
Response of Wheat and Its Relatives to Low Temperatures

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Low-temperature (LT) adaptation is determined by a complex quantitative system that is expressed by plants in anticipation of and during exposure to temperatures that approach freezing. It is controlled by a highly integrated genetic system that is regulated by environmentally responsive, complex pathways. In the last decade, a virtual flood of genetic and genomic information on LT adaptation has arisen from investigations using model plant systems and tools with an unprecedented level of sophistication for analyses. This greater appreciation of the interactions that determine crop adaptation has provided us with the ability to design strategies to minimize the risk of LT damage in different stages of crop development. For example, our ability to manipulate the differences in genetic and environmental responses has allowed us to identify the genetic factors that determine LT-tolerance gene expression and to successfully transfer the superior frost-tolerance genes from a hardy winter wheat variety into spring wheat. The superior LT-tolerance genes have also been tagged using molecular markers that allow plant breeders to select hardy spring and winter habit lines without having to wait for a test frost in the field. However, even with the opportunities offered by advances in technology, we have been unable to produce super hardy cultivars. As a result, while the genes within cereals have a high degree of similarity and the regulation of LT tolerance is operational across species, we have not been able to successfully exploit the superior LT tolerance of rye for cultivar improvement in related cereal species like wheat. Progress in this area will have to wait for a much clearer understanding of the plants LT-response mechanisms and the genetic and environmental interactions that control their expression. This interdisciplinary effort will be expensive and immediate breakthroughs should not be expected, but progress to date suggests that we now have the tools to identify the pieces of the LT-tolerance puzzle.

Low temperature acclimation

To be successful in cool season and high winter stress climates, plants must be programmed to recognise and respond to temperatures that are favourable for growth and the environmental cues that signal seasonal changes typical of the regional environment for which they were selected or in which they evolved. In regions with cold winters, vernalization requirement is an important adaptive feature that delays heading by postponing the transition from the vegetative to the reproductive phase. Similarly, photoperiod requirement is an adaptation that allows the plant to flower at the optimum time. Cool season plants also have the ability to low temperature (LT) acclimate.

In wheat and its relatives, LT acclimation is a cumulative process that is initiated once temperatures drop below 10 to 15°C (Fowler et al., 1999). There is an inverse relationship between temperature and acclimation rate and, when plants are grown at constant temperatures in the acclimation range, the most rapid changes in LT tolerance occur during the initial stages of

acclimation. Exposure of hardened plants to higher temperature results in de-acclimation, but the process of LT acclimation can be re-initiated by exposing plants that are still in the vegetative stage to inducing temperatures. Time sequence studies have shown that LT-induced gene expression in cereals is developmentally regulated (Fowler et al., 1996b) and there is an over-winter decline in LT response due to an inability to maintain LT tolerance genes in an up-regulated state once vernalization (Fowler et al., 1996a) and photoperiod (Mahfoozi et al., 2000; 2001a; b; Fowler et al., 2001) requirements have been satisfied. Consequently, the ability to anticipate and respond to environmental cues is dependent upon a highly integrated system of structural, regulatory, and developmental genes (Figure 1).

The factors responsible for LT tolerance interfere with active growth and the linkage of LT-tolerance gene expression to phenological development means that significant quantities of the protective compounds are only present when they are required in the plants life cycle. The ability to tolerate LT in the seedling stage allows for a longer period of growth and establishment thereby providing the plant with a competitive advantage and increased production potential. However, because LT gene expression can only be sustained when the plant is in the vegetative stage, it also means that the genetic potentials of both spring and winter habit cultivars are not given an opportunity to be fully expressed once the plant enters the reproductive stage.

Vernalization requirements extend the vegetative stage thereby allowing LT-tolerance genes to be expressed for a longer period of time during plant establishment in the autumn and in preparation for below freezing winter temperatures (Fowler et al. 1996a; b). Similarly, photoperiod sensitivity allows plants to maintain LT-tolerance genes in an up-regulated state for a longer period of time under short day compared to long day conditions (Mahfoozi et al., 2000; 2001a; b; Fowler et al., 2001) experienced in regions with long mild winters and in the early spring. These observations explain why winter habit genotypes eventually lose their ability to maintain a high level of LT-tolerance under acclimating temperatures and only limited levels of LT tolerance have been observed in spring habit cultivars. Spring habit plants do not have a requirement for a high level of LT tolerance, as they complete their life cycle during the summer and early fall, and a high level of LT tolerance is no longer required in over-wintering cereals after the onset of warm conditions in the spring when rapid growth and reproduction begin. In fact, for species adapted to regions with long, mild winters, a high level of freezing tolerance is often less important than a rigorous photoperiod, dormancy, or vernalization requirement that prevents plants from entering the extremely cold-sensitive reproductive growth stage until the risk of LT damage has passed.

Based on the above observations the evolution of and selection for genetic options that permit extensive modification of thermosensitive metabolic processes and critical structural components should not come as a surprise, especially in winter annual and perennial plants that must adapt to a wide range of seasonal stresses. Because LT response is determined by a highly integrated system of structural and developmental genes regulated by environmentally responsive, complex pathways that allow full expression of LT induced genes only when they are required in the life cycle (Figure 1), it has been difficult to separate cause and effect adjustments to LT and other environmental cues that signal seasonal changes (Fowler et al., 1999). For this reason, the long term research challenge has been to isolate the different variables

involved in the expression of plant LT adaptation so that the critical responses to environment can be identified and exploited in crop improvement programs.

Developmental regulators

According to the developmental theory (Fowler et al. 1999), the developmental genes (vernalization, photoperiod, etc) determine the *duration* of expression of LT-induced structural genes (Fowler et al. 1996a; b; Mahfoozi et al. 2000) while the *rate* of LT acclimation is determined by genotype dependent expression levels of structural genes. In other words, the developmental genes act as the switches controlling the duration of expression of LT-induced structural genes (Fowler et al. 1996a; b) while the *rate* component determines the degree that the structural genes are up regulated (Figure 1).

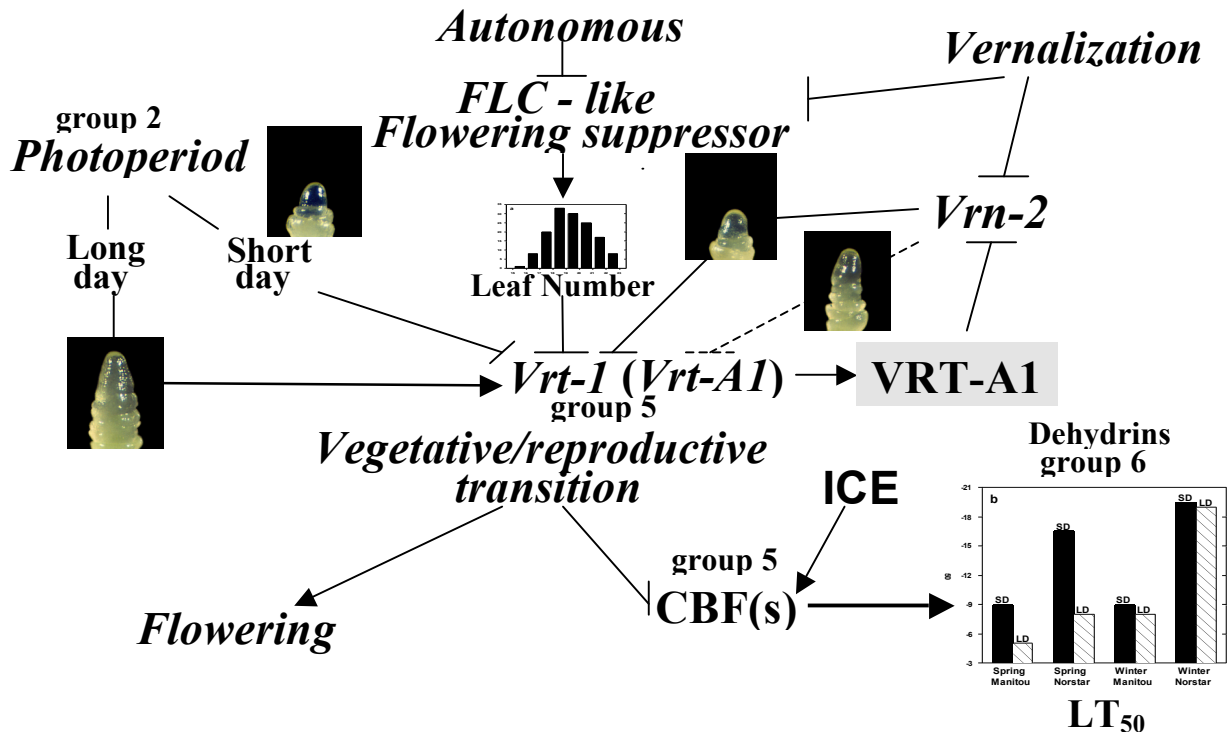


Figure 1. Flowering pathway and regulation of LT tolerance gene expression in common wheat (*T. aestivum*) - from Fowler and Limin, 2006. *Vrt-1* = *Vrn-1* = Vegetative reproductive transition genes = Meristem identity.

We have produced two reciprocal near isogenic lines (NILs), Winter Manitou and Spring Norstar, from a Norstar x Manitou backcross program (Limin and Fowler, 2002). Winter Manitou has the Manitou genetic background and the vernalization (winter growth habit) allele from Norstar (*vrn-A1*) while Spring Norstar has the Manitou spring habit allele (*Vrn-A1*) and the Norstar genetic background. Detailed evaluations of these NILs for the vernalization locus (Figure 2) have shown the cold tolerant ‘Norstar’ winter wheat genetic background achieves greater LT tolerance than that of spring ‘Manitou’ due to a faster *rate* of acclimation (Fowler and Limin, 2004). A vernalization requirement allows virtually full expression of the LT tolerance potential of both Norstar and Manitou genetic backgrounds under a 20-h day length (Norstar and Winter Manitou). Similarly, almost full expression of LT tolerance potential can be achieved by

photoperiod responsive genotypes like Spring Norstar in the absence of a vernalization requirement when grown under an 8-h day length (Figure 3; Limin and Fowler, 2006). The critical observation in these studies is that plant development toward flowering progressively reduces LT acclimation ability and the *duration* of time in early developmental stages determines the degree to which the LT-tolerance genetic potential is expressed. This makes the expression of LT-tolerance genes pathway-dependent rather than a result of the action of single genes operating in isolation. These interactions also make LT-tolerance-related characters appear to be associated with genes that determine flowering time, explaining the pleiotropic effect (growth habit and LT tolerance) attributed to genes like *vrn-A1* (Brule-Babel and Fowler, 1988).

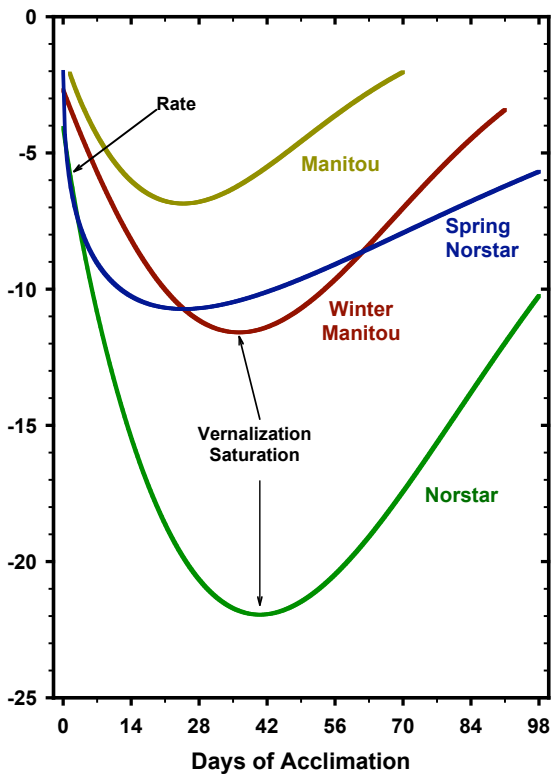
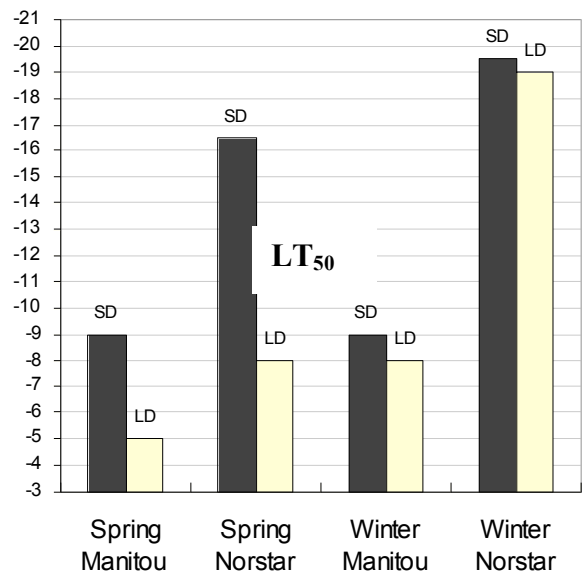


Figure 2. Norstar winter wheat and Manitou spring wheat and the near isogenic lines Spring Norstar and Winter Manitou acclimated at 6°C and a 16 hour day length for 0 to 98 days. Note the limited ability of Manitou to acclimate compared to Spring Norstar, which has the superior *rate* of acclimation genes from Norstar, and Winter Manitou, which has the vernalization (*duration*) gene from Norstar. A longer *duration* of acclimation due to a vernalization requirement plus a superior *rate* of acclimation results in the very hardy Norstar. **From:** Fowler and Limin, 2004.

Figure 3. LT tolerance (LT_{50}) of Norstar x Manitou near isogenic lines: Winter Norstar and Spring Norstar, Spring Manitou and Winter Manitou, following 28 days acclimation at 4°C. Short-day (SD) = 8 h, long-day (LD) = 20 h. A strong photoperiod requirement and superior *rate* of acclimation genes allow Spring Norstar to achieve hardiness levels approaching Winter Norstar when seeded under the short days experienced in the fall. **From:** Limin and Fowler, 2006.



Duration and Rate of Low-Temperature Gene Expression

Molecular studies have demonstrated that the photoperiod and vernalization genes influence the expression of LT-induced genes in cereals through separate pathways that eventually converge to activate genes controlling plant development (Fowler et al., 2001; Figure 1). In both instances, delay in the transition from the vegetative to the reproductive stage causes increased LT tolerance that is sustained for longer in plants that have a vernalization or photoperiod requirement.

Detailed analyses of flowering pathways using the *Arabidopsis* model indicate that multiple developmental and environmental cues and pathways regulate the vegetative/reproductive transition. There are at least 80 genes and loci and a number of genetic pathways known to affect flowering time in *Arabidopsis* (Simpson et al., 1999; 2002) and as complex, or more complex, regulatory system likely exists in wheat (Fowler and Limin, 2003). Early indications are that there are differences in the evolution of mechanisms regulating the flowering pathways between dicots and monocots as well as within the *Triticeae* group and it is likely that a much more complex system will eventually be revealed in cereals. In addition, it appears that the descriptive name associating vernalization directly with the function of *vrn-A1* is misleading and should be corrected. The *vrn-A1* locus functions as part of the vegetative/reproductive complex that is suppressed by the action of upstream vernalization responsive genes. Current theory has spring habit genotypes arising from a mutation(s) resulting in the loss of recognition of a suppressor of flowering and, as such, it would be more appropriate if *vrn-1* was designated *vrt-1* (vegetative reproductive transition - 1) to reflect its true function at this location in the flowering pathway (Figure 1). The orthologous (related gene) in *Arabidopsis* is AP1, a flowering gene not involved in the vernalization response. The accumulated evidence indicates that the *vrn-A1* region in wheat is also a convergence point, or master switch, for pathways that determine the vegetative/reproductive transition thereby giving it a direct influence on the *duration* of expression of the *rate* determining LT-tolerance genes. These distinctions become important as a clear understanding of the gene networks and complex interactions that determine LT tolerance is required before effective strategies can be designed for the identification and selection of the factors influencing this character of major economic importance in field crops.

A large number of chromosomes have been shown to influence LT tolerance in conventional, non-molecular genetic studies and, for this reason, it has been generally assumed that a large number of genes with small effects and complex interactions determine the phenotypic expression of LT tolerance. As indicated above, evaluation of the near isogenic lines (NILs) for the non-hardy spring habit (*Vrn-A1*) cultivar ‘Manitou’ and the very cold-hardy winter habit (*vrn-A1*) cultivar ‘Norstar’ (Limin and Fowler, 2002) has shown that both *duration* and *rate* of acclimation contributed significantly to the 13.8°C difference in minimum survival temperature between Norstar and Manitou (Fowler and Limin, 2004). These studies also established that genes responsible for the *duration* of expression of LT-tolerance genes can be separated from genes determining the *rate* of acclimation (Figure 2).

In *Arabidopsis*, *Cbf* genes are transcriptional factors that are rapidly up-regulated in response to LT treatment and are activators of cold regulated genes (Thomashow et al., 2001).

The *Cbf* genes are induced in turn by *ICE* transcriptional activators (Figure 1), which have a complex, wide ranging transcriptome. Transcripts encoding CBF-like proteins have been shown to accumulate rapidly in response to LT in 'Puma' rye and Norstar wheat (Jaglo et al., 2001) suggesting that a similar mechanism operates in cereals and tandem clusters of *Cbf* genes map to the same positions as the cold hardiness genes in barley (Skinner et al., 2005) and wheat (Vagujfalvi et al., 2005) indicating that CBF-like genes are primary candidates for the frost tolerance genes. As transcriptional activators for LT tolerance associated genes, the CBF-like genes are also prime candidates for the *rate* genes (Figure 2).

Pathway Interactions

A plant must be programmed to recognize and respond to temperatures that are favorable for growth and to the environmental cues that signal seasonal changes. In this environmentally responsive system there is also a need for the plant to record the progress of seasons so that it can properly anticipate the normal periods of LT stress and commit fully to growth and reproduction once the weather is favorable. The fact that both LT acclimation and vernalization have similar above freezing activation ranges suggests the likelihood of an extensive integration of LT-sensing mechanisms. These complicated time/temperature relationships and unexplained genetic interactions indicate that detailed functional genomic or phenomic analyses of natural allelic variation will be required to identify the critical genetic components of the highly integrated systems for LT adaptation that are regulated by environmentally-induced complex pathways.

Phenotypic and molecular studies have shown that the *duration* of LT tolerance is determined by how fast phenological development proceeds and the time to vegetative /reproductive transition, which in turn is a function of a) vernalization requirements, b) photoperiod requirements, c) leaf number, d) length of phyllochron (Limin and Fowler, 2002) and e) low temperatures that delay reproductive transition in plants that have reached the stage of competence to flower (Fowler and Limin, 2004). Related studies have also shown that the mechanism regulating the level of expression of LT-induced genes is associated with a gene(s) integrated into the developmental pathway and the *rate* of acclimation is determined by a) acclimation temperature and b) LT tolerance genetic potential (Fowler et al., 1999; Fowler and Limin, 2004). Consequently, given the right combinations of time, temperature and day length, LT-tolerance genes will locate to molecular map positions associated with variability in phenological development.

Simulation Model

A basic understanding of the LT responses found in cereals has allowed the construction of a LT tolerance simulation model for winter cereals (Fowler et al., 1999). The model is based on a series of equations that describe acclimation, dehardening, and damage due to LT stress that are consistent with recent interpretation of LT gene regulation. LT tolerance is estimated on a daily basis relative to stage of phenological development and cultivar cold hardiness potential. The model has been field validated for cereals over wintered at Saskatoon and it has application in the simulation of LT responses for a broad range of species and climates. In this model, the developmental genes (vernalization, photoperiod, etc) are assumed to be responsible for the *duration* of expression of LT-induced structural genes (Fowler et al., 1996a; b; Mahfoozi et al.,

2000) while the *rate* of LT tolerance acquisition is determined by genotypic differences in cold-hardiness potential. Vernalization requirements prevent the plant from going reproductive during favorable periods for growth and development in the fall and early winter (Fowler et al., 1996a; b) and photoperiod sensitivity allows plants to maintain LT genes in an up-regulated state for a longer period of time under short day compared to long day environments (Mahfoozi et al., 2001). In both instances, the delay in the transition from the vegetative to the reproductive stage produces increased LT tolerance that is sustained for a longer period of time.

An interactive web-based version of the model has been developed for use by farmers, extension workers, and researchers interested in estimating winter survival in cereals (http://www.usask.ca/agriculture/plantsci/winter_cereals/WWModel.php; Fowler and Greer, 2003). A crop variety menu offers the choice of a wide range of winter cereal species and cultivars. The LT50 and vernalization options allow the user to expand on these choices and experiment with different values. The data files contain soil temperature records for selected years and locations that can be expanded when new data becomes available. The present files include examples from Canada (Saskatoon and Indian Head, Saskatchewan and Oak River, Manitoba) and Prague, Czech Republic. We also have weather data from Maragheh, Iran (Mahfoozi et al., 2005) that will be added once the day length response variable has been satisfactorily modeled. Soil temperatures for the current year are added as the winter progresses thereby allowing interested users to monitor the predicted condition of the present crop in the Saskatoon region. In addition, a Management Impact Calculator allows users to evaluate the effects of sub-optimal seeding date, seeding depth and phosphorous and nitrogen fertilization on the winter hardiness of crops grown in western Canada. A large database that can be quickly and easily supplemented combined with a flexible, interactive model which complies with the known LT responses of cereals creates a teaching tool that allows production risks, cause-and-effect processes, and genetic theories to be systematically investigated by users throughout the world.

Progress in Breeding for Low Temperature Adaptation in Cereals

The importance of reliable crop management resources and the ability to take advantage of small improvements in cultivar LT tolerance and growing season adaptation has been clearly demonstrated by winter wheat growers in the

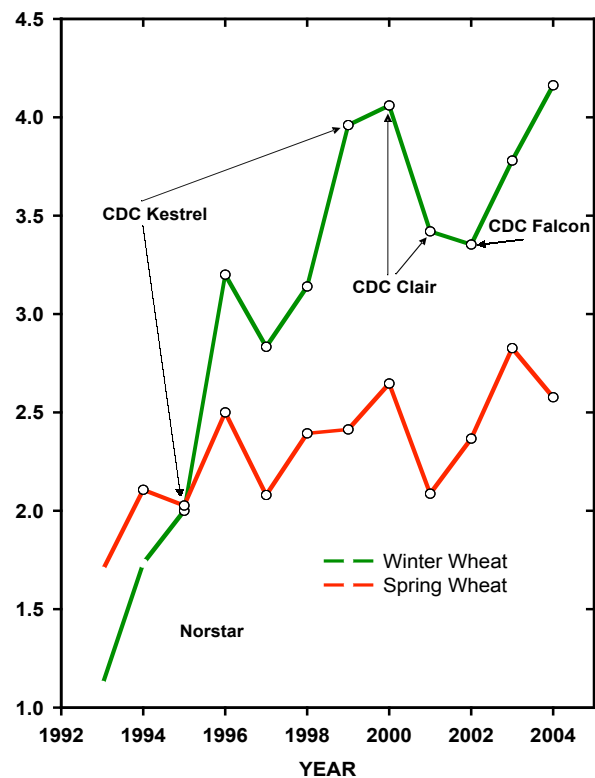


Figure 4. Average spring and winter wheat grain yield in Manitoba for the period 1992 to 2004 (source - Statistics Canada). Arrows indicate the dominant cultivar grown by farmers. CDC – Crop Development Centre University of Saskatchewan.

eastern prairies who have recently over-wintered crops that yielded up to 6 tonne/ha. This is a region where only 15 years ago most critics claimed that winter wheat could not be successfully grown in a normal year, let alone one with record low winter temperatures. The high level of success achieved in this example is best demonstrated in Manitoba where the availability of better adapted cultivars and the adoption of improved management practices allowed farmers to increase their average grain yield of winter wheat from 1.1 in 1992 to 4.2 tonne/ha in 2004 (Figure 4). This single example demonstrates that there is still a considerable opportunity to further adapt our cropping systems to the environmental extremes experienced in western Canada. The degree of future success that can be realized in this area will be dependent on our ability to unravel the pieces of the LT-tolerance puzzle and how effectively we are able to use this information in the development cultivars with superior LT tolerance.

In a 1929 publication, Quisenberry and Clarke noted that “The possibility of developing hardier varieties through breeding has been recognized for years”. However, the reality is that the maximum cold hardiness potential of most cereal crops has reached a stubborn plateau that has not been breached for decades. In fact, all the efforts of modern science have been unable to produce the super hardy cultivars needed to expand winter crop production into regions requiring a level of cultivar LT tolerance superior to that found in the land races selected by early farmers indicating that improvements in LT adaptation do not come easily. In contrast, the last 80 or more years have seen improvements in agronomic practices within most established production areas that have allowed plant breeders to reduce their selection pressure for LT tolerance. Consequently, while plant breeding efforts over the years have created cultivars with a high level of adaptation, there is still considerable potential for improvement in LT tolerance of cultivars available for most of the current winter wheat production areas around the world. Unfortunately, this is not the case for winter cereals other than rye in most of western Canada.

Our search for superior LT tolerance genes has been expanded to include attempts at interspecific and intergeneric transfers. There are considerable differences in the maximum LT tolerances found in different winter cereals (Fowler and Carles, 1979; Limin and Fowler, 1981; 1982; 1984) and the possibility that genes can be transferred between species to increase the genetic variability available to winter cereal breeding programs has been explored. However, these attempts have done little more than demonstrate the difficulties that must be overcome before the full potential of superior species-specific LT-tolerance gene expression can be captured through interspecific gene transfers in breeding programs.

The superior LT tolerance of rye was found to be suppressed when combined in tetraploid (Limin et al., 1985) and hexaploid (Dvorak and Fowler, 1978) wheat backgrounds. Artificially synthesized ABD genome hexaploid wheat (Limin and Fowler, 1982) also demonstrated the nonadditivity of closely related genomic systems. Further investigation of LT gene expression in hybrids among Triticeae species (Limin and Fowler, 1988; 1989) led to the conclusion that chromosome dosage or ratios influence LT tolerance by shifting competitively balanced systems toward the parent with the greatest chromosome number. Molecular investigations of these hybrids has subsequently revealed that highly conserved and coordinately regulated LT-induced gene families of both species are expressed in interspecific crosses (Limin et al., 1995; 1997). However, these genes were not expressed independently and the degree of LT gene expression in interspecific crosses was regulated at the transcriptional level by the

higher ploidy parent. These observations indicate that, before we can successfully exploit alien genetic variability for LT tolerance, we must first acquire a greater understanding of the complex genetic mechanisms that plants have evolved for the efficient integration of LT responses into the daily processes of survival, growth, and reproduction.

The linkage of LT tolerance expression to phenological development adapts the plant to the environment for which it was selected or in which it evolved. For example, a high level of LT tolerance is no longer required after the onset of warm conditions in the spring when rapid growth and reproduction begin. Consequently, satisfaction of vernalization and photoperiod requirement results in a decline in LT tolerance of over-wintering plants. This results in complicated phenological development x LT tolerance interactions that must be optimized for each production area if cultivars are to be successful. Consequently, because individual genes are part of a complex system, a better understanding of the LT response mechanisms will greatly assist plant breeders in designing strategies to significantly improve the LT adaptation of important economic crops. For example, we have been able to successfully transfer the superior frost tolerance genes from a hardy winter wheat cultivar (Norstar) into a spring wheat line (Spring Norstar) demonstrating that the LT tolerance of spring habit wheat genotypes can be significantly improved by the inclusion of LT tolerance *rate* determining gene(s) from Norstar (Figure 2; Fowler and Limin, 2004). When the superior *rate* determining gene(s) were combined with a rigorous photoperiod requirement (Limin and Fowler, 2006), Spring Norstar was able to achieve a winter hardiness level approaching that of winter Norstar and survive the high stress winters of 2003-04 and 2004-05 in western Canada when sown in the fall at the recommended seeding date for winter wheat (Figure 5).

Figure 5. When combined with a photoperiod requirement that increases its *duration* of acclimation, the superior *rate* of acclimation genes in Spring Norstar allow it to achieve winter hardiness levels approaching that of winter Norstar when seeded in the fall. The Spring Norstar in this photo was seeded September 7, 2004 and the picture was taken April 29, 2005



An one or two degree improvement in low-temperature tolerance of non-acclimated plants combined with a very rapid initial rate of acclimation once temperatures drop below 8 to 10°C also indicates that spring seeded Spring Norstar should suffer less damage than current spring wheat cultivars when exposed to late spring frost during the growing season. The winter wheat varieties grown in western Canada must have a very high level of cold tolerance in order to survive our winters and Norstar, which is a parent in most of our current winter wheat varieties, ranks amongst the hardiest. In contrast, spring wheat varieties grown in western

Canada are damaged by exposure to even a slight frost. In the past century, plant breeding efforts that target the western Canadian prairies have created cultivars with a high level of adaptation, but we still lose millions of dollars each year in potential crop productivity and market quality due to frost damage. Consequently, the advantage offered by spring habit varieties with improved frost tolerance would be expected to produce a multi-million-dollar return on years when frost occurs during the growing season. On more average years, production of spring habit varieties with improved frost tolerance would provide additional economic opportunities by extending our growing season, allowing more flexibility in our management choices, providing opportunities to reduce herbicide costs through better crop competition, increasing crop moisture utilization, lowering energy requirements, and increasing productivity while using more environmentally friendly farming systems.

The superior frost tolerance of Spring Norstar still needs to be combined with the disease resistance, agronomic performance, and market acceptability of current spring wheat varieties. Since individual genes are part of a complex system, it will be necessary to determine the best combinations that will maximize the improvement of frost resistance in commercial spring varieties. The superior frost-tolerance genes of Norstar have been tagged using molecular markers (Baga et al., 2006), which will assist this process by allowing wheat breeders to select the critical genes without having to wait for a test frost in the field. This will greatly speed up the selection for cold hardiness in both spring and winter wheat breeding programs and significantly increases the chances of having the right lines in the field for evaluation when a damaging frost that permits field evaluation occurs.

While over-winter LT damage in the seedling stage is primarily a concern in temperate climates, frost damage during the reproductive stage can cause severe economic losses in most wheat producing regions of the world. Widely fluctuating late afternoon and early morning temperatures make the timing and severity of LT stress important considerations during the active growing season. Both spring and winter habit genotypes can cold acclimate after reproductive transition and before heading demonstrating that the vegetative/reproductive transition does not act as an off switch for LT-tolerance genes (Fowler et al., 1996b). However, plants have only a limited ability to cold acclimate during this period and they reach their maximum level of LT tolerance very quickly once they are exposed to temperatures in the acclimation range indicating that a short, rapid LT response mechanism is functional up to the time of heading. The lack of progress in selecting for frost resistance after head (ear) emergence suggests that LT tolerance expression is minimal at these stages. Selection for resistance after head emergence becomes a much more complex problem because avoidance mechanisms like supercooling play a greater role when plants are exposed to temperatures just below freezing during this period. Also, LT acclimation is a cumulative process and we do not have a clear understanding of how responsive plants are after head emergence indicating that more detailed studies are required to establish if the limited LT tolerance after heading is due to insufficient induction time or an inability to respond to temperatures in the acclimation range.

In the last decade, a virtual flood of genetic and genomic information has arisen from investigations using model plant systems and tools with an unprecedented level of sophistication for the analysis of the transcriptome, proteome and metabolome. However, a large gap exists between these basic scientific developments and the utilization of this knowledge in crop

improvement programs that focus on breeding for complex traits like LT tolerance. Progress has been made in the mapping, isolation, and characterization of the major LT adaptation genes that will allow for the more rapid and directed incorporation of LT tolerance genes using marker assisted backcrossing and other molecular techniques. Advances in biotechnology have provided even greater opportunities for plant breeders to expand their attack on the LT tolerance barrier that has frustrated them for so long. However, exploitation of this new technology to produce adapted, super-hardy cultivars will require close co-operation between plant breeders and biotechnologists. This interdisciplinary effort will be expensive and immediate breakthroughs should not be expected, but progress to date suggests that we now have the tools to identify the pieces of the LT-tolerance puzzle.

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References

Båga, M.; Chodaparambil, S.V.; Limin, A.E.; Pecar, M.; Fowler, D.B. and Chibbar, R.N. 2006. Identification of quantitative trait loci for low temperature tolerance in cold-hardy winter wheat. *Functional and Integrative Genomics*. (submitted).

Brule-Babel, A.L. and Fowler, D.B. 1988. Genetic control of cold hardiness and vernalization requirement in winter wheat. *Crop Sci.*, 28: 879-884.

Dvorak J. and Fowler D.B. 1978. Cold hardiness potential of triticale and tetraploid rye. *Crop Sci.* 17: 477-478.

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

Fowler D.B. and Carles R.J. 1979. Growth, development, and cold tolerance of fall-acclimated cereal grains. *Crop Sci.* 19: 915-922.

Fowler, D.B.; Chauvin, L.P.; Limin, A.E. and Sarhan, F. 1996a. The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. *TAG.*, 93: 554-559.

Fowler, D.B. and Greer, K. 2003. A Web-based Model for Estimating Winter Survival in Cereals. 6 pages. Soils and Crops Workshop, University of Saskatchewan, Saskatoon, Saskatchewan.

Fowler, D.B. and Limin, A.E. 2003. Functional genomics of low-temperature stress. Proc. 10th International Wheat Genetics Symp. Paestum, Italy (949-951).

Fowler, D.B. and Limin, A.E. 2004. Interactions among factors regulating phenological development and acclimation rate determine low-temperature tolerance in wheat. *Ann. Bot.*, 94: 717-724.

Fowler, D.B. and Limin, A.E. 2006. Progress in Breeding Wheat with Tolerance to Low Temperature in different Phenological Developmental Stages. Proceedings 7th International Wheat Conference, Mar del Plata, Argentina. (In press).

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

Fowler, D.B.; Limin, A.E.; Wang, S.Y. and Ward, R.W. 1996b. Relationship between low-temperature tolerance and vernalization in wheat and rye. *Can. J. Plant Sci.*, 76: 37-42.

Jaglo, K.R.; Kleff, S.; Amundsen, K.L.; Zhang, X.; Haake, V.; Zhang, J.Z.; Deits, T. and Thomashow, M.F. 2001. Components of the Arabidopsis C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. *Plant Physiol.*, 127: 910-917.

Limin, A.E.; Danyluk, J.; Chauvin, L-P.; Fowler, D.B. and Sarhan, F. 1997. Chromosome mapping of low-temperature induced *Wcs 120* family genes and regulation of cold-tolerance expression in wheat. *MGG*, 253: 720-727.

Limin, A.E.; Dvorak, J. and Fowler, D.B. 1985. Cold hardiness in hexaploid triticale. *Can. J. Plant Sci.*, 65: 487-490.

Limin, A.E.; Houde, M.; Chauvin, L.P.; Fowler, D.B. and Sarhan, F. 1995. Expression of the cold-induced wheat gene *Wcs120* and its homologs in related species and interspecific combinations. *Genome* 38:1023-1031.

Limin, A.E. and Fowler, D.B. 1981. Cold hardiness of some relatives of hexaploid wheat. *Can. J. Bot.* 59: 572-573.

Limin, A.E. and Fowler, D.B. 1982. The expression of cold hardiness in Triticum species amphiploids. *Can. J. Gen. Cytol.* 26: 405-408.

Limin, A.E. and Fowler, D.B. 1984. Cold hardiness in Triticum and Aegilops species. *Can. J. Plant Sci.*, 65: 71-77.

Limin, A.E. and Fowler, D.B. 1988. Cold hardiness expression in interspecific hybrids and amphiploids of the Triticeae. *Genome* 30: 361-365.

Limin, A.E. and Fowler, D.B. 1989. The influence of cell size and chromosome dosage on cold hardiness expression in the Triticeae. *Genome* 32: 667-671.

Limin, A.E. and Fowler, D.B. 2002. Developmental traits affecting low-temperature tolerance response in near-isogenic lines for the vernalization locus *Vrn-A1* in wheat (*Triticum aestivum* L. em Thell). *Ann. Bot.*, 89: 579-585.

Limin, A.E. and Fowler, D.B. 2006. Low-temperature tolerance genetic potential in wheat (*Triticum aestivum* L.): Response to photoperiod, vernalization, and plant development. *Planta*, (accepted).

Mahfoozi, S.; Limin, A.E.; Ahakpaz, F.; Roustaii, M.; Ketata, H. and Fowler, D.B. 2005. Regulation of cold tolerance in barley under field conditions in north-west Iran. *Can. J. Plant Sci.*, 85: 597-592.

Mahfoozi, S.; Limin, A.E. and Fowler, D.B. 2001a. Developmental regulation of low-temperature tolerance in winter wheat. *Ann. Bot.* 87: 751-757.

Mahfoozi, S.; Limin, A.E. and Fowler, D.B. 2001b. Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. *Crop Sci.*, 41: 1006-1011.

Mahfoozi, S.; Limin, A.E.; Hayes, P.M.; Hucl, P. and Fowler, D.B. 2000. Influence of photoperiod response on the expression of cold hardiness in cereals. *Can. J. Plant Sci.*, 80:721-724.

Quisenberry, K.S. and Clarke, J.A. 1929. Breeding hard red winter wheats for winter hardiness and yield. *USDA Tech. Bull.* No.136.

Simpson, G.G.; Gendall, A.R. and Dean, C. 1999. When to switch to flowering. *Ann. Rev. Cell Develop. Biol.*, 15: 519-550.

Simpson, G.G. and Dean, C. 2002. Arabidopsis, the Rosetta stone of flowering time? *Science*, 296: 285-289.

Skinner, J.S.; Szűcs, P.; von Zitzewitz, J.; Marquez-Cedillo, L.; Filichkin, T.; Thomashow, M.F.; Stockinger, E.J.; Chen, T.H.H. and Hayes, P.M. 2005. Mapping of barley homologs to genes that regulate low temperature tolerance in Arabidopsis. *TAG*, (accepted).

Thomashow, M.F.; Gilmour, S.J.; Stockinger, E.J.; Jaglo-Ottosen, K.R. and Zarka, D.G. 2001. Role of the *Arabidopsis* CBF transcriptional activators in cold acclimation. *Phys. Planta*, 112:171-175.

Vagujfalvi, A.; Aprile, A.; Miller, A.; Dubcovsky, J.; Delugu, G.; Galiba, G. and Cattivelli, L. 2005. The expression of several *Cbf* genes at the *Fr-A2* locus is linked to frost resistance in wheat. *MGG*, (In Press).