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Faculty of Science

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DYNAMICS OF COLD REGULATED PROTEINS DURING COLD ACCLIMATION IN CEREALS

PhD Thesis

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This PhD thesis is dedicated to my beloved wife
Markéta

Abstract

The aim of this dissertation was to study the mechanism of cold acclimation via the dynamics of cold regulated proteins (such as WCS120 or DHN5) in different frost-tolerant wheat and barley cultivars. Mass spectrometry analysis of a total sample of proteins, soluble upon boiling, showed qualitative differences between cold-acclimated (e.g., 7 COR proteins) and non-acclimated (e.g., only 3 COR proteins) samples of the winter wheat Mironovskaya 808. Furthermore, by 2-DE or W-blot analysis, there were found quantitative differences in the accumulation of WCS120 proteins between cultivars, grown under different time, photoperiod, and/or temperature conditions. The higher levels of WCS120 proteins are associated with higher frost tolerance of cultivars, grown under constant and low temperature. However, the dynamics of WCS120 proteins during long-term cold-acclimation, with periods of de-acclimation and re-acclimation, demonstrated that plants with the same level of frost tolerance could be distinguished by the level of accumulation of the WCS120 proteins. These results indicated that developmental genes influence the ability to re-accumulate WCS120 proteins by the partial vernalization of plants, while the ability to induce high frost tolerance was only influenced by the saturation of vernalization. Using five wheat and two barley cultivars with different abilities to resist frost, it was also shown that dry weight content, frost tolerance and accumulation of dehydrins (WCS120 in wheat, or DHN5 in barley) in the leaves is both tolerance- and temperature-dependent.

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Introduction

The aim of this study was to determine the relationships between induction and regulation of the level of frost tolerance and COR (cold-regulated) proteins (especially WCS120 proteins) in wheat cultivars of differing frost tolerance, grown under different time, photoperiod, and/or temperature conditions. Moreover, the question if such related species as wheat and barley have interspecies differences in their induction and regulation of these two traits, was investigated.

Before this current work began, it was known that under cold treatment WCS120 proteins accumulated more rapidly in higher frost-tolerant cultivars; and therefore, it had been suggested that WCS120 proteins could be used as a marker of frost tolerance (Houde *et al.* 1992, Sarhan *et al.* 1997). However, up until this study, the differences in the amounts of WCS120 proteins (or in the levels of *wcs120* mRNA) had only been found between spring (frost-sensitive) and winter (frost-tolerant) cultivars (e.g. Houde *et al.* 1992, Fowler *et al.* 1996). In other words, the differences in the expression of the *wcs120* genes were only found in those cultivars with extreme differences in frost tolerance.

Therefore, the first aim, in part, was to find if two highly frost-tolerant winter wheat cultivars differentiated by their levels of accumulation of WCS120 proteins. Moreover, due to the opportunity to use a gel-free proteomic analysis (LC-MS/MS; Liquid Chromatography-Tandem Mass Spectrometry) during my stay in Denmark, the changes in protein patterns between non- and cold-acclimated samples could be studied. The detailed description of this part of the dissertation is described in Paper 3.

After that first partial aim was solved, the next question emerged: how did the environmental changes, such as the different time-courses of cold treatments (cold-acclimation), followed by different periods of de-acclimation at high temperature, and ending with a cold treatment again (re-acclimation), influence the levels of WCS120 proteins, dry weight content, time to heading (i.e., development), as well as the level of frost tolerance? The previous studies had shown a rapid decrease of WCS120 proteins during de-acclimation (e.g., Kobayashi *et al.* 2004). Other studies had compared the levels of frost tolerance with the levels of WCS120 proteins, during long-term cold acclimation (e.g., Fowler *et al.* 1996), and with the vernalization requirement in re-acclimated plants (e.g., Prášil *et al.*, 2004). However, up until now, no one had tried to compare all the traits (frost tolerance, vernalization, dry-weight content and WCS120 proteins) in one experiment. The new and interesting results obtained by this approach are described in Paper 4.

During the work, we found a slight accumulation of WCS120 at 17°C in winter wheat cultivars. These results led us to the question: if the expression of dehydrins, related to cold-acclimation, is induced at higher temperature in highly frost-tolerant cultivars; while at a lower temperature and with a lower rate in low-tolerant cultivars? Up until now, there has not been any published evidence that the different frost-tolerant cultivars have a different temperature threshold for the induction of dehydrins. However, differences were found for another COR proteins in cereals grown at different low temperatures. The threshold temperature for the induction of barley chloroplast-targeted protein, COR14, and his ortholog in wheat has been observed (e.g., Crosatti *et al.* 1995, Giorni *et al.* 1999, Vágújfalvi *et al.* 2000, Francia *et al.* 2004). Yet, nobody had compared either the amount of COR14 with the actual DWC and FT of sampled leaves, nor the barley and wheat cultivars in the same experiment, as are described in Paper 5.

The dehydrins and their role in the cold responses of plants are reviewed in Paper 1; the approach to the plant proteomes in the research are reviewed in Paper 2; while Papers 3, 4, and 5 describe the experimental results of this dissertation. All chapters represent papers previously submitted or published, and therefore the text and references have different formats between chapters, due to the different specific requirements of the journals.

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Conclusion

In this PhD study, I focused upon one group of the COR (cold-regulated) proteins, accumulated during cold-acclimation in wheat (*Triticum aestivum*) – the WCS120 (wheat cold specific) proteins. The aim of this study was to determine the relationship between frost tolerance and the accumulation of WCS120 proteins in different cultivars, grown under different conditions.

Protein gel blot analysis, mass spectrometry, and image analysis of 2-DE (two-dimensional gel electrophoresis) gels demonstrated the differences in the protein patterns of the samples. In all analyses, the fraction of proteins soluble upon boiling, was used.

In Paper 3, we reported the finding that three-week cold-acclimated Mironovskaya 808 (LT_{50} = -20.8ºC) had higher accumulations of three members of the WCS120 proteins (WCS120, WCS66 and WCS40), than did Bezostaya 1 (LT_{50} = -18.6ºC), grown under the same conditions. Moreover, we observed that both cultivars accumulated a very low level of WCS120 protein at 17ºC. The qualitative differences in protein patterns between cold-acclimated and non-acclimated samples of the cultivar Mironovskaya 808 were demonstrated by LC-MS/MS. For instance, the mass spectrometry analysis showed 7 COR proteins in the cold-acclimated plants, in contrast to only 3 COR proteins found in the non-acclimated plants.

In Paper 4, we observed that long-term cold acclimation (up to 112 days), and either short (5 days) or long (14 days) de-acclimation, followed by re-acclimation of the winter wheat Mironovskaya 808, affected the levels of WCS120 proteins, dry-weight content (DWC), and the frost tolerance in the leaves. During long-term cold acclimation, it was demonstrated that the maximum DWC and accumulation of the WCS120 proteins were reached at about the same time as the vernalization saturation of Mironovskaya 808; while the maximum of the frost tolerance of plant leaves was about 14 days earlier. The DWC content of the WCS120 proteins, as well as the level of frost tolerance rapidly decreased after plant de-acclimation. The longer the de-acclimation, the greater were the decrease of these traits. After cold-re-acclimation, the DWC and frost tolerance reached a similar level, as in those plants with the same time of cold treatment, but without de-acclimation; while the WCS120 proteins accumulated at a lower level. Therefore, the hypothesis has been formulated that the developmental genes influence the ability to re-accumulate WCS120 proteins by partial vernalization of plants, while the ability to re-induce high FT is only influenced by the saturation of vernalization.

In Paper 5, we observed that dry weight content, frost tolerance, induction of dehydrins, and their accumulation in the leaves is both time- and temperature-dependent. The result, that dehydrins were undetected at 25°C, confirmed cold-regulated induction of these proteins, and also indicated that the threshold temperature for this induction is at a lower temperature. However, the wheats were already differentiated at a higher temperature (17°C); while the barleys only at a lower temperature (9°C). The result, that highly frost-tolerant wheat cultivars (M808 and Šárka) grown at 17°C accumulated a higher level of WCS120 proteins (although their levels of FT and DWC were insignificant from low-tolerant cultivars), indicates that the threshold temperature for WSC120 proteins is higher, than the temperature where it is possible to differentiate the wheats by a frost test. The winter and spring wheat cultivars were differentiated according to the threshold temperatures for the induction of expression (i.e., response: present or absent), as well as for accumulation (i.e., response: greater or less) of the dehydrins (WCS120 proteins); while the barleys only were differentiated according to the thresholds temperature for the accumulation of the dehydrin (DHN5). These results indicate that different mechanisms can also occur for COR protein induction between these two members of *Triticae*.

In conclusion, the results indicated that cold regulation of the COR proteins and frost tolerance can be different, and that the level of the studied dehydrins could be associated, not only with the level of frost tolerance, but also with the developmental stage and differences in the growth temperature of plants.

List of Included Papers

Journals:

Paper 1

Kosová K., **Vítámvás P.**, Prášil I.T. (2007) The role of dehydrins in plant response to cold. *Biologia Plantarum. Accepted.* I was responsible for the wheat portion of this review, and the database searching done.

Paper 2

Vítámvás P., Kosová K., Prášil I.T. (2007) Proteome analysis in plant stress research. *Czech Journal of Genetics and Plant Breeding. Accepted.* I was responsible for both the writing and preparation of the manuscript.

Paper 3

Vítámvás P., Saalbach G., Prášil I. T., Čapková V., Opatrná J., Jahoor A., 2007, WCS120 protein family and proteins soluble upon boiling in cold-acclimated winter wheat. *Journal of Plant Physiology,* DOI 10.1016/j.jplph.2006.06.011. I was responsible for the preparation of all samples, protein gel blot analysis, 2-DE analysis, interpretation of results obtained by LC-MS/MS and image analysis, as well as for manuscript writing and preparation.

Paper 4

Vítámvás P., Prášil I.T. WCS120 protein family and frost tolerance during cold-acclimation, de-acclimation and re-acclimation of winter wheat. *Submitted 2007.* I was responsible for all phases of the research presented and for the manuscript writing and preparation.

Paper 5

Vítámvás P., Kosová K., Prášil I.T. Temperature-dependent accumulation of dehydrins in wheat and barley cultivars with different frost tolerance. *Submitted 2007.* I was responsible for all phases of the research presented and for the manuscript writing and preparation.

Meetings & Conferences:

Vítámvás P., Kosová K., Prášil I.T., 2006, Proteome and protein analysis in plant stress research. *In: Advances in Plant Molecular Biology: New Approaches in Plant Genome Analysis, November 29, 2006, Prague, pp. 5-13*

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