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Research article

WCS120 protein family and frost tolerance during cold acclimation, deacclimation and reacclimation of winter wheat

Pavel Vítámvás, Ilja Tom Prášil*

Laboratory of Plant Proteomics and Stress Physiology, Crop Research Institute, Drnovská 507, 161 06 Prague, Czech Republic

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ABSTRACT

We studied how long-term cold acclimation of winter wheat (variety Mironovskaya 808), interrupted by deacclimation and then followed by reacclimation, affected the levels of cold-induced WCS120 proteins, dry-weight content, and frost tolerance in leaves. Two experiments were performed: (1) plants undergoing long-term cold acclimation (up to 112 days) were quickly deacclimated (for 5 days), and then reacclimated again to cold; (2) plants vernalized for varying periods of time in an early stage of their development were, after a longer deacclimation of about 14 days, exposed for the same time period to cold. Five members of the WCS120 protein family were detected and quantified by image analysis in protein gel blots (in the first experiment); as well as in two-dimensional electrophoresis gels (in the second experiment). In both experiments, partially vernalized plants, after reacclimation, re-established their frost tolerance to levels similar to plants having had the same duration of cold treatment, but without deacclimation. On the other hand, these partially and fully vernalized plants reaccumulated WCS120 proteins to lower levels than plants that were not deacclimated. Further, using a mathematical model (the peak four-parameter Weibull equation), the same type of response curve was observed during plant cold treatment not only for the level of frost tolerance, but also for dry-weight content and accumulation of WCS120 proteins, with the maximum values reached at about the same time as vernalization saturation.

1. Introduction

Both vernalization and frost tolerance (FT) in winter wheat are controlled by exposure to cold temperatures. Vernalization requires long-term exposure to cold temperatures, while FT is induced immediately after the plant's exposure to cold treatment, and it is lost very quickly after transfer back to normal conditions [5,18,19]. Vernalization prevents overwintering wheat from entering the frost-sensitive reproductive phase. Winter wheat in the vegetative phase is able to maintain a high level of FT during the winter. Spring wheat, without a vernalization requirement, enters the reproductive phase very quickly and is able to develop only a limited level of FT [24].

Cold acclimation involves several biochemical and physiological changes. The result of these changes is not only a high level of FT, but also a decrease in water content (e.g., [20]), as well as the accumulation of several compounds protecting plant cell structures during cold-induced dehydration stresses [27]. Accumulation of some *Cor* (cold-regulated) transcripts and COR proteins during cold acclimation is positively correlated with the level of FT in wheat as well as in other cereals (e.g., [8,10,13,21]). The *wcs120* (wheat cold-specific) gene family belongs to the Cor/Lea superfamily [14,27], and encodes a group of highly abundant proteins ranging in molecular weight (MW) from 12 to 200 kDa; among these, the five major members, WCS200 (MW = 200 kDa), WCS180 (180 kDa), WCS66 (66 kDa), WCS120 (50 kDa), and WCS40 40 kDa), are inducible by cold treatment [9,26]. The WCS120 protein family members share homology with the Lea D11 dehydrins, are rich in both glycine and threonine, are highly hydrophilic and soluble upon boiling, and have a p*I* above 6.5 [5,14,27]. This protein family is coordinately regulated by low temperature and accumulates to high levels in wheat plants exhibiting FT, as well as in other frost-tolerant species of the *Poaceae* [5,26,28]. However, it has mainly been the most abundant WCS120 protein that has been used for comparison of FT level and protein accumulation between spring- and winter-habit wheats [5,13,26]. During deacclimation of cold-acclimated wheat, a rapid decrease in the WCS120 protein is observed [13]. However, the levels of these five WCS120 proteins have not been studied

Abbreviations: 2-DE, two-dimensional gel electrophoresis; COR, cold-regulated; DWC, dry-weight content; FT, frost tolerance; LEA, late embryonic abundant; LT_{50}, lethal temperature; MW, molecular weight; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; WCS, wheat cold-specific.

* Corresponding author. Tel.: +420 2 3302 2440; fax: +420 2 3302 2286.
E-mail address: prasil@vurv.cz (I.T. Prášil).

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previously during long-term cold acclimation interrupted by deacclimation, followed by reacclimation.

The dynamics of FT in wheat grown in a controlled environment under uniform conditions have been described in a number of studies [5,17,24]. Plants exposed to low temperatures show a rapid increase in FT. The rate at which FT develops gradually slows until the maximum level of acclimation is reached. After that, there is a gradual loss of FT. Fowler et al. [5,6] and Mahfoozi et al. [18] both observed that wheat plants reached the highest FT at about the same time as vernalization saturation (measured by final leaf number) occurred. Moreover, the accumulation of WCS120 proteins in cold-acclimated winter wheat plants also peaked at the same time [5]. These observations supported the hypothesis that genes for the vernalization requirement (which control the transition from the vegetative to the reproductive phase) also act to control expression of low temperature-induced genes (including the WCS120 family) associated with the acquisition of FT [3,4].

Wheat plants, under natural winter conditions, are exposed to changes in the surrounding temperature, which affects their level of FT. During lower temperatures, a greater level of FT is observed, and during warmer periods, some FT is lost [7]. The ability to reinduce FT during a cold period following a warmer period is termed reacclimation. Reacclimation studies (i.e., when the deacclimation of cold-acclimated plants is interrupted by another cold period) have shown that winter wheat plants are able to re-establish a high level of FT during reacclimation, but only before their vernalization requirement has been satisfied [7,12,18,24]. After vernalization saturation, their ability to reacclimate (i.e., gain high FT) is gradually lost. This phenomenon was used to study the relationship between the levels of WCS120 proteins, dry-weight content (DWC), and FT. The aim of this study was to determine whether accumulation of the WCS120 proteins is related to the level of FT during plant acclimation, deacclimation and reacclimation, as well as whether the levels of these proteins are altered in winter wheat that has been partially vernalized. As most of the recently published papers have concentrated on determination of only the WCS120 protein, we evaluated all five members of the WCS120 protein family that are regulated by cold.

2. Results

2.1. WCS120 proteins, lethal temperature (LT_{50}) and vernalization of wheat during acclimation and reacclimation (Experiment I)

Plants were cold acclimated for 112 days. At 21, 42 and 63 days of acclimation, a subsample of the plants was returned to 17 °C for 5 days (deacclimation) and then exposed again to cold (reacclimation). The accumulation of WCS120 proteins during cold acclimation, deacclimation (excluding the sample deacclimated after 21 days of acclimation), and reacclimation was studied by protein blot analysis of proteins soluble upon boiling (Fig. 1B). These five protein bands were identified as WCS proteins according to their MW - WCS200 (MW 200 kDa), WCS180 (MW 180 kDa), WCS66 (MW 66 kDa), WCS120 (MW 50 kDa) and WCS40 (MW 40 kDa); all five of the most abundant members of the WCS120 protein family were detected in plants exposed to cold. Furthermore, the WCS120 proteins were also very noticeable on one-dimensional silver-stained gels (Fig. 1A). Before cold acclimation (day = 0), samples showed very low levels of WCS120 protein. In all plants from the cold treatment, the WCS120 protein showed the highest accumulation level, WCS66 was second, and WCS40 had the least accumulation (Fig. 2). Both of the more abundant proteins (WCS120 and WCS66) contributed the most to the total WCS120 protein. The other proteins (WCS200, WCS180, and principally the minor WCS40 protein) changed less markedly; however, the total WCS120 protein differed significantly between treatments.

During acclimation, all members of the WCS120 protein family showed very similar trends in accumulation, with the maximum level occurring at around 63 days (Fig. 2). Thereafter, the levels decreased up to 112 days of acclimation. After 5 days of plant deacclimation, the amount of the WCS120 proteins decreased rapidly in all treatments. Deacclimated leaf samples all had WCS120 proteins; however, these proteins were present only in

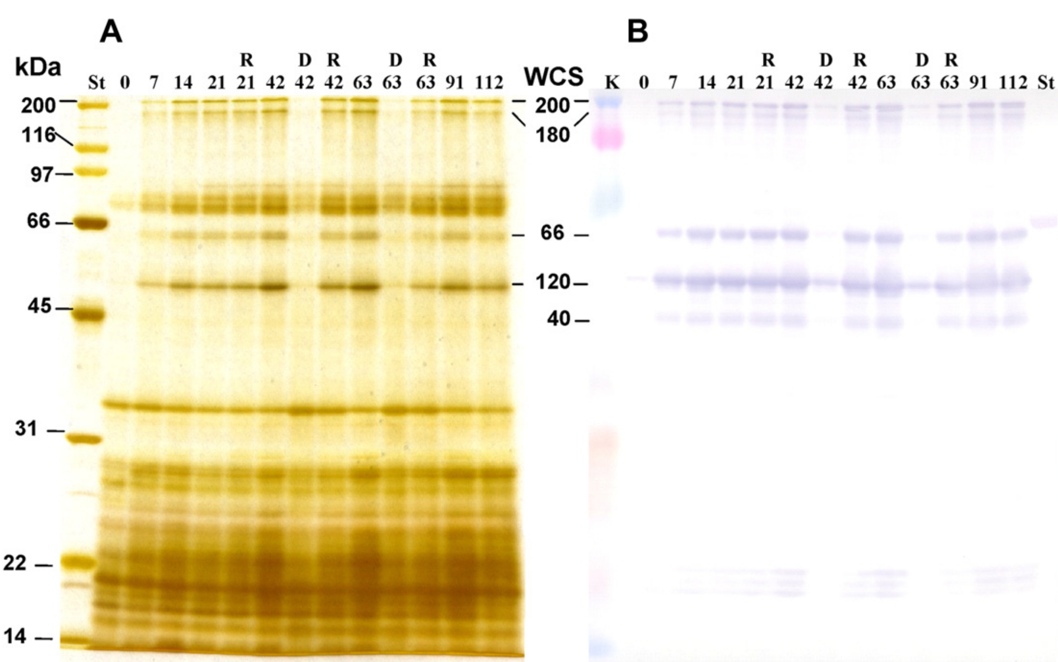

Fig. 1. Silver stained gel (left) and Western blot with anti-dehydrin antibody (right) of leaf heat-stable proteins sampled during cold acclimation (0–112 days at 2 °C), deacclimation (5 days at 17 °C) and reacclimation (21 or 28 days at 2 °C) of Mironovskaya 808 plants. K, Kaleidoscope prestained standards, broad range (Bio-Rad); St, SDS–PAGE standards, broad range (Bio-Rad); 0–112 indicates number of days of cold acclimation, D42 and D63, deacclimation after 42 and 63 days of cold acclimation, and R21, R42 and R63, reacclimation after 21, 42 and 63 days of cold acclimation and 5 days of deacclimation. WCS label indicates wheat cold-specific proteins.