

Minireview

The wheat *wcs120* gene family. A useful model to understand the molecular genetics of freezing tolerance in cereals

Fathey Sarhan, François Ouellet and Alejandro Vazquez-Tello

Sarhan, F., Ouellet, F. and Vazquez-Tello, A. 1997. The wheat *wcs120* gene family. A useful model to understand the molecular genetics of freezing tolerance in cereals. – *Physiol. Plant.* 101: 439–445.

Winter, as compared with spring cereals, possess better acclimation mechanisms that allow them to overwinter and survive freezing temperatures. This difference is genetically programmed and involves a complex genetic system. To understand the nature of this system and its regulation by low temperature, genes associated with freezing tolerance in wheat (*Triticum aestivum* L.) were identified and characterized. Among these, the *wcs120* gene family encodes a group of proteins ranging in size from 12 to 200 kDa. As shown by biochemical, immunohistochemical, molecular and genetic analyses, this gene family is specific to the Poaceae, highly abundant and coordinately regulated by low temperature. Furthermore, accumulation of WCS protein is directly correlated with the development of freezing tolerance. These analyses also revealed a regulatory control of the vernalization process over low temperature gene expression in winter cereals. Recent studies suggest that the molecular mechanisms controlling the expression of these genes involve negative regulatory factors that are modulated by phosphorylation.

Key words – Chromosome mapping, cold acclimation, cryoprotection, dehydrins, desiccation, freezing tolerance, low temperature regulation, Poaceae, transcriptional repressors, Triticeae, *Triticum aestivum*, vernalization, *wcs120*, wheat.

F. Sarhan (corresponding author, e-mail sarhan.fathey@uqam.ca) et al., *Départ des Sciences biologiques, Univ. du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, PQ, H3C 3P8, Canada.*

Introduction

Low temperature (LT) acclimation allows hardy plants to develop efficient tolerance mechanisms needed for winter survival. During this period, numerous biochemical, physiological and metabolic functions are altered in plants. These changes are regulated by LT at the gene expression level. To understand the molecular basis of adaptation to LT, efforts were focused on identifying LT-responsive genes. Cold-induced genes and their products have been isolated and characterized in many species (Hughes and Dunn 1996). However, the difficulty has been to separate genes associated with metabolic adjustment to LT from those responsible for cold acclimation and freezing tolerance (FT). In this respect,

the use of genotypes of the same species that differ in their capacity to develop FT provides valuable tools to distinguish between stress and acclimation genes.

In wheat and other cereals, the expression of several genes during cold acclimation was found to be positively correlated with the capacity of each genotype to develop FT. Among these, the *wcs120* gene family in wheat encodes a group of highly abundant proteins ranging in size from 12 to 200 kDa. This protein family is coordinately regulated by LT and accumulates to high levels in freezing-tolerant members of the Poaceae (Houde et al. 1992b, Limin et al. 1995). Although their precise function is still unknown, their high hydrophilicity, stability upon boiling, and structural similarity with some dehydrins suggest that they may be associated with the pro-

Received 6 December, 1996; revised 10 March, 1997

tection of cells against desiccation or other stresses caused by freezing. In this review, we will summarize the general characteristics of this gene family and discuss their putative function during cold acclimation. We will also review the recent progress in understanding the molecular and genetic mechanisms controlling their expression at LT.

Abbreviations – CA, cold-acclimated; EMSA, electrophoretic mobility shift assay; FT, freezing tolerance; LEA, late embryogenesis abundant; LT, low temperature; LT₅₀, 50% killing temperature; NA, nonacclimated; PD₅₀, concentration required to give 50% residual enzymatic activity after freezing and thawing; WCOR, wheat cold-regulated; WCS, wheat cold specific.

Molecular characterization and biochemical properties

The WCS120 group of proteins was identified in wheat using polyclonal antibodies directed against a major LT-induced protein of 50 kDa encoded by the *wcs120* gene (Houde et al. 1992a,b). The WCS120 family consists of five major proteins with molecular masses of 200, 180, 66, 50 and 40 kDa. A prolonged exposure of the immunoblots revealed the presence of two more immunologically-related proteins with molecular masses of 21 and 12 kDa. Sequence analysis of 5 members of this family has revealed the presence of two repeated domains: a lysine-rich domain (GEKKGVMENIKEK-LPG) and a glycine-rich domain (TGGTYGQQGHT-GTT) (Chauvin et al. 1994, Danyluk 1996, Houde et al. 1992a, Ouellet et al. 1993), which vary in number according to the molecular mass of the protein. The WCS120 proteins are rich in glycine and threonine (>39%), highly hydrophilic, soluble upon boiling and have a pI above 6.5 (Tab. 1). Another common feature of this group of proteins is the discrepancy between the calculated and apparent molecular masses on SDS-PAGE, the apparent molecular masses being much higher than the calculated ones. This discrepancy is now a common

observation for other stress proteins with skewed amino acid composition, and may result from the unusually high binding of SDS by some amino acids (Weretilnyk and Hanson 1990). The WCS66, WCOR80 and WCOR726 proteins share, respectively, 89.7, 77.7 and 67.5% identity at the amino acid level with WCS120. The strong homology between these proteins is due to the presence of the repeated domains and may explain the cross-reactivity of the anti-WCS120 antibody. The WCS120 protein family shares homology with the D11 dehydrin family (Dure 1993). However, apart from the presence of the repeated elements, the WCS120 family does not share the other characteristics found in several members of the D11 family, such as a high content of charged amino acids, a serine stretch and the N-terminal conserved sequence DEYGNP (Danyluk 1996). The WCS120 protein shows the highest identity with DHN5 from barley and its homolog in *Triticum durum*. A recent study of dehydrin gene expression during cold acclimation in barley showed that *dhn5* is the major cold-induced dehydrin gene (Close et al. 1995). This interesting observation confirms the finding that the WCS120 family is a major group of proteins specifically induced by LT in tolerant species of the Poaceae. In spite of the presence of lysine- and glycine-rich repeats in dehydrins from freezing-tolerant dicotyledonous species, there was no cross hybridization or reactivity with either *wcs* cDNA or antibodies. This may suggest that FT in monocotyledons and dicotyledons involves different proteins with similar biochemical properties.

The accumulation of the WCS120 family in more than 20 genotypes of wheat and other cereals during the acclimation period is positively correlated with the capacity of each genotype to develop FT (F. Sarhan, L. P. Chauvin, A. Limin and B. Fowler, unpublished results). This correlation supports the suggestion of Houde et al. (1992b) to use the antibody as a molecular marker to select for the FT trait in the Poaceae. In fact, the anti-WCS120 antibody is currently used to assess the

Tab. 1. Characteristics of the wheat *wcs120* gene family. Molecular mass determined by SDS-PAGE and (calculated from the cDNA sequence); ND, not determined.

Gene	Molecular mass of encoded protein (kDa)	Isoelectric point	No. of repeats		Induction	Chromosomal location	Reference and accession number
			Lysine	Glycine			
<i>wcs200</i>	200 (ND)	6.50	ND	ND	cold	6AL	Ouellet et al. 1993, Limin et al. 1997
<i>wcs180</i>	180 (ND)	6.50	ND	ND	cold	6DL	Houde et al. 1995, Limin et al. 1997
<i>wcs66</i>	66 (46.8)	7.28	7	4	cold	6BL	Chauvin et al. 1994, Limin et al. 1997; L27516
<i>wcs120</i>	50 (39.0)	7.77	6	11	cold	6DL	Houde et al. 1992a, Limin et al. 1997; M93342
<i>wcs40</i>	40 (ND)	7.30	ND	ND	cold	ND	Houde et al. 1995
<i>wcs726</i>	21 (17.7)	7.04	3	2	cold, drought	ND	Danyluk 1996; U73213
<i>wcs80</i>	12 (9.6)	8.05	2	2	cold, drought	ND	Danyluk 1996; U73212

segregation of FT in different crosses and to study the inheritance of the members of this protein family in wheat and rye. This antibody is a valuable tool to classify cereal cultivars according to their capacity to develop FT. The method of western blotting is rapid and economical compared with the invasive, time consuming methods currently used to evaluate the LT₅₀.

Abundance, tissue distribution and cellular localization

The total WCS120 protein family accumulates to 72 $\mu\text{g g}^{-1}$ fresh weight in the winter wheat seedlings (cvs Fredrick and Norstar) after 21 days of cold acclimation. This represents ca 0.9% of the total soluble proteins and a calculated cellular concentration of 1.34 μM (based on an 85% cellular water content). This amount reaches 1% at 49 days of acclimation, a period which coincides with the maximum FT. The 50- and 66-kDa proteins are the most abundant and represent 64% of this protein family (Houde et al. 1995). The significance of the differential expression and accumulation of the members of this family is not known. However, the presence of proteins of variable molecular sizes having a similar antigenicity and coordinately regulated by LT is novel and suggests that their function is determined through the common lysine- and glycine-rich repeated elements within their structure. It is thus likely that the number of repeats within each protein, and the amount of proteins synthesized may have an important role in defining their function during the induction of FT.

The meristematic crown, the most freezing-tolerant tissue, accumulates more of these proteins than the basal region of the crown, shoot and roots (Houde et al. 1992b). This observation is consistent with the fact that winter wheat survival is determined by the capacity of the crown meristematic tissue to survive the winter (Tanino and McKersie 1984). Immunocytochemical localization showed that these proteins accumulate to high levels in the vascular transition zone with no detectable expression in mature xylem, in the shoot apical meristem or lateral root primordia (Houde et al. 1995). This differential tissue expression suggests that sensitive cells in this region require a higher amount of the WCS120 proteins. The proteins may help alleviate the dehydration stress associated with freezing, thus resulting in increased tolerance to mechanical damage due to freezing. The high level of accumulation of the WCS120 proteins in the vascular tissues may also play a role in maintaining or improving metabolite transport at LT. The superior growth performance and photosynthetic capacity of freezing-tolerant plants support this hypothesis (Huner et al. 1993). Interestingly, the accumulation of this protein family in the companion cells coincides with the localization of sucrose synthase (Crespie et al. 1991, Nolte and Koch 1993). This enzyme, which has greater activity during cold acclimation, is involved in the regulation of sucrose-starch interconversion and could thus be a

key enzyme in the accumulation of sucrose as a cryoprotectant during cold acclimation (Guy et al. 1992, Perras and Sarhan 1984). Although the physiological significance of sucrose synthase in transport tissues is unclear, it is suggested that it may provide the UDP-glucose needed to repair injuries caused by freezing. The presence of this precursor may promote callose formation which could reduce the size-exclusion limit of sieve-plate pores in sieve tubes, leading to their rapid sealing in case of injury (Nolte and Koch 1993). A parallel can be made with cryptobiotic organisms where dehydrins are induced concomitantly with sucrose phosphate synthase and sucrose synthase, leading to sucrose accumulation (Bartels et al. 1993).

Immunogold localization and biochemical fractionation have confirmed that the WCS120 proteins are present both in the cytoplasm and the nucleoplasm of cold-acclimated crowns. They are not found in cell walls, organelles or in dense chromatin. Similarly, the ABA-induced protein RAB17 (Goday et al. 1994) and the dehydrins (Close 1996) are localized in the cytoplasm and nucleus of several cell types of maize. The presence of these proteins in high amounts in the nucleus indicates that they may protect or stabilize the transcriptional machinery from inactivation. However, it remains unclear how these proteins are targeted to the nucleus because sequence analysis did not reveal the presence of a known nuclear signal peptide. The WCS120 protein family is also devoid of the serine-tract found in RAB proteins that is believed to be involved in nuclear import (Goday et al. 1994). On the other hand, it was suggested that the basic nature of the lysine-rich repeat may play a role in nuclear import (Danyluk 1996). It is also possible that the WCS120 proteins could be co-transported to the nucleus through protein-protein interactions.

In vitro cryoprotection assays indicated that the WCS120 protein (PD₅₀ of 10 $\mu\text{g ml}^{-1}$ or 0.2 μM) is as effective as BSA and sucrose (at 250 mM) in protecting lactate dehydrogenase against freezing denaturation (Houde et al. 1995). In similar studies, Kazuoaka and Oeda (1994) obtained equivalent cryoprotection with COR85, a cold-responsive protein from spinach homologous to WCS120. Considering the fact that the WCS120 protein family is expressed only in specific cells, the WCS120 protein concentration is probably much higher than the calculated 1.34 μM . Houde et al. (1995) suggested that these proteins surround vital cellular proteins and protect them from unfolding or aggregating during freezing or dehydration. A similar explanation was given for the cryoprotective role of BSA in protecting enzyme preparations during storage at LT (Tamiya et al. 1985).

Chromosome mapping of the *wes120* gene family

Common wheat (*Triticum aestivum* L.) is a hexaploid species comprised of three genomes designated A, B and D (AABBDD, 42 chromosomes). Two of the diploid

species contributing these genomes are *T. monococcum* (A genome) and *T. tauschii* (D genome). The origin of the B genome is uncertain and could be multispecific due to interspecific genome hybridization involving members of a similar taxonomic group (Kimber and Sears 1987). In order to map the *wcs120* gene family to specific chromosome arms in this complex genome, Limin et al. (1997) used a combination of Southern and western analyses on the ditelocentric series in the hexaploid Chinese Spring wheat, in which one homologous pair of chromosome arms is missing in each line. Using this approach, *wcs120*, *wcs200* and *wcs66* were mapped to 6DL, 6AL and 6BL, respectively (Tab. 1). Although the other members of this family are associated with the group 6 chromosomes, it was not possible to map them to specific chromosome arms.

Diploid species of the A and D genomes as well as tetraploid AB species were screened by western analysis to confirm the genomic origin of these proteins (Limin et al. 1997). The tetraploid AB genome species were used to help ascertain the origin of the B genome constituents because the diploid origin of this genome is uncertain. A 180-kDa protein and a more prominent 200-kDa protein are present in the A genome species, and a 180-kDa protein is prominent in the D genome species. A major difference in the tetraploid AB species is the variable expression of a 66-kDa protein not found in either of the A or D genome groups. On the basis of the 14 *T. aestivum* cultivars examined to date, it is possible that the B genome progenitor may have expressed this protein because all hexaploids express the 66-kDa protein and the other related proteins. Members of the related dehydrin gene family (*dhn3*, *dhn4* and *dhn5*) in *Hordeum vulgare*, another species belonging to the Triticeae, have also been mapped to homologous chromosome 6 in barley (Close and Chandler 1990, Pan et al. 1994). These group 6 chromosomes therefore appear to be the location of many structural genes involved in LT and dehydration stress responses (Close 1996, Limin et al. 1997, Welin et al. 1994).

Gene regulation

Freezing tolerance and accumulation of the WCS120 proteins were evaluated in the chromosome substitution series in which one pair of specific chromosomes from Cheyenne (CNN) substitutes for the homologous pair in Chinese Spring (CS) (Limin et al. 1997). As expected, protein levels were much higher in the hardy winter cultivar CNN than in the less hardy CS wheat during LT exposure. In the substitution series, only the 5A line showed a WCS120 protein accumulation higher than the level observed in CS during cold acclimation. Interestingly, two more wheat LT-responsive genes, *wcor410* and *wcor719* (Danyluk 1996, Danyluk et al. 1994, 1996), were also found to be regulated by factors on 5A. This chromosome is most frequently found to have the greatest effect on cold tolerance (Galiba et al. 1993,

Roberts 1990, Sutka 1994). In barley, Hayes et al. (1993) found the largest quantitative trait loci (QTL) for winter field survival, LT₅₀, growth habit, and crown fructan content on the long arm of chromosome 7 (equivalent to chromosome 5 in wheat). It is possible that barley chromosome 7 acts in the same manner as wheat chromosome 5A in that it may influence growth habit and regulate the expression of LT-induced genes such as *dhn5*, which is located on the same homologous group 6 chromosome arm of barley as are the *wcs120* family genes. It appears from these analyses that chromosome 5A carries the regulatory gene(s) that control the expression of the *wcs120* gene family and of other LT-responsive genes correlated with FT. The identification of the regulatory mechanism(s) will certainly improve our understanding of how LT regulates gene expression and FT in cereals.

Vernalization control

The *wcs120* gene family was used to investigate the relationship between LT gene expression and the vernalization response at the molecular level in wheat and rye (Fowler et al. 1996a). The data showed that spring habit cultivars that do not have a vernalization response are unable to maintain LT-induced gene expression in an up-regulated condition when exposed to 4°C. Consequently, they are unable to achieve levels of FT similar to those of winter habit cultivars. A close association between the point of vernalization saturation and the start of a decline in *wcs120* family mRNA and protein levels indicates that vernalization genes have a regulatory influence on LT gene expression in winter cereals. These observations support the hypothesis that the vernalization genes play a key role in determining the duration of expression of the FT-associated genes, which suggests an explanation for the apparent pleiotropic effect that the *vrn1* gene has on both FT and the vernalization response in wheat (Brule-Babel and Fowler 1988, Roberts 1990, Sutka 1994). It appears that any factor that delays the transition from vegetative to reproductive stages, such as a vernalization or photoperiod requirement for flowering, should be expected to increase the level of expression of FT genes in cereals exposed to acclimating temperatures (Fowler et al. 1996b).

There is genetic evidence suggesting that *vrn1* is homologous to other spring habit genes in wheat and related species. This group includes the vernalization genes *vrn1*, *vrn4* and *vrn3* on chromosomes 5A, 5B and 5D of wheat, *sh2* on chromosome 7 of barley, and *sp1* on chromosome 5 of rye (Galiba et al. 1995, Laurie et al. 1995, Pan et al. 1994, Plaschke et al. 1993). A close linkage has been reported between several of these vernalization loci and FT genes (Hayes et al. 1993, Pan et al. 1994, Roberts 1990), suggesting that at least some of the genes conditioning LT responses in cereals are present in clusters (Fowler et al. 1993). While the above data provide strong support for the hypothesis that the vernalization genes are pleiotropic, affecting both

growth habit and FT in wheat and rye, the likelihood of close genetic linkage of genes conditioning the LT responses is not ruled out. In fact, the role that vernalization genes play in determining FT may simply be restricted to a regulatory influence on the duration of expression of LT-induced genes.

Molecular mechanism of gene regulation

To understand the regulatory mechanisms controlling the expression of the *wcs120* gene at LT, the promoter region was identified and characterized (Ouellet et al. 1995, Vazquez-Tello et al. 1996). Transient expression experiments indicated that the *wcs120* promoter confers a LT response to the luciferase reporter gene. Sequence analysis showed that the core element CANNTG is repeated 8 times within the *wcs120* promoter. This motif is also present in the promoter region of several light- and ABA-responsive genes (Guiltinan et al. 1990, Williams et al. 1992) and was identified as the preferred binding site for the basic helix-loop-helix (b-HLH) proteins, a large family of transcription factors playing a key role in cell progression and developmental gene regulation (Anthony-Cahill et al. 1992, Blackwell and Weintraub 1990). Furthermore, the common plant regulatory factors (CPRFs) and G-box binding factors (GBFs) belonging to the basic leucine zipper (bZIP) class of proteins interact specifically with this motif (Harter et al. 1994, Weisshaar et al. 1991). Sequence comparison between the *wcs120* promoter and those of several genes regulated by either LT, drought, salinity or ABA did not reveal significant homologous regions, except in the case of the promoter of the *dhn5* gene, the barley homolog of *wcs120* (Close et al. 1995). Vazquez-Tello et al. (1996), using short promoter fragments in Electrophoretic Mobility Shift Assays (EMSA), revealed the presence of multiple DNA-binding proteins in nuclear extracts from nonacclimated (NA) plants. In contrast, no DNA-binding activity was observed in the nuclear extracts from cold-acclimated (CA) plants. In vitro dephosphorylation of CA nuclear extracts with alkaline phosphatase prior to the EMSA restored the binding activity. Moreover, okadaic acid (a potent phosphatase inhibitor) markedly stimulated the in vivo accumulation of the WCS120 family of proteins, suggesting that protein phosphatases PP1 and/or PP2A negatively regulate the expression of the *wcs120* family. In addition, the endogenous total nuclear kinase activity was found to be significantly higher in the CA nuclear extracts. Western analysis showed that a PKC γ -like protein (84 kDa) is selectively translocated into the nucleus in response to LT. This work, though preliminary, provides the first molecular evidence that the expression of a LT-responsive gene is regulated by nuclear negative regulatory factors whose DNA-binding activity is modulated by a phosphorylation/dephosphorylation mechanism. The presence of multiple and distinct *cis*- and *trans*-elements suggest a complex mechanism of transcriptional regulation. Based on the avail-

able data, we propose a hypothetical model describing how LT regulates gene expression (Fig. 1). At nonacclimating temperatures, repressor factors are not phosphorylated or are maintained dephosphorylated by nuclear protein phosphatases (PP). This state allows them to bind to the *wcs120* promoter region, possibly blocking the assembly of the transcriptional machinery (TM). Following exposure to LT, both Ca²⁺-dependent and Ca²⁺-independent protein kinases are stimulated (Monroy and Dhindsa 1995; F. Ouellet, A. Vazquez-Tello and F. Sarhan, unpublished results), leading to the phosphorylation and inactivation of the putative repressors. This would allow the transcriptional machinery to assemble and begin transcription.

Conclusions

The function of the WCS120 proteins in the development of FT is still not clearly defined. However the physiological, biochemical, molecular and genetic char-

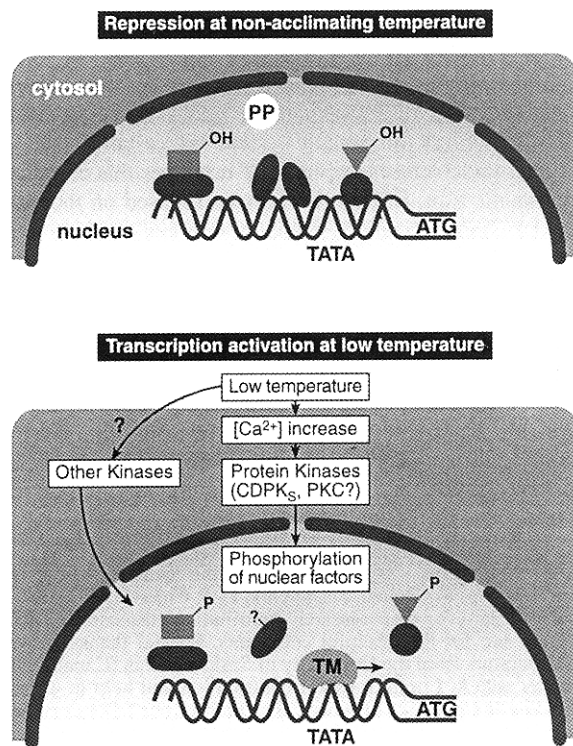


Fig. 1. Hypothetical model describing how low temperature regulates the expression of low temperature-responsive genes. At nonacclimating temperatures, negative regulatory factors (ovals and circle, DNA-binding subunits; square and triangle, regulatory subunits) are not phosphorylated or are maintained dephosphorylated by nuclear phosphatases (PP). This state allows them to bind to the *wcs120* promoter region, thus blocking the assembly of the transcriptional machinery. Following exposure to low temperature, both Ca²⁺-dependent and Ca²⁺-independent protein kinases are stimulated leading to the phosphorylation and inactivation of the putative repressors. This allows the transcriptional machinery (TM) to assemble and begin transcription.

acterization provides valuable information that contributes to the understanding of their function and their regulation. The abundance of these proteins in the crown vascular transition zone in hardy cereal cultivars indicates a major cryoprotective role during LT acclimation. Producing transgenic plants that express these proteins will certainly help to verify their direct role in improving FT. The *wcs120* gene has been successfully transferred to tobacco, strawberry and alfalfa, and the analysis of these transgenes is in progress. One possible obstacle we may encounter when transferring genes from one species to another is correct targeting. It is possible that targeting a gene product to specific tissues or cell compartments may require transport systems or a particular microenvironment that are not present in all plants. These questions will be addressed after the analysis of transgenic plants from different species is completed.

The use of integrated molecular and cytogenetic approaches was very successful in mapping the *wcs120* genes to group 6 of wheat and led to the identification of the chromosome carrying the major regulatory gene(s). Chromosome 5A was found to regulate the expression of the *wcs120* genes as well as other LT-responsive genes correlated with FT (e.g. *wcor410* and *wcor719*). This finding suggests that a major regulatory switch governs the expression of LT-responsive genes in wheat. The identification of this genetic system will certainly lead to a better understanding of how LT regulates this complex multigenic trait. Efforts can now be focused on the molecular characterization of this system and on the elucidation of the interaction occurring between this regulator and the promoter regions of LT-responsive genes. The data generated from these studies may offer information leading to the genetic manipulation of FT in plants. Indeed, the identification of a major FT determinant may allow the development of a single-gene transformation strategy. This would, without a doubt, simplify the tedious task of multiple gene transformation proposed to improve multigenic traits in agronomically important crops.

Acknowledgments – We are grateful to the members of our research group, M. Houde, J. Danyluk, L. P. Chauvin, S. Dallaire and F. Allard for their research contributions discussed in this review and for their helpful comments. Special thanks to our collaborators from the University of Saskatchewan (Canada), B. Fowler and A. Limin, for their contribution and help in understanding the genetics of wheat. Research in our laboratory has been supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche of the Ministère de l'Éducation du Québec.

References

- Anthony-Cahill, S. J., Benfield, P. A., Fairman, R., Wasserman, Z. R., Brenner, S. L., Stafford, W. F., Altenbach, C., Hubbell, W. L. & Degrado, W. F. 1992. Molecular characterization of helix-loop-helix peptides. – *Science* 255: 979–983.
- Bartels, D., Alexander, R., Schneider, K., Elster, R., Velasco, R., Alamillo, J., Bianchi, G., Nelson, D. & Salamini, F. 1993. Desiccation-related gene products analyzed in a resurrection plant and in barley embryos. – *In* Plant Responses to Cellular Dehydration during Environmental Stress (T. J. Close and E. A. Bray, eds), pp. 119–127. American Society of Plant Physiologists, Rockville, MD. ISBN 0-943088-26-7.
- Blackwell, T. K. & Weintraub, H. 1990. Differences and similarities in DNA-binding preferences of MyoD and E2A protein complexes revealed by binding site selection. – *Science* 250: 1104–1110.
- Brule-Babel, A. L. & Fowler, D. B. 1988. Genetic control of cold hardiness and vernalization requirement in winter wheat. – *Crop Sci.* 23: 879–884.
- Chauvin, L.-P., Houde, M. & Sarhan, F. 1994. Nucleotide sequence of a new member of the freezing tolerance-associated protein family in wheat. – *Plant Physiol.* 105: 1017–1018.
- Close, T. J. 1996. Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. – *Physiol. Plant.* 97: 795–803.
- & Chandler, P. M. 1990. Cereal dehydrins: Serology, gene mapping and potential functional roles. – *Aust. J. Plant Physiol.* 17: 333–344.
- , Meyer, N. C. & Radik, J. 1995. Nucleotide sequence of a gene encoding a 58.5 kDa barley dehydrin that lacks a serine tract. – *Plant Physiol.* 107: 289–290.
- Crespi, M. D., Zabaleta, E. J., Pontis, H. G. & Salerno, G. L. 1991. Sucrose synthase expression during cold acclimation in wheat. – *Plant Physiol.* 96: 887–891.
- Danyluk, J. 1996. Identification et caractérisation moléculaire de gènes induits au cours de l'acclimatation au froid chez le blé (*Triticum aestivum*). – Ph. D. Thesis, Université de Montréal, Canada.
- , Houde, M., Rassart, É. & Sarhan, F. 1994. Differential expression of a gene encoding an acidic dehydrin in chilling sensitive and freezing tolerant Gramineae species. – *FEBS Lett.* 344: 20–24.
- , Carpentier, É. & Sarhan, F. 1996. Identification and characterization of a low temperature regulated gene encoding an actin-binding protein from wheat. – *FEBS Lett.* 389: 324–327.
- Dure, L. III. 1993. Structural motifs in lea proteins. – *In* Plant Responses to Cellular Dehydration during Environmental Stress (T. J. Close and E. A. Bray, eds), pp. 91–103. American Society of Plant Physiologists, Rockville, MD. ISBN 0-943088-26-7.
- Fowler, D. B., Limin, A. E., Robertson, A. J. & Gusta, L. V. 1993. Breeding for low-temperature tolerance in field crops. – *Int. Crop Sci.* 1: 357–362.
- , Chauvin, L. P., Limin, A. E. & Sarhan, F. 1996a. The regulatory role of vernalization in the expression of low-temperature induced genes in wheat and rye. – *Theor. Appl. Genet.* 93: 554–559.
- , Limin, A. E., Wang, S. Y. & Ward, R. W. 1996b. Relationship between low-temperature tolerance and vernalization response in wheat and rye. – *Can. J. Plant Sci.* 76: 32–42.
- Galiba, G., Tuberosa, R., Kocsy, G. & Sutka, J. 1993. Involvement of chromosomes 5A and 5D in cold-induced abscisic acid accumulation in and frost tolerance of wheat calli. – *Plant Breed.* 110: 237–242.
- , Quarrie, S. A., Sutka, J., Morgounov, A. & Snape, J. W. 1995. RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. – *Theor. Appl. Genet.* 90: 1174–1179.
- Goday, A., Jensen, A. B., Culiñez-Marcia, F. A., Albà, M. M., Figueras, M., Serratos, J., Torrent, M. & Pagès, M. 1994. The maize abscisic acid-responsive protein Rab17 is located in the nucleus and interacts with nuclear localization signals. – *Plant Cell* 6: 351–360.
- Guiltinan, M. J., Marcotte, W. R. & Quatrano, R. S. 1990. A plant leucine zipper protein that recognizes an abscisic acid responsive element. – *Science* 250: 267–271.
- Guy, C. L., Huber, J. L. A. & Huber, S. C. 1992. Sucrose phosphate synthase and sucrose accumulation at low temperature. – *Plant Physiol.* 100: 502–508.

- Harter, K., Kircher, S., Frohnmeyer, H., Krenz, M., Nagy, F. & Schäfer, E. 1994. Light-regulated modification and nuclear translocation of cytosolic G-box binding factors in parsley. – *Plant Cell* 6: 545–559.
- Hayes, P. M., Blake, T., Chen, T. H. H., Tragoonrung, S., Chen, F., Pan, A. & Lui, B. 1993. Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome 7 associated with components of winter hardiness. – *Genome* 36: 66–71.
- Houde, M., Danyluk, J., Laliberté, J.-F., Rassart, É., Dhindsa, R. S. & Sarhan, F. 1992a. Cloning, characterization, and expression of a cDNA encoding a 50 kilodalton protein specifically induced by cold acclimation in wheat. – *Plant Physiol.* 99: 1381–1387.
- , Dhindsa, R. S. & Sarhan, F. 1992b. A molecular marker to select for freezing tolerance in Gramineae. – *Mol. Gen. Genet.* 234: 43–48.
- , Daniel, C., Lachapelle, M., Allard, F., Laliberté, S. & Sarhan, F. 1995. Immunolocalization of freezing-tolerance-associated proteins in the cytoplasm and nucleoplasm of wheat crown tissues. – *Plant J.* 8: 583–593.
- Hughes, M. A. & Dunn, M. A. 1996. The molecular biology of plant acclimation to low temperature. – *J. Exp. Bot.* 47: 291–305.
- Huner, N. P. A., Öquist, G., Hurry, V. M., Krol, M., Falk, S. & Griffith, M. 1993. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. – *Photosynth. Res.* 37: 19–39.
- Kazuoka, T. & Oeda, K. 1994. Purification and characterization of COR85-oligomeric complex from cold-acclimated spinach. – *Plant Cell Physiol.* 35: 601–611.
- Kimber, G. & Sears, E. R. 1987. Evolution in the genus *Triticum* and the origin of cultivated wheat. – *In* *Wheat and Wheat Improvement*, 2nd Ed. (E. G. Heyne, ed.), pp. 154–164. American Society of Agronomy, Madison, WI. ISBN 0-89118-091-5.
- Laurie, D. A., Pratchett, N., Bezant, J. H. & Snape, J. W. 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. – *Genome* 38: 575–585.
- Limin, A. E., Houde, M., Chauvin, L. P., Fowler, D. B. & Sarhan, F. 1995. Expression of the cold-induced wheat gene *Wcs120* and its homologs in related species and interspecific combinations. – *Genome* 38: 1023–1031.
- , Danyluk, J., Chauvin, L. P., Fowler, D. B. & Sarhan, F. 1997. Chromosome mapping of low-temperature induced *Wcs120* family genes and regulation of cold-tolerance expression in wheat. – *Mol. Gen. Genet.* 253: 720–727.
- Monroy, A. F. & Dhindsa, R. S. 1995. Low-temperature signal transduction: Induction of cold acclimation-specific genes of alfalfa by calcium at 25°C. – *Plant Cell* 7: 321–331.
- Nolte, K. D. & Koch, K. E. 1993. Companion-cell specific localization of sucrose synthase in zones of phloem loading and unloading. – *Plant Physiol.* 101: 899–905.
- Ouellet, F., Houde, M. & Sarhan, F. 1993. Purification, characterization and cDNA cloning of the 200 kDa protein induced by cold acclimation in wheat. – *Plant Cell Physiol.* 34: 59–65.
- , Vazquez-Tello, A. & Sarhan, F. 1995. Analysis of the promoter region of the cold-regulated wheat gene *Wcs120*. – *In* *Proceedings of the Annual Meeting of the Canadian Society of Plant Physiologists*, Guelph. ISSN 0842-0602.
- Pan, A., Hayes, P. M., Chen, F., Chen, T. H. H., Blake, T., Wright, S., Karsai, I. & Bedo, Z. 1994. Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L.). – *Theor. Appl. Genet.* 89: 900–910.
- Perras, M. & Sarhan, F. 1984. Energy state of spring and winter wheat during cold hardening. Soluble sugars and adenine nucleotides. – *Physiol. Plant.* 60: 129–132.
- Plaschke, J., Börner, A., Xie, D. X., Koebner, R. M. D., Schlegel, R. & Gale, M. D. 1993. RFLP mapping of genes affecting plant height and growth habit in rye. – *Theor. Appl. Genet.* 85: 1049–1054.
- Roberts, D. W. A. 1990. Identification of loci on chromosome 5A of wheat involved in control of cold hardiness, vernalization, leaf length, rosette growth habit, and height of hardened plants. – *Genome* 33: 247–259.
- Sutka, J. 1994. Genetic control of frost tolerance in wheat (*Triticum aestivum* L.). – *Euphytica* 77: 277–282.
- Tamiya, T., Okahashi, N., Sakuma, R., Aoyama, T., Akahane, T. & Matsumoto, J. J. 1985. Freeze denaturation of enzymes and its prevention with additives. – *Cryobiology* 22: 446–456.
- Tanino, K. K. & McKersie, B. D. 1984. Injury within the crown of winter wheat seedlings after freezing and icing stress. – *Can. J. Bot.* 63: 432–436.
- Vazquez-Tello, A., Ouellet, F. & Sarhan, F. 1996. The expression of the cold-responsive *Wcs120* gene is upregulated by phosphorylation of nuclear repressors. – *In* *Proceedings of the Annual Meeting of the Canadian Society of Plant Physiologists*, Communications FSAA: LL-96-05-01, Université Laval, Québec.
- Weisshaar, B., Armstrong, G. A., Block, A., Da Costa e Silva, O. & Hahlbrock, K. 1991. Light-inducible and constitutively expressed DNA-binding proteins recognizing a plant promoter element with functional relevance in light responsiveness. – *EMBO J.* 10: 1777–1786.
- Welin, B. V., Olson, Å., Nylander, M. & Palva, E. T. 1994. Characterization and differential expression of *dhn1/leal/rab*-like genes during cold acclimation and drought stress in *Arabidopsis thaliana*. – *Plant Mol. Biol.* 26: 131–144.
- Weretilnyk, E. A. & Hanson, A. D. 1990. Molecular cloning of a plant betaine-aldehyde dehydrogenase, an enzyme implicated in adaptation to salinity and drought. – *Proc. Natl. Acad. Sci. USA* 87: 2745–2749.
- Williams, M. E., Foster, R. & Chua, N. H. 1992. Sequences flanking the hexameric G-box core CACGTG affect the specificity of protein binding. – *Plant Cell* 4: 485–496.