteins as well as dehydrins are characterized by thermostability and are extremely hydrophilic (Close 1996). A polypeptide with a molecular mass of 29 kDa is likely to be homologous to responsible to abscisic acid, which is apparently specifically activated by abscisic acid. A protein spectrum of winter rye contains a polypeptide with a molecular mass of 55.3 kDa (Fig. 2*A*,*B*,*C*,*D*), which is likely to be one of acidic wcor410 dehydrins. Western-blot analysis of

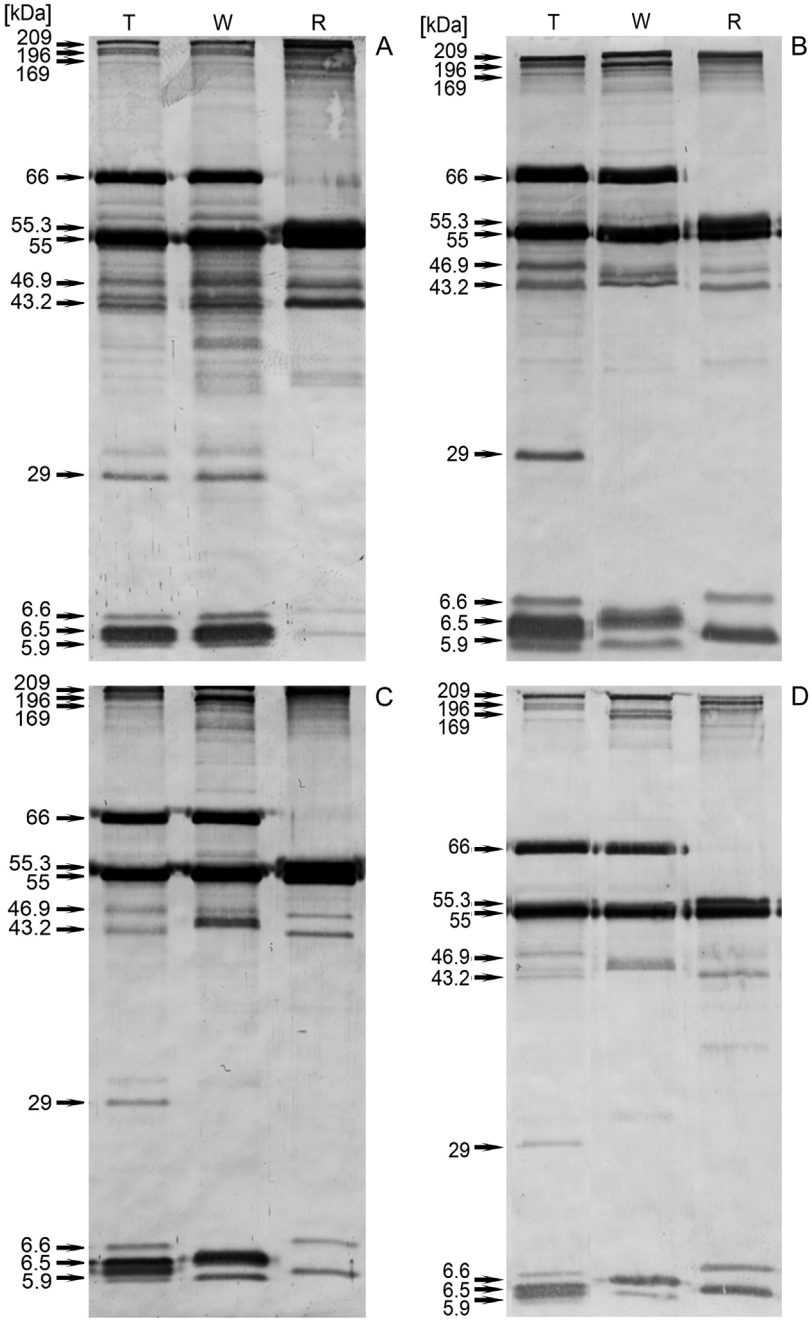

Fig. 2. Dehydrins extracted from a crown protein thermostable fraction. The samples from winter crops were collected at 25 November (*A*), 20 February (*B*), 3 March (*C*), and 30 March (*D*). Immunoblotting on 12 % (m/v) SDS-PAGE (15 μg of protein per lane). T - winter triticale, W - winter wheat, and R - winter rye. The figure presents a typical immunoblotting picture, which was completed in three replicates for each sampling time in every observation year (2010 - 2013).

dehydrin composition of the winter cereal crowns allowed to identify low-molecular proteins with molecular masses 6.6, 6.5, and 5.9 kDa (Fig. 2*A*,*B*,*C*,*D*). Our results demonstrate that this group of low-molecular proteins was intensely expressed throughout the overwintering period. The origin of these peptides is unknown, but they could be earlier uncharacterized immunologically connected ones with 12 (9.6) kDa peptides identified by Sarhan *et al*. (1997). The identified low-molecular polypeptides most likely do not correspond to wcs726 and wcs80 proteins of the WCS120 family.

As it has been shown, wheat cultivars with a higher frost tolerance can be differentiated from less-tolerant ones by evaluation of WCS120 protein content when grown at temperatures higher than those that are normally used in cold-acclimation treatments (Vítámvás *et al*. 2010). Frost-tolerant wheat cultivars are characterized by the ability to induce expression of several dehydrin genes (*WCS120*, *WCS66*, and *WCS40*) during cold acclimation (Vítámvás *et al*. 2007). Our results demonstrate that the winter cereal species with a different frost tolerance were also differentiated from each other by qualitative dehydrin composition: rye contained a unique polypeptide of 55.3 kDa, whose presence can probably explain the highest freezing tolerance of this species, and winter wheat differred from triticale by the absence of a 29 kDa dehydrin during winter (Fig. 2*B*,*C*,*D*). We assume that aforementioned two dehydrin peptides play a crucial role in establishment of winter resistance of the investigated cereal species. Further investigation of these proteins will enable to identify their protective function under low-temperature stress more precisely.

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