Wheat production in a changing environment - low temperature adaptation

Fowler DB

Department Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada

Wheat (*Triticum aestivum* L.) is one of the most widely adapted food crops in the world. Its continued dominance in area harvested relative to the other major cereal crops is due to an ability to cope with abiotic stresses, particularly low temperature (LT). Wheat and its relatives have evolved a broad range of complex systems that are expressed in anticipation of and during exposure to temperatures that approach freezing. These highly responsive mechanisms have been exploited by early farmers and later by plant breeders to develop a wide range of successful cultivars that have played, and will continue to play, critical roles in feeding an ever-expanding human population. However, the highly integrated quantitative systems of structural, regulatory, and developmental genes that activate and control the LT protective mechanisms which have made wheat such a widely adapted crop are also responsible for a large number of changes in morphological, biochemical, and physiological characteristics. As a result, separation of cause-and-effect adjustments has been a challenge and the genetic control of LT tolerance has been difficult to ascertain.

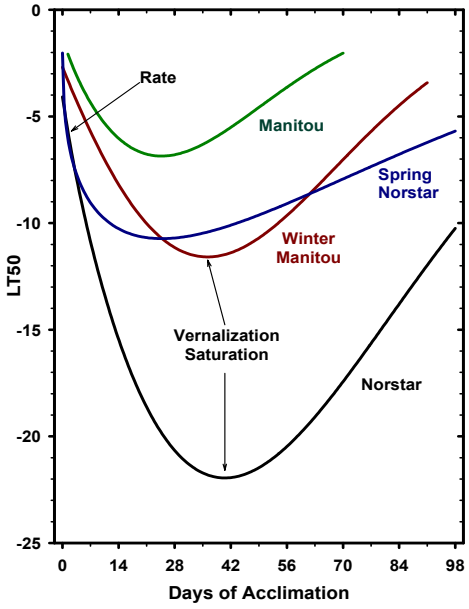

Figure 1. *LT tolerance (LT_{50}) of Norstar and Manitou and the NILs spring Norstar and winter Manitou acclimated at 6 °C for 0 to 98 days (17). Note the limited ability of Manitou to acclimate compared to spring Norstar, which has the superior rate of acclimation genes from Norstar, and winter Manitou, which has the vernalization (duration) gene from Norstar.*

Phenotypic and molecular studies have shown that the LT induced protective mechanisms in wheat are developmentally regulated and involve acclimation processes that can be stopped, reversed, and restarted (19). Acclimation is a genotypic dependent cumulative process that is activated once temperatures fall below a threshold level. Below the threshold there is an inverse relationship between temperature and acclimation rate (13, 19) and, when plants are grown at constant temperatures, the most rapid changes occur during the initial stages of acclimation (Fig. 1). Full expression of cold hardiness genes only occurs in the vegetative stage (34) and exposure of hardened plants to temperatures that promote active growth results in rapid de-acclimation. Plants that are still in the vegetative stage have the ability to re-acclimate following periods of exposure to warm temperatures (20) while plants in the reproductive phase have only a limited ability to re-acclimate (19). These relationships are reflected in the developmental model of LT tolerance gene regulation (19) where *duration* and *rate* of gene expression determine the level of LT tolerance. In this model, the developmental genes that determine the length of the vegetative stage control the *duration* of LT tolerance gene expression (16, 20) while a threshold temperature dependent *rate* component (13) regulates the degree to which LT induced genes are up-regulated (Fig. 1, 17).

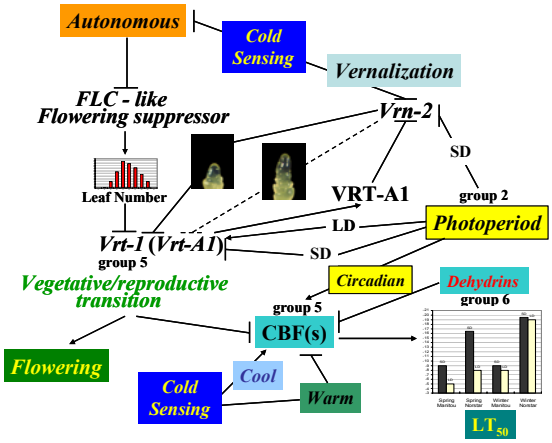

Figure 2. *Flowering pathway and regulation of LT tolerance gene expression in wheat - adapted from (18). Vrt-1 = Vrn-1 = Vegetative reproductive transition genes = Meristem identity.*

Developmental Regulation

Plants must be able to respond to environmental cues and record the progress of seasons so that they can properly anticipate the normal periods of LT stress and

commit fully to growth and reproduction once the weather is favourable. The linkage of LT tolerance expression to phenological development provides plants with an effective means of reacting to the seasonal changes for which they were selected or in which they evolved. To facilitate this environmentally responsive system and allow sufficient time to build up resources, a battery of genes has arisen that delay the expression of meristem identity genes and flowering even under inductive conditions by repressing the autonomous or earliness pathway (Fig. 2). By lengthening the vegetative stage, these mechanisms also allow the plant to optimize its competitive advantage and reproductive capacity at the start of the growing season.

Winter habit genotypes normally have the greatest LT challenge to overcome when they are in the seedling stage and the more severe the LT stress anticipated, the greater the LT tolerance that must be achieved. The acclimation process and the struggle to survive require energy and, as a result, healthy plants that enter the winter with well-developed crowns are in the best position to withstand LT extremes and regenerate roots and leaves in the spring. For this reason, in high stress environments like that found in western Canada, optimum planting dates for winter wheat are early enough to allow for a 4 to 5 week establishment period during which there is active growth at warm temperatures followed by another 4 to 7 weeks acclimation before the soil freezes for the winter. A vernalization requirement maintains the plant in the vegetative stage during this autumn establishment period (Fig. 2) and winter wheat normally does not realize its maximum cold hardiness potential until after the soil is frozen in the late autumn. Once cold acclimation has been completed, winter wheat genotypes can maintain a high level of cold hardiness provided crown temperatures remain below freezing.

For genotypes adapted to regions with long mild winters, a high level of freezing tolerance is often less important than a rigorous photoperiod or vernalization requirement that prevents plants from entering the extremely cold-sensitive reproductive growth stage until the risk of LT damage has passed. As a consequence, winter habit genotypes adapted to these conditions normally have both strong vernalization and photoperiod requirements. A vernalization requirement delays the transition from the vegetative to the reproductive phase in the autumn and early winter. By the time vernalization saturation is achieved, day length is short enough that a photoperiod requirement further delays the transition from the vegetative to the reproductive stage (Fig. 2). Once the long days that accompany the approach of spring arrive and rapid growth resumes, a high level of LT tolerance is no longer required and winter wheat responds similarly to spring wheat.

While over-winter LT damage in the seedling stage is primarily a concern with winter wheat, economic losses from frost damage after the vegetative/reproductive transition is a significant risk factor in many of the 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 this period. Both spring and vernalized winter habit genotypes have a limited ability to cold acclimate and they reach their maximum level of LT tolerance very quickly when exposed to temperatures in the acclimation range (20). While of major importance, the small genetic differences that are expressed after the vegetative/reproductive transition make selection for LT tolerance at this stage very difficult in breeding programs.

Low-temperature tolerance QTL: Several reputed homoeologous LT tolerance genes have been mapped to the group 5 chromosomes of wheat. The first group was mapped to positions 2 (*Fr-A1*), 10 (*Fr-D1*), and 40 (*Fr-B1*) cM from the homoeologous *Vrn-1* (spring/winter determining) loci (23, 46). Subsequently, a second frost resistance locus designated *Fr-A2* mapped to the long arm of *Triticum monococcum* chromosome 5 (52), 30 cM proximal to the RFLP marker *Xwg644* that is known to be tightly linked to *Vrn-A1* and the *Fr-A1* locus (23). Marker *Xgwm639-5B*, which mapped near the peak of the *Fr-B1* QTL (46), is closely linked to *Xbcd508,* which is located at the peak of *Fr-A2* (52) indicating that *Fr-B1* is an ortholog of *Fr-A2* not of *Fr-A1*. Because the *Fr-A1* has never been isolated or sequenced and was mapped at contradicting locations proximal and distal to *Vrn-A1*, its existence as a separate locus from *Vrn-A1* must be considered inconclusive (34).

Given the close associations between the vernalization and the LT tolerance genes, it is possible that vernalization and LT responses are interrelated and *Vrn-A1* may be pleiotropic, regulating both phenological development and the expression of LT tolerance (4, 19, 41, 43). Most of the initial mapping studies utilized spring by winter comparisons, which include the confounding effects of duration of LT gene expression due to differences at the *Vrn* region, while the early winter by winter comparisons used parents with minor differences in LT tolerance. As previously indicted, the genetic potentials of both spring and winter habit genotypes are restricted once the plant enters the reproductive stage (Fig. 2). The influence of the spring habit alleles in limiting the expression of LT tolerance genes starts very early and is reflected in the threshold temperature at which LT tolerance genes are induced (13). Consequently, this leaves the impression that the spring habit *Vrn1* allele has a dominant pleiotropic effect for frost susceptibility.

Vernalization and Photoperiod**:** Genes for vernalization are found on the 4th, 5th, and 1st group chromosomes of the Triticeae (37). *Vrn-A1* is homoeoallelic to locus *Sh2* (*Vrn-H1*) in barley and *Sp1* (*Vrn-R1*) in rye. In common wheat (ABD genome), the three major vernalization determining loci have been mapped to the long arms of chromosomes 5A (*Vrn-A1*), 5B (*Vrn-B1*), and 5D (*Vrn-D1*) (37). *Vrn-A1* does not require a vernalization treatment while *Vrn-B1* and *Vrn-D1* have short vernalization requirements and winter habit genotypes are recessive for all three genes. The main developmental genes in the diploid species, barley (44) and *T. monococcum* (A genome, 47), are *Vrn-1* and *Vrn-2*. The dominant *Vrn-1* is responsible for spring habit while *Vrn-2* is dominant for winter habit (10) and there

is an epistatic interaction between *Vrn-1* and *Vrn-2* (Fig. 2). *Vrn-2* is a repressor of flowering that is down-regulated by vernalization, so a loss of function of *Vrn-2* results in spring habit. Initiation of transcription of the dominant *Vrn-1* allele down-regulates *VRN-2* (35) accelerating time to flowering. Multiple copies of *Vrn-1* and *Vrn-2* in tetraploid and hexaploid wheat have complicated efforts to identify allelic variation for *Vrn-2* in these species. However, a down-regulation in *VRN-2* transcript in hexaploid wheat by RNA interference (RNA::*VRN-2*) has resulted in an up-regulation of *VRN-1* and a reduction in vernalization requirement (Fig. 2, 53). The phenomenon of short-day vernalization, which acts through short-day down-regulation of the *VRN-2* flowering repressor, has been reported for wheat and barley (11, 49). However, normal development and flowering is associated with long-day acceleration in cereals while short days lengthen the vegetative phase by acting as a direct repressor of the *Vrn-1* complex (7, 11). A vernalization requirement allows virtually full expression of the LT tolerance potential in cereals grown under a 20-h day length. Similarly, almost full expression of LT tolerance potential can be achieved by short-day responsive genotypes in the absence of a vernalization requirement when grown under an 8-h day length (34).

Positional cloning studies (54) have shown that *Vrn-A^{m}1* is completely linked to MADS-box gene *AP1*. Concurrently, a gene designated *TaVRT-1* (7) was cloned, characterized, and localized to the *Vrn-1* regions on the long arms of homoeologous group 5 chromosomes in common wheat. The level of expression of *TaVRT-1* was associated with the vernalization response and transition from the vegetative to reproductive phase, a finding supported by the results for the *WAP1* gene (39). *TaVRT-1* has very close sequence homology and similar expression patterns to *Vrn-A^{m}1* and the barley homolog *HvBM5* (7, 48). Molecular studies have demonstrated that the *TaVRT-1* and *HvBM5* genes are both regulated by photoperiod and cumulative low temperatures and that the accumulation of their encoded products is associated with the progressive repression of cold-induced genes and a decrease in LT tolerance (7). In winter-habit genotypes, photoperiod sensitivity influences LT tolerance gene expression even before vernalization saturation (36), implying that vernalization is progressive and that plant development can be influenced by photoperiod during vernalization.

Master Switches**:** Photoperiod and vernalization responses have been shown to influence the expression of LT induced genes in cereals through separate pathways that eventually converge at the *Vrn-1* complex to activate genes controlling plant development (Fig. 2, 14). Model systems have demonstrated that the flowering pathways in plants are much more complicated and, as gateways for the vegetative /reproductive transition, the *Vrn-1* complex would also be expected to act as a master switch that integrates the responses to a much longer list of environmental and genetic factors. Current theory has spring habit genotypes arising from a mutation(s) resulting in the loss of recognition of a suppressor(s) of flowering (9). This places the *vrn-1* loci downstream from the vernalization machinery and, as such, the descriptive name associating vernalization directly with the function of *vrn-1* genes in cereals is misleading and should be amended (18). As convergence points or master switches for pathways that determine the vegetative/reproductive transition, it would be more appropriate if *vrn-1* was designated *vrt-1* (vegetative-reproductive transition-1) to reflect their true role in the flowering pathway (Fig. 2).

In this system, the point of transition from the vegetative to the reproductive growth stage is pivotal in determining the expression of LT tolerance genes (19) and development toward flowering progressively reduces the plant's ability to acclimate (Fig. 1 and 2). Consequently, the *duration* of time in early developmental stages establishes the degree to which the LT tolerance genetic potential is expressed (34), which in turn is a function of a) vernalization requirement, b) photoperiod requirement, c) leaf number, d) length of phyllochron (33) e) LT that slows development in the absence of a vernalization requirement (13), and f) other factors that extend the length of the vegetative stage (17). Related studies have also shown that the mechanism regulating the level of expression of LT tolerance is associated with genes integrated into the developmental pathway and the *rate* of acclimation is determined by a) acclimation temperature and b) LT tolerance genetic potential (17, 19). This makes the expression of LT tolerance genes pathway dependent rather than due to the action of single genes operating in isolation. As a result, LT tolerance QTLs associated with variation in phenological development are only revealed in mapping populations under the appropriate conditions of time, temperature, and day length. 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.

Low-Temperature Sensing and Gene Induction

Most LT research is carried out in controlled environments where plants are moved from conditions favourable for active growth and establishment, e.g., near 20^{o}C, directly into temperatures well into the acclimation range, e.g., 2 to 6 ^{o}C. Differences in genetic potential are quickly magnified under these conditions and an inverse relationship between exposure temperature and LT tolerance indicates that cereals are able to monitor temperature with a high level of precision (19). Plants grown under field conditions are normally subjected to a broad range of continually changing environmental cues and adapted cultivars have been selected to utilize these signals to anticipate and prepare for periods of LT stress. Activation of the LT sensing mechanisms becomes the first line of response and from a practical standpoint a warmer threshold temperature allows winter habit genotypes a longer time to prepare for the extremes of winter. Better preparation also places both spring and winter habit genotypes in a

more favourable position to cope with unexpected frosts during the growing season.

Because differences in LT genetic potential are poorly expressed and difficult to measure during the initial stages of acclimation (5), we have a limited understanding of how plants sense cold and the temperatures at which the acclimation mechanisms are activated. In wheat and its relatives, the threshold acclimation temperature has been generally accepted as approximately 10°C. However, there are recognized differences in the temperatures at which cereal genotypes start to acclimate under field conditions (15) and the expression of some LT regulated genes (51) has been reported at temperatures warmer than those normally considered within the induction range.

Recent attempts to quantify differences in cold sensing have shown that LT acclimation is induced at temperatures ranging from 8°C for tender spring wheat to 15°C for hardy winter wheat demonstrating that there is important variability in the mechanisms by which genotypes monitor and respond to temperature (13). When exposed to constant temperatures approaching the threshold level, plants often require a week or more before they start to acclimate suggesting that activation of the LT tolerance machinery is a measured response. This delayed response resulted in an average 2°C warmer induction temperature after 7 compared to 2 days indicating that very subtle differences in time and temperature, or some other environmental factor, can eventually trigger the acclimation process. Once acclimation starts, the differences in genetic potentials are quickly magnified with the result that genotypes with warmer threshold temperatures have the most rapid LT responses. The large differences in threshold induction temperatures (13) and rapid initial changes in LT tolerance that are inversely related to the exposure temperature (19) support the notion that the cold sensing mechanism and responses in the early stages of acclimation play a critical role in determining plant cold acclimation potential.

Reciprocal near-isogenic lines (NILs) for the *Vrn-A1* locus of tender spring habit (Manitou - *Vrn-A1*) and cold hardy winter habit (Norstar - *vrn-A1*) cultivars (33) have been used to quantify the effects of threshold temperature (13) and duration (17) components of the developmental model for LT tolerance gene regulation (Fig. 1 and 2). An average 5.7°C warmer activation temperature for Norstar and spring Norstar (*Vrn-A1*) compared to Manitou and winter Manitou (*vrn-A1*) demonstrated the range of induction temperatures expected when the vernalization requirement due to *vrn-A1* was neutralized (13). Comparison of the spring and winter habit NILs (Norstar vs spring Norstar and Manitou vs winter Manitou) also revealed that a vernalization requirement increased the induction temperature by an average of 1.5°C indicating that the early commitment of spring habit genotypes to the vegetative/reproductive transition lowers the threshold temperature. In addition to emphasizing the importance of threshold temperature and length of the vegetative stage, these observations also demonstrate the importance of genotype x environment interactions in determining LT tolerance.

Results of mapping studies indicate that a single QTL, designated as *Fr2*, determines a large part of the phenotypic variation for LT tolerance in cereals. *Fr2* has been mapped to chromosome 5A of diploid (52) and hexaploid (1) wheat and orthologous locus in barley (22) and rye (2). Differences in the initial rate of acclimation have also been mapped to the *Fr-A2* QTL (1) establishing that this region is directly involved in the temperature-sensing mechanism of wheat. Clusters of CBFs have been located in the *Fr-2* QTL in diploid and hexaploid wheat and in barley (1, 22, 38, 42) suggesting that CBF-like genes are candidates for the *Fr-2* frost tolerance genes (50). Recent studies have identified polymorphisms in the *TmCBF* genes that map to the *Fr-2* locus as possible candidates in determining LT tolerance and *COR14b/DHN5* transcript levels (29).

Molecular studies in *Arabidopsis* have shown that the cold signalling system requires a cascade of transcriptional regulators in which the *Cbf* genes play a central role in the activation of downstream LT regulated *COR* genes and LT responses (27, 45). Transcripts encoding CBF-like proteins have also been shown to accumulate rapidly in response to LT in Puma rye and Norstar wheat (27) suggesting that a similar mechanism operates in cereals (Fig. 2). While increased expression of CBFs has been linked to increased cold tolerance (28), constitutive expression of the CBF regulon has been shown to have a deleterious influence on growth and development (25) in *Arabidopsis* and the transcription factors that induce LT tolerance expression in wild type plants are repressed at warm temperatures (Fig. 2). This repressor is down-regulated by LT thereby activating *COR* genes downstream in the LT response pathway (21).

The dehydrin families (e.g. WCOR*410*, WCS120, DH5, and others) have received the most attention among the LT induced proteins. These proteins concentrate in different cellular compartments and their properties, abundance, and localization suggest that they are involved in the protection of critical membranes by replacing water and stabilizing membranes against freezing or dehydration stress (8). The *vrn-1* complex on the wheat group 5 chromosomes has been shown to regulate the expression of a least four gene families correlated with LT tolerance that have been mapped to the group 6 chromosomes (8, 30). Expression studies with the LT induced *Wcs120* and *Wcor410* families indicate that, even though there are large differences in LT tolerance, similar proteins are expressed by spring and winter-habit cultivars within species (8, 16, 26). Cold hardy genotypes produce more of the same dehydrins than tender genotypes indicating a common regulatory control of these structural genes.

The complex regulatory control and large genotype by environment interactions found in the LT tolerance pathways make it difficult to sort out cause-and-effect adjustments, especially as one moves upstream from the final molecular targets and physiological responses. In wheat, differences in *COR*

gene expression are related to differences LT tolerance when genotypic differences are large, especially during the initial stages of acclimation. When exposed to temperatures in the acclimation range levels of *COR* genes that are up-regulated by LT induced activators peak very early (less than 2 days) followed by a decline (24) indicating that there is also repression of this pathway as cold tolerance accumulates (Fig. 2). As a result, genotypic differences become less evident over time and *COR* gene expression levels are often divergent enough for genotypes with intermediate hardiness that comparisons among tissues and/or acclimation times can give variable interpretations. Earlier studies (31) indicated a translocatable substance that promotes cold acclimation in different plant parts, such as leaves, crowns and roots, is not produced when winter wheat plants are exposed to acclimating temperatures. Consequently, the cold-hardiness level and *COR* gene expression in different tissues is dependent upon interacting circuitries and the environmental conditions to which each tissue has been exposed creating sampling difficulties that can obscure our view of the LT tolerance picture (24). As a further complexity, the LT response pathways are gated by the circadian clock (21) and expression levels of LT induced genes often follow different daily patterns (Fig. 2).

While considerable emphasis has been placed on the detection of frost tolerance QTL and explaining the role of CBFs in determining phenotypic variation in LT tolerance, QTLs associated with the upstream cold sensors that activate this induced system have not been identified in cereals and the cold sensing mechanism itself remains very much a mystery. Changes in membrane fluidity, exoskeleton rearrangement, and calcium influxes are thought to play a role in the activation of LT responses in plants (3). The *ICE1* genes (6), which are constitutively expressed in *Arabidopsis*, and cold shock proteins (40) have also been implicated in the regulation of cold response transcriptional activators. These observations support the perception that the temperature monitoring mechanism involves post-transcriptional modification of constitutively expressed gene products whose efficiency in regulating the expression of downstream activators is directly related to temperature. They also suggest that the mechanisms responsible for cold sensing and activation of the acclimation processes are quite complex, possibly involving multiple sensors and signalling pathways. This apparent complexity remains to be reconciled with the reality that cereals are able to monitor and rapidly respond to temperature changes with a high level of precision and large genotypic differences in LT tolerance among and within cereal species can be readily quantified (13).

Low Temperature Adaptation

The maximum cold hardiness potential of wheat has reached a stubborn plateau that has not been breached for decades. Given this restriction, the recent expansion of winter wheat production into the high LT stress regions like western Canada has had to rely on the use of no-till management systems that maintain a protective snow cover during the winter months (12). In lower stress regions of the world, the last 100+ years have also seen improvements in agronomic practices that have allowed breeders to reduce their selection pressure for LT tolerance. Consequently, while selection has created cultivars with a high level of adaptation, there is still considerable potential for improvement in LT tolerance within most low and intermediate stress production areas. In the case of spring wheat, the successful transfer of the superior rate determining frost tolerance genes from a hardy winter wheat cultivar into tender spring wheat lines has demonstrated that the LT tolerance of spring wheat can be significantly improved (17). When the superior rate determining gene(s) are combined with a rigorous photoperiod requirement (34), these spring genotypes are able to achieve a winter hardiness level approaching that of hardy winter wheat sown in the autumn.

The possibility that genes can be transferred between species to increase the genetic variability available to winter wheat breeding programs has been explored (32). However, these attempts have done little more than demonstrate the difficulties that must be overcome before the full potential of superior species-specific cold-tolerance gene expression can be captured through interspecific gene transfers. For example, while the structural genes within the Triticeae have a high degree of homology and the regulation of LT tolerance is operational across genomes, we have not been able to successfully exploit the superior LT tolerance of rye for improvement of related cereal species. The major *Fr* QTLs are located on the long arms of the group 5 chromosomes in cereals but the substitution of rye chromosome 5R from the hardy rye cultivar ‘Puma” for 5A of the tender wheat cultivar ‘Chinese Spring” and the 5RL translocation of 5A of the hardy winter wheat cultivar ‘Norstar’ did not improve the LT tolerance compared to the wheat parent. Also, the superior LT tolerance of rye has not been expressed when combined in tetraploid and hexaploid wheat backgrounds. The complex interactions that have stymied these attempts should be expected as there is evidence that regulatory mechanisms, such as species specific tRNA and mRNA promoters and interacting transcription factors, often co-evolve. As a result, progress in this area will have to wait for a clearer understanding of the signal transduction and genetic cascade controlling LT gene expression.

An increased gene dosage and the ability to accommodate mutations in a polyploidy background has greatly expanded the genetic options in hexaploid wheat and provided unique opportunities for LT adaptation fine tuning for a wide diversity of environments that extend from the northern limits for crop production in the temperate zone to higher elevations in tropical regions. However, the complicated phenological development by LT tolerance interactions that have allowed for wide adaptation must be optimized for each new production area and/or change in environment if cultivars are to be successful. A better understanding of the critical junctions in the pathways that determine LT tolerance should produce molecular markers for use in marker

assisted breeding to accelerate the selection process. As these and other molecular tools are improved, our increased ability to exploit the inherent plasticity of wheat will re-enforce its important role in continuing efforts to respond to new and changing environments, including those associated with global warming.

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