Quantitative Expression of Cold-Acclimation Genes in Wheat (*Triticum aestivum* L.).

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Key Words: gene expression analysis, cold regulated genes (COR genes), quantitative real-time PCR, wheat, winter survival

Abstract

Winter wheat (*Triticum aestivum* L.) is seeded in the fall, regrowth resumes in spring, culminating in an early summer harvest. Yield is generally 20-25% higher than spring wheat. However, winter damage/kill can reduce yield. The low fall temperature allows the wheat plant to cold acclimate – a process during which physiological and biochemical changes occur resulting in the plant being able to withstand freezing temperatures. Specific cold regulated (COR) genes, such as *Wcs120*, are involved in these changes. Expression of COR genes are induced by transcriptional activators, such as *WCBF1*, in response to low temperature (LT). However, winter damage can still occur due to genetic differences limiting low temperature acclimation. An understanding of this cold acclimation/tolerance process will allow for better breeding strategies to improve winter wheat survival. Thus, the objective of this study was to determine the quantitative expression of some COR genes from field and growth chamber-grown winter and spring wheats using quantitative real-time PCR and establish their correlation, if any, to LT_{50} values (temperature at which 50% of plants are killed). Winter wheat (Norstar), spring wheat (Manitou) and two near-isogenic lines (Spring Norstar and Winter Manitou derived from reciprocal crosses of the two varieties) were used. Leaves were sampled on three dates (Sept. 29, Oct. 12 and Oct. 26, 2004) for the field grown plants and after 0, 2, 7, 14, 21, 28, 42, 56, 70, 84 and 98 days of LT acclimation for the growth chamber-grown plants. Relative expression of *Wcs120* and *WCBF1* genes were determined. Initial expression was high for both genes upon exposure to low temperature for all four lines from the growth chamber experiment. Expression decreased upon longer acclimation periods. The winter hardy wheat, Norstar, showed highest relative expression for both genes compared to the three other lines. This research implies that response to LT is very rapid and that accumulated LT tolerance (LT_{50}) and LT tolerance gene translation, as revealed by accumulation of *Wcs120*, lags considerably.

Introduction

Winter wheat (*Triticum aestivum* L.) is a fall planted crop that resumes growth early in the spring. Yield is generally 20-25% higher than spring wheat, but a major disadvantage is that winter wheat, without proper management, can still be damaged by low temperatures (LT). Winter wheat can survive low temperatures by a process called cold acclimation, which results in physiological and biochemical changes within the plant and induction of genes related to cold

tolerance, such as *WCBF*1 (Stockinger et al., 1997) and *Wcs120* (Houde et al. 1995). *WCBF1* is expressed upon exposure of the plant to LT and it in turn induces expression of other genes conferring LT tolerance. *Wcs120* is a cold-regulated (COR) gene expressed during cold acclimation and is thought to be involved in protection of plant cells against freezing damage. Accumulation levels of the *Wcs120* protein have been closely associated with LT tolerance in wheat. An understanding of the cold acclimation/tolerance process will allow better breeding strategies to improve winter wheat survival.

Objectives

The objectives of this study were to determine the LT_{50} values (temperature at which 50% of wheat plants are killed in a controlled freeze test) of field- and growth chamber-grown wheat plants and to determine the quantitative expression of *WCBF1* and *Wcs120* genes in plants sampled at intervals of time during the course of the study.

Materials and Methods

Plant growth and treatments

The genetic stocks used in this experiment were: Norstar (winter wheat), Manitou (spring wheat), Spring Norstar and Winter Manitou (two near-isogenic lines, derived from reciprocal crosses of Norstar and Manitou (Fowler and Limin 2004)). Following 2 weeks of growth at 20ºC plants were grown in a growth chamber maintained at 6ºC and sampled at different intervals (0, 2, 7, 14, 21, 28, 42, 56, 70, 84 and 98 days). The leaf samples were stored at -80ºC. For the field study, trials were seeded on September 7, 2004 and plants were sampled on September 29, October 12 and October 26, 2004. Leaves were collected and stored at -80ºC. Plant crowns from the growth chamber and field studies were used for the freeze tests in order to determine the LT_{50} of each line on each sampling date. For the freeze tests, 5-6 crowns of each line were grouped together for each test temperature. Crowns were placed in a freezer at -3ºC for 12 hours and the temperature was decreased at a rate of 2ºC/hour until -17ºC. Groups of crowns were removed at each pre-selected temperature and stored at 4ºC overnight. The crowns were then planted in soil and the numbers of live plants were counted after 3 weeks to determine the LT_{50} values.

Real-time PCR

Total RNA was extracted from the leaves using a Trizol™ (Invitrogen Life Technologies) method. 1 μg of total RNA was reverse-trancribed using Superscript III (Invitrogen Life Technologies) and gene specific primers. A 1/5 dilution of the cDNA was used for real-time PCR. The PCR reaction consisted of the Full Velocity™ SYBR Green QPCR Master Mix kit (Stratagene), which contained the buffer, dNTPs and SYBR Green I. Real-time PCR was performed on the MX3000P real time PCR machine (Stratagene). Data was analyzed using the $2^{-\Delta\Delta Ct}$ method to determine the relative expression.

Results and Discussion

LT50 values of field- and growth chamber-grown plants

The minimum LT_{50} value for winter Norstar acclimated in the growth chamber was -23ºC. Spring Norstar also acclimated rapidly for 1 week and then suddenly slowed after 1 to 2 weeks reaching a minimum LT_{50} of -10.5ºC. The winter Manitou, on the other hand, achieved greater low temperature tolerance compared to the spring habit Manitou (Fig. 1). The LT_{50} values from the field (Fig. 2) showed that the low temperature tolerance of Norstar, Spring Norstar and Winter Manitou increased over the three sampling dates, but the low temperature tolerance of Manitou increased over the two sampling dates and decreased by the third sampling date.

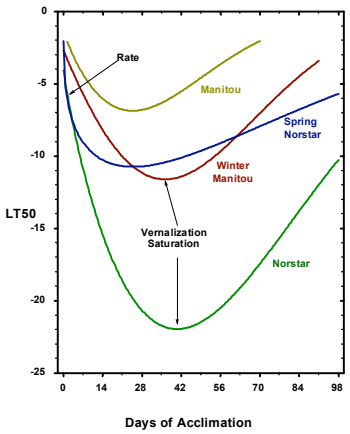

Figure 1: LT_{50} values for Norstar, Manitou, Spring Norstar and Winter Manitou low temperature acclimated in the growth chamber at 6ºC.

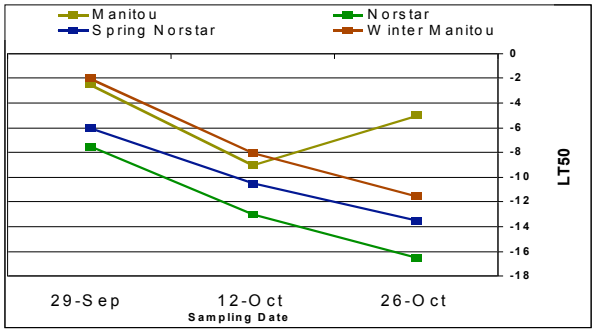

Figure 2: LT_{50} values for Norstar, Manitou, Spring Norstar and Winter Manitou sampled on September 29, October 10 and October 26, 2004 in the field at Saskatoon.

Relative expression of Wcs120 using real-time PCR

The expression of *Wcs120* in all four lines was highest at 2 days. However, the expression of *Wcs120* in Norstar was higher and more sustained than Spring Norstar, or any other line, over the sampling days up to 28 days (Fig. 3). Spring Norstar retained some of the high pattern of expression evident in Norstar, but only up to 14 days. Manitou showed the lowest amount of expression. The amount of expression in Winter Manitou did not improve significantly when compared to the spring habit Manitou although the initial 2-day and the 28-day values suggest a slightly different pattern of response. The low expression of *Wcs120* in spring Manitou was expected and is a reflection of its inability to tolerate freezing temperatures.

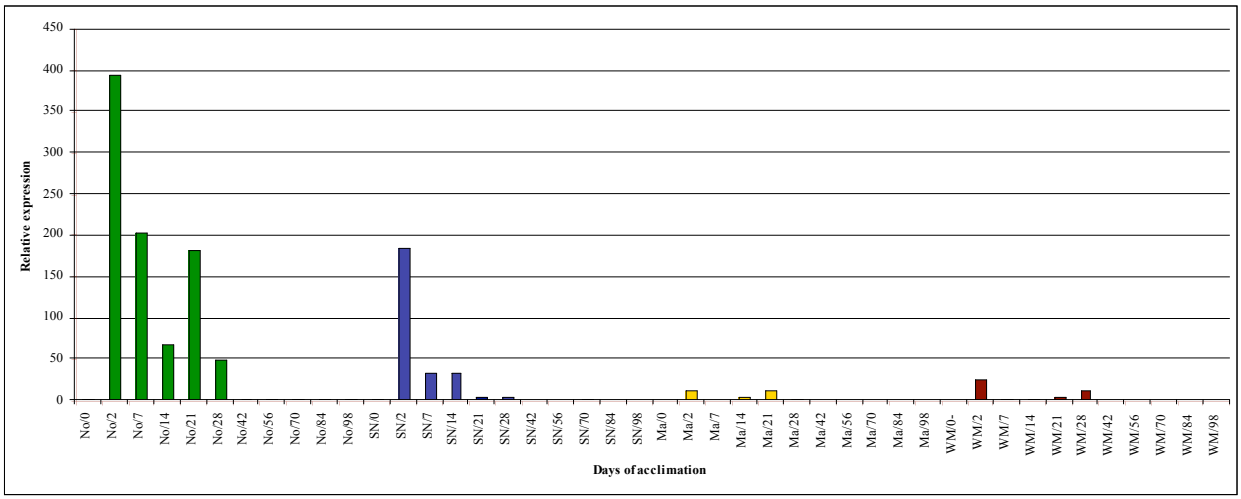

Figure 3: Relative expression (real-time PCR) of *Wcs120* from the growth chamber samples for Norstar (No), Spring Norstar (SN), Manitou (Ma) and Winter Manitou (WM).

Relative expression of WCBF1

Norstar showed the highest expression of *WCBF1* at 2 days, with a subsequent decrease to 28 days (Fig. 4). Spring Norstar had a high level of expression at 2 days, but this expression was lower than the winter Norstar. However, there was a sharp decrease at 7 days, with gradual decease thereafter up to 28 days. Out of all four lines, the spring habit Manitou showed the lowest level of expression. Winter Manitou showed an increase in expression compared to the spring habit Manitou.

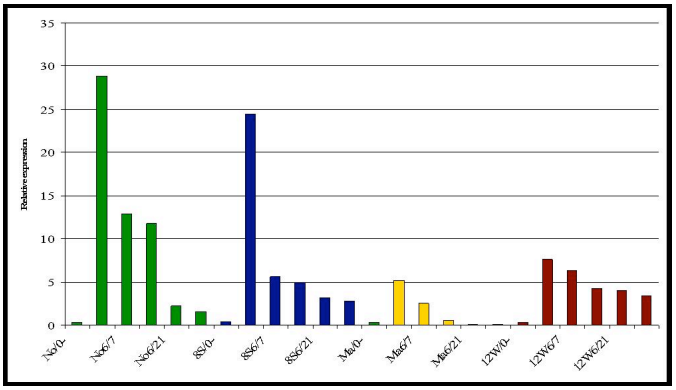

Figure 4: Relative expression of *WCBF1* from the growth chamber samples for Norstar (No), Spring Norstar (8S), Manitou (Ma) and Winter Manitou (12W).acclimated at 6ºC for 0, 2, 7, 14, 21, 28 days.

The level of expression of *Wcs120* in Norstar was highest and was maintained at a higher level compared to the other three lines; which corresponds with its greater LT tolerance as measured by LT_{50}. Spring Norstar showed a high initial response but quickly dropped off reflecting the LT_{50} response pattern in Fig. 1. The expression of *WCBF1* correlates quite well with an increase in *Wcs120* expression suggesting that *Wcs120* may be regulated at least in part by *WCBF1*. The expression level of *WCBF1* in Winter Manitou related well with its greater LT tolerance, which was maintained for longer compared to Spring Manitou. These early results suggest that CBF expression in response to LT is very rapid and that LT tolerance expression lags considerably behind LT tolerance gene translation as revealed by accumulation of *Wcs120* protein (Fowler et al. 1996) and as measured by LT_{50}. Real-time PCR for both *Wcs120* and *WCBF1* on field samples is in progress.

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