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ORIGINAL ARTICLE

Allen E. Limin · D. Brian Fowler

Low-temperature tolerance and genetic potential in wheat (*Triticum aestivum* L.): response to photoperiod, vernalization, and plant development

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Abstract It is frequently observed that winter habit types are more low-temperature (LT) tolerant than spring habit types. This raises the question of whether this is due to pleiotropic effects of the vernalization loci or to the linkage of LT-tolerance genes to these vernalization loci. Reciprocal near-isogenic lines (NILs) for alleles at the *Vrn-A1* locus, *Vrn-A1* and *vrn-A1*, determining spring and winter habit respectively, in two diverse genetic backgrounds of wheat (*Triticum aestivum* L.) were used to separate the effects of vernalization, photoperiod, and development on identical, or near identical, genetic backgrounds. The *vrn-A1* allele in the winter lines allowed full expression of genotype dependent LT tolerance potential. The winter allele (*vrn-A1*) in a very cold tolerant genetic background resulted in 11°C, or a 2.4-fold, greater LT tolerance compared to the spring allele. Similarly, the delay in development caused by short-day (SD) versus long-day (LD) photoperiod in the identical spring habit NIL resulted in an 8.5°C or 2.1-fold, increase in LT tolerance. The duration of time in early developmental stages was shown to underlie full expression of genetic LT-tolerance potential. Therefore, pleiotropic effects of the vernalization loci can explain the association of LT tolerance and winter habit irrespective of either the proposed closely linked *Fr-A1* or the more distant *Fr-A2* LT-tolerance QTLs. Plant development progressively reduced LT-acclimation ability, particularly after the main shoot meristem had advanced to the double ridge reproductive growth stage. The *Vrn-1* genes, or other members of the flowering induction pathway, are discussed as possible candidates for involvement in LT-tolerance repression.

A. E. Limin (✉) · D. B. Fowler
Department of Plant Sciences, University of Saskatchewan,
51 Campus Drive, S7N 5A8 Saskatoon , SK, Canada
E-mail: allen.limin@usask.ca
Fax: +1-306-9665015

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Abbreviations *AP1*: APETALA 1 · CBF: C-repeat/dehydration-responsive element binding factor · *Cor*: Cold-responsive · DR: Double ridge · LD: Long day · LT: Low-temperature · NIL: Near-isogenic line · SD: Short day · *TaVRT-1*: *Triticum aestivum* vegetative to reproductive transition-1

Introduction

The interrelationship between vernalization requirement and cold tolerance has always been unclear. Early wheat breeders (Hayes and Aamodt 1927; Quizenberry 1931) found that winter survival and growth habit were significantly correlated traits and spring habit genotypes were generally less hardy than winter genotypes, this relationship was not however absolute. Brule-Babel and Fowler (1988) noted that, although there appeared to be a pleiotropic effect of the *Triticum aestivum* L. *Vrn-A1* gene, affecting both cold tolerance and vernalization response, there were hardy spring habit lines. Blake et al. (1993), working with doubled haploid lines of barley (*Hordeum vulgare* L.) from a winter by spring cross, found that the largest factor affecting winter survival effectively segregated as a single Mendelian gene associated with delayed flowering on chromosome 7 (5H), this location corresponds to the *Sh2* (*Vrn-H1*) gene on chromosome 5H. A subsequent publication from this same group (Hayes et al. 1993) suggested that this may not be the pleiotropic effect of a single locus but was more likely to be due to linkage. The observed relationship of vernalization requirement and LT tolerance is further obscured by the fact that both are induced in

the same approximate temperature range (Rawson et al. 1998; Fowler and Limin 2004).

In *T. aestivum*, spring/winter growth habit is determined by the homoeologous *Vrn-1* series of genes of which *Vrn-A1* is the strongest inducer of spring growth habit. Using positional cloning Yan et al. (2003) identified the probable ortholog of *Vrn-A1*, *Vrn-A^{m}1*, in the A genome carrying diploid wheat *T. monococcum L.* Concurrently, Danyluk et al. (2003) cloned and characterized a gene named *T. aestivum* vegetative to reproductive transition-1 (*TaVRT-1*) and localized this gene to the *Vrn-1* regions on the long arms of homoeologous group 5 chromosomes, regions that are associated with vernalization and freezing tolerance in 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 of Murai et al. (2003) for the *WAP1* gene. *TaVRT-1* has very close sequence homology and similar expression patterns to *Vrn-A^{m}1* and to the barley (*Hordeum vulgare* L.) homolog *HvBM5* (Danyluk et al. 2003; Trevaskis et al. 2003).

The *TaVRT-1* and *HvBM5* genes associated with the vernalization response and reproductive transition in wheat and barley have both been found subject to photoperiod regulation (Danyluk et al. 2003). Response to photoperiod has been shown to affect accumulation of LT tolerance in spring (Mahfoozi et al. 2000; Fowler et al. 2001) and winter habit cereals particularly after, or shortly before, vernalization saturation. Both photoperiod sensitivity and vernalization are responses to environmental stimuli affecting the developmental pathway (flowering pathway) in plants (Welch et al. 2004; Henderson et al. 2003) ultimately affecting timing of the reproductive transition and thereby the duration of expression of LT tolerance genes (Mahfoozi et al. 2001a).

The relationship between the ability of cereals to express LT-tolerance genes and the vernalization response has been clarified on a physiological basis. Based on observations made on a large number of wheat (*T. aestivum* L.) and rye (*Secale cereale* L.) cultivars, Fowler et al. (1996b) reported a very close association between the point of vernalization saturation and a decline in LT tolerance. Expression levels of the LT-induced wheat gene, *Wcs120*, (Houde et al. 1992) was found to be highly correlated with LT tolerance and a decline in *Wcs120* mRNA levels was associated with both a decline in the protein product and the point of vernalization saturation (Fowler et al. 1996a). The duration of expression of LT-tolerance genes, as governed by vernalization requirement, was also found to have a very significant effect on the accumulation of LT tolerance in near-isogenic lines of *T. aestivum* developed in both a spring and a winter wheat genetic background (Limin and Fowler 2002).

In this study we reveal the level of LT tolerance in wheat relative to genetic potential, vernalization requirement, and plant development. Previously produced reciprocal NILs (Limin and Fowler 2002) for the spring (*Vrn-A1*) and winter (*vrn-A1*) alleles in genetic backgrounds with highly dissimilar LT-tolerance potential were used as the genetic tools. Plant development was manipulated with photoperiod, vernalization requirement, and time. Development was monitored by dissecting main stem shoot apices. The expression of LT tolerance (LT_{50}) after these genetic and environmental manipulations is then discussed in relation to the genetic potential for this character.

Materials and methods

Plant material

Two Canadian (publicly available) wheat (*T. aestivum* L.) cultivars, 'Manitou' spring wheat and the very cold tolerant 'Norstar' winter wheat were used as the parental materials. Differences in spring/winter growth habit have been shown to be determined by the *Vrn-A1* locus (Brule-Babel and Fowler 1988). Reciprocal near-isogenic lines (NILs) for the spring (*Vrn-A1*) and winter (*vrn-A1*) alleles, derived from Manitou and Norstar, respectively, in the genetic backgrounds of each parent (Limin and Fowler 2002) were used as the genetic stocks. These NILs have been further purified and advanced to the BC10 generation giving 99.95% theoretical recovery of each parental genotype. The two sets of NILs have been designated in the text as 'spring or winter Norstar' and 'spring or winter Manitou'.

Plant growth, LT acclimation, and freeze-testing conditions

Plants were grown hydroponically as described in detail in a previous report (Limin and Fowler 2002). Seedlings were grown for 10 days in hydroponics tanks filled with continuously aerated one-half strength modified Hoagland's solution (Brule-Babel and Fowler 1988) at 20°C, 16-h days at 275 μmol m^{-2} s^{-1} PPFD, by which time they had three or four fully expanded leaves and visible crowns. They were then transferred to 2 or 4°C LT-acclimation chambers and the designated photoperiod at 220 μmol m^{-2} s^{-1} PPFD. Each photoperiod treatment remained constant in all experiments during both warm growth conditions and LT acclimation.

The procedure outlined by Limin and Fowler (1988) was used to determine the LT_{50} (temperature at which 50% of the plants are killed by LT stress) of each genotype at the end of each LT acclimation period. The shoot apex of the main stem from a minimum sample of three plants was dissected to determine the representative stage of development prior to freeze-testing at the times indicated in Figs. 1 and 2.

Three experiments were conducted to investigate the affect of vernalization, photoperiod, and development

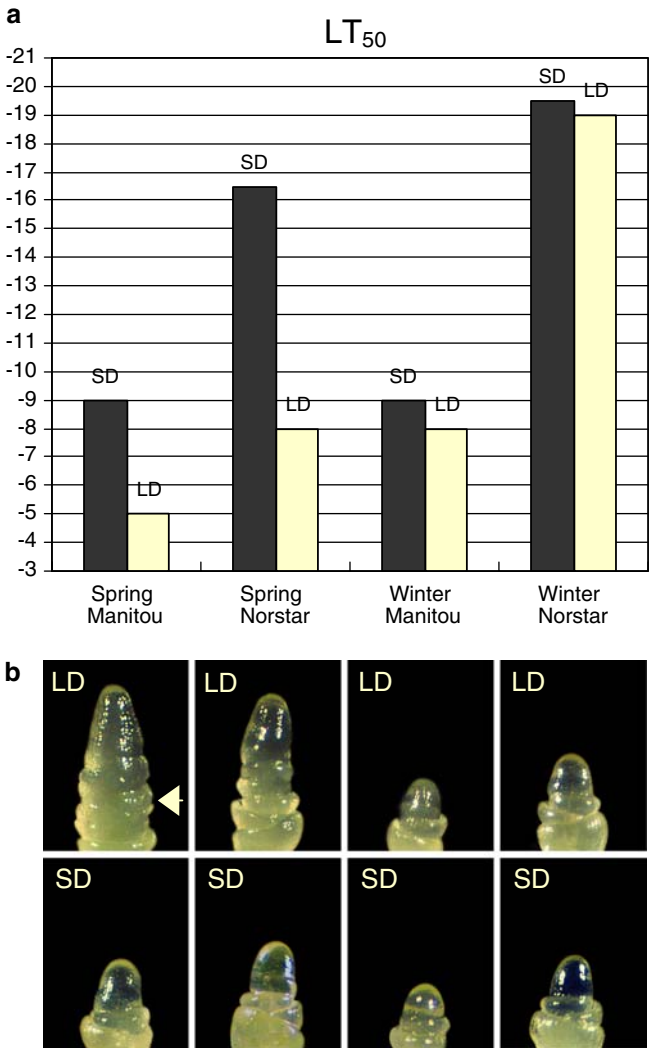

Fig. 1 **a**, **b** Low-temperature (*LT*) tolerance (LT_{50}) in the NILs (**a**): winter Norstar and spring Norstar, spring Manitou and winter Manitou, following 28 days LT acclimation at 4°C. Short day (*SD*) = 8 h, long day (*LD*) = 20 h; SE = 1.6. **b** Shoot apex development in the NILs following 14 days growth at 20°C plus 28 days vernalization/acclimation at 4°C. *Arrow* indicates double ridge

on LT-tolerance. (1) The affect of photoperiod on development and LT_{50} was determined for plants grown under long (LD, 20-h day) and short day (SD, 8-h day) for 28 days at 4°C LT acclimation (Fig. 1). (2) The affect of plant development prior to LT acclimation was determined for plants grown from 7 to 42 days at 20°C and 16-h day (photoperiod was not a variable) prior to 2°C LT acclimation (Fig. 2). In this experiment, germination was synchronized so that plants of different ages were acclimated for the same lengths of time and evaluated in the same freeze-test. (3) The effect of plant age and development on LT-acclimation ability (Fig. 3) was determined on plants grown at 20°C and 16-h day for 42 days and then LT acclimated at 2°C from 0 (unacclimated) to 42 days The experimental design in each experiment was a two factor factorial (NILs × acclimation regimes) in a two replicate randomized complete block design.

Results and discussion

Effect of photoperiod and vernalization on plant development and LT tolerance

The LT50 values for all NILs are shown in Fig. 1a following treatment of 14 days of growth at 20°C and 28 days of LT acclimation/vernalization at 4°C under both 20 and 8-h day lengths. Dissection of shoot apices indicated that winter Manitou and winter Norstar had been maintained in the early stages of phenological development by their vernalization requirement (Fig. 1b). Photoperiod had no discernable effect on development of these winter habit genotypes. In the spring habit genotypes, the LD treatment resulted in rapid development as shown by DR formation in Manitou and shoot apex elongation in spring Norstar. The SD treatment resulted in substantially delayed development vis-à-vis the LD treatment (Fig. 1b). It has been previously shown that spring Norstar produces two more leaves than spring Manitou (Limin and Fowler 2002), which accounts for the delay in development to DR stage in this line even under LD conditions.

Vernalization requirement in the winter lines appeared to allow full expression of the genetic LT tolerance potential of the Manitou and Norstar genotypes. In contrast, photoperiod did not have a significant ($P > 0.05$) effect on LT tolerance in the winter habit genotypes (Fig. 1a). Spring Manitou and spring Norstar had large photoperiod responses and when grown under SD had a much longer vegetative phase and significantly ($P < 0.05$) greater LT tolerance than under LD conditions. The SD treatment was as effective as a long vernalization requirement in facilitating expression of LT tolerance in NILs with the Manitou genetic background (SD spring Manitou and winter Manitou, Fig. 1a). The SD effect was most apparent in spring Norstar, probably because of the much greater LT-tolerance potential of the Norstar genotype. The SD effect was large enough to bring the spring Norstar LT_{50} to within 2.5–3°C of the very cold-tolerant winter cultivar Norstar (Fig. 1a).

These observations underscore the relationship of adapted plants to their environment and emphasize that an overriding factor in accumulation of LT tolerance in cereals is the point of vegetative to reproductive transition. This transition is determined primarily by components of the flowering pathway. Cereals are known to vary in their response to the major environmental cues of temperature (vernalization response) and photoperiod (Mahfoozi et al. 2001a, b; Fowler et al. 2001). Genetic differences in LT-tolerance potential clearly existed between the winter Manitou and Norstar lines (Fig. 1a), which were given equal opportunity to LT acclimate prior to full vernalization saturation (Limin

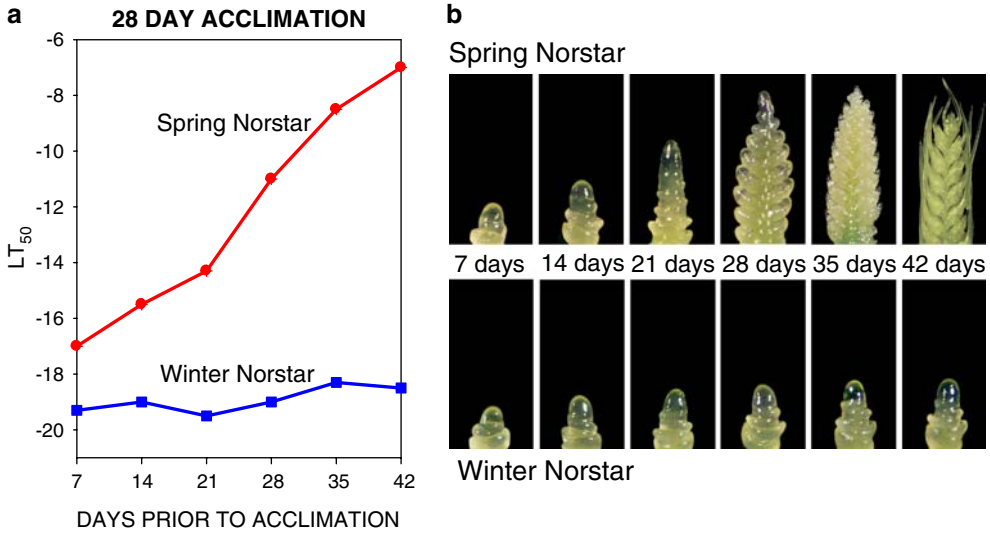

Fig. 2 **a**, **b** Norstar NILs (winter Norstar and spring Norstar) were grown at 20°C and 16-h day from 7 to 42 days and then LT acclimated at 2°C for 28 days. **a** LT_{50} versus plant age. SE = 0.57. **b** Dissected shoot apices indicating stage of development at the start of LT acclimation

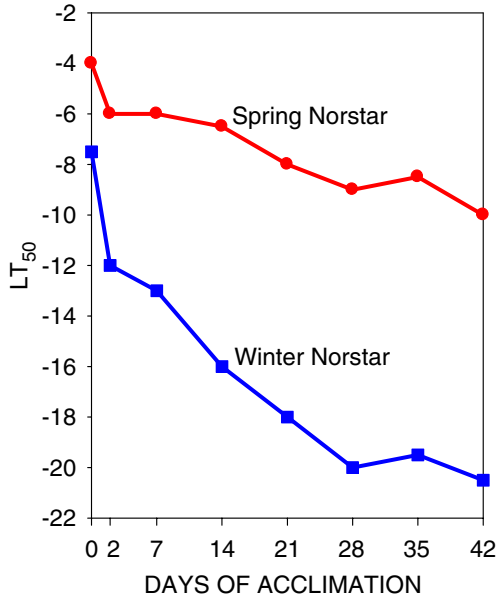

Fig. 3 Acclimation profiles of the Norstar NILs, 0–42 days at 2°C and 16-h day. Plants were grown 42 days under a 16-h day prior to acclimation. See Fig. 2b for developmental stage. SE = 0.42

and Fowler 2002) and DR formation (Fig. 1b). This is the developmental window that allows full expression of LT-induced genes known to be associated with LT tolerance in cereals (Fowler et al. 1996b). Spring habit genotypes grown under LD photoperiods advanced rapidly toward DR formation (the morphological indicator of reproductive transition, Fig. 1b) limiting their ability to accumulate LT tolerance. SD photoperiod repressed reproductive development in the spring genotypes (Fig. 1b) and allowed LT tolerance to accumulate to levels equal, or near equal, to their genetic LT-tolerance potential as seen in the vernalization requiring winter genotypes (Fig. 1a).

The winter season is preceded by shortening day length and sensing of this change permits plants with minimal or no vernalization requirement to utilize a LD photoperiod requirement (SD sensitivity) to remain in the vegetative growth phase thereby allowing for longer and greater expression of their LT-tolerance genetic potential. Adapted plants could, therefore, use one or both methods to maintain the vegetative phase and allow expression of LT tolerance genes. Karsai et al. (2001) reported that, amongst 39 barley varieties of diverse origin, photoperiod sensitivity could be as effective as a strong vernalization requirement in allowing acquisition of LT tolerance. The photoperiod sensitivity of the NILs used in this study have also revealed that vernalization is not necessarily essential in the development of LT-tolerant wheat genotypes.

Effect of plant development on LT acclimation

The Norstar NILs (winter Norstar and spring Norstar) were grown at 20°C and 16-h day for 7–42 days before LT acclimation to determine the effect of plant development on LT-acclimation ability (Fig. 2a, b). Shoot meristem development in vernalization requiring winter Norstar showed only a very slow elongation with time (Fig. 2b) and apical development did not reach the DR stage over the duration of the experiment. Spring Norstar was similar to winter Norstar in its stage of apical development from 7 to 14 days of growth at 20°C, but by 21 days of growth it had advanced to the very early stages of double ridge formation (Fig. 2b). Subsequently, floral organ development was rapid with spikelet and even awn formation well advanced by 35 and 42 days (Fig. 2b).

The LT tolerance of winter Norstar did not change significantly ($P > 0.05$) whether it was grown for 7 or 42 days under a 16-h day length at 20°C prior to LT acclimation (Fig. 2a), indicating that the strong vernalization requirement was effective in maintaining the plants in an early state of development. The LT-accli-

mation profile of spring Norstar versus chronological stage of development indicates nearly full expression of the Norstar LT-tolerance genes, as determined by LT_{50} measurement, in the early stages of development. It was, however, apparent that as development progressed prior to the cold-acclimation treatment, the spring Norstar plants progressively lost the ability to respond to the LT treatment (Fig. 2a). The powerful influence of the spring/winter determining system in *T. aestivum*, keyed in this instance by the *vrn-A1* allele, showed the strong influence conferred by the vernalization requirement in keeping plants in a very early state of development. Conversely, the spring habit *Vrn-A1* allele allowed unfettered development in the near-isogenic spring line. These observations again confirm the importance of stage of development in determining a plant's ability to respond to temperature.

The response of cold-hardy winter Norstar to LT acclimation has been well documented (Fowler et al. 1996a, b; Fowler and Limin 2004). These responses to LT acclimation have, however, been determined in the early seedling stages. Plant age is rarely considered in relation to LT tolerance because 'winter' cereals are seeded in the fall and are subjected to low temperatures and shortening day lengths. Plants with a vernalization requirement or SD photoperiod sensitivity remain vegetative under these conditions. Though little explored, it is taken as 'common knowledge' that advanced development prevents LT acclimation. Figure 2 illustrates this common wisdom but also shows that the loss of cold acclimation ability is *progressive*, in step with ongoing phenological development. This was particularly true after DR formation, reaffirming the importance of the vegetative/reproductive transition in LT acclimation (Fowler et al. 1996a, b; Mahfoozi et al. 2001a, b; Danyluk et al. 2003). Implicit in these observations is that, although the genetic LT-tolerance potential is present, as in spring Norstar, it will remain hidden if development is allowed to proceed.

Kinetic acclimation profiles of Norstar NILs following 42 days of growth and development

Response of the winter Norstar and spring Norstar NILs to LT acclimation was examined after 42 days of growth and development at 20°C and 16-h day; the point of maximum separation of the LT acclimation curves as determined in Fig. 2a. The acclimation curves (Fig. 3) for both lines were then developed for periods of LT acclimation from 0 to 42 days under a 16-h day length to compare their response over time as opposed to the single LT_{50} determined after 28 days acclimation shown in Fig. 2a. The acclimation curve for winter Norstar (Fig. 3) was similar to that previously described for this cultivar when acclimated at 2°C but with a pre-acclimation growth period of only 14 days (Fowler and Limin 2004). The acclimation curve of spring Norstar showed less responsiveness to LT than winter Norstar throughout the 42 day acclimation period (Fig. 3) as expected based on the developmental stages of the plants at the start of the acclimation period (Fig. 2b)

We had a limited prior understanding of the LT responses of spring and winter habit genotypes as phenological development progressed. Even though the shoot apex of winter Norstar showed slight elongation after 42 days of growth and development at 20°C and a 16-h day (Fig. 2a, b), its vernalization requirement held it in a developmentally early stage such that it responded like a recently emerged seedling (Fig. 3). Interestingly spring Norstar, although in a very advanced stage of development and with a limited ability to accumulate LT tolerance, was relatively hardy when unacclimated (day 0, Fig. 3) and was able to respond quickly to 2 days of acclimation. This was somewhat surprising given such an advanced stage of development (Fig. 2b), although some ability to acclimate has been shown in spring habit genotypes (Fowler et al. 1996b; Prasil et al. 2004). The potential may therefore exist for superior LT-tolerance gene sets to be residually expressed in spring habit genotypes. Further research is underway to verify this potential.

Genetic and environmental interactions determining LT

This study has verified that developmental stage is a critical component of LT-tolerance acquisition in cereals. Plant development toward flowering progressively reduces the ability of wheat to acclimate to LT particularly after the main shoot meristem has advanced to the DR (reproductive) growth stage. The LT-tolerance potential of a genotype can be retained by either a vernalization requirement or photoperiod sensitivity that maintains the plant in the early stages of phenological development. Incorporation of the spring habit allele *Vrn-A1* into winter Norstar, which has superior LT tolerance, rapidly advanced development under LD. However, either LT acclimation shortly after germination or growth under SD photoperiod maintained these plants in the early developmental stages and allowed for rapid expression of the superior LT-tolerance potential in spring Norstar.

We have previously shown (Fowler et al. 1999) that genes responsible for phenological development (vernalization and photoperiod genes) determine the duration of expression of LT-tolerance genes and that these genes can be separated (Fowler and Limin 2004) from genes determining the rate of acclimation (LT-tolerance genes per se). In this regard several presumed homoeologous LT-tolerance QTLs (designated *Fr-1* for frost resistance 1) have been mapped to positions 2 (*Fr-A1*), 10 (*Fr-D1*), and 40 (*Fr-B1*) cM from the homoeologous *Vrn-1* (spring/winter determining) loci (Galiba et al. 1995; Toth et al. 2003) on the group-5 chromosomes of *T. aestivum*. A second frost resistance locus designated *Fr-A2* has been reported on *T. monococcum* chromosome 5 (Vagujfalvi et al. 2003). *Fr-A2* mapped to the

long arm of chromosome 5A, 30 cM proximal to the RFLP marker *Xwg644* that is known to be tightly linked to *Vrn-A1* and the proposed *Fr-A1* locus (Vagujfalvi et al. 2003; Galiba et al. 1995).

The presumed homoeologous *Fr-1* QTLs were mapped to varying distances from the *Vrn-1* vernalization loci before the existence of *Fr-A2* was known. In this regard, marker *Xgwm639-5B* which mapped near the peak of the *Fr-B1* QTL (Toth et al. 2003) is closely linked to *Xbcd508*, which is located at the peak of *Fr-A2* (Vagujfalvi et al. 2003) implying that *Fr-B1* is likely an ortholog of *Fr-A2* not of *Fr-A1* (Catalogue of gene symbols for wheat: 2004, http://www.wheat.pw.usda.gov/ggpages/wgc/2004upd.html). Unfortunately, *Fr-A1* with the closest reported linkage to *Vrn-A1*, has never been isolated or sequenced and has been mapped at contradicting locations proximal and distal to *Vrn-A1* (Galiba et al. 1995; Toth et al. 2003). The existence of *Fr-1* as a separate locus from *Vrn-1* is therefore not conclusive.

The CBF-like barley gene, *Cbf3*, was mapped on the peak of the *Fr-A2* QTL for frost tolerance, a similar location to chilling-induced *HvCbf3* in barley (Choi et al. 2002) and to *Rcg1* (regulator for *Cor 14b*) in *T. aestivum* (Vagujfalvi et al. 2000). These observations suggest that *Cbf3* might be a candidate gene for *Fr-A2* (Vagujfalvi et al. 2003), although there is a cluster of *CBF*-like transcriptional activators in this region (Francia et al. 2004). This is an intriguing finding since the *CBF* transcriptional factors in *Arabidopsis* appear to be an integral part of the LT-tolerance pathway and have been shown to activate *Arabidopsis Cor* genes even in the absence of LT (Jaglo-Ottosen et al. 1998; Gilmour et al. 2000). Transcripts encoding CBF-like proteins were also found to accumulate rapidly in response to LT in 'Puma' rye (*Secale cereale* L.) and Norstar wheat (Jaglo et al. 2001). This suggests that a similar mechanism operates in *T. aestivum* where the group 5 chromosomes, 5A in particular, have been shown to affect LT_{50} levels and regulate the LT-tolerance associated *Wcs120* gene family located on the group 6 chromosomes of all three hexaploid wheat genomes (Limin et al. 1997).

In the present study, the effects of the winter allele (*vrn-A1*) versus the spring allele (*Vrn-A1*) were isolated under LD conditions where photoperiod did not interfere with development (Fig. 1). The winter allele in the cold tolerant Norstar genetic background resulted in an 11°C or a 2.4-fold greater LT tolerance compared to the spring allele. The delay in development of the spring habit (*Vrn-A1*) NIL caused by short-day (SD) versus long-day (LD) photoperiod resulted in an 8.5°C or 2.1-fold increase in LT-tolerance (Fig. 1). These results emphasize the very large effect of the *Vrn-A1* alleles and the response to photoperiod on the plants developmental stage and its relationship to LT tolerance in plants sharing identical (SD versus LD spring Norstar) or very near identical (spring Norstar versus winter Norstar NILs) LT-tolerance genes and genetic backgrounds.

The developmental effects were also clearly demonstrated when spring Norstar was acclimated after just 7 days of growth at 20°C (Fig. 2a, b). In this early developmental stage the difference in LT_{50} between spring and winter Norstar was only 2.3°C, however, this difference increased to 11.5°C after 42 days at 20°C before LT acclimation. These results emphasize the effect that developmental regulators like the photoperiod (*Ppd*) and vernalization (*Vrn*) genes have on LT tolerance. They also provide an explanation of why winter habit and LT tolerance are so often associated. The corollary of this association is, of course, that spring habit genotypes show less ability to LT acclimate. However, if LT acclimation begins at an early developmental stage or the plant is maintained in this early developmental stage by photoperiod sensitivity, near full expression of the LT tolerance potential can be achieved. Therefore, pleiotropic effects of the vernalization loci can explain the association of LT tolerance and winter habit (Brule-Babel and Fowler 1988) irrespective of LT-tolerance gene linkage to the *Vrn-1* loci. However, the existence of such a linkage cannot be ruled out nor can linkage of other flowering pathway genes that may affect timing of the reproductive transition.

The results of the experiments described in this study establish that the duration of time in early developmental stages underlies full expression of LT tolerance genetic potential. The consequence of this relationship between stage of phenological development and LT-tolerance gene expression is the frequent observation that winter genotypes tend to be more cold-tolerant than spring genotypes. We have shown that the change in ability of plants to accumulate LT tolerance occurs at both the transcriptional (Fowler et al. 1996a; Danyluk et al. 2003) and translational (accumulation of WCS120 LT-tolerance associated protein) levels (Fowler et al. 1996a) through repression of the LT-tolerance pathway. A strong vernalization requirement maintained winter Norstar plants in an early vegetative stage allowing them to consistently respond to LT over the full length of the experiment (Fig. 2a) illustrating the effect of the two allelic forms of *Vrn-A1* on the Norstar genotype. Since these 'vernalization' genes are orthologs of *Arabidopsis AP1* (Danyluk et al. 2003; Amasino 2004), which plays a central role in the flower induction pathway (Levy and Dean 1998; de Folter et al. 2005), very early up-regulation of the *Vrn-1* genes in spring types and their up-regulated expression coincident with vernalization saturation in winter habit types (Danyluk et al. 2003) make these genes key candidates for the LT-tolerance repression pathway. However, the mechanism of repression could also involve members of the flowering pathway other than *Vrn-1* itself. This repression coincides with reproductive competence when an adapted plant would no longer have a need for LT tolerance, as this would be expected to be a period of phenological development when environmental conditions are ideal for rapid growth, flowering, and seed formation, not a time of preparation for LT-stress.

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